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

RECOMMENDATIONS FOR THE LABORATORY TESTING OF BIVALVE MOLLUSCS FOR THE CLASSIFICATION OF BIVALVE MOLLUSC HARVESTING AREAS UNDER REGULATION (EC) No 854/2004

1. Method for the enumeration of *Escherichia coli*

The method for *E. coli* to be used for testing for the classification of bivalve mollusc harvesting areas in the UK is that described in ISO TS 16649-3 'Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* – Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide.', used in a 5x3 MPN format. This technical specification cross-refers to the MPN tables in ISO 7218 and therefore these should be used instead of any others. It should be noted that while 5x3 MPN tables are given in the current (2007) version of ISO 7218, they are not given in any earlier versions which laboratories may have in their possession. There are slight differences between the tables given in ISO 7218 and those previously used in the UK and thus laboratories that have included MPN tables in their Standard Operating Procedures, or other laboratory documentation, should make sure that these are consistent with the tables given in the standard.

2. Sample receipt

(a) Sample container

A sample must be received in an intact food grade plastic bag. The container/bag should be labelled with the sender's reference number and any other relevant information (e.g. species). Samples should not be examined if they are received unlabelled.

(b) Temperature on receipt

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 shellfish sample temperature should be recorded. In this case, the temperature should be measured by placing the thermometer or probe in the centre of the bagged shellfish sample. Samples should not be frozen at any stage between sampling and commencement of the test. Any samples that are received frozen should not be tested.

The temperature of the air immediately surrounding the sample(s) should be taken as soon as the coolbox is opened. If this temperature exceeds 8°C then the sample temperature should be taken immediately with the thermometer placed inside the sample bag in the centre of the sample – between the shellfish in the bags. The temperatures should be

recorded. Alternatively, the laboratory may request the submitting authority to place a plastic universal bottle containing approximately 25 ml of water at ambient temperature 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. The laboratory should take the temperature of the water sample immediately after the cool box is opened.

It has been shown that shellfish samples properly packed in a cool box should be able to reach a temperature of less than 8°C within 4 hours and then maintain this for at least the maximum elapsed period specified in the sampling protocol. With this in mind, the following criteria should be used to determine whether samples are satisfactory with regard to temperature on arrival at the laboratory:

Elapsed time between sample collection and receipt is 4 hours or more

If internal air temperature is measured

If the internal air temperature exceeds 8°C then the between sample temperature should be checked. If this is between 1 and 8°C, the samples are satisfactory. If this is above 8°C, they are not satisfactory.

If water sample temperature is measured

If the temperature of the water sample is between 1 and 8°C, the samples are satisfactory. If it is above 8°C, they are not satisfactory.

Elapsed time between sample collection and receipt is less than 4 hours

The air and contents (or water sample) will not necessarily have reached 8°C or less within this period but should be less than the temperature at the time of sampling.

(c) Condition of sample

A sample is also considered unsatisfactory on receipt when:

- The sample bag is received leaking such as to lead to potential contamination of that or other samples
- The shellfish are immersed in water or mud/sand

If samples are received in a state in which they are considered unsatisfactory, a note should be recorded to this effect and the sending authority should be informed that this may be a factor affecting the quality of the result.

3. Number of shellfish to be tested

The following numbers of individuals by species per sample are recommended:

King scallops (<i>Pecten maximus</i>)	10-12
Queen scallops (<i>Aequipecten opercularis</i>)	15-30
Oysters (<i>Crassostrea gigas</i> and <i>Ostrea edulis</i>)	10-12
Hard clams (<i>Mercenaria mercenaria</i>)	10-12
Manila clams (<i>Tapes philippinarum</i>)	15-30
Palourdes (<i>Tapes decussatus</i>)	15-30
Thick trough shells (<i>Spisula solida</i>)	30-50
Sand Gapers (<i>Mya arenaria</i>)	10-12
Razor clams (<i>Ensis</i> spp.)	10-12
Mussels (<i>Mytilus</i> spp.)	15-30
Cockles (<i>Cerastoderma edule</i>)	30-50
Whelks (<i>Buccinum undatum</i>)	10-12
Periwinkles (<i>Littorina littorea</i>)	30-50
Abalone (<i>Haliotis</i> spp.)	10-12

No test should be undertaken on less than 10 shellfish due to the variation in *E. coli* content seen from one animal to another (as stated in Commission Regulation [EC] No 2073/2005 on microbiological criteria). It is recommended that at least 50 g of flesh and intravalvular fluid be used.

4. Quality control

The recommended method specifies positive and negative control strains. Experience has shown that weakly β -glucuronidase positive strains may show as negative on some BCIG agars depending on the exact formulation of the medium. It is therefore further recommended that a weakly positive strain be included as a control organism in the test procedure. NCTC 13216 is a suitable strain of *E. coli* for this purpose.

5. Interpretation of MPN tube combinations

The most probable number (MPN) tables published in the standard method contain only category 1 and 2 values, that is only those values that have a high probability of being correct. Values that do not appear in the tables are not acceptable and should not be used. If an unacceptable value is obtained, report the result as void.

6. External quality assurance

Laboratories undertaking testing for classification purposes in the UK must take part in the Shellfish EQA Scheme organised by the Health Protection Agency (HPA) in conjunction with Cefas. Results from classification testing laboratories are made available to the National Reference Laboratory (NRL) on an anonymous basis so that performance can be monitored. Further information on the scheme can be found on the Internet at the following site:

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1200055669446?p=1200055669446>

Laboratories undertaking testing for classification purposes in the UK must also take part in any relevant ring trials organised by the NRL.

7. Accreditation

Laboratories in the UK that undertake testing of samples for the classification of harvesting areas must be accredited by the United Kingdom Accreditation Service (UKAS) for the specific *E. coli* method (i.e. this requirement is not met by the laboratory having accreditation for another method used for the enumeration of *E. coli* in general food samples).

8. Additional information

Additional information on the microbiological testing of shellfish with respect to the UK may be found on the Internet at the following site: www.nrlcefas.org Information can also be obtained from Dr Ron Lee at the Cefas Weymouth Laboratory:

Telephone: 01305 206656; e-mail: ron.lee@cefas.co.uk