

# Department for Environment Food & Rural Affairs

1.	Purchase Order	To be provided once contract is in place		
	Number	Assessment of the presence of aquatic antimicrobial resistance (AMR) across England		
		- Lot 2		
2.	Customer	Environment Agency		
		Horizon House		
		Deanery Ro Bristol		
		BS1 5AH		
3.	Contractor(s)	Bangor University		
		Bangor		
		Gwynedd		
		LL57 2DG		
		Registration number: 1141565		
		Charity overview, BANGOR UNIVERSITY - 1141565, Register of Charities - The Charity		
		Commission		
4.	Co-Funder(s)	N/A		
F	Defre Orer	The following Defre Crown members will receive the herefit of the Deliverships. Environment		
5.	Defra Group	Agency		
	Weiliber S	Agency		
6.	The Agreement	This Order is part of the Agreement and is subject to the terms and conditions appended at		
		Appendix 1 and shall come into effect on the Start Date.		
		Unless the context otherwise requires, capitalised expressions used in this Order have the		
		same meanings as in the terms and conditions.		
		The following documents are incorporated into the Agreement. If there is any conflict, the following order of precedence applies (in descending order):		
		a) this Order;		
		b) the terms and conditions at Appendix 1; and		
		c) the remaining Appendices (if any) in equal order of precedence.		
7.	Deliverables	Goods:		
		None		
		Services:		
8.	Milestone Delays	N/A		
•••	(Clause 18.2.10)			
9.	Start Date	2 <sup>nd</sup> Sentember 2024		
10.	Expiry Date	20 <sup>th</sup> December 2024		
11.	Extension Period			
	(Clause 5.2)	N/A		
12.	Charges	The Charges for the Deliverables shall be as set out in Appendix 3 – Charges. Unless and to		
		the Agreement.		

13. Payment including Payment by Co- funder(s)	Payments will be made in pounds (GBP) by BACS transfer using the details provided by the supplier on submission of a compliant invoice.		
Tunder(3)	It is our preference that all invoices are sent electronically, quoting a valid Purchase Order		
	<ul> <li>Any invoices that are submitted that do not meet the following criteria will not be processed: <ul> <li>1 PDF per invoice (no larger than 4mb in size) – all supporting documentation must be included in that PDF (no additional separate supporting documentation as a separate file).</li> <li>Multiple invoices can be attached to one email; however, as above we can only accept 1 invoice per PDF (and no additional supporting files).</li> <li>Invoices must be dated.</li> <li>Invoices must quote a valid Purchase Order.</li> <li>Invoices must have a breakdown of what is being billed.</li> <li>Invoices must include the total before and after VAT.</li> </ul> </li> </ul>		
14. Customer's Authorised	For general liaison your contact will continue to be		
Representative(s)			
	or, in their absence,		
15. Contractor's	For general liaison your contact will continue to be		
Authorised Representative			
	or, in their absence,		
16. Co-funder's	N/A		
Authorised Representative			
17. Optional Intellectual Property Rights ("IPR") Clauses	The Customer has chosen Option <b>B</b> in respect of intellectual property rights provisions for the Agreement as set out in the terms and conditions.		
18. Contractor's general liability cap	The liability of the Contractor as set out in Clause 16.2.1 of the terms and conditions is limited to the greater of:		
	(a) an amount equal to 150% of the Charges paid or payable to the Contractor; and		
	(b) £5,000,000		
19. Progress Meetings			
and Progress Reports	The Contractor shall attend progress meetings and provide reports to the Customer as outlined in the specifications		
20. Address for notices	Customer: Contractor:		

21.	Key Personnel of the Contractor	Key Personnel Role: Key Personnel Name: Contact Details: Contractor's Project Manager			
22.	Procedures and Policies	For the purposes of the Agreement:			
		The Customer's security / data security requirements are: Contractor must comply with all UK legal requirements regarding GDPR, and data security and commercial confidentiality where applicable			
		The Customer's additional sustainability requirements are: <u>Environment Agency quality policy and commitment to sustainable business - GOV.UK</u> (www.gov.uk)			
		The Customer's equality and diversity policy/requirements and instructions related to equality Law [and] environmental policy is: <u>Equality and diversity - Environment Agency - GOV.UK (www.gov.uk)</u>			
		The Customer's health and safety policy is: Contractor must comply with all UK Health and Safety legal requirements			
23.	Commercial	Clause 11 (Commercial Exploitation) shall apply to this Agreement:			
	Exploitation	Yes: 🗆			
	(Clause 11)	No: 쯔			
24.	Special Terms	N/A			
25.	Additional	N/A			
	Insurance				
26.	Protection	The further data protection provisions as contained at Annex 1 of the Terms and Conditions			
	Provisions	Yes:			
		No: 🛱			
L					

