

Summary of Results

External Quality Assessment of Water Microbiology Wastewater Scheme

Distribution Number: WW001 Sample Numbers: WW001A & WW001B

Distribution Date:	28 July 2025
Results Due:	29 August 2025
Report Date:	24 September 25
Samples prepared and quality control tested by:	Divya George Afifa Halim Nafeesa Hussain Cansev Katar Sabine Naujokat Zak Prior Jake Videlefsky
Data analysed by:	Nita Patel Zak Prior
Report compiled by:	Nita Patel Zak Prior
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**UK Health Security Agency
Food and Environmental Proficiency Testing Unit (FEPTU)**
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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 using proficiency testing samples, ensures the accuracy and reliability of wastewater analysis, a cornerstone of effective environmental protection.

FEPTU Quality Control:

To demonstrate homogeneity of the sample, a minimum of 10 freeze-dried vials, selected randomly from a batch, are tested in duplicate for selected parameters requiring enumeration or detection.

To demonstrate stability of the sample, a minimum of six freeze-dried vials, selected randomly from a batch, are examined throughout the distribution period for enumeration/detection parameters.

UKHSA uses methods stipulated in the Standing Committee of Analysts series of documents for methods stipulated in the Microbiology of Recreational and Environmental Waters: [Microbiology Working Group – Standing Committee of Analysts](#)

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 [Scheme Guide - Food and Environmental Proficiency Testing Unit](#)

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 distribution was produced using the principles and practices within ISO/IEC 17043:2023.

Total sent samples	18
Non returns	2

Sample: WW001A

Contents: *Escherichia coli* (4.5×10^5) (wild strain), *Pantoea agglomerans* (1.0×10^4) (wild strain), *Enterococcus faecium* (6.8×10^6) (wild strain), *Pseudomonas aeruginosa* (1.0×10^3) (wild strain) SARS-CoV-2 Omicron B.1.1.529 (quantification: undetermined by FEPTU)

Expected results:

All counts are expressed as colony forming units (cfu) per 100 mL (or genome copies per 100mL for SARS-CoV-2) unless otherwise stated

Guide to Scoring and Statistics:

Please note that there are not enough data results (<10) to calculate robust statistics and scores. Whilst this data has been presented as a guide to provide some indication of your performance, they should be interpreted with caution. Your results can be found on pages 5 and 6 of this report and to note the z-scores have been calculated using the wider fixed standard deviation 0.55.

Parameter	Coliform	<i>Escherichia coli</i>	Enterococci	<i>Salmonella</i> spp.	SARS-CoV-2
FEPTU median	4.1×10^5 (5.61 log ₁₀)	4.1×10^5 (5.61 log ₁₀)	6.0×10^6 (6.78 log ₁₀)	Not detected	Detected
No. results returned	5	8	8	4	7 (6 quantification)
Assigned value (Participants median all results)	1.95×10^6 (6.29 log ₁₀)	1.02×10^6 (6.01 log ₁₀)	6.40×10^6 (6.81 log ₁₀)	Not detected	9.47×10^5 (5.98 log ₁₀)
Minimum and maximum values	5.50×10^5 – 2.41×10^6	5.00×10^5 – 1.53×10^6	4.20×10^6 – 6.80×10^6		7.40×10^4 – 7.50×10^6
Expected range	6.17×10^5 – 6.17×10^6	2.93×10^5 – 3.54×10^6	2.02×10^6 – 2.02×10^7		2.36×10^4 – 3.79×10^7
Standard deviation* (log ₁₀)	0.11	0.27	0.03		0.80
Uncertainty of assigned value ($U(X_{pt}) = \log_{10}$)	0.07	0.13	0.01		0.41
Total number of censored values (greater than)	1	1	3		0
False Positives	N/A	N/A	N/A	0	N/A
False Negatives	0	0	0	N/A	0
Not examined	11	8	8	12	9

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

Sample: WW001B

Contents: *Citrobacter braakii* (2.1×10^2) (wild strain), *Enterococcus faecalis* (6.0×10^3) (wild strain), *Salmonella* Wentworth 11;Z₁₀;1,2 (4.9×10^3 per vial) (wild strain) and SARS-CoV-2 Beta B.1.351 (quantification: undetermined by FEPTU)

Expected results:

All counts are expressed as colony forming units (cfu) per 100 mL (or genome copies per 100mL for SARS-CoV-2) unless otherwise stated

Guide to Scoring and Statistics:

Please note that there are not enough data results (<10) to calculate robust statistics and scores. Whilst this data has been presented as a guide to provide some indication of your performance, they should be interpreted with caution. Your results can be found on pages 5 and 6 of this report and to note the z-scores have been calculated using the wider fixed standard deviation 0.55.

