



# **Overview of sampling, pre-enrichment and laboratory analysis of samples for EFSA Aquaculture Baseline Survey**

**DRAFT**

**April 2026  
Version 1**

HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Approval
1	New document		April 2026	

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## Background

To harmonise the antimicrobial resistance (AMR) monitoring systems in the European Union (EU), the European Commission (EC) has adopted legislation (1) laying down detailed rules for the monitoring and reporting of AMR in zoonotic and commensal bacteria by Member States (MSs). The legislation provides a framework for routine monitoring of AMR in bovine animals, pigs and poultry. Complementary to that, the European Food Safety Authority (EFSA) has additionally recommended to undertake baseline surveys (BLSs) to assess the epidemiological situation on specific AMR issues (2). One such highlighted topic is the assessment of the prevalence of AMR in bacteria from aquaculture animals. This is of importance, as, at present, there are only limited data on the occurrence of AMR in aquaculture production in Europe and the data that are available cannot easily be compared due to methodological differences.

To address this need, in accordance with Article 31 of Regulation (EC) No 178/2002, EFSA was requested to provide technical and scientific support for the development of a BLS on the prevalence of AMR in bacteria isolated from EU-produced aquaculture animals, including proposed harmonised approaches for the collection and analysis of AMR data from aquaculture animals (3). The target organisms selected for the BLSs include *Aeromonas* isolated from finfish, as well as commensal *Escherichia coli*, *Klebsiella pneumoniae* (optional), ESBL-, AmpC- and/or carbapenemase-producing *E. coli*, *Enterococcus faecalis* and *E. faecium*, as well as *Vibrio parahaemolyticus* and *V. alginolyticus*, isolated from mussels (3).

The present document provides an overview of the workflow for the sampling and analysis of the samples from aquaculture animals. The individual analyses are described in detail in a series of protocols that together with the “Technical specifications for a EU-wide baseline survey of antimicrobial resistance in bacteria from aquaculture animals” (3) aim to provide harmonised methods for AMR monitoring of bacteria in aquaculture productions, including BLSs.

Specifically, the following protocols should be used in conjunction with the baseline survey of antimicrobial resistance in bacteria from aquaculture animals:

- Susceptibility testing by MIC of bacteria from aquaculture animals
- Isolation of *Aeromonas* from finfish
- Isolation of *Escherichia coli* and *Klebsiella pneumoniae* from mussels
- Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from mussels
- Isolation of *Enterococcus faecalis* and *E. faecium* from mussels
- Isolation of *Vibrio parahaemolyticus* and *V. alginolyticus* from mussels
- Identification of bacteria with MALDI-TOF (Matrix-Assisted Laser Desorption-Ionization Time Of Flight)
- Identification of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* by PCR

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Procedure	Theory/comments
<p><b>1. Finfish: sample collection and analysis</b></p>	<p>The present document is not meant to serve as a detailed protocol on sampling but rather to provide an overview of the workflow for collecting and processing samples for aquaculture BLS.</p> <p>For details on sampling please refer to section 6 of the document “Technical specifications for a baseline survey on AMR in aquaculture animals” (3). General instructions on sampling in that document include the following:</p> <p>“Sampling should be planned at the required frequency at the selected locations (e.g. production units, dispatch centres and production areas). As far as possible, the locations should be visited on different days in the week and month over the period of the survey. Consideration should also be given to the requirement for initial processing of the sample by the laboratory to be performed within 72 h of taking the sample, and for samples to arrive at the laboratory during the working week. For example, sampling on Friday should be avoided without prior agreement with the laboratory.” (3)</p> <p>Figure 1 shows an overview of the analysis of samples from finfish. The samples will be analysed for isolation and AST of <i>Aeromonas</i>.</p>
<p>1.1. Collect gill samples from five finfish, amounting to at least 25 g of pooled sample, as described in the technical specifications (3).</p> <p>Samples should be transported to the laboratory at a temperature between 0 and 8 °C. Samples should arrive at the laboratory and be initially processed within 48 hours of sampling, where possible. The maximum acceptable time for initial processing is 72 hours from the time of sampling.</p> <p>Upon receiving the samples at the laboratory, inspect them visually and discard any samples with damaged packaging or incorrect labelling. Discard any samples that did not arrive to the laboratory within 72 hours of sampling as well as any samples which have not been kept at the appropriate temperature during transport or storage.</p>	<p>The BLSs will focus on samples from healthy aquaculture animals.</p> <p>For instructions on sampling, please refer to section 6 of the document “Technical specifications for a baseline survey on AMR in aquaculture animals” (3), including the following excerpt:</p> <p>“For each randomly selected epidemiological unit (production batch) of finfish, five marine finfish (seabass and salmon) or freshwater finfish (trout) are sampled, from which all gill arches are collected bilaterally and pooled. A grammage of at least 25 g per pooled sample of gills deriving from the five finfish sampled should be reached. Gills are relevant samples with respect to sampling standardisation, availability and easiness to collect. Regarding the sampling stage, samples are taken at harvesting, i.e. either at post-harvest on the farm or after euthanasia at the</p>