Signed for and on behalf of the **Customer** 

Signed for and on behalf of the Contractor

#### Appendix 1: R&D Terms and Conditions

The terms and conditions applicable to this requirement can be found on the website below

Research and development terms and conditions - GOV.UK (www.gov.uk)

# Appendix 2: Specification/Description

# 1. Background to the requirement

The Environment Agency is undertaking a research project to understand the spatial presence and distribution of antimicrobial resistance (AMR) in the aquatic environment across in England.

In order to investigate the presence of AMR in the aquatic environment we will carry out sampling and analysis across a variety of surface waters in three co-aligned campaigns.

- Campaign A: A national survey of sample sites (rivers & streams) across England sampled once.
- Campaign B: A focused study of selected coastal bathing waters in one area sampled six times.
- Campaign C: A focused study of a stretch of the river Trent sampled in a single day at 18 closely located sites.

The procurement of this work is split into two lots:

- Lot 1: Sampling and distribution (SC240029) of surface waters, required to be conducted across England over the autumn from September November 2024
- Lot 2: Lab analysis (SC240030) to be conducted by a laboratory based in mainland GB from September December 2024

# This specification relates to Lot 2: Lab analysis (SC240030). The contractor will receive samples from the supplier procured for Lot 1: Sampling and distribution (SC240029).

# 2. Specific deliverables: aquatic AMR analysis

#### 2.1. Sampling receipt

Grab samples of surface waters will be sampled, bottled and transported to the contracted lab for analysis (delivered by sampling contractor/ courier; SC240029). Surface water samples will include freshwater river water **and** saline coastal water. A spreadsheet detailing sample IDs will be provided.

#### 2.2. Sample number, frequency and limits

Overall, 219 water samples are to be received across all three campaigns. Specifically,

- Campaign A: 165 samples
- Campaign B: 36 samples
- Campaign C: 18 samples

Sampling will commence from 15<sup>th</sup> September 2024 and complete within a maximum of 8 weeks (by 6<sup>th</sup> November 2024) with samples arriving at labs by 12PM the next day.

Sampling for any campaign will take place between Sunday to Wednesday, with Monday and Tuesday sampling preferred.

Across all sampling teams, on a given day a maximum of 20 samples and minimum of 6 samples may be taken (to maintain lab capacity). Please advise in your response if lab capacity can achieve or exceed these proposed limits (bearing in mind 20 samples equates to 60 filtrations and DNA extractions).

Therefore, this programme will require a minimum of 11 and maximum of 32 sampling days depending on team sampling effort. Samples will arrive at labs by 12PM after each sampling day.

Upon award a detailed sampling plan with specific dates and sample numbers will be provided to the analysis contractor by 10th September 2024 to enable preparations to receive samples, as advised by the contractor for lot 1 (SC240029).

Samples from Campaign C are to be collected during a dry period when the river level is below a certain threshold, therefore the date for the arrival of these samples will be confirmed closer to the sampling week (this will be a day within the period 15/9/24 - 6/11/24).

#### 2.3. Sample volume

For each sample, bottles will be filled with water for analysis – see table below for details.

Bottle	Туре	Volume to collect	Purpose
WQ	Plastic	1 litre	Water quality analyses
Bac	Sterile, plastic	2 litres	Bacterial phenotypes
DNA	Sterile, plastic	3 litres	DNA analyses

In total, 6 litres of water will be collected for each sample.

For each day a maximum of 20 collections of 6 litres are to be received (120 litres per day). And in total across all 3 campaigns, 219 collections of 6 litres are to be received (1,314 litres).

