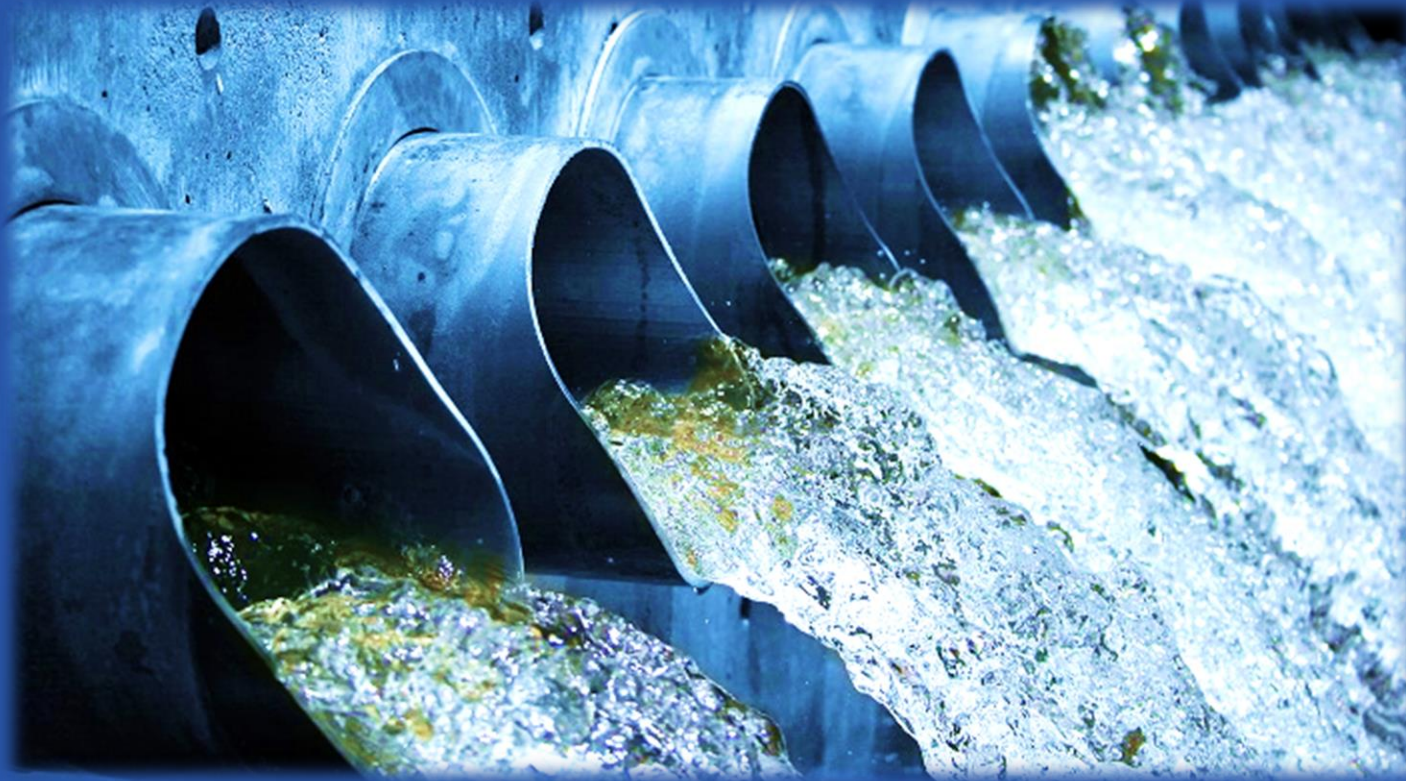




FEPTU

Food & Environmental
Proficiency Testing Unit

Operated by UK Health Security Agency



Waste pilot distribution update

Zak Prior & Nita Patel, October 2024

Contents

- What is Wastewater?
- Background
- Sample design
- Results
- Questionnaire
- Summary



