INTERNATIONAL STANDARD

ISO 6887-3

Second edition
2017-03

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

Microbiologie de la chaîne alimentaire — Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique —

Partie 3: Règles spécifiques pour la préparation des produits de la pêche

Reference number
ISO 6887-3:2017(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword ..... **v**

1 Scope **1**

2 Normative references **2**

3 Terms and definitions **2**

4 Principle **2**

5 Diluents **2**

6 Apparatus **2**

7 Sampling and sample types **3**
7.1 General procedures 3
7.2 Specific procedures for sampling bivalve molluscs, echinoderms and tunicates from primary production 3
7.2.1 General 3
7.2.2 Sampling and laboratory sample transport 3
7.2.3 Sampling method 3
7.2.4 Size and number of individuals per sample 4
7.2.5 Temperature control during transport 4
7.3 Specific procedures for sampling bivalve molluscs, gastropods, echinoderms and tunicates placed on the market 4

8 General procedures **4**

9 Specific procedures **4**
9.1 Raw fishery products, including fish, crustaceans, molluscs, tunicates and echinoderms (see Annex A) 4
9.1.1 Whole fresh fish (more than 15 cm in length) 4
9.1.2 Whole fresh fish (less than 15 cm in length) 5
9.1.3 Sliced fish, fillets and steaks 5
9.1.4 Whole and sliced cephalopods 5
9.1.5 Whole crustacea such as crabs 5
9.1.6 Shelled crustacea flesh 5
9.1.7 Crustacea such as prawns, crayfish, and lobsters 6
9.1.8 Live bivalve molluscs 6
9.1.9 Echinoderms 7
9.2 Processed products 7
9.2.1 Whole smoked fish 7
9.2.2 Smoked fish fillets and slices, with or without skin 8
9.2.3 Whole cooked molluscs in the shell 8
9.2.4 Fish and fish-based multi-component products (e.g. pre-prepared fish taco, mixed seafood selections, mixed fish ball) 8
9.2.5 Cooked or precooked shelled bivalves 8
9.2.6 Salted or pickled products (including fish eggs/roe such as caviar) 8
9.2.7 Dried fish including dried salted fish 9
9.2.8 Fermented products 9
9.2.9 Marinated products 9
9.2.10 Breaded products 9
9.3 Frozen fish, crustacea, molluscs, tunicates, and echinoderms 9
9.3.1 Fish fillets, large fish pieces frozen in blocks, frozen small parts and single portions 9
9.3.2 Shelled crustacea (such as prawns) frozen in blocks 9
9.3.3 Whole crustacea (such as prawns) frozen in blocks 9
9.3.4 Flaked crustacean flesh (such as crab meats) frozen in blocks 9
9.3.5 Molluscs (whole cephalopods, bivalve molluscs and gastropods) 10

10 Further dilutions **10**

Annex A (informative) **Classification of major taxa** **11**

Annex B (informative) **Recommended number of individual live bivalve molluscs to be submitted to the laboratory** **12**

Annex C (informative) **Additional guidance for small fish, crabs and lobsters** **13**

Bibliography **16**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 6887-3:2003), which has been technically revised.

A list of all parts in the ISO 6887 series can be found on the ISO website.

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

WARNING — The use of this document may involve hazardous materials, operations and equipment. It is the responsibility of the user of this document to establish appropriate safety and health practices and to determine the applicability of regulatory limitations before use.

1 Scope

This document specifies rules for the preparation of fish and fishery product samples and their suspension for microbiological examination when the samples require a different preparation from the methods described in ISO 6887-1. ISO 6887-1 defines the general rules for the preparation of the initial suspension and dilutions for microbiological examination.

This document includes special procedures for sampling raw molluscs, tunicates and echinoderms from primary production areas.

NOTE 1 Sampling of raw molluscs, tunicates and echinoderms from primary production areas is included in this document, rather than ISO 13307, which specifies rules for sampling from the terrestrial primary production stage.

