



National Reference Laboratory  
for monitoring bacteriological and viral  
contamination of bivalve molluscs

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## **RECOMMENDATIONS FOR THE COLLECTION AND TRANSPORT OF BIVALVE MOLLUSCS FOR THE CLASSIFICATION OF BIVALVE MOLLUSC HARVESTING AREAS UNDER REGULATION (EC) No 854/2004**

### **1. Introduction**

Regulation (EC) No 854/2004 of 29 April 2004 requires the designation of bivalve mollusc production areas. This necessitates the sampling and testing of bivalve molluscs in order to determine the extent of sewage (or other faecal) contamination. This sampling only needs to be undertaken for areas from which bivalves are being taken to be placed on the market for human consumption.

### **2. Time of sampling**

Sampling should be undertaken, where practical, on as random a basis as possible with respect to likely influencing environmental factors e.g. tidal state, rainfall, wind etc so as to avoid introducing any bias to the results.

### **3. Sampling method**

Wherever possible, species should be sampled by the method normally used for commercial harvesting as this can influence the degree of contamination. For samples taken as part of the harvesting area classification programme, the sampling officer should take the temperature of the surrounding seawater at the time of sampling and record this on the collection form. Where intertidal shellfish are sampled dry, the temperature of the shellfish sample should be recorded immediately after collection. In this case, the temperature should be measured by placing the thermometer or probe in the centre of the bagged shellfish sample.

### **4. Size of individual animals**

Samples should only consist of animals that are within the normal commercial size range. Immature/juvenile bivalve molluscs may give *E.coli* results that are unrepresentative of mature stock that will be harvested for commercial sale/human consumption. In circumstances where less mature stock is being commercially harvested for human consumption then samples of these smaller bivalves may be gathered for analysis.

## 5. Sample composition

The following sample sizes (in terms of number of individuals by species) are recommended for submission to the laboratory:

King scallops ( <i>Pecten maximus</i> )	12-18
Queen scallops ( <i>Aequipecten opercularis</i> )	18-35
Oysters ( <i>Crassostrea gigas</i> and <i>Ostrea edulis</i> )	12-18
Hard clams ( <i>Mercenaria mercenaria</i> )	12-18
Manila clams ( <i>Tapes philippinarum</i> )	18-35
Palourdes ( <i>Tapes decussatus</i> )	18-35
Thick trough shells ( <i>Spisula solida</i> )	35-55
Sand Gapers ( <i>Mya arenaria</i> )	12-18
Razor clams ( <i>Ensis</i> spp.)	12-18
Mussels ( <i>Mytilus</i> spp.)	18-35
Cockles ( <i>Cerastoderma edule</i> )	35-55
Whelks ( <i>Buccinum undatum</i> )	12-18
Periwinkles ( <i>Littorina littorea</i> )	35-55
Abalone ( <i>Haliotis</i> spp.)	12-18

There is an absolute lower number of shellfish (10) and a minimum requirement of 50 g of flesh and intravalvular fluid for the test undertaken by the laboratory. The number of animals given above is intended to satisfy these requirements and to include a small additional allowance in case animals become moribund during transit.

## 6. Preparation of samples

Any mud and sediment adhering to the shellfish should be removed. This is best achieved by rinsing/scrubbing with fresh water of potable quality or seawater from the immediate area of sampling. Do not totally re-immerses the shellfish in water as this may cause them to open. Allow to drain before placing in a food grade plastic bag. The container/bag should be labelled with the sender's reference number and any other relevant information (e.g. species).

## 7. Sample transport

Samples should be transported in cool boxes at a temperature between 1°C and 8°C. **Samples should not be frozen** and freezer packs should not come into direct contact with the samples or sample bags. Analysis should be undertaken as soon as practically possible after sampling with a normal limit of 24 hours after sampling. Where specific geographical considerations make it impossible to consistently comply with the 24 hour limit, the upper limit may be extended to 48 hours if a validation study shows that this does not affect the *E. coli* concentration in the shellfish.

The cool boxes used for such transport should be validated using appropriate temperature probes, to ensure that the recommended temperature is achieved and maintained for the appropriate period. The number and arrangement of freezer packs, and the sample packing procedure, shown to be effective in the validation procedure should be followed during routine use. Where validation data already exists for a specific type of cool box, there is no need to undertake a local revalidation.

Where the receiving laboratory has indicated that it wishes to measure the temperature of the received material by means of a water sample, a plastic universal bottle containing approximately 25 ml of water at ambient temperature should be placed amongst the bagged samples at the time the last sample is placed in the cool box. The bottle should be clearly marked as being for temperature measurement.

## **8. Submission form**

Sample point identification number and name, map co-ordinates, time and date of collection, species sampled, method of collection (hand-picked, dredged, etc) and seawater temperature should be recorded on the submission form. Any other information deemed relevant (e.g. unusual events, adverse weather conditions etc) should also be recorded.

## **9. Receiving laboratory**

The laboratory to which the samples are sent must be part of the network identified by the competent authority. It must be UKAS-accredited for the testing of shellfish for *E. coli* by ISO 16649-3 and must take part in both the HPA/Cefas Shellfish EQA scheme and appropriate NRL ring trials.

## **10. Additional advice**

Additional advice on sampling can be obtained from Mr Andy Younger at the CEFAS Weymouth Laboratory: telephone: 01305 206695; e-mail: [andrew.younger@cefas.co.uk](mailto:andrew.younger@cefas.co.uk)