What is Wastewater

- It is used water from homes, industrial and commercial sources
- Effluents can be treated or untreated and released to a variety of environments, such as lakes, ponds, streams, rivers, estuaries, oceans, storm runoff
- Made up of human feces, protein, fat, vegetable and sugar material from food preparation
- Faecal coliform bacteria in human waste is typically harmless, but there are pathogens that cause human disease
- Disease-causing pathogens in wastewater can pollute beaches and contaminate shellfish populations. These can be bacteria or viral

Background

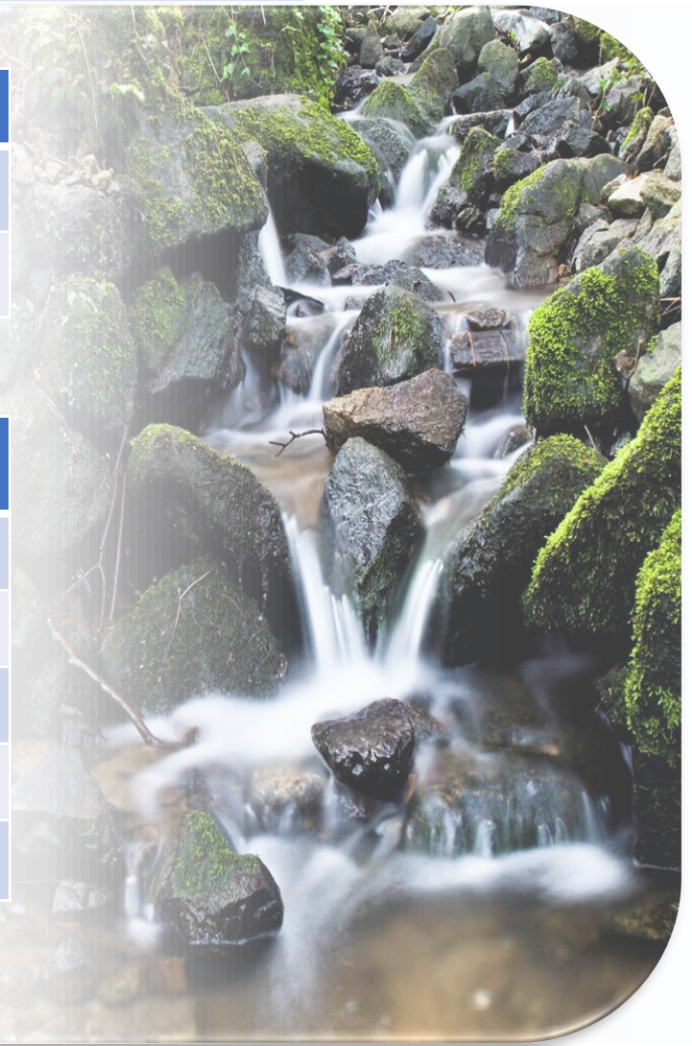
- We launched a pilot to see if there was an interest in such a scheme
- We also wanted to learn how to best provide such a scheme to our participants
- Samples were designed, prepared and tested by in UKHSA microbiologists
- Realistic levels of target organisms with background flora representative of real samples
- Samples were made in a freeze-dried matrix to accommodate high levels
- Quality control tests for stability and homogeneity to reflect commonly used methods



Sample design

Sample	Contents
WW00PA	<i>E. coli</i> , <i>Citrobacter koseri</i> , <i>Enterococcus faecalis</i> and <i>Salmonella</i> Enteritidis
WW00PB	<i>E. coli</i> , <i>Pantoea agglomerans</i> , <i>Enterococci faecium</i> , <i>Pseudomonas aeruginosa</i> and SARS-CoV-2 Omicron (B.1.1.529)

Examinations	WW00PA FEPTU Median	WW00PB FEPTU Median
<i>Escherichia coli</i> in 100mL	8.9x10 ⁶	8.8x10 ⁶
Total coliforms in 100mL	1.8x10 ⁷	8.8x10 ⁶
Enterococci in 100mL	8.5x10 ⁵	5.7x10 ⁶
<i>Salmonella</i> spp. per L	Detected	Not detected
SARS-CoV-2 per 200mL	Not detected	Detected