This document excludes preparation of samples for both enumeration and detection test methods where preparation details are specified in the relevant International Standards (e.g. ISO/TS 15216-1 and ISO/TS 15216-2 for determination of hepatitis A virus and norovirus in food using real-time RT-PCR).

This document is intended to be used in conjunction with ISO 6887-1. It is applicable to the following raw, processed or frozen fish and shellfish and their products (see Annex A for classification of major taxa):

a) Raw fishery products, molluscs, tunicates and echinoderms including:
   - whole fish or fillets, with or without skin and heads, and gutted;
   - crustaceans, whole or shelled;
   - cephalopods;
   - bivalve molluscs;
   - gastropods;
   - tunicates and echinoderms.

b) Processed products including:
   - smoked fish, whole or prepared fillets, with or without skin;
   - cooked or partially cooked, whole or shelled crustaceans, molluscs, tunicates and echinoderms;
   - cooked or partially cooked fish and fish-based multi-component products.

c) Raw or cooked frozen fish, crustaceans, molluscs and others, in blocks or otherwise, including:

— fish, fish fillets and pieces;

— whole and shelled crustacean (e.g. flaked crab, prawns), molluscs, tunicates and echinoderms.

NOTE 2 The purpose of examinations performed on these samples can be either hygiene testing or quality control. However, the sampling techniques described in this document relate mainly to hygiene testing (on muscle tissues).

2 Normative references

The following documents are referred to in text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6887-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

4 Principle

The general principles for sample preparation and subsequent steps are described in ISO 6887-1. This document describes specific measures for fish and fishery products, including raw, processed and frozen products.

5 Diluents

Diluents for general use and special purposes are described in ISO 6887-1 and there are no additional specific requirements for fish and fishery products.

6 Apparatus

Usual microbiological laboratory equipment for general use (ISO 7218 and ISO 6887-1) and in particular, the following:

6.1 Homogenizer.

6.1.1 Rotary homogenizer (blender), as specified in ISO 7218, but if a large test portion is used, the equipment should include a 1 l bowl.

6.1.2 Peristaltic homogenizer, as specified in ISO 7218.

6.2 Sterile instruments suitable for dissecting samples and opening shells (e.g. oyster knives, hammer, pliers, adjustable vice, oyster cracker, sterile scissors, shellfish picker, winkle picker, scalpels and butcher's knives).

6.3 Sterile forceps (small and large), **spatulas** and **spoons**.

6.4 Small stiff brush, for scrubbing shells.

6.5 Electric drill, equipped with sterile wood bit (14 mm or 16 mm diameter).

6.6 Sterile gauze sheets, suitable for preventing splintering when breaking up shells.

6.7 Food grade plastic bags with waterproof labels, suitable as sampling containers.

6.8 Gloves, strong, suitable to protect operator from injury.

7 Sampling and sample types

7.1 General procedures

Carry out sampling in accordance with the instructions given in this clause for samples from the primary production stage (7.2) or products placed on the market (7.3). For products not detailed here, carry out sampling according to the specific standard appropriate to the product concerned or see ISO/TS 17728. If specific sampling instructions are not available, it is recommended that agreement be reached on this subject by the parties concerned.

7.2 Specific procedures for sampling bivalve molluscs, echinoderms and tunicates from primary production

7.2.1 General

The design and implementation of environmental sampling programmes will affect the results obtained from microbiological examinations. Where the results of this testing are used in microbiological monitoring programmes, particularly for official controls such as classification and monitoring of marine production areas, special consideration should be given to formal recording of sampling plans, species selection and spatial and temporal aspects of sampling design.[6]

7.2.2 Sampling and laboratory sample transport

A sampling protocol containing details of sampling methods, cleaning, packing and transport requirements should be agreed by the parties concerned.