Parameter	Coliform	<i>Escherichia coli</i>	Enterococci	<i>Salmonella</i> spp.	SARS-CoV-2
FEPTU median	2.4×10^2 (2.38 log ₁₀)	0	5.9×10^3 (3.77 log ₁₀)	Detected	Detected
No. results returned	5	8	8	4	7 (6 quantification)
Assigned value (Participants median all results)	6.49×10^2 (2.81 log ₁₀)	-	8.44×10^3 (3.93 log ₁₀)	Detected	3.22×10^6 (6.51 log ₁₀)
Minimum and maximum values	1.40×10^2 – 3.00×10^3	-	6.40×10^3 – 1.30×10^4		6.69×10^4 – 2.06×10^7
Expected range	1.32×10^2 – 3.17×10^3	-	2.67×10^3 – 2.67×10^4		2.05×10^5 – 5.17×10^7
Standard deviation* (log ₁₀)	0.34	-	0.09		0.60
Uncertainty of assigned value ($U(X_{pt}) = \log_{10}$)	0.19	-	0.04		0.31
Total number of censored values (greater than)	0	-	0		0
False Positives	N/A	0	N/A	0	N/A
False Negatives	0	N/A	0	N/A	0
Not examined	11	8	8	12	9

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

Summary of all the participant's results for WW001A and WW001B for *Escherichia coli*

Lab	Results WW001A	UKHSA score	Z-score	Results WW001B	UKHSA score	Z-score
	1553100	2	0.33	0	2	N/A
	500000	2	-0.56	0	2	N/A
	540000	2	-0.50	0	2	N/A
	1080000	2	0.05	0	2	N/A
	1413600	2	0.26	<1	2	N/A
	>100000	2	N/A	0	2	N/A
	1020000	2	0.00	0	2	N/A
	531818	2	-0.51	0	2	N/A

Summary of all the participant's results for WW001A and WW001B for coliform

Lab	Results WW001A	UKHSA score	Z-score	Results WW001B	UKHSA score	Z-score
	1732900	2	-0.09	649	2	0.00
	2200000	2	0.09	3000	2	1.21
	550000	0	-1.00	140	2	-1.21
	2419600	2	0.17	1022	2	0.36
	>100000	2	N/A	380	2	-0.42

Summary of all the participant's results for WW001A and WW001B for Enterococci

Lab	Results WW001A	UKHSA score	Z-score	Results WW001B	UKHSA score	Z-score
	6800000	2	0.05	8500	2	0.00
	6400000	2	0.00	13000	2	0.34
	6600000	2	0.02	6700	2	-0.18
	>1000000	2	N/A	8400	2	0.00
	TNTC	N/A	N/A	9804	2	0.12
	>100000	2	N/A	6400	2	-0.22
	4200000	2	-0.33	7500	2	-0.09
	6150000	2	-0.03	9350	2	0.08

Summary of all the participant's results for WW001A and WW001B for *Salmonella* spp.

Lab	Results WW001A	UKHSA score	Z-score	Results WW001B	UKHSA score	Z-score
	Not detected	2	0	Detected	2	0
	Not detected	2	0	Detected	2	0
	Not detected	2	0	Detected	2	0
	Not detected	2	0	Detected	2	0

Summary of all the participant's results for WW001A for SARS-CoV-2

Lab	Results WW001A	UKHSA score	Z-score	Quantification results WW001A	UKHSA score	Z-score
	Detected	2	0	7.40E+04	2	-2.01
	Detected	2	0	5.11E+06	2	1.33
	Detected	2	0	7.50E+06	2	1.63
	Detected	2	0	4.24E+05	2	-0.64
	Detected	2	0	6.28E+05	2	-0.32
	Detected	2	0			
	Detected	2	0	1.43E+06	2	0.32

