Annex A - Sample Submission Instructions

Types and condition of shellfish accepted for testing

Samples may be submitted as <u>live shellfish or, for biotoxin and testing only, also as shucked</u> 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:

Microbiological test		Biotoxin testing			
Shellfish species	E. coli Salmon ella spp.	Vibrio Parahae molytic us	ASP	PSP	Lipophilic toxins (OA,DTX,PTX,AZA, YTX)
Oysters (Crassostrea gigas and Ostrea edulis)	Υ	Y	Y	Y	Υ
Mussels (Mytilus spp.)	Υ	Υ	Υ	Υ	Υ
Cockles (Cerastoderma edule)	Υ	Y	Υ	Υ	Υ
Hard clams (Mercenaria mercenaria)	Υ	Y	Υ	Υ	N
King scallops (Pecten maximus)	Y	Y	Y (whole, adductor only or adductor+roe)		
Queen scallops (Aequipecten opercularis)	Y	Y	Y (whole only)		
Manila clams (Tapes philippinarum)	Y	Y	Y	Y	Υ
Palourdes (Tapes decussatus)	Y	Y	Υ	Y	Υ
Razor clams (<i>Ensis</i> spp.)	Υ	Y	Υ	Υ	Υ
Sand gapers (Mya arenaria)	Υ	Y	N	N	N
Surf clams (Spisula solida)	Y	Y	Y	Υ	Υ
Otter clams (Lutraria lutraria)	Y	Y	Υ	Y	Υ
Other species	Y		N	N	N

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

We ask that for each analysis you submit the number of individual shellfish stipulated in the table below. As a minimum, we will require the following:

Biotoxin testing enough individual shellfish to yield at least **50 grams** of meat.

Bacterial testing minimum 12 animals.

Number of individual shellfish to send	Microbiological test			
depending on species (or minimum weight in shell)	E. coli/Salmonella spp. And V. parahaemolyticus	Biotoxin Testing		
Oysters	12-18	30-35		
Mussels	15-30	1 kg		
Cockles	35-55	1 kg		
Hard clams	12-18	30		
King scallops, Razor clams & otter clams	12-18	15		
Queen scallops, Manila clams & palourdes	18-35	30-50		
Surf clams	35-55	1 kg		
Sand gapers	12-18	N/A		
Abalone	12-18	N/A		
Sea urchins	12-60 depending on diameter – please contact us	N/A		
Others	Please contact us to discuss	N/A		

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.co.uk before shipping materials.

Packaging & Transport

Customers should send samples using an overnight courier service to ensure that samples arrive at the laboratory to enable testing to commence within 24h of sample collection. Further advice on packing samples, see our packaging guidance below.

Biotoxin testing

Live, shucked or frozen shellfish can be tested for biotoxins. Fresh or refrigerated should be transported in a cool box (or similar) and kept between 1 to 10°C during transit. It is recommended that precautions are taken to ensure that frozen fishery products do not thaw during transport.

Bacterial testing

Only live shellfish samples can be tested. Shellfish samples should be transported in a cool box (or similar) and kept between 1 to 10°C during transit. Samples should be received at the laboratory live and not frozen (freezing can alter the levels of bacteria in the sample).

Packaging Guidance

Samples should be shipped in suitable insulated packages. It is recommended that any cool packs are frozen in a freezer for a minimum of 24 hours prior to use. Samples must remain chilled between 1°C and 10°C during transport. Special care should be taken when submitting frozen samples to ensure that they do not thaw during transport.

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 filled in and placed inside a separate polythene bag and secured to the sample. Both shellfish sample and information sheet should be placed inside the shipping box.

Care must be taken to correctly place any additional cooling packs to ensure that the shellfish sample does not come into direct contact with the cooling 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).

Once correctly assembled, secure the box lid with adhesive tape to prevent leakage and send the sample via Royal Mail Special Delivery or similar carriers/couriers.

Samples must be delivered to the laboratory as soon as possible and preferably within **24** hours of collection. If short term (up to 24h only) storage prior to dispatch/delivery is absolutely necessary, live or fresh shellfish should be stored at 1 to 10°C.

Important notes:

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

Submission to the laboratory

Each individual sample must be accompanied with a completed sample submission form (see template below). Unlabelled samples arriving at the laboratory with incomplete paperwork or those in poor condition will not be tested.

Samples should be addressed to:

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.co.uk. It is also recommended that you advise the laboratory by emailing cst@cefas.co.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

10:00 on Tuesday to Friday inclusive. Samples arriving after 10:00 will be stored until the following working day. Samples which arrive on Mondays will be stored until the following working day.

Bacterial testing

10:00 on Monday to Thursday inclusive. Samples arriving after 10:00 Monday to Wednesday will be tested the following day. Samples arriving after 10:00 Thursday will not be tested, unless exceptional circumstances and subject to prior agreement.

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

Your reference:		This reference number must be uniq ue to your sample. Please ensure that you do not provide information on the exact geog raphical origin of your sample					
Contact name:							
Senders address:							
Contact phone number(s):	Landline			Mok	oile		
Reporting email:							
Date & time of collection:			Date	e of dispate	ch:		
		Tick box f	or analys	es required			
Bivalve Species		ASP	PSP	LT	E.coli	E.coli & sal. Vibi	rio
Mussels (<i>Mytilus sp.</i>)							
Pacific oysters (C.gigas)]
Native oysters (O. edulis)]
Cockles (C. edule)]
King scallops - Whole (P. maximus	5)]
King scallops - Shucked (P. maxii	nus)]
Queen scallops (A. opercularis)]
Razor clams (Ensis sp.)]
Hard clams (M. mercenaria)							
Surf clams (S. solida)]
European otter clams (L. lutraria)							
Palourdes/Carpet shell clams (<i>T. decussatus</i>)]
Manila clams (<i>T. philippinarum</i>)]
Other species - by prior request on	ly]

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.co.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 μ g/kg for OA/DTX/PTX (exact value dependant on shellfish species tested).

Measurement uncertainties (which qualifies the uncertainty around the reported values) and full details of the method's performance will be provided with the test results. For further information, please contact cst@cefas.co.uk.

Bacterial testing

E.coli - the EU reference method for *E. coli* in bivalve shellfish: **ISO TS 16649-3 -** Microbiology of food and animal feedings stuffs - Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* - Part 3: Most probable number technique using5-bromo-4-chloro-3-indolyl- β -D glucuronide. Test results will be reported as Ecoli MPN per 100g shellfish flesh and and intravalvular fluid

Salmonella spp. - the EU reference method for *Salmonella* in bivalve shellfish: **ISO 6579 -** Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. Test results will be reported as detected or not detected in 25g shellfish flesh and intravalvular fluid

V. parahaemolyticus – ISO 21872

For further information in relation to *E.coli* and *Salmonella*, please contact cst@cefas.co.uk

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:

E. coli 95%	% of results reported within	3 WD of sample recei	pt – 100% within 5 WD
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Salmonella spp.

95% of negative results reported within 4 WD of sample receipt $-\ 100\%$ within 5 WD

95% of positive results reported within 5 WD of sample receipt – 100% within 7 WD

Biotoxins

ASP & PSP (screen) - 80% of results reported within 2 WD of sample receipt - 100% within 5 WD

PSP quantitation – 80% of results reported within 3 WD of sample receipt – 100% within 5 WD. Customers will be informed if such test is required (no further charge will apply). LT – 70 % of results reported within 2 WD of sample receipt – 100% within 5 WD

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.

Important notes:

- The KPIs given above are valid for a minimum volume of 20 tests per financial year per analysis type.
- 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.