### 2.4. Sample logistics

Samples will be transported for analysis in refrigerated transport and will arrive for analysis within 24 hours (i.e. to arrive by 12PM the next day). Therefore, samples may arrive to the contractor for analysis commencing on Monday, Tuesday, Wednesday and Thursday each week.

To ensure delivery within 24 hours, the analysis lab must be located in mainland GB.

Samples will be stored in appropriate containers for microbiological, molecular and nutrient analysis.

All samples will be labelled with a unique barcode, identifying location (site ID), date and sample ID. Samples will use the sample ID format:

- PSSS\_N\_X
  - Where "P" is the sampling Campaign program (A, B or C)
  - Where "SSS" is the 3-digit sampling site ID with leading zeroes (e.g. 001, 002... 018 ... 165 etc)
  - Where "N" corresponds to the nth time this site has been sampled in the campaign.
  - Where "X" is a code corresponding to the label of the sampling bottle series (i.e. "WQ" for WQ/nutrient analysis, "Bac" for Bacteria, "DNA" for DNA extraction).

Please note that for all samples in Campaign A and C, the N value will be 1 as they are only being sampled once. While for samples in Campaign B, N will range from 1-6 (because 6 samples will be taken at each site).

For example, for campaign A at site A028, the first time the site is sampled, the 2-litre bottle for bacterial phenotypic analysis would be labelled: A028\_1\_Bac

All analysis is to be completed within 48 hours of receiving the sample.

Prior to kick-off meeting (by 6<sup>th</sup> September 2024), the contractor will develop/ provide documented standards and protocols for analysis. No work may be subcontracted to another party unless with the express permission of the Environment Agency.

### 2.5. Water filtration

- Samples with high levels of suspended solids should be passed through a Seward stomacher strainer bag (pore size of 0.5mm) and the resulting liquid filtrate should be treated as with other surface water samples with low levels of suspended solids as below.
- Samples of surface water should be examined via membrane filtration using 0.45 µm poresize gridded sterile cellulose ester or PES 47 mm diameter membranes (e.g., Pall GN-6 membranes, #66068) in sterile water funnels on a vacuum pump membrane filtration manifold for a range of volumes specified in section 2.7.
- For sample volumes ≤10 ml, additional Peptone Saline Diluent (Thermofisher, UK) should be added to the funnel prior to the surface water sample being added (to result in a final filtered volume of ~50 ml) to enable even distribution of micro-organisms across the membrane.

### 2.6. Water quality analysis

For water quality analysis, each 1 litre sample, labelled 'WQ' (see 2.3 for details), is required to be tested for:

- pH
- Turbidity
- Suspended solids
- Chemical oxygen demand (COD) (settled)
- Total Nitrogen as N
- Ammoniacal Nitrogen as NH4
- Nitrite as N
- Nitrate as N
- Orthophosphate as P
- Alkalinity to pH 4.5 as CaCO<sub>3</sub>
- Conductivity at 25 °C

Analysis data is to be reported within 1 week of receiving the sample. Reports will be in a spreadsheet format prescribed by the Environment Agency. Where recorded, any raw data (scanned in-lab record sheets, data files, image files) should also be provided.

### 2.7. Bacterial phenotypic analysis

Bacterial phenotypic analysis detailed in the following table is required for each sample taken from the bulk 2 litre (bottle 'Bac') sample provided.

	Organism Agar		Plates of surface water to test	
1	Total <i>E. coli</i> Tryptone Bile Glucuronic Ag (TBX)		1 plate per volume filtered* (Volumes tested to cover measurement range from 1 - 100,000 cfu)	
2	Putative ESBL <i>E. coli</i>	TBX + 4mg/L cefotaxime	5 plates per volume filtered (Volumes tested to cover measurement, range from 1 - 10,000 cfu)	

\*For example, if four volumes of 0.01ml, 0.1ml, 1ml and 10ml were used (to capture the full measurement range) 4x1 plates would be required per sample for the total *E. coli* measurement.

# 2.7.1. Protocol information

# (1) Total *E. coli*

For bacterial colony counts of total *E. coli* are to be taken from filter membranes of water placed on Tryptone Bile Glucuronic Agar (TBX) and incubated at 37°C for 4 hours and 44°C for 14-16 hours. Colony confirmation is to be undertaken on Blue/green colonies, and results reported as colony forming units (cfu) per 100 ml.