Results WW00PA

Test Parameter	<i>Escherichia coli</i>	Total coliforms	Faecal coliforms	Enterococci	<i>Salmonella</i> spp.	SARS-CoV-2	Lab ID	<i>Salmonella</i> spp. result		
FEPTU median	8.9x10 ⁶	1.8x10 ⁷	8.9x10 ⁶	8.5x10 ⁵	Detected	Not detected	9	Detected		
No. results returned	11	9	8	11	7	2	Lab ID	Sars-Cov-2 result	CT value	Genomic units
Assigned value (Participants median all results)	Not determined due to insufficient data				Detected	Not detected	1853	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	2332	Not Detected	N/A	<7 copies/mL
Standard deviation* (log ₁₀)	Not determined due to insufficient data				N/A	N/A	1519	Detected		
Total number of censored values (greater than)	5	5	4	5	N/A	N/A	1784	Detected		
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				

Results WW00PB

Parameter	<i>Escherichia coli</i>	Total coliforms	Faecal coliforms	Enterococci	<i>Salmonella</i> spp.	SARS-CoV-2
FEPTU median	8.8x10 ⁶	8.8x10 ⁶	8.8x10 ⁶	5.7x10 ⁶	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

Lab ID	<i>Salmonella</i> spp. result		
9	Not Detected		

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
1459	Not Detected		
1519	Not Detected		
1784	Not Detected		

Questionnaire

- Standards

***E. coli*, coliform and faecal coliform**

Of the ten responses received, the majority used microbiology of drinking water part 3 and/or 4 guidelines or standards

Enterococci

Of the ten responses received, the majority used ISO 7899-1 or 2:2000 Water quality – Detection and enumeration of enterococci. Most laboratories used multiple guidelines or standards