Summary of all the participant's results for WW001B for SARS-CoV-2

Lab	Results WW001B	UKHSA score	Z-score	Quantification results WW001B	UKHSA score	Z-score
	Detected	2	0	6.69E+04	1	-3.06
	Detected	2	0	1.49E+07	2	1.21
	Detected	2	0	2.06E+07	2	1.47
	Detected	2	0	2.29E+06	2	-0.27
	Detected	2	0	2.79E+06	2	-0.11
	Detected	2	0			
	Detected	2	0	3.72E+06	2	0.11

General comments

Scoring of this scheme:

The expected range for the quantification/enumeration results reported have been calculated using the median absolute deviation from the median (*MADe***) values in whole numbers, which are determined from the median result reported by participants' and take into account the following criteria:

- (1) median ± 2 *MADeS**
- (2) median ± 3 *MADeS**
- (3) median $\pm 0.5 \log_{10}$ units

If the ranges in (1) and/or (2) are less than the value of the median $\pm 0.5 \log_{10}$ units then the expected range is extended as described in (3).

******The median absolute deviation from the median value is a robust estimate of the standard deviation (*S**). It is only possible to calculate a precise estimate of the *S** if more than 20 participants report a value.

	Score
Expected range within the range according to criteria (1)	2
Outlying results (1) within the range of criteria (2) but not within criteria (1)	1
Outlying results (2) outside the range of criteria (2)	0

Statistical evaluation

Participants are advised that for a robust statistical evaluation at least 20 reported results are required for a parameter. When statistical calculation is based on 10 – 19 results, they should be interpreted with caution as they may be overly influenced by outlying results. This is the reason why the standard deviation of the enumeration results reported can be wide.

Information of importance

Please refer to this website to obtain the latest information for your proficiency testing:
<https://www.feptu.org.uk/> or use the following links for more specific detail:

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](#)

Questionnaire analysis

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 for comparing data.

The data shown below is for information only and does not attempt to compare the performance of the various methods used by laboratories in this PT distribution. Not all laboratories provided information to all the questions.

The below table provides information on the published methods followed and the number of users for coliform, *Escherichia coli*, Enterococci and *Salmonella* spp. examinations.

Coliform		<i>Escherichia coli</i>		Enterococci		<i>Salmonella</i> spp.	
ISO 9308-2:2012	3	ISO 9308-2:2012	1	ISO 7899-1:1998	1	ISO 19250:2010	1
SCA: Microbiology of recreational and Environmental Waters Part 3	2	ISO 9308-1:2014	1	ISO 7899-2:2000	2	SCA: Microbiology of recreational and Environmental Waters Part 8	3
		SCA: Microbiology of recreational and Environmental Waters Part 3	5	SCA: Microbiology of recreational and Environmental Waters Part 4	4		
				Enterolert-E	1		

SCA: Standing committee of analysts: [Microbiology Working Group – Standing Committee of Analysts](#)

The below table provides information on the type of methods used and the number of users for coliform, *Escherichia coli*, Enterococci and *Salmonella* spp. examinations.

Method type	Coliform	<i>Escherichia coli</i>	Enterococci	<i>Salmonella</i> spp.
Membrane filtration	3	6	7	
Most probable numbers	2	1		
Membrane filtration followed by enrichment - XLD				4

The following method analysis is for the SARS-CoV-2 examination as provided by five laboratories:

Standard guideline followed:

The Lab followed the indications from the OMS and the Portuguese Health General-Directorate Center for Disease Control and Prevention
In-house method
RT-qPCR for detection of SARS-CoV-2 in wastewater V.2 dx.d

In-house validated method: <https://doi.org/10.1128/msystems.00353-21>

In house methodology developed as part of the Northern Ireland wastewater surveillance programme.

The volume examined varied with 15mL, 20mL, 80mL, 100mL and 200mL.

The volume concentrated varied with 0.5mL, 1.2mL, 15mL and 100mL.