# (2) Putative ESBL E. coli

Putative extended spectrum beta-lactamase (ESBL) producing *E. coli*, hereafter referred to as ESBL *E. coli*, are to be detected from filter membranes over 5 plates using TBX with **4 mg/L cefotaxime** agar (as suggested by the WHO Tricycle protocol for ESBL *E. coli* (https://www.who.int/publications/i/item/9789240021402).

To aid recovery of putative ESBL *E. coli* from the water, these media are to be incubated at 37°C for 18-20 hours rather than 44°C as per ISO standard and WHO tricycle method (<u>https://www.who.int/publications/i/item/9789240021402</u> and <u>https://www.iso.org/standard/85903.html</u>). Potential colonies are then to be sub-cultured to fresh agar plates and incubated at 44°C to confirm identity of blue/green colonies, and results reported as colony forming units (cfu) per 100ml.

### 2.7.2. Phenotypic data/ analysis reporting & isolate (putative ESBL *E. coli*) storage

For each filtration the following data is required: volume filtered (in ml)

For each agar plate counted the number of colonies is required in cfu/ml. Where multiple plates are counted, please provide values from each plate so variation can be determined.

Analysis data will be reported within 1 week of sample receipt.

Up to 5 resistant, putative ESBL *E. coli* isolates per sample are to be stored in appropriate medium for storage (frozen) & for later whole genome analysis. Stored isolates are to be assigned an isolate number and labelled accordingly with a numeric suffix. E.g. Five isolates from Sample ID "A028\_1\_Bac" labelled as:

- A028\_1\_Bac1
- A028\_1\_Bac2
- A028\_1\_Bac3
- A028\_1\_Bac4
- A028\_1\_Bac5

Details about each isolate is to be recorded (e.g. sample ID, volume filtered for plate, isolate ID, etc.). Reports will be in a spreadsheet format prescribed by the Environment Agency.

#### 2.8. DNA extraction

From each bulk sample of 3L (bottle 'DNA'), it is required that the water is to be filtered by three 1L filtrations for subsequently three DNA extractions. Samples should be labelled with a numeric suffix. For example, the 3L bottle for "A028\_1\_DNA" would lead to three extractions labelled: "A028\_1\_DNA1", "A028\_1\_DNA2" and "A028\_1\_DNA3".

Filtrations are required to use 0.45 µm gridded sterile Polyethersulfone (PES) 47 mm diameter membranes. Where water samples contain high levels of sediment and do not pass easily through the filter membranes, smaller volumes are to be filtered and the volume recorded.

Filters should be stored at -20°C in sterile bags until DNA extraction takes place.

#### 2.8.1. DNA extraction, quantification and normalization

DNA should be extracted from replicates in separate runs to avoid any reagent/ extraction errors affecting all replicates of the same sample.

To ensure randomised analysis, an order for replicate submission will be provided by the Environment Agency project team.

DNA should be extracted from filters using the DNeasy PowerWater kit (Qiagen, 14900-100-NF).

The elution volume should be recorded.

After extraction, DNA should be quantified (in  $ng/\mu L$ ) and DNA quality assessment using NanoDrop, with DNA quality of 260/280 score recorded.

On each day on which DNA extraction is performed a reagent blank control sample should be provided. It is anticipated no more than 15 blank samples will be submitted, please advise if more will be required.

Please label blank DNA extracts as: BLANK\_XX, where XX corresponds to the day of DNA extraction (from 01 to 15).

Following DNA quantification an aliquot of DNA is required to be stored in a separate tube for qPCR (suffix Q) with the remaining DNA in the original tube (suffix R). For example, the DNA extract "A028\_1\_DNA1" would be split into vials for "A028\_1\_DNA1Q" and "A028\_1\_DNA1R".

Where the DNA concentration is less than 10 ng/ $\mu$ L, 50  $\mu$ L of the DNA extract should be added to numbered safe-lock 1.5 mL microtubes (<u>NOT</u> PCR tubes or 96-well plates) to avoid evaporation or spilling during transportation.