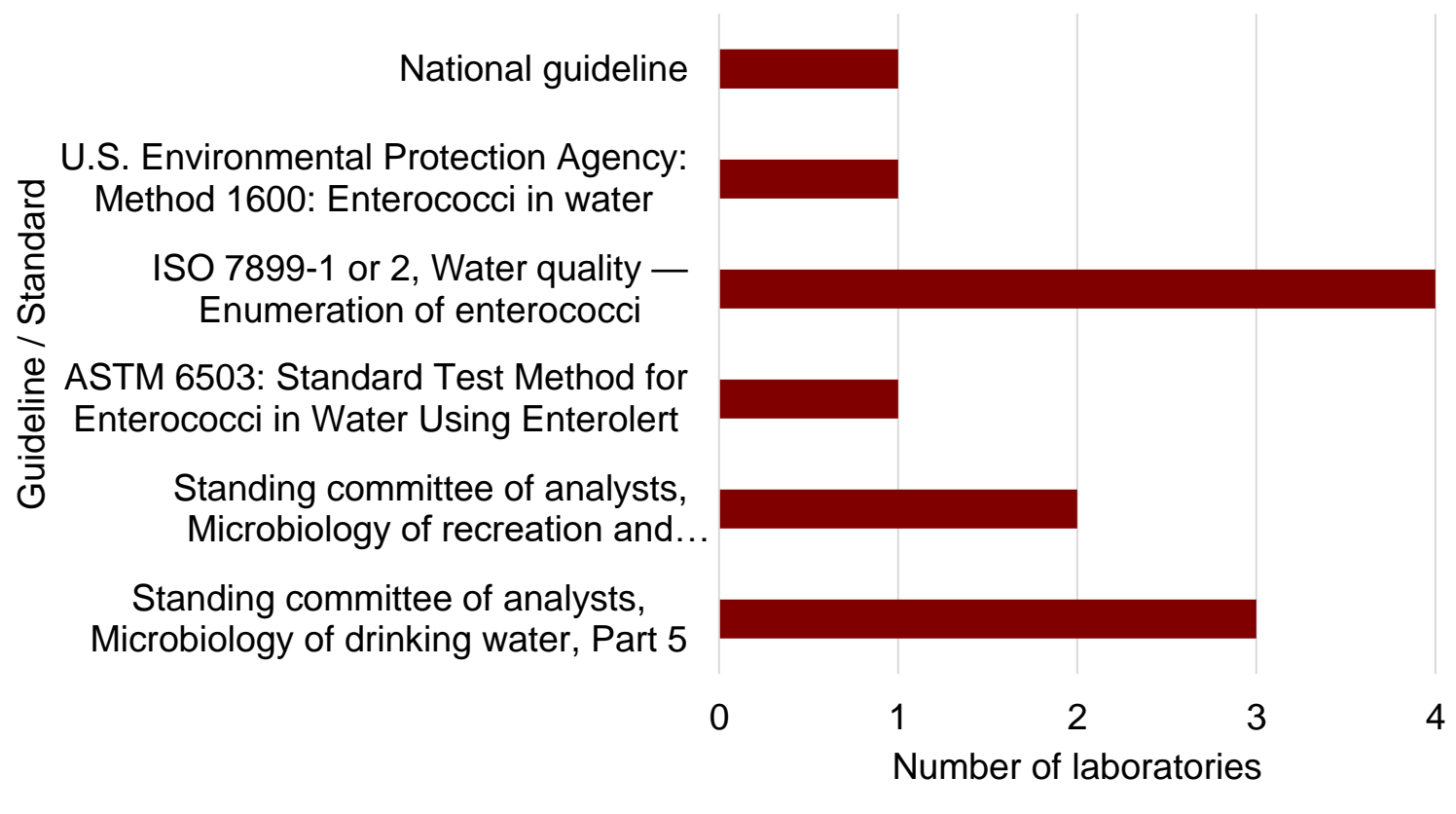
***Salmonella* spp.**

Of the six responses three laboratories used Microbiology of Recreational and environmental water part 3:19250:2010 Water quality - Detection of *Salmonella* spp.

SARS-CoV-2

Of the two responding laboratories, neither used any specific guidelines or standards

