



Salmonella detection in matrix shellfish

Analysis of live bivalve molluscs

The procedure to be followed for the detection of *Salmonella* spp. in live bivalve molluscs is EN ISO 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. Follow the parts of this ISO document for detection of *Salmonella* in food samples. This method is suitable for analysis of raw and cooked shellfish.

For the preparation of the test samples reference is made to EN ISO 6887-3:2017. Further details on sample transport, storage and sample preparation are described below.

Sample transport and receipt

Samples of bivalve molluscs should be packed immediately after sampling in such a way that the shellfish reach a temperature between 0 °C and 10 °C within 4 hours and maintain this temperature for at least 24 hours. Samples must be received at the laboratory in an intact food grade plastic bag with a temperature between 0 °C and 10 °C. For samples where less than 4 hours have passed between sampling and arrival at the laboratory, the internal air temperature should be less than the starting temperature at time of sampling. Samples should be regarded as unsatisfactory if on receipt at the laboratory the sample is frozen, the container is leaking, the shellfish are covered in mud or immersed in water or mud/sand.

Sample selection

Discard all dead and damaged shellfish. Select shellfish that are healthy and alive according to the following points:

- Movement of any kind of the shellfish flesh upon touching
- Shellfish open and close of their own accord
- Tapping on the shell causes closing or movement
- Tightly closed shellfish

Select the appropriate number, but at least 10 individual animals depending on the species (See Appendix 1). Mud and sediment should be removed prior to opening by rinsing or scrubbing under cold, running tap water of potable quality. Shellfish should not be re-immersed in water as this may cause them to open.

References

EN ISO 6887-3:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 3: Specific rules for the preparation of fish and fishery products. International Organization for Standardization, Geneva.

EN ISO 6579-1:2017. 'Microbiology of food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp'. International Organization for Standardization, Geneva.

APPENDIX 1: RECOMMENDED NUMBER OF INDIVIDUAL LIVE BIVALVE MOLLUSCS AS SAMPLE PORTION

(Table adapted from CEFAS protocol: Detection of *Salmonella* spp. in bivalve molluscan shellfish, issue 3 25.05.2017)

Name ^{A B}	Latin name ^C	Sample size ^D
Saint James Scallop	<i>Pecten jacobaeus</i>	10 - 12
King scallops	<i>Pecten maximus</i>	10 - 12
Razor clams	<i>Ensis spp.</i>	10 - 12
Soft shell clams (Sand Gapers)	<i>Mya arenaria</i>	10 - 12
Northern horse mussels	<i>Modiolus</i>	10 - 12
Abalone	<i>Haliotis spp</i>	10 - 12
Whelks	<i>Buccinum undatum</i>	10 - 15
Variegated scallop	<i>Chlamys varia</i>	10 - 18
Oysters	<i>Crassostrea gigas and Ostrea edulis</i>	10 - 18
Noah's Ark shells	<i>Arca noae</i>	10 - 25
Mediterranean mussels	<i>Mytilus galloprovincialis</i>	10 - 30
Smooth clams	<i>Callista chione</i>	10 - 30
Purple or green Sea urchins – Adults (juvenile)	<i>Paracentrotus lividus</i>	10 – 30 (40 – 80)
Hard clams	<i>Mercenaria</i>	12 - 18
Dog winkles	<i>Thais haemastoma</i>	15 - 25
Ark clams	<i>Barbatia barbata</i>	15 - 25
Bearded horse mussels	<i>Modiolus barbatus</i>	15 - 30
Queen scallops	<i>Aequipecten opercularis or Chlamys opercularis</i>	15 - 30
Warty venus clams	<i>Venus verrusosa</i>	15 - 30
Mussels	<i>Mytilus spp.</i>	15 - 30
Manila clams	<i>Tapes philippinarum</i>	18 - 35
Palourdes (Grooved carpet shell clams)	<i>Tapes decussatus (Venerupis decussata)</i>	18 - 35
Rayed artemis	<i>Dosinia exoleta</i>	18 - 35
Clam	<i>Venerupis rhomboides</i>	20 - 25
Pullet carpet shell	<i>Venerupis senegalensis</i>	20 - 25
Cockles	<i>Cerastoderma edule</i>	30 - 50
Turbinate monodont	<i>Phorcus turbinatus</i>	30 - 50
Atlantic surf clams (Thick trough shells)	<i>Spisula solida</i>	30 - 50
Periwinkles	<i>Littorina littorea</i>	30 - 50
Bean clams	<i>Donax spp.</i>	30 - 50
Wedge shell clams	<i>Donax trunculus</i>	40 - 80
Striped venus clams	<i>Chamelea gallina</i>	40 - 80
Cut trough shells	<i>Spisula subtruncata</i>	70 - 90

^A Species of relevance provided by EU NRLs.

^B The list given in Appendix 1 includes bivalve molluscs, echinoderms, tunicates and marine gastropods. Commission Regulation (EU) No 505/2010 amending 854/2004 does not foresee a requirement for classification of marine gastropods.

^C Sample sizes given in this table have been provided by former NRLs for live bivalve molluscs. The weight of shellfish flesh and liquor should be at least 50g for the *Salmonella* method (for very small species such as the *Donax* spp. a minimum amount of 25g is permitted). For species not given in the table, sufficient shellfish should be opened to achieve this minimum weight of flesh and liquor, with the provision that a minimum of ten animals should be used for very large species. In general, the more shellfish that are included in the initial homogenate, the less the final result will be influenced by the inherent animal-to-animal variation in *Salmonella* concentration.

^D Number of shellfish to be tested for *Salmonella* spp. Regulation (EU) No. 2073/2005 specifies a minimum of 10 animals of any species should be examined.