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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

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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.