Annex A – Biotoxin Sample Submission Instructions

Types and condition of shellfish accepted for testing

Samples may be submitted as live shellfish or also as shucked uncooked/unprocessed chilled/frozen shellfish.

Prior to submitting live shellfish, mud and sediment adhering to the shellfish should be removed (where possible). This is best achieved by rinsing/scrubbing with clean sea water or freshwater of potable quality. If these are not available, the seawater from the immediate area of sampling may be used instead. Do not totally re-immerse the shellfish in water as this may cause them to open. Allow them to drain before placing in the sample container.

Samples should arrive at the laboratory in good condition so opened, gaping or damaged shells should not be included in the sample. Samples should only consist of animals that are within the normal commercial size range. Immature/juvenile animals may provide results that are unrepresentative of mature stock harvested for human consumption.

The laboratory will only accept and test the following shellfish types:

Species	**Biotoxin testing**			
	ASP	**PSP**	**LT (DSP)** (OA, DTX, PTX), AZP (AZA), YTX)	**Cyanotoxin**
Pacific oysters *Magallana (Crassostrea) gigas*	Y	Y	Y	Y
Native oysters *Ostrea edulis*	Y	Y	Y	Y
Mussels *Mytilus spp.*	Y	Y	Y	Y
Cockles *Cerastoderma edule*	Y	Y	Y	Y
King scallops *Pecten maximus*	Y (whole, adductor only or adductor + roe)			
Queen scallops *Aequipecten opercularis*	Y (whole, shucked / unshucked or processed shucked)			
Razor clams *Ensis spp.*	Y	Y	Y	Y
Hard clams *Mercenaria mercenaria*	Y	Y	Y	Y
Surf clams *Spisula solida*	Y	Y	Y	Y
European otter clams *Lutraria lutraria*	Y	Y	Y	Y
Palourdes clams *Ruditapes (Tapes) decussatus*	Y	Y	Y	Y
Manila clams *Ruditapes (Tapes) philippinarum*	Y	Y	Y	Y
Water	N	N	N	Y
Other species - by prior request only	Depending on species, we may be able to undertake unaccredited tests. Please contact to discuss.			
Cefas is a UKAS accredited testing laboratory No. 2293 and the methods and species listed above are included within our scope of accreditation.				

Important note:

- Sample(s) consisting of other types of shellfish or of processed shellfish will be rejected on arrival, unless an exception has been agreed with the laboratory in advance of the sample(s) being submitted.

Minimum samples size

The number of individual shellfish required for testing is dependent on the species. Cefas require the customer to submit the number of individual shellfish or minimum weight (in the shell) stipulated. The table below indicates recommendations of weight/quantities, in order to meet these minimum requirements.

For representative testing to be undertaken, you will need to supply sufficient numbers of individual shellfish to yield at least 50 grams of meat. As a minimum quantity, regardless of the weight, 10 viable animals will be required to undertake testing.

Species	**Biotoxin Testing** **ASP, PSP, LT (DSP (OA, DTXs, PTXs), AZP (AZAs), YTXs), Cyanotoxins** *Recommended number of individual shellfish to fulfil weight requirement (50 g) / weight to fulfil quantity requirement (10) unless otherwise specified*
Pacific oysters *Magallana (Crassostrea) gigas*	15 - 20
Native oysters *Ostrea edulis*	30 - 35
Mussels *Mytilus spp.*	1 kg
Cockles *Cerastoderma edule*	1 kg
King scallops *Pecten maximus*	15
Queen scallops *Aequipecten opercularis*	30 - 50
Razor clams *Ensis spp.*	10
Hard clams *Mercenaria mercenaria*	30
Surf clams *Spisula solida*	1 kg
European otter clams *Lutraria lutraria*	15
Palourdes clams *Ruditapes (Tapes) decussatus*	30 - 50
Manila clams *Ruditapes (Tapes) philippinarum*	30 - 50
Other species - by prior request only	Please contact to discuss.

Important notes:

- If you are sending samples for more than one type of analysis, we will require you to submit the minimum number of shellfish necessary for **each** test. Please ensure that these are provided in separate bags and are clearly labelled.
- It may be possible to test a sample comprising fewer than 10 individual animals. For advice**,** please **contact cst@cefas.gov.uk before shipping materials.**

- ***Minimum sample sizes (water):*** For cyanotoxin testing, water volumes should range from between 100 mL to 1000 mL. Less volume is required when the cyanobacterial biomass is present at dense levels in the surface waters.

Packaging & Transport

Customers should send samples in suitable insulated packages labelled **"PERISHABLE"** using an overnight courier service to ensure that samples arrive at the laboratory as soon as possible.

For biotoxin testing, rapid sample delivery maintains sample suitability. All packaging used should be sufficient to prevent leakage of the contents, leaking packages may be rejected by the courier firm prior to delivery or when received at the lab if there is a health and safety or biosecurity risk.

