

European Union Reference Laboratory (EURL) Proficiency Testing Schemes Instruction sheet for shellfish samples

Supplementary information for whole live bivalve molluscan shellfish proficiency testing schemes

Samples:

- Individual samples in this proficiency testing (PT) scheme each comprise a single species of live bivalve molluscs.
- Commission Regulation EC (No) 2073/2005 on microbiological criteria stipulates the use of a pooled sample of a minimum number of 10 individual animals for enumeration of *Escherichia coli* in live bivalve molluscs. This is to reduce the potential for individual animal-to-animal variation in microbiological content to bias the sample result.
- Samples provided as part of this PT scheme comprise at least 12 animals in the case of smaller species additional animals are provided to ensure that sufficient flesh and intravalvular fluid is provided for the analyses. The PT sample should comprise at least the following numbers of individuals:

• Oysters (<i>Crassostrea gigas</i> and <i>Ostrea edulis</i>)	12 - 18
• Mussels (<i>Mytilus</i> spp.)	18 - 35
• Hard shell clams (<i>Mercenaria mercenaria</i>)	12 - 18
• Manila clams (<i>Tapes philippinarum</i>)	18 - 35
• King scallops (<i>Pecten maximus</i>)	12 - 18
• Queen scallops (<i>Aequipecten opercularis</i>)	18 - 35
• Cockles (<i>Cerastoderma edule</i>)	35 - 55
• Razor clams (<i>Ensis</i> spp.)	12 - 18
• Palourdes (<i>Tapes decussatus</i>)	18 - 35

• Sample temperature

- The internal air temperature should be recorded immediately on arrival. The temperature should not exceed 10°C.
- The sample temperature should be recorded, the sample temperature should be between 1-10°C, if sample temperature exceeds 10°C or is below 1°C **please contact the EURL immediately. Samples should not be frozen.**
- Where possible samples should be processed within 24 hours of dispatch, if not processed immediately samples should be stored at 3±2°C.
- Before commencement of analysis shellfish should be examined visually, only animals that are alive according to any of the following criteria should be chosen:
 - Any exposed flesh should react to touch.
 - Shellfish should open and close of their own accord.
 - They should respond to percussion.
 - Tightly closed shellfish.
- Dead or damaged shellfish should be discarded.
- Samples should not be re-immersed in water.
- If the samples are considered unsatisfactory please contact the EURL immediately.

Methods:

- Samples should be processed in duplicate according to your routine procedures.
- The number of dilutions required for the sample is indicated on the results form, to avoid making insufficient dilutions please refer to the form prior to initiating the test.
- The reference method for the enumeration of *E. coli* in live bivalve molluscs is ISO TS 16649-3:2005. 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.

- The reference method for detection of *Salmonella* spp. in live bivalve molluscs is ISO 6579:2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Reporting:

- Results should be reported on the form supplied by the EURL.
- Both the MPN *E. coli* per 100g and the numerical combination used to determine the MPN should be recorded on the form.
- MPNs should be determined using 5 x 3 MPN tables provided in ISO 7218. ISO 7218 Microbiology of food and animal feeding stuffs - General recommendations and guidance for microbiological examinations.
- For examination for *Salmonella* spp. result should be recorded as presence or absence in 25g.
- The laboratory ID number should be clearly recorded on the form.
- Results should be returned by the date stipulated on the base of the form.
- Results can be faxed, emailed or posted, please ensure to return the form to the nominated named member of the EURL indicated on the base of the form.
- **Results received after the deadline cannot be included in the proficiency testing report.**

Useful references:

- ISO TS 16649-3:2005 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. Geneva, Switzerland.
- The EURL standard operating procedure based upon the above standard can be accessed via the EURL website http://www.crlcefas.org/InformationCentre/docs/EURL_SOP_E_coli_07_02_11_FINAL.pdf
- Anon. 2002. ISO 6579:2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Geneva, Switzerland.
- The EURL standard operating procedure based upon the above standard can be accessed via the CRL website. http://www.crlcefas.org/InformationCentre/docs/CRL_SOP_SALMONELLA_17_11_07.pdf
- ISO 7218:2007 Microbiology of food and animal feeding stuffs - General recommendations and guidance for microbiological examinations. Geneva, Switzerland.
- http://www.crlcefas.org/InformationCentre/docs/854_h3oregulation.pdf
- http://www.crlcefas.org/InformationCentre/docs/882_Official_food_feed_controls.pdf
- http://www.crlcefas.org/InformationCentre/docs/20732005_microcriteria.pdf

Useful contacts:

Troubleshooting and microbiological advice: Louise Stockley

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General comments, including problems with receipt of samples: Louise Stockley
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