The concentration methods used is shown in the table below:

Samples are centrifuged for 10 minutes at 10000xg to remove solid wastes. 1×10^5 copies of IBV is added (used as an extraction control). A first filtration is performed using a 0.45 μ m filter. The filtrate is submitted to a new filtration using a 1 μ m filter. The filter is removed and placed in a 15 mL tube that contains a 2 mL saline solution. For RNA extraction the lab used 200 μ L of the suspension.	Microfiltration
Filtration concentration using Vivaspin units, (Vivaspin® 20 10 kDa MWCO filtration units).	Ultrafiltration
20mLs of sample is centrifuged then 15mLs of supernatant is placed in an Amicon filter and centrifuged again. 180 μ L of the concentrate extracted. 20 μ L of the PRRSV internal control is added to the 180 μ L concentrate prior to extraction.	Ultrafiltration
Size-exclusion Filter centrifugation Briefly, following removal of solids by centrifugation at 2,000 $\times$ g, raw sewage was filtered through a 0.45- μ m filter (Nalgene 100-ml Rapid-Flow) and concentrated down to 1.2 ml using Vivaspin 20 centrifugal concentrators with a 10 kDa molecular weight cutoff and following manufacturer's instructions.	Ultrafiltration
Concentrated using InnovaPrep CP Select Concentrating Pipette (https://www.innovaprep.com/products/cp-select). Ultrafilter tips (150 kDa MWCO), purged using an elution buffer containing 0.075% Tween 20 in 25 mM Tris.	Ultrafiltration

The primers used is shown below:

Primers used: N1FW and N1RV, as well as IBVFW and IBVRV
¢ Forward primer (N1-F): GACCCCAAATCAGCGAAAT (50 μM stock) ¢ Reverse primer (N1-R): TCTGGTTACTGCCAGTTGAATCTG (50 μM stock) ¢ Probe (N1-P): ACCCCGCATTACGTTTGGTGGACC with FAM-MGB (5 μM stock)
Primer name Sequence (5'-3') 2019-nCoV_N1 - Forward Primer GAC CCC AAA ATC AGC GAA AT 2019-nCoV_N1 - Reverse Primer TCT GGT TAC TGC CAG TTG AAT CTG 2019-nCoV_N1 - Probe FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1 PRRSV1 - Forward Primer CAG GAC TTC GGA GCC TCG T PRRSV1 - Reverse Primer AGC AAC TGG CAC AGT TGA TTG A PRRSV1 - Probe /5Cy5/ACG AGC TGT /TAO/TAA ACG AGG A/3IAbRQSp/

in house
Commercial kit - Qiagen SARS-CoV-2 N1+N2 Assay Kit (https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/sars-cov-2-assay-kit).

Information on the RNA details is shown below:

RNA extraction reagents	RNA extraction equipment	RNA volume
Kits used for RNA extraction: GeneJET RNA Purification Kit; RTP® Pathogen Kit	Centrifuges, filtration equipment, micropipettes. The amplification was performed in a CFX Real Time equipment.	5 µL
Maxwell® RSC GMO authentication kit cartridges Promega Lysis Buffer MC501	Maxwell® RSC 48 Instrument	5µl
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	Kingfisher Flex instrument with 96 well head.	5µL
MagMax Viral RNA extraction kit	Kingfisher Duo	5uL out of 90uL extracted
Roche MagNA Pure 96 DNA and Viral NA Large Volume Kit	Roche MagNA Pure 96 Instrument	Three PCR reps of 3µL and three of 6µL

Details on the mastermix is shown below:

Mastermix: NZYSpeedy One-step RT-qPCR Probe Master Mix (NZYTech - 10 µL; 1 µL primer; 4 µL H2O; Total - 15 µL reaction volume)
For each 20µl reaction = - 7.08 µl nuclease-free water - 5.00 µl TaqMan Virus Fast one-step qRT-PCR mix - 1.00 µl BSA (from RNA Ultrasense kit) - 0.32 µl MgSO₄ (from RNA Ultrasense kit) - 0.20 µl N1-F primer (50 µM stock) †' Final: 0.5 µM - 0.40 µl N1-R primer (50 µM stock) †' Final: 1.0 µM - 1.00 µl N1-P probe (5 µM stock) †' Final: 0.25 µM Total master mix volume per reaction: 15µl + 5µl of sample RNA
Reagent Luna Warm Start RT Enzyme Mix 10 µL Primer Warm Start RT Enzyme Mix 1 µL Primer/Probe Mix 1.5 µL BSA (20µg/µL) 0.1 µL Water 2.4 µL Primer and probe stocks are at 100µM
For Primary Reaction (Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase) 2xReaction Mix: 12.5uL