Cyanotoxin water testing: Once water/bacterial biomass samples are collected, the containers should be sealed, and placed into a container for overnight shipment to the Cefas laboratory. No temperature control is necessary during the shipment stage, and frozen ice blocks should be avoided to prevent unwanted cell lysis within the samples. Algal powders can be sent in a sealed container with no temperature control necessary.

Biotoxin and cyanotoxin shellfish testing: Live, shucked or frozen shellfish can be tested for biotoxins. Fresh or refrigerated shellfish samples should be transported in a cool box (or similar) and must be kept between 2 and 10°C during transit. Frozen samples should be packaged to maintain temperatures below 0 °C. It is recommended that precautions are taken to ensure that frozen fishery products do not thaw during transport.

It is recommended that any cool packs are frozen in a freezer for a minimum of 24 hours, prior to use.

Shellfish to be tested should be placed inside a polythene bag, which is then securely tied (double bagging is recommended to prevent puncture of the bag(s) by the shells). The Sample Submission Form must be fully completed and placed inside a separate polythene bag and secured to the sample. Both the sample and information sheet should be placed inside the cool box (or similar).

Care must be taken when packing the samples to ensure the shellfish sample does not come into direct contact with the cool pack(s). Should one sample not fit into one box, please split the sample into two boxes, ensuring that each sample is accompanied by a Sample Submission Form (marked 1 of 2 and 2 of 2).

If submitting more than one sample in the same shipping box, each sample must be accompanied by its own Sample Submission Form. Care should be taken to ensure the Sample Submission Form remains attached to the relevant sample so that samples are easily identifiable on arrival at the laboratory.

Once correctly assembled, secure the cool box lid with adhesive tape to prevent leakage and send the sample via Royal Mail Special Delivery or similar carriers/couriers. Please note it is essential that the packaging guidance provided is strictly followed. Failure to adhere to these guidelines may result in the refusal of samples.

Samples must be delivered to the laboratory as soon as possible and preferably within 24 hours of collection. If short term (up to 24 h only) storage prior to dispatch/delivery is absolutely necessary, live or fresh shellfish should be stored between 0 °C and 10 °C.

Please note, the laboratory does not return transport boxes provided by the customer, unless agreed in advance with the customer. Please note a charge will apply (please consult price

list). Cefas will not be held liable for the condition of the transport box once the courier has received it. Any issues relating to damages should be raised with the courier in question.

Submission to the laboratory

Delivery will be at the expense of the customer. It will be the responsibility of the customer to ensure samples are delivered in good condition and clearly labelled for identification, and please label as "**PERISHABLE**". Please ensure that samples are sent with an accompanying Sample Submission Form. Unlabelled samples arriving at the laboratory with incomplete paperwork or those in poor condition will not be tested and will be destroyed. Samples should be mailed to the address below and must indicate the relevant department (biotoxin/cyanotoxin):

Cefas Shellfish Testing
Barrack Road
Weymouth
Dorset
DT4 8UB, UK

It is requested that, where possible, a minimum of one week notice is provided to the laboratory prior to sample submission. Notice should be in writing to cst@cefas.gov.uk. It is also recommended that you advise the laboratory by emailing cst@cefas.gov.uk when samples are posted as this will help staff identify which samples are due and whether these have been delayed in the post.

For testing to be initiated on the day of receipt, we ask that the samples arrive at the laboratory by the cut off times stated below:

Biotoxin testing:	**Before 10:00am on Tuesday to Thursday inclusive.** Samples arriving after 10:00 will be stored until the following working day. Samples which arrive on Monday or Friday will be stored until the following Tuesday.
Cyanotoxin testing:	Due to the nature of the service, cyanotoxin samples cannot be tested on the day of receipt. Please contact cst@cefas.gov.uk for further information.

Important notes:

- Please note that the laboratory is closed on bank holidays and for two weeks during the Christmas period. The laboratory may also only operate a minimum testing service on Civil Service privilege days. These will be advised with a minimum 2 weeks notice.

Sample Submission Form – Biotoxins and Cyanotoxins

Sample Reference Number *:	
Contact name:	
Senders address:	
Contact number (landline/mobile):	
Reporting email:	
Date & time of collection:	
Dispatch date:	
Sample quantity (Number of individual animals in sample)	

*** This reference must be unique to each sample and should be a maximum of ten characters. Please ensure you do not refer to the exact geographical origin of your sample.**
Please note that we will only test species listed in the table below. Samples containing any other types of shellfish/processed shellfish will be rejected on arrival, unless agreement is reached with Cefas in advance.

Analysis required - Tick the box that corresponds to the type of test required & species being tested. Please also indicate whether the sample is shucked or unshucked (S/U) & sample temperature, at time of collection.