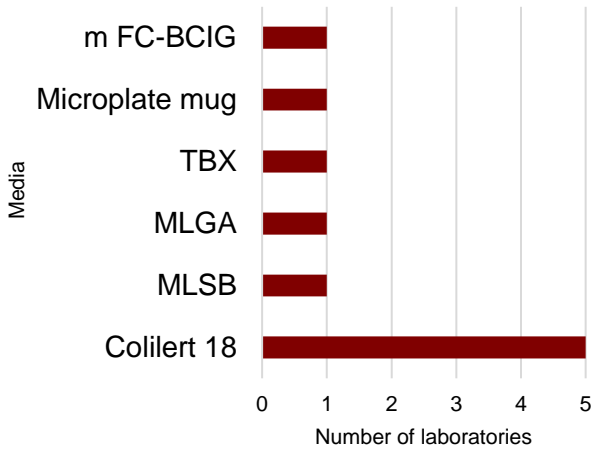
Graph 1. Enterococci



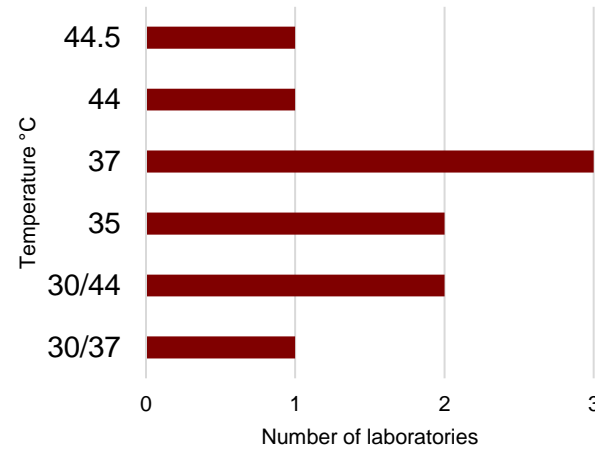
Questionnaire - methods

Enumeration e.g. *E. coli*

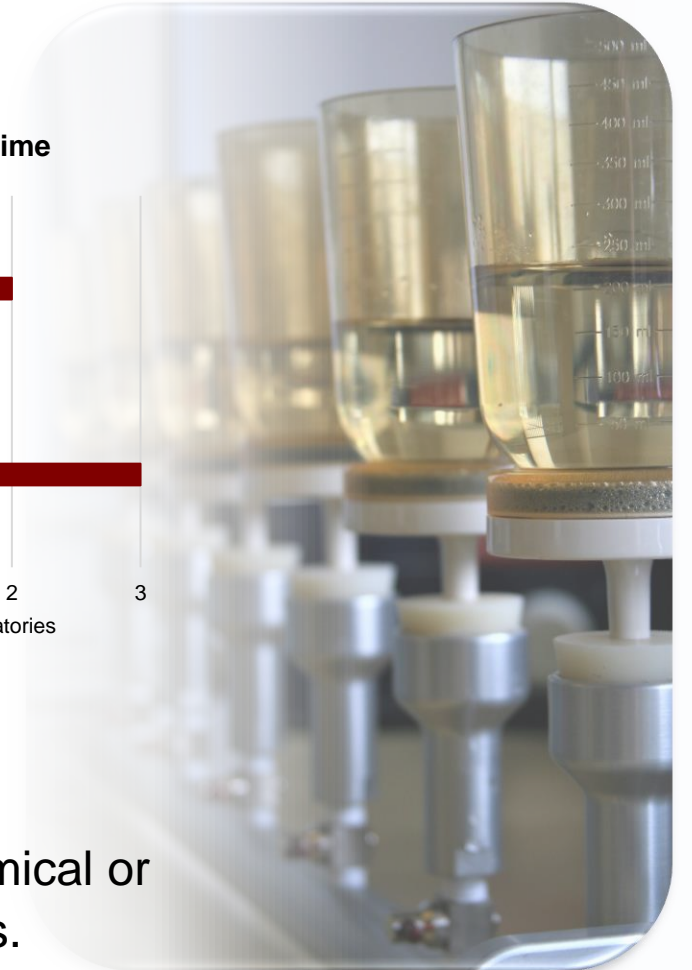
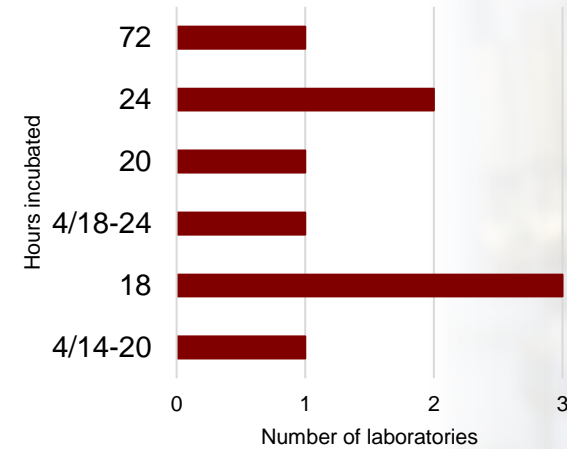
Graph 2a: Media used



Graph 2b: Temperature



Graph 2c: Incubation time



- 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.