7.2.3 Sampling method

The species under examination should be sampled, as far as possible, using the method employed for commercial harvesting. Equipment used for sampling shall be restricted to that used for this purpose. To avoid contamination by micro-organisms adhering to marine sediments, disturbance of surrounding sediments shall be avoided. Once removed from water and having closed, animals shall be cleaned by rinsing or scrubbing with clean seawater or fresh potable water. Animals shall not be re-immersed in water.

Individual laboratory samples shall be placed in separate undamaged food grade plastic bags (6.7) or equivalent, with waterproof labels containing information to ensure sample traceability.

Where sampling using the commercial harvesting method is not possible, unprocessed animals harvested for commercial purpose should be taken periodically as checks to show that results for laboratory samples collected using the alternative sampling method are acceptable.

7.2.4 Size and number of individuals per sample

Laboratory samples should comprise individuals within the normal commercial size range. A pooled sample comprising a minimum of 10 animals with a minimum amount of flesh and intravalvular liquid of 50 g should be used (for very small species such as *Donax* spp. a minimum amount of 25 g is permitted). Additional animals shall be collected to allow for a proportion of individuals received at the laboratory being in a moribund state. Recommended numbers of individuals for some species are given in Annex B.

7.2.5 Temperature control during transport

The temperature of the sample (either the laboratory sample or the surrounding seawater) should be recorded immediately after collection.

Transport temperature shall be between 0 °C and 10 °C and the equipment used shall be capable of achieving this temperature range within 4 h of sample packing and maintaining it for at least 24 h. If cool packs are used, laboratory samples shall not come into direct contact with their surfaces. Samples shall not be frozen.

Internal air temperature of the temperature controlled unit shall be recorded on receipt at the laboratory.

For samples where less than 4 h have elapsed between collection from the production area and receipt at the laboratory, internal air/sample temperature should be less than the temperature recorded at the time of sampling.

Microbiological examination should be initiated within 24 h of collection of the sample from the production area. If testing cannot be initiated within 24 h or if sample temperatures between 0 °C and 10 °C cannot be achieved, data should be generated to verify that the use of alternate transport and storage conditions does not affect the microbiological content of the sample.

NOTE Studies have shown that *E. coli* will not significantly increase in mussels (*Mytilus edulis*) or Pacific oysters (*Crassostrea gigas*) at temperatures of 15 °C or less for up to 48 h.[8]

7.3 Specific procedures for sampling bivalve molluscs, gastropods, echinoderms and tunicates placed on the market

Use the specific sampling procedures given in 7.2.4.

8 General procedures

All preparations and manipulations shall be carried out using aseptic techniques and sterile equipment (ISO 7218).

9 Specific procedures

9.1 Raw fishery products, including fish, crustaceans, molluscs, tunicates and echinoderms (see Annex A)

9.1.1 Whole fresh fish (more than 15 cm in length)

The gills and the anus shall be covered with sterile cotton wool, drenched in alcohol at a volume fraction of 70 %. Disinfect the surface of the dorsal region (using cotton wool with alcohol at a volume fraction

of 70 %) and remove and discard a section of the skin using sterile forceps (6.3) and scalpel (6.2). Take a cube-shaped sample of dorsal muscle, dice it and break up in an appropriate diluent.

If the fish is eviscerated, the gills shall be covered with sterile cotton wool, drenched in alcohol at a volume fraction of 70 %, and a cube-shaped sample of dorsal muscle shall be removed from inside the body cavity.

Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

Add diluent to make a 1 in 10 suspension and blend in a rotary or peristaltic homogenizer (6.1) as necessary.

9.1.2 Whole fresh fish (less than 15 cm in length)

Using sterile scissors (6.2) and forceps (6.3), remove a portion of fish just anterior to the tail insertion by making two cuts to produce transverse sections, the first cut to remove the tail and tail insertion and the second to remove a steak.

Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

Add diluent to make a 1 in 10 suspension and blend in a rotary or peristaltic homogenizer (6.1) as necessary.

NOTE Additional guidance for small fish up to 15 cm in length is given in Annex C.