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	<p>slaughterhouse, depending on the size of the production unit (farm) and the structure of the production sector considered. The time elapsed between killing and sampling should be short. Each sample should be labelled with a unique number which should be used from sampling to testing. The use of unique numbering system at the country level is recommended.” (3)</p> <p>It is necessary to keep the samples at a temperature approaching that of melting ice to avoid unreliable results. Samples should not be frozen.</p> <p>It is important that sample processing be initiated as soon as possible and no later than 48 h after sampling to optimise recovery of <i>Aeromonas</i>.</p> <p>During transport and storage prior to analysis, samples should be handled according to the ISO 7218 standard: “Microbiology of the food chain – General requirements and guidance for microbiological examinations”.</p> <p>For further details, refer to the Guidance document on official controls under Regulation (EU) 2017/625 (4), and the Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 that lays down specific hygiene rules for food of animal origin (5).</p>
<p>1.2. Store samples at the laboratory between 0 and 8 °C until microbiological analysis. Analyse samples within 72 h of sampling, preferably immediately after samples are received and within 48 hours of sampling. It must be ensured that the cold chain is maintained at all times between sample collection and analysis.</p>	<p>For storage prior to analysis, samples should be handled in accordance with the ISO 7218 standard: “Microbiology of the food chain – General requirements and guidance for microbiological examinations”.</p> <p>Samples should not be frozen.</p>
<p>1.3. Initiate sample analysis by following the protocol “Isolation of <i>Aeromonas</i> from finfish”. The isolation process entails homogenisation of the gill sample in buffered peptone water (BPW), followed by isolation of putative colonies on glutamate starch phenol red agar (GSP) plates.</p> <p>The genus of putative colonies should then be confirmed through species (ID), as described in section 2 of the <i>Aeromonas</i> isolation protocol.</p>	<p>The analysis of samples of gills from finfish is separate from the analysis of samples of mussels and should proceed independently.</p> <p>The only targeted bacterial indicator in the case of finfish is <i>Aeromonas</i>, at the genus level (Figure 1).</p>

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<p>At least one confirmed <i>Aeromonas</i> colony should then be subcultured to purity and stored.</p> <p>Perform antibiotic susceptibility testing (AST), as described in the protocol “Susceptibility testing by MIC of bacteria from aquaculture animals”.</p> <p>The flowchart for analysis of gill samples from finfish is shown in Figure 1.</p>	
<p><b>2. Mussels: sample collection and analysis</b></p>	<p>The present document is not meant to serve as a detailed protocol on sampling but rather to provide an overview of the workflow for collecting and processing samples for aquaculture BLS.</p> <p>For details on sampling please refer to section 6 of the document “Technical specifications for a baseline survey on AMR in aquaculture animals” (3). General instructions on sampling in that document include the following:</p> <p>“Sampling should be planned at the required frequency at the selected locations (e.g. production units, dispatch centres and production areas). As far as possible, the locations should be visited on different days in the week and month over the period of the survey. Consideration should also be given to the requirement for initial processing of the sample by the laboratory to be performed within 72 h of taking the sample, and for samples to arrive at the laboratory during the working week. For example, sampling on Friday should be avoided without prior agreement with the laboratory.” (3)</p> <p>The mussel samples will be analysed for the isolation and AST of i) commensal <i>Escherichia coli</i>, ii) <i>Klebsiella pneumoniae</i> (optional), iii) ESBL-, AmpC- and/or carbapenemase-producing <i>E. coli</i>, iv) <i>Enterococcus faecalis</i> and <i>E. faecium</i>, v) <i>Vibrio parahaemolyticus</i> and <i>V. alginolyticus</i>.</p> <p>Figures 2 and 3 show an overview of the analysis of samples of mussels.</p>
<p>2.1. Collect two sets of samples from 15 mussels, with each pooled sample amounting to at</p>	<p>The BLSs will focus on samples from healthy, live mussels.</p>