Questionnaire

- methods continued

***Salmonella* spp vs SARS-CoV-2**

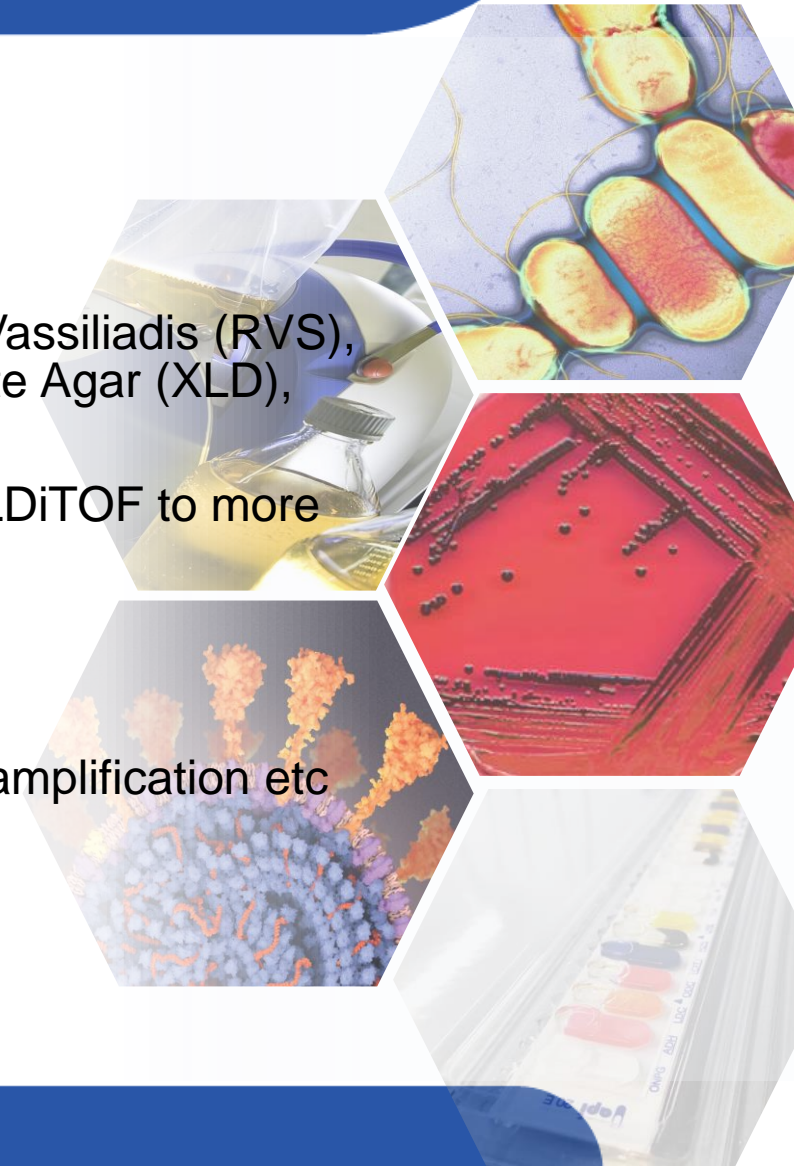
Salmonella methods are very similar amongst labs

- The processing consisting of using 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.
- However, the confirmations vary between rapid methods such as PCR and MALDI-TOF to more traditional culture such as serology and API20Es.

SARS-CoV methods vary amongst labs

- From sample size tested (1.2 – 50 mL), concentration step, extraction method, amplification etc
- However, Both used in house 'N' gene targeting assays.

Probably due to Standard methods availability



Summary

- Overall participants did well in the pilot in the detection of target organisms
- Difficult to conclude on enumeration results due to low participation
- There are many standard and in house methods, maybe because wastewaters are difficult to determine as a water type
- Due to this study, we will be launching wastewaters as a non-accredited scheme next year
- Introduce a guide for dilutions to reduce the number of censored values
- We will amend the reconstitution instructions to reduce risk of spillage
- For more detail we have a report which is available on request

Thank you for your attention

WASTEWATER SCHEME

(non accredited)

water and environmental microbiology examinations

Please note this scheme is being offered for one year to establish the demand – this will be reviewed once more information is known

Sample schedule for 1 April 2025 to 31 March 2026

Distribution number	Sample numbers	Dispatch date	Date results due by	Examinations and enumerations <u>required</u>
				<i>Salmonella</i> spp. per 1L SARS-CoV-2 per 1mL All other counts are per <u>100mL</u>
WW001	WW001A WW001B	28/07/2025	29/08/2025	<i>Escherichia coli</i> Total coliforms Faecal coliforms Enterococci <i>Salmonella</i> spp. detection SARS-CoV-2 detection or enumeration

Email: foodeqa@ukhsa.gov.uk