9.1.3 Sliced fish, fillets and steaks

No specific requirements; treat in accordance with ISO 6887-1.

9.1.4 Whole and sliced cephalopods

Disinfect the surface of the skin and suckers (using cotton wool with alcohol at a volume fraction of 70 %). Remove the skin and suckers with sterile forceps (6.3) and a scalpel (6.3) and discard. Take cube-shaped samples of dorsal muscles and pieces from the tentacles. Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

The flesh from cephalopods is relatively firm; grind up the test portion in diluent using a rotary homogenizer (6.1.1) or cut it into fine pieces. Add further diluent to make a 1 in 10 suspension and blend in a rotary or peristaltic homogenizer (6.1) as necessary.

9.1.5 Whole crustacea such as crabs

Disinfect the surface (using cotton wool with alcohol at a volume fraction of 70 %) and with sterile hammer (6.2), pliers (6.2) or forceps (6.3) remove or break the carapace (see C.2) and claws to extract the maximum amount of flesh for testing. For large claws, an oyster cracker (6.2) can be used to break open the shell before extracting the flesh.

Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

Add diluent to make a 1 in 10 suspension and blend in a rotary or peristaltic homogenizer (6.1) as necessary.

9.1.6 Shelled crustacea flesh

Take the amount of flesh required in the test method, make the initial 1 in 10 suspension in a diluent and blend in a rotary or peristaltic homogenizer (6.1) as necessary.

Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

9.1.7 Crustacea such as prawns, crayfish, and lobsters

9.1.7.1 Species where tails only are consumed

Disinfect the surface (using cotton wool with alcohol at a volume fraction of 70 %). Break the crustacean at the junction between the cephalothorax and abdomen (see Figure C.3). Using sterile forceps (6.3) pull the edible portion of flesh from the cephalothorax and butt end of the abdomen.

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Add the necessary quantity of diluent to give a 1 in 10 suspension.

Blend in a rotary or peristaltic homogenizer (6.1) as necessary.

9.1.7.2 Species consumed whole

Take the entire animal for examination. Use sufficient material from the laboratory sample to give a representative test portion as specified in the test method.

Add the necessary quantity of diluent to give a 1 in 10 suspension.

Blend in a rotary or peristaltic homogenizer (6.1) as necessary.

9.1.8 Live bivalve molluscs

9.1.8.1 General

On arrival at the laboratory, the internal air temperature of the transport container shall be recorded. For samples where more than 4 h have elapsed between collection and receipt, the internal air temperature should be between 0 °C and 10 °C. If the internal air temperature of the transport container is greater than 10 °C, the sample temperature should be measured; this should not exceed 10 °C. For samples where less than 4 h have elapsed between collection and receipt, internal air/sample temperature should be less than the temperature recorded at the time of sampling.

Laboratory samples shall be stored at 3 °C ± 2 °C.

The animals shall be alive. Discard individuals with open or damaged shells. A representative test sample shall contain at least 10 individuals[7] and shall be at least 50 g (25 g for small animals, e.g. *Donax* spp.) as detailed in 7.2.4 above. Testing of bivalves includes both the flesh and intravalvular water; open sufficient shellfish to yield the amount of flesh and intravalvular fluid specified in the test method.

Microbiological examination should be initiated within 24 h of collection of the sample. If testing cannot be initiated within 24 h or if sample temperatures of 0 °C and 10 °C cannot be achieved, data should be generated to verify that the use of alternate transport and storage conditions does not affect the microbiological content of the sample.

NOTE Studies have shown that *E. coli* will not significantly increase in mussels (*Mytilus edulis*) or Pacific oysters (*Crassostrea gigas*) at temperatures of 15 °C or less for up to 48 h.[8]

9.1.8.2 Methods requiring a 1 in 10 initial suspension

Wash and brush (6.4) each shell under running water of potable quality, especially around the hinge or opening.

Drain the cleaned bivalves and put them on a clean surface.