If the DNA concentration exceeds 10 ng/ $\mu$ L an appropriate dilution should be made to produce a 50  $\mu$ L aliquot at a normalised concentration of 10 ng/ $\mu$ L. For this the dilution ratio and volume of DNA used should be recorded.

For the blank samples please also provide a 50  $\mu$ L aliquot with suffix Q and remainder (suffix R) DNA regardless of DNA concentration.

#### 2.8.2. DNA storage

These Q-suffixed 50uL aliquot tubes should be numbered on the lid according to an order provided by the Environment Agency project team. It is anticipated that replicates will be numbered differently to separate them on further onwards analysis.

The 50  $\mu$ L aliquot tubes at or below 10 ng/ $\mu$ L should be stored frozen in trays until sending for Environment Agency NLS Starcross lab (address will be provided).

The remaining volume of DNA extract (sample ID suffix R) (expected to be up to 50  $\mu$ L if the full elution volume is 100  $\mu$ L) is to be recorded (approx. volume with concentration and quality from above). This is also to be stored frozen (ideally at -80°C) for sending to Environment Agency NLS Starcross lab.

#### 2.8.3. DNA extraction reporting

For each filtration and extraction steps, the following data is required:

- Volume filtered (in mL)
- Elution volume (in µL)
- DNA dilution ratio (decimal)
- DNA volume diluted (in µL)
- DNA extraction day number (corresponding to the blank sample)

Analysis data will be reported by 6<sup>th</sup> December 2024 and reports will be in a spreadsheet format prescribed by the Environment Agency.

### 2.8.4. Distribution of isolates and DNA extract

- The analysis lab contractor will be responsible for arranging and paying for the transport of isolate and DNA extract samples to the Environment Agency lab (Starcross, Devon).
- Samples (isolate and DNA extract) will arrive at the lab by 4th December 2024.

# 3. Timeframes and deadlines

Task No.	Deliverable	Responsible party	Format / Compatibility Requirements	Date of completion, end:
1	Attend kick-off meeting	Contractor & Environment Agency	Virtual meeting (1 x 1 hrs)	By 2 <sup>nd</sup> September 2024
2	Detailed analysis plan (e.g. protocols for sample processing and each analysis method)	Contractor	In advance of kick off meeting	By 6 <sup>th</sup> September 2024
3	Attend pre- sampling meeting	Contractor & Environment Agency	Virtual meeting (1 x 1 hrs)	By 13 <sup>th</sup> September 2024
4	Sample receipt and filtering	Contractor with guidance by EA (for sample delivery)	Samples to be received from courier within 24 hours of samples being taken (i.e. to arrive by 12PM next day).	By 8 <sup>th</sup> November 2024
5	Water quality analysis	Contractor	Samples will be prepared & analysed within 48 hours of sampling.	By 8 <sup>th</sup> November 2024
6	Bacterial Phenotypic Analyses	Contractor	Samples will be prepared & analysed within 48 hours of receipt.	By 8 <sup>th</sup> November 2024
7	Reports on water quality and bacterial phenotypic analysis	Contractor with guidance by EA	To be distributed on conclusion of the final analysis.	Updates weekly & final reports by 15 <sup>th</sup> November 2024
8	Storage of isolates for potential future WGS analysis	Contractor	Isolates to be stored following phenotypic analysis.	By 29 <sup>th</sup> November 2024
9	DNA extraction	Contractor	Samples will be filtered within 48 hours of sampling.	By 29 <sup>th</sup> November 2024
10	Distribution of: - DNA (Q and R suffix samples) - stored E. coli isolates to Environment Agency NLS (Starcross, Devon)	Contractor with guidance by EA	To be distributed on conclusion of the final extraction and quantification.	By 4 <sup>th</sup> December 2024

11	Report of DNA extraction, stored isolate information and raw data	Contractor with guidance by EA	To be distributed on conclusion of the final analysis.	By 6 <sup>th</sup> December 2024
12	Attend project closure meeting/ debrief	Contractor & Environment Agency	Virtual meeting (1 x 1 hrs)	By 13 <sup>th</sup> December 2024

# Costing table attached:



# Appendix 4: Processing Personal Data

#### Authorised Processing Template

N/A