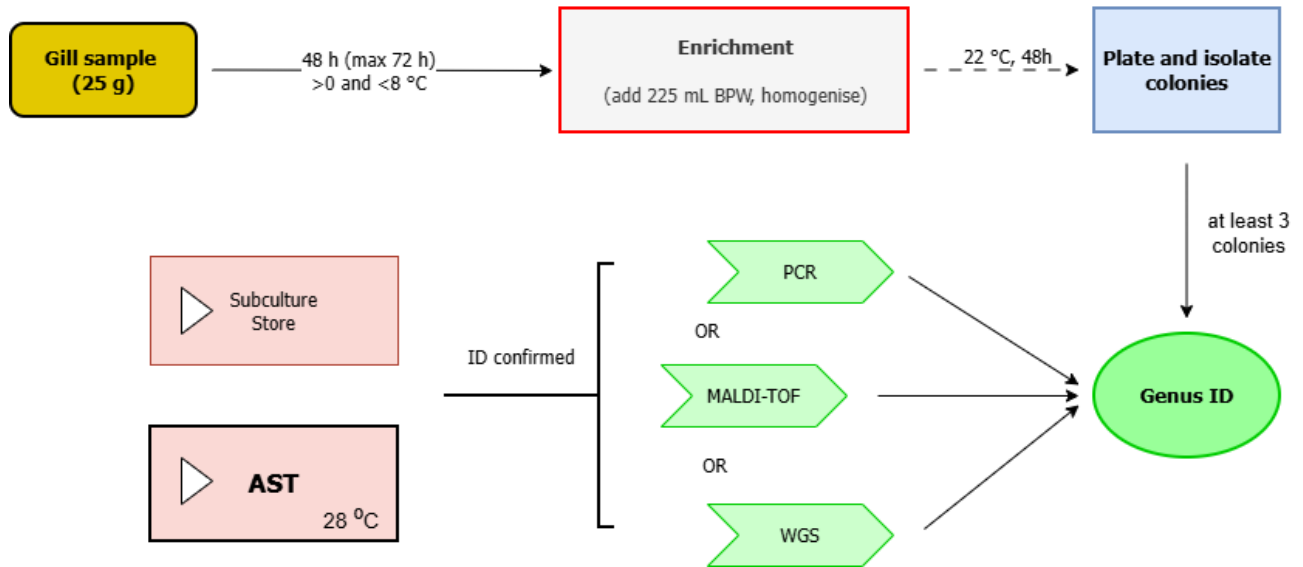
Procedure	Theory/comments
<p>least 25 g, as described in the technical specifications (3).</p> <p>Samples should be transported to the laboratory at a temperature between 0 and 8 °C. Samples should arrive at the laboratory and be initially processed within 24 hours of sampling, where possible. The maximum acceptable time for initial processing is 72 hours from the time of sampling.</p> <p>Upon receiving the samples at the laboratory, inspect them visually and discard any samples with damaged packaging or incorrect labelling. Discard any samples that did not arrive to the laboratory within 72 hours of sampling as well as any samples which have not been kept at the appropriate temperature during transport or storage.</p>	<p>For instructions on sampling, please refer to section 6 of the document “Technical specifications for a baseline survey on AMR in aquaculture animals” (3), including the following excerpt:</p> <p>“The locations within a geographical region visited at the same time should be varied. Where more than one mussel species is present at the sample site, a sample of only one mussel species is required for the survey.</p> <p>A sample of 15 live mussels should be taken and dispatched to the designated laboratory for pooled analysis. During sampling, precautions should be taken in order to avoid any activity that could affect the levels of bacterial contamination or result in a sample that is unsuitable for laboratory analysis. The mussels are to be placed in an intact food grade plastic bag or box (single use), securely packaged and dispatched to the laboratory.</p> <p>Representative sampling points within production/harvesting areas: Where multiple representative sampling points are present in the same production area, the representative sampling point, where mussel species are present, with the highest levels of <i>E. coli</i> contamination based on the routine monitoring performed under Regulation 2019/627 should be used to obtain the sample of mussels. It is important that the mussels sampled have been growing in the selected production area for more than 28 days, in order to be representative of this area and not a previous one.</p> <p>Approved dispatch centres: The sample should be taken from one batch of live mussels present on the premises at the time of visit. Fifteen mussels should be selected from the boxes on the packing line (representative of the sizes and grades of animals in the batch). The sample should not contain a mix of mussel species.” (3)</p> <p>It is necessary to keep the samples at a temperature approaching that of melting ice to avoid unreliable results. Samples should not be frozen.</p> <p>During transport and storage prior to analysis, samples should be handled according to the ISO 7218 standard: “Microbiology of the food chain –</p>