If there is a byssus muscle, do not tear it away; cut it with sterile scissors, knife or scalpel (6.2) before fully opening.

As each shell is opened, collect the flesh and intravalvular water in a sterile container suitable for blending. Bivalves that have lost their intravalvular water may be used if they are still alive when the shell is opened.

Add one part of flesh and intravalvular water to two parts of diluent. Blend with a rotary homogenizer (6.1.1) for 30 s to 2 min depending on the homogenizer used (ISO 7218). A peristaltic homogenizer (6.1.2) may be used but note that shell splinters can puncture plastic bags. Double- or triple-bagging can help to prevent leaking and the risk of contamination.

In this way, an approximate 1 in 3 suspension is obtained to which the required amount of diluent is added to obtain an accurate 1 in 10 initial suspension.

9.1.8.3 Methods requiring a 1 in 2 initial suspension

Proceed as in 9.1.8.2 but use one part of flesh and intravalvular water to one part of diluent to produce an accurate initial 1 in 2 suspension.

NOTE An initial suspension of 1 in 2 is required for official control testing of bivalve shellfish, marine gastropods and echinoderms according to ISO 16649-2 or other applications where a level of detection of ≤200 cfu per 100 g product is required.

9.1.9 Echinoderms

9.1.9.1 Echinoderms such as sea urchins

Wash at least 10 individuals under running potable water, and place them on a sterile tray.

Hold the sea urchin with forceps (6.3) or wear a strong clean glove (6.8) and cut off a piece of the ventral surface with sterile sharp scissors (6.2) to expose the flesh. Collect the whole flesh and fluid in a sterile container suitable for blending.

Prepare an initial suspension of approximately 1 in 3 in diluent, homogenize in a rotary or peristaltic homogenizer (6.1) as necessary and then add the required amount of diluent to obtain an accurate 1 in 10 suspension.

9.1.9.2 Echinoderms such as holothurians (e.g. sea cucumbers) and tunicates

Wash at least 10 individuals under running potable water, and place them on a sterile tray.

Cut individuals into fine pieces with sterile scissors (6.2).

Prepare an initial suspension of approximately 1 in 3 in diluent, homogenize in a rotary or peristaltic homogenizer (6.1) as necessary and then add the required amount of diluent to obtain an accurate 1 in 10 suspension.

9.2 Processed products

9.2.1 Whole smoked fish

If the whole fish is eaten, then the skin shall be included in the sample. If the skin is not eaten then the skin shall be excluded. Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

The test portion shall be taken from the dorsal area and the flesh cut, diced and homogenized using a rotary or peristaltic homogenizer (6.1) as necessary in diluent to obtain a 1 in 10 suspension.

9.2.2 Smoked fish fillets and slices, with or without skin

Take pieces of the fillet and dice them, under sterile conditions, without removing the skin. Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using either a rotary or peristaltic homogenizer (6.1) as necessary in diluent to obtain a 1 in 10 suspension.

9.2.3 Whole cooked molluscs in the shell

9.2.3.1 Cooked or partially cooked gastropods

Remove the operculum with a sterile scalpel (6.2) then extract the body using forceps (6.3), a winkle picker or shellfish picker (6.2).

Alternatively, carefully crush the shells open using a hammer (6.2) without damaging the flesh.

Remove any shell debris with sterile forceps (6.3) and dice the flesh.

Prepare an initial suspension of approximately 1 in 3 in diluent, homogenize, and then add the required amount of diluent to obtain an accurate 1 in 10 suspension.

9.2.3.2 Cooked or partially cooked bivalves

Extract the body from the shell using sterile forceps (6.3), scalpel and oyster knife or shellfish picker (6.2).

Dice the flesh.

Prepare an initial suspension of approximately 1 in 3 in diluent, homogenize in a rotary or peristaltic homogenizer (6.1), and then add the required amount of diluent to obtain an accurate 1 in 10 suspension.

9.2.3.3 Whole cooked or partially cooked crustacea

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Add the necessary quantity of diluent to give a 1 in 10 suspension.