Enzyme: 1uL
Sense primer: 0.5uL
Antisense primer: 0.5uL
Nuclease free Water: 5.5uL

Nested Reaction
Dream Taq Master Mix: 12.5uL
Sense primer: 1uL
Antisense primer: 1uL
Nuclease free Water: 8.5uL

Applied Biosystems AgPath One-Step RT PCR reagent
(<https://www.thermofisher.com/order/catalog/product/AM1005>).

Per reaction: 12.5 µL of 2x RT-PCR buffer, 1 µL of 25x Enzyme mix, 0.25 µL of BSA, DEPC - 4.25 µL per sample for 6 µL RNA input, 7.25 µL per sample for 3 µL RNA input.

The cycle run details is shown below:

50°C - for 20 sec
95°C - 3.00 m
95°C - 10 sec
58°C - 45 sec
x40

Reverse Transcription: 50°C for 30 min (1 cycle)
Initial Denaturation: 95°C for 20 s (1 cycle)
PCR Cycling (45 cycles):
Denaturation: 95°C for 3 s
Annealing/Extension: 60°C for 45 s (with data collection)

Temperature Time Cycles
55°C 10 min 1
95°C 1 min
95°C 10 sec 40
55°C 30 sec

For Primary Reaction
50°C for 30 minutes
94°C for 2 minutes
40 cycles 94°C for 15 seconds
50°C for 30 seconds
68°C for 1 minute
68°C for 5 minutes
10°C Hold

For Nested Reaction
94°C for 2 minutes
40 cycles 94°C for 15 seconds
50°C for 30 seconds
68°C for 30 seconds
68°C for 5 minutes
10°C Hold

1x cycle of reverse transcription at 50oC for 10 mins, 1x cycle of RT denaturation at 95oC for 10 mins, 45x cycles of denaturation at 95oC for 10 secs and combined annealing and extension at 60oC for 30 secs.

The amplification platform used along the lower limit of detection (LOD) and quantification (LOQ) is shown in the table below:

Amplification platform	LOD	LOQ
Real Time PCR CFX	100 copies/100 mL	100 copies/100 mL
Stratagene Mx3005P	Not established	Not established
ABi 7500	1,316 gc/L (based on original SEPA data)	11,368 gc/L (based on original SEPA data)
Mastercycler Nexus X2	Information not accessible at the moment	Information not accessible at the moment
Roche LightCycler 480 II.	2 gene copies per reaction.	3.5 gene copies per reaction.

Details about other viruses tested is shown below:

Norovirus GI and norovirus GII in wastewater
Poliovirus, non polio enteroviruses, influenzavirus - Hepatitis E and Norovirus and work in progress
Rotavirus, Norovirus (GI,GII,GIV,GIX), Hepatitis A virus (HAV), Hepatitis E virus (HEV), Enterovirus A71, Enterovirus D68, Sapovirus, Aichivirus, Astrovirus.
Influenza, Norovirus, RSV, EV-D68, Polio, HAdV-F41.

1/5 of the laboratories stated they already participate in a SARS-CoV-2 proficiency testing scheme.

Conclusions on this PT exercise is:

- Due to the low number of data sets reported for each examination, the performance of a laboratory must be interpreted with caution.
- The low number of data sets used for statistical calculations can result in wider expected range, standard deviation and uncertainty.
- The variability of how laboratories examine wastewater for SARS-CoV-2 is clear from the analysis undertaken on the methods, this is expected as there is no approved published method for laboratories to follow.
- In general the overall performance of laboratories in this PT exercise is very good with >95% of the results being correct.

The next distribution for the wastewater scheme is scheduled for 16 February 2026.

The organisers would like to thank the participants for providing additional methodology information and to David Walker at Cefas for providing expert comments and for supporting this scheme.

End of report