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	<p>General requirements and guidance for microbiological examinations”.</p> <p>For further details, refer to the Guidance document on official controls under Regulation (EU) 2017/625 (4), and the Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 that lays down specific hygiene rules for food of animal origin (5).</p>
<p>2.2. Store samples at the laboratory between 0 and 8 °C until microbiological analysis. Analyse samples within 72 h of sampling, preferably immediately after samples are received and within 24 hours of sampling. It must be ensured that the cold chain is maintained at all times between sample collection and analysis.</p>	<p>For storage prior to analysis, samples should be handled in accordance with the ISO 7218 standard: “Microbiology of the food chain – General requirements and guidance for microbiological examinations”.</p> <p>Samples should not be frozen.</p>
<p>2.3. The two samples of mussels will be analysed separately (Figure 3):</p> <ul style="list-style-type: none"> <li>• First sample: Add 25 ± 0.5 g of mussel sample to 225 mL of buffered peptone water (BPW) and homogenise. Incubate the pre-enrichment culture at 37 ± 1 °C for 18-22 h.</li> </ul> <p>This pre-enrichment culture will be used for isolation of i) commensal <i>Escherichia coli</i>, ii) <i>Klebsiella pneumoniae</i> (optional), iii) ESBL-, AmpC- and/or carbapenemase-producing <i>E. coli</i>, and iv) <i>Enterococcus faecalis</i> and <i>E. faecium</i>.</p> <p>Continue with step 2.4.</p> <ul style="list-style-type: none"> <li>• Second sample: Add 25 ± 0.5 g of mussel sample to 225 mL of the primary enrichment medium alkaline saline peptone water (ASPW), preheated to 37 ± 1 °C. Homogenise the suspension of the primary enrichment culture and incubate at 37 ± 1 °C for 6 ± 1 h.</li> </ul> <p>The primary enrichment culture will be used for isolation of <i>Vibrio parahaemolyticus</i> and <i>V.</i></p>	<p>The first step of analysis for both samples is pre-enrichment. However, the pre-enrichment of the first sample will be done in 225 mL of buffered peptone water (BPW), whereas the other in 225 mL of alkaline saline peptone water (ASPW).</p> <p>The first sample will be used for isolation of commensal <i>E. coli</i>, <i>Klebsiella pneumoniae</i> (optional), ESBL-, AmpC- and/or carbapenemase-producing <i>E. coli</i>, and <i>Enterococcus faecalis</i> and <i>E. faecium</i>, whereas the second sample will be used for isolation of <i>Vibrio parahaemolyticus</i> and <i>V. alginolyticus</i>.</p> <p>All isolation protocols, including additional details on this step, can be found on the EURL-AMR website.</p>

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<p><i>alginoliticus</i>. Continue the analysis with the secondary enrichment step, isolation steps, species ID, subculturing and storage, as described in the protocol “Isolation of <i>Vibrio parahaemolyticus</i> and <i>V. alginolyticus</i> from mussels”.</p>	
<p>2.4. <u>For first sample only, pre-enriched in BPW:</u></p> <p>Streak one loopful (10 µL inoculation loop) as follows:</p> <p><b>For <i>E. coli</i>:</b> streak onto a MacConkey agar plate. Incubate at 37 ± 1 °C for 18-22 h.</p> <p><b>For <i>K. pneumoniae (optional)</i></b> streak onto a MacConkey agar plate and, optionally, also onto a Simmons citrate agar plate with inositol. Incubate at 37 ± 1 °C for 18-22 h.</p> <p><b>For ESBL-, and/or AmpC-producing <i>E. coli</i>:</b> streak onto a MacConkey agar plate containing 1 mg/L of cefotaxime (CTX). Incubate at 37 ± 1 °C for 18-22 h.</p> <p><b>For carbapenemase-producing <i>E. coli</i>:</b> streak onto suitable selective agars. Incubate as instructed by the manufacturer of the plates.</p> <p><b>For <i>Enterococcus faecalis</i> and <i>E. faecium</i>:</b> streak onto a Slanetz and Bartley agar plate. Incubate at 37 ± 1 °C for 48 h.</p> <p>Continue with the next isolation steps, species ID, subculturing, and storage as described in the respective isolation protocols.</p>	<p>An overview of the different plates can be found in Figure 3.</p> <p>For details on this step, please refer to the respective isolation protocols, which are available on the EURL-AMR website. The description here is only meant to provide a descriptive overview of the isolation process.</p>
<p>2.5. Perform antimicrobial susceptibility testing (AST), as detailed in the protocol “Susceptibility testing by MIC of bacteria from aquaculture animals”.</p>	<p>The protocol “Susceptibility testing by MIC of bacteria from aquaculture animals” will be available on the EURL-AMR’s website.</p>