Blend in a rotary or peristaltic homogenizer (6.1).

9.2.4 Fish and fish-based multi-component products (e.g. pre-prepared fish taco, mixed seafood selections, mixed fish ball)

Take representative parts of each component in proportion to the amounts in the whole product. Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Add the necessary quantity of diluent to give a 1 in 10 suspension.

Blend in a rotary or peristaltic homogenizer (6.1).

9.2.5 Cooked or precooked shelled bivalves

No specific requirements; treat in accordance with ISO 6887-1.

9.2.6 Salted or pickled products (including fish eggs/roe such as caviar)

Treat as dehydrated or acidic products in accordance with ISO 6887-1.

9.2.7 Dried fish including dried salted fish

Treat as dehydrated products in accordance with ISO 6887-1.

9.2.8 Fermented products

Treat as acidic products in accordance with ISO 6887-1.

9.2.9 Marinated products

Treat as acidic products in accordance with ISO 6887-1.

9.2.10 Breaded products

No specific requirements; treat in accordance with ISO 6887-1.

9.3 Frozen fish, crustacea, molluscs, tunicates, and echinoderms

9.3.1 Fish fillets, large fish pieces frozen in blocks, frozen small parts and single portions

Either take a test portion from the frozen block using a drill with a sterile bit (6.5) or defrost at ambient temperature (18 °C to 27 °C) for approximately 60 min but no more than 3 h. Remove pieces with sterile pliers or forceps. Leave to defrost further if necessary until soft enough to cut into smaller pieces with a sterile knife (6.2) and forceps (6.3).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Blend the pieces in a rotary or peristaltic homogenizer (6.1) with diluent to obtain a 1 in 10 suspension.

9.3.2 Shelled crustacea (such as prawns) frozen in blocks

Leave the laboratory sample to defrost for approximately 60 min but no more than 3 h at ambient temperature (18 °C to 27 °C) until the block breaks. Carefully separate the block into pieces using a sterile hammer or butcher's knife (6.2) and take pieces of flesh with sterile forceps (6.3) or pliers (6.2). Alternatively remove the test portion from the frozen block using a drill with a sterile bit (6.5).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

9.3.3 Whole crustacea (such as prawns) frozen in blocks

Leave to defrost for approximately 60 min but no more than 3 h at ambient temperature (18 °C to 27 °C) until the block breaks. Extract the individual animals with sterile pliers (6.2) or forceps (6.3). Allow to defrost so cephalothorax and abdomen (see Annex C) may be separated and the edible portion removed with sterile forceps (6.3).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

9.3.4 Flaked crustacean flesh (such as crab meats) frozen in blocks

Remove the test portion from the frozen block using a drill with sterile bit (6.5) or defrost at ambient temperature (18 °C to 27 °C) for approximately 60 min but no more than 3 h until the block breaks. Remove pieces of flesh with sterile pliers (6.2) or forceps (6.3).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

9.3.5 Molluscs (whole cephalopods, bivalve molluscs and gastropods)

9.3.5.1 Whole cephalopods frozen in blocks

Remove material using a drill with a sterile bit (6.5) or defrost at ambient temperature (18 °C to 27 °C) for approximately 60 min but no more than 3 h. Cut off pieces with sterile scissors or butcher's knife (6.2).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

9.3.5.2 Whole gastropods and bivalve molluscs frozen in blocks

Leave to defrost for approximately 60 min but no more than 3 h at ambient temperature (18 °C to 27 °C) until the block breaks. Extract the individual animals with sterile pliers (6.2) or forceps (6.3). Leave to defrost further if necessary until soft enough to extract the body from the shell using sterile forceps (6.3), scalpel and oyster knife or shellfish picker (6.2).

Alternatively crush the shells open using a sterile hammer (6.2) without damaging the flesh.

Remove any shell debris with sterile forceps (6.3) and dice the flesh.