Species name	Cyanotoxin	ASP	PSP	LT (DSP, AZP, YTX)	Shucked/ Unshucked	Sample temp (° C)
Pacific oysters *Magallana (Crassostrea) gigas*	☐	☐	☐	☐		
Native oysters *Ostrea edulis*	☐	☐	☐	☐		
Mussels *Mytilus spp.*	☐	☐	☐	☐		
Cockles *Cerastode rmaedule*	☐	☐	☐	☐		
King scallops *Pecten maximus*	☐	☐	☐	☐		
Queen scallops *Aequipecten opercularis*	☐	☐	☐	☐		
Razor clams *Ensisspp.*	☐	☐	☐	☐		
Hard clams *Mercenaria mercenaria*	☐	☐	☐	☐		
Surf clams *Spisula solida*	☐	☐	☐	☐		
European otter clams *Lutraria lutraria*	☐	☐	☐	☐		
Palourdes clams *Ruditapes (Tapes) decussatus*	☐	☐	☐	☐		
Manila clams *Ruditapes (Tapes) philippinarum*	☐	☐	☐	☐		
Other species - by prior request only	☐	☐	☐	☐		
Water	☐					
If agreed prior to submission, please include details of "other species" in box to the right. (include genus & species)						
Sample state - Specify the state of the sample (e.g., whole or homogenized). If homogenized, provide details such as whether it includes the whole animal (including guts) or just the adductor muscle and roe.						

Annex B – Testing and Reporting

Testing methods and reporting format

Samples will be checked on receipt and prior to analysis for their suitability for testing. Any sample found unsuitable or insufficient for testing will be reported to the customer without undue delay.

The tests undertaken by the laboratory on each sample will be as specified on the sample submission form completed by the customer (assuming sufficient material is provided for the full suite of requested tests).

For further information regarding the methods used for analysis, please contact cst@cefas.gov.uk. Specifically, samples will be tested using the following methods:

Biotoxin testing

The methods approved by FSA and used for official control testing in the UK:

ASP – the EU reference method: **liquid chromatography method with UV detection**, based on Quilliam et al., 1995. Test results will be expressed as below the limit of quantitation (<LOQ – LOQ=1 mg/kg flesh) or as a total toxin concentration in mg/kg of shellfish flesh.

PSP – the EU approved method: **AOAC 2005.06 liquid chromatography method with fluorescence detection**, used as a qualitative screen or quantitative method. Test results will be expressed as not detected or when toxins have been detected as either the total PSP toxin concentration (expressed as µg saxitoxin equivalents [STX eq]/kg shellfish flesh or as below the reporting limit if the total PSP content is below 160 µg STX eq/kg. A semi-quantitative method may be used to screen samples containing low toxin levels. Results in this circumstance will be expressed as <400 µg STX eq/kg.

Lipophilic toxins (okadaic acid, dinophysistoxins and pectenotoxins; azaspiracids and yessotoxins) – the EU reference method: **liquid chromatography-tandem mass spectrometry method**. Test results will be reported as the actual toxin concentration determined in the sample for each of the three regulated toxin groups. This will be expressed either

- in µg OA or AZA equivalents and mg YTX equivalents per kg of shellfish flesh or
- as below the reporting limit (<RL), if the toxin contents are found to be below:
- 14 µg/kg for AZA
- 0.1-0.2 mg/kg for YTX (exact value dependant on shellfish species tested)
- and below 14-58 µg/kg for OA/DTX/PTX (exact value dependant on shellfish species tested).

Turnaround Times & Reporting of Results

Results will be reported electronically to the contact person whose details are provided on the sample submission form. If you require the results to be sent to additional or different contacts arrangements must be agreed in advance. Please indicate this on the form or **contact us**.

Results will be reported within the following timescales:

Biotoxin testing:

ASP & PSP (screen) – 100% of results reported within 7 WD of sample receipt

PSP quantitation – 100% of results reported within 7 WD of sample receipt. Customers will be informed if such test is required (no further charge will apply).

LT – 100 % of results reported within 7 WD of sample receipt

Test results that breach the EU regulatory limits set out in 853/2004 (as amended) will be reported as soon as the results are available.

Cyanotoxin testing:

100% results are reported within 7 WD of sample receipt.

Reporting timescales may be subject to adjustments due to public holidays and privilege days. If samples are submitted within 7 days or fewer before a public holiday or privilege day, the guaranteed turnaround time should be extended by the number of holidays.

Important notes:

- The timeframes given above are for samples submitted by the cut-off times and on the testing days specified in Appendix A. A one working day delay will apply to samples received after the specified cut-off times. The laboratory will advise if samples have been received late.
- Occasionally, quality control issues can arise. If this occurs, it will not always be possible to report results within the timeframes given above. In a small proportion of cases properties inherent to the sample may mean that it is not possible to obtain a valid result even after retesting. In these events we will contact you to discuss available options.