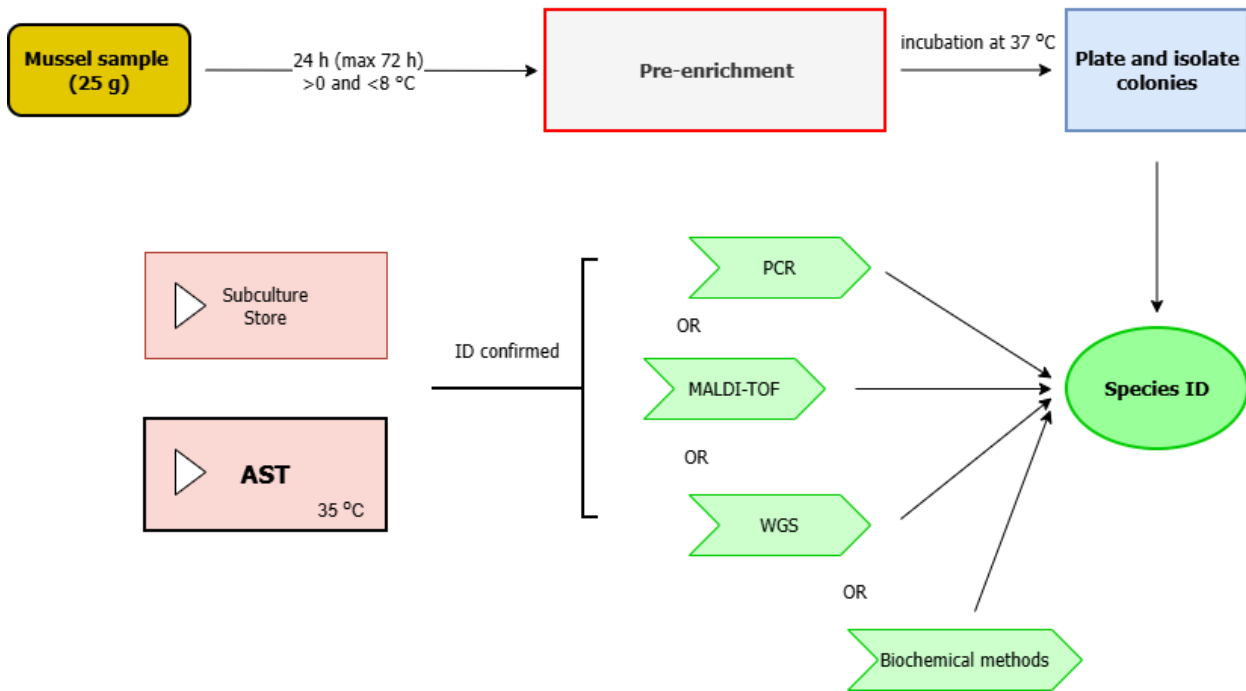
## Figures

### Fish samples - *Aeromonas*



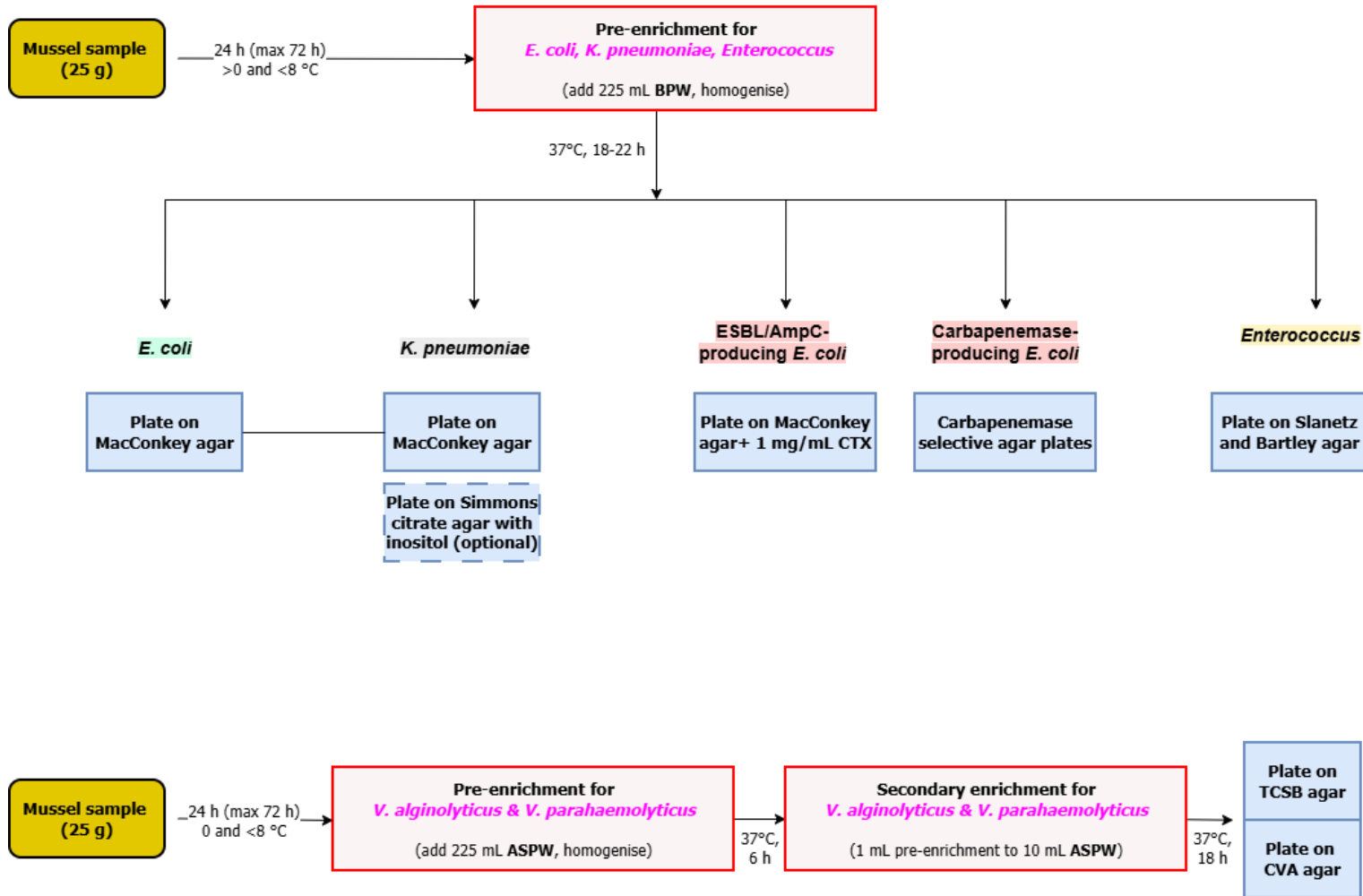
**Figure 1:** Flowchart for analysis of gill samples from finfish, with the aim of isolating *Aeromonas* and testing it for antimicrobial susceptibility. It has not yet been decided whether the analysis will involve incubation of the enrichment culture or plating from it directly.

## Mussel samples - *E. coli*, *K. pneumoniae*, *Enterococcus*, *Vibrio*



**Figure 2:** Flowchart for analysis of samples of mussels. Detailed instructions on isolation, species ID and AST can be found in the respective protocols.

### Mussel samples - Overview



**Figure 3:** Overview of analysis of samples of mussels. Only the first steps of the isolation process are shown. Detailed instructions on isolation and analysis for each of the indicator organisms can be found in the respective isolation protocols.

## References

- (1) Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU. [https://eur-lex.europa.eu/eli/dec\\_impl/2020/1729/oj/eng](https://eur-lex.europa.eu/eli/dec_impl/2020/1729/oj/eng)
- (2) Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food, EFSA Journal Volume 17, Issue 6, e05709, Jun 2019.  
DOI: <https://doi.org/10.2903/j.efsa.2019.5709>
- (3) Technical specifications for a EU-wide baseline survey of antimicrobial resistance in bacteria from aquaculture animals. EFSA Journal Volume 22, Issue 7, e8928, Jul 2024.  
DOI: <https://doi.org/10.2903/j.efsa.2024.8928>
- (4) Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation)  
<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02017R0625-20250105>
- (5) Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.  
<https://eur-lex.europa.eu/eli/reg/2004/853/oj/eng>