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

9.3.5.3 Cooked or partially cooked, shelled molluscs such as gastropods and bivalve molluscs frozen in blocks

Leave to defrost for approximately 60 min but no more than 3 h at ambient temperature (18 °C to 27 °C) until the block breaks. Extract the individual animals with sterile pliers (6.2) or forceps (6.3).

Use sufficient material from the edible portion of the laboratory sample to give a representative test portion as specified in the test method.

Homogenize using a rotary or peristaltic homogenizer (6.1) in diluent to obtain a 1 in 10 suspension.

10 Further dilutions

Prepare further dilutions in accordance with ISO 6887-1.

Annex A
(informative)

Classification of major taxa

Taxonomical Division		Examples
Phylum — Chordata	Class — Myxini	Hagfish, Nuta-unagi, Meokjango, Yu sheng
	Class — Petromyzontida	Lamprey
	Class — Chondrichthyes	Whitefish, Makorepe, ghost shark
	Class — Elasmobranchii	Sharks, flake, sora, rays, skates
	Class — Actinopterygii	Fin fish
Phylum — Arthropoda, subphylum — Crustacea		Crayfish, yabby, marron, scampi, clawed lobster, spiny lobster, langoustines, shrimp, prawns, crabs,
Phylum — Mollusca	Class — Cephalopoda	Octopus, squid, cuttlefish, nautilius.
	Class — Bivalvia	Oysters, mussels, scallops, clams, cockles
	Class — Gastropoda	Abalone (paua), conch, periwinkles, whelks, limpets, sea slugs, snails
Phylum — Chordata, subphylum — Tunicata		Sea squirts, sea pork, sea tulips, sea violet, piure
Phylum — Echinodermata	Class — Holothurian	Sea cucumber, trepan, sea slug
	Class — Echinoidea	Sea urchins (hota, ututuk, kina, uni) star fish

Annex B
(informative)

Recommended number of individual live bivalve molluscs to be submitted to the laboratory

Species		Number
Scientific name	**Common name (English)**	
Pecten maximus	King scallop	12 to 18
Aequipecten opercularis	Queen scallop	18 to 35
Crassostrea gigas	Pacific oyster	12 to 18
Ostrea edulis	Flat oyster	12 to 18
Mercenaria mercenaria	Hard clams	12 to 18
Tapes philippinarum	Manilla clam	18 to 35
Ruditapes decussatus	Grooved carpet shells	18 to 35
Spisula solida	Thick trough shells	35 to 55
Mya arenaria	Sand gapers	12 to 18
Ensis spp.	Razor clams	12 to 18
Mytilus spp.	Mussels	18 to 35
Cerastoderma edule	Cockles	35 to 55
Donax spp.	Bean clams	40 to 70

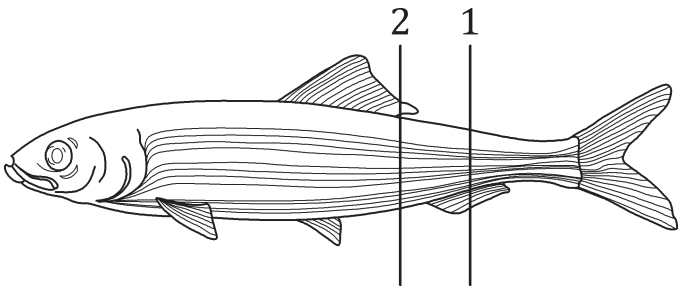
Annex C
(informative)

Additional guidance for small fish, crabs and lobsters

C.1 Small fish (up to 15 cm long)

Using sterile scissors and forceps, remove a portion of the fish just anterior to the tail insertion by making two cuts to produce transverse sections; the first cut to remove the tail and the tail insertion and the second to remove a steak (see Figure C.1).

Take care not to remove any viscera or gut contents.



Key

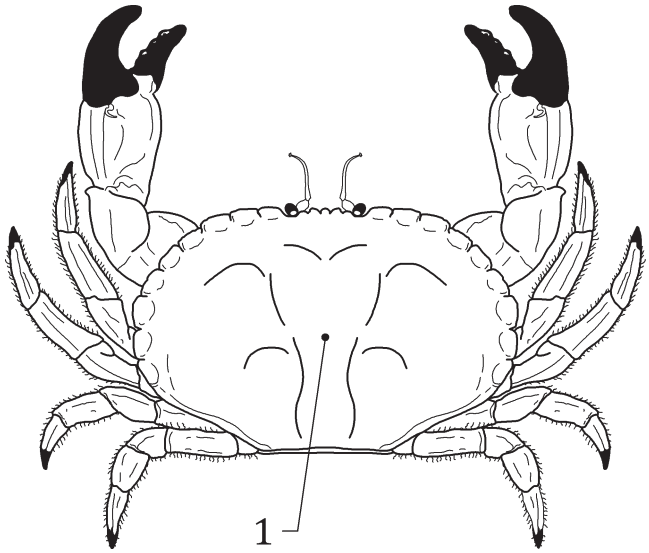
1 cut 1

2 cut 2

Figure C.1 — Example of test sampling of a fish up to 15 cm in length

C.2 Crabs

Lift off carapace (see Figure C.2) with sterile forceps and crack claws. Using sterile forceps, take sufficient flesh to yield the amount specified in the test method.



Key

1 carapace

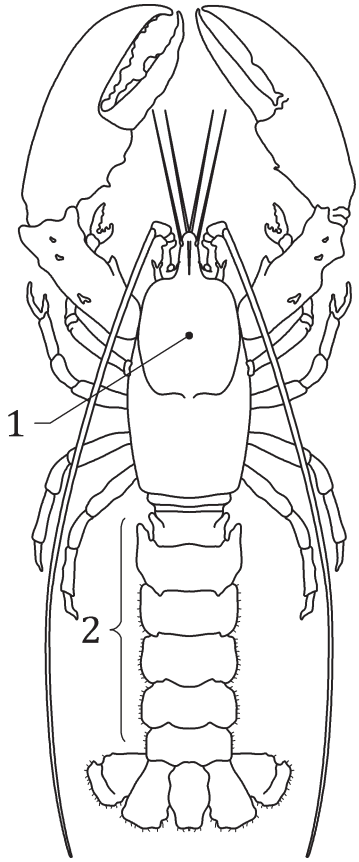
Figure C.2 — Carapace of a crab

C.3 Flesh from lobsters and crayfish

Break the crustacean at the junction between the cephalothorax and abdomen (see Figure C.3).

Using sterile forceps, pull the flesh from the cephalothorax and butt end of the abdomen (this includes a little gut, which is usually eaten).

Take sufficient flesh to yield the amount specified in the test method.



Key

1 cephalothorax

2 abdomen

Figure C.3 — Cephalothorax and abdomen of a lobster

Bibliography

[1] ISO 13307, *Microbiology of food and animal feed — Primary production stage — Sampling techniques*

[2] ISO/TS 15216-1, *Microbiology of the food chain — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR — Part 1: Method for quantification*

[3] ISO/TS 15216-2, *Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR — Part 2: Method for qualitative detection*

[4] ISO 16649-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli* — Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

[5] ISO/TS 17728,*Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples*

[6] European Union Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs. Microbiological monitoring of bivalve mollusc harvesting areas, guide to good practice: Technical application (2014). Available at: https://eurlcefas.org/media/13828/gpg_issue-5_final_all.pdf

[7] European Communities 2005. Commission Regulation (EC) 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Communities* L338, 22.12.05: 1-26

[8] Centre for Environment Fisheries and Aquaculture Science (CEFAS). Fish Health Inspectorate (FHI) Annual Report (2015). Published: 21 May 2015. Updated: 23 February 2016. Available at: https://www.gov.uk/government/publications/fish-health-inspectorate-reports-2015

ICS 07.100.30

Price based on 16 pages