EAST AFRICAN COMMUNITY

EDICT OF GOVERNMENT

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EAS 217-1-4 (2008) (English): Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1-4: Specific rules for the preparation of fish and fishery products

ISO INSIDE

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EAS 217-1-4:2008
ICS 07.100.30

EAST AFRICAN STANDARD

Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1-4: Specific rules for the preparation of fish and fishery products

EAST AFRICAN COMMUNITY

First Edition 2008

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that “Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose”.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

This East African Standard has been revised and aligned to ISO 6887-3:2003, *Microbiology of food and animal feeding stuffs — 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 STANDARD

ISO 6887-3

First edition
2003-08-01

Microbiology of food and animal feeding stuffs — 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 des aliments — 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 6887-3:2003(E)

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Published in Switzerland

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 6887-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 6887 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — 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*

— *Part 2: Specific rules for the preparation of meat and meat products*

— *Part 3: Specific rules for the preparation of fish and fishery products*

— *Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

Microbiology of food and animal feeding stuffs — 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 standard may involve hazardous materials, operations and equipment. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior the use.

1 Scope

This part of ISO 6887 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 method described in ISO 6887-1. ISO 6887-1 defines the general rules for the preparation of the initial suspension and decimal dilutions for microbiological examination.

This part of ISO 6887 only describes methods of preparation that are applicable to several microorganisms simultaneously. It excludes the preparations that only apply to the detection and/or enumeration of a single microorganism where the methods of preparation are described in the relevant standard concerning that microorganism, for example *Vibrio parahaemolyticus*.

This part of ISO 6887 is applicable to the following raw, processed, cooked or frozen fish and shellfish and their products:

a) RAW fish, crustaceans, molluscs and others, including

- fish, whole or fillets, with or without skin and heads, and gutted,
- fish, salted, dried smoked or pickled,
- cephalopods, whole or sliced,
- crustaceans, whole, including prawns, crayfish, lobsters, crabs and Norway lobsters,
- live gastropods, bivalves, echinoderms and tunicates, and
- snails;

b) PROCESSED fish, crustaceans, molluscs and others, including

- dried, smoked, marinated, salted, pickled and breaded fish or shellfish,
- fish, whole or prepared fillets, with or without skin,
- surimi and delicatessen fish products,

— whole or shelled crustaceans and molluscs, and crustacean and mollusc flesh,

— cooked fish, crustaceans, molluscs, holothurians, tunicates, shellfish and snail-based dishes;

c) FROZEN fish, crustaceans, molluscs and others, in blocks or otherwise, including

— fish, fish fillets and pieces,

— whole and shelled prawns,

— flaked crab,

— cephalopods, and

— shelled cooked shellfish and shelled snails.

NOTE 1 Milk and milk products are dealt with in ISO 8261.

NOTE 2 The purpose of the analysis performed on these test samples may be either hygiene testing or quality control. However, the sampling techniques described in this part of ISO 6887 relate mainly to hygiene testing (on muscle tissues).

2 Normative references

The following referenced documents are indispensable for the application 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 6887-1:1999, *Microbiology of food and animal feeding stuffs — 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 rules for microbiological examinations.*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
laboratory sample
sample prepared for sending to the laboratory and intended for inspection or testing

[ISO 7002]

3.2
test portion
measured (volume or mass) representative sample taken from the laboratory sample for use in the preparation of the initial suspension

3.3
initial suspension
primary dilution
suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed with, normally, a nine-fold quantity of diluent, allowing large particles, if present, to settle

3.4
further decimal dilutions
suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.3) with a nine-fold volume of diluent and by repeating this operation with further dilutions, until a decimal dilution series, suitable for the inoculation of culture media, is obtained

4 Principle

An initial suspension (3.3) is prepared to obtain as uniform a distribution as possible of the microorganisms contained in the test sample.

A pre-enrichment or enrichment suspension is prepared in the same way, using the medium recommended by the method of analysis concerned, except in the special cases mentioned in each product section of this part of ISO 6887.

If necessary, decimal dilutions (3.4) are prepared in order to reduce the number of microorganisms per unit volume to allow, after incubation, observation of any growth (in the case of liquid media) or colonies (in the case of agar plates), as stated in each specific standard.

In order to restrict, if required, the range of enumeration to a given interval, or if high numbers of microorganisms are foreseen, it is possible to inoculate only the necessary decimal dilutions (at least two successive dilutions) needed to achieve the enumeration according to the calculation described in ISO 7218.

5 Diluents

5.1 Basic materials

See ISO 6887-1.

When examining raw, unprocessed marine fish for their natural marine (halophilic) microbial flora, the use of a 3,5 % to 4 % solution of sodium chloride (i.e. isotonic to seawater), for example, is recommended.

5.2 Diluents for general use

5.2.1 Peptone salt solution

See ISO 6887-1:1999, 5.2.1.

5.2.2 Buffered peptone water

See ISO 6887-1:1999, 5.2.2.

5.3 Diluents for special purposes

5.3.1 Peptone-salt solution with Bromocresol purple

5.3.1.1 Composition

Peptone salt solution (see 5.2.1)	1 000 ml
Bromocresol purple (0,04 % alcohol solution, e.g. ethanol solution)	0,1 ml

5.3.1.2 Preparation

Add 0,1 ml of Bromocresol purple to 1 000 ml of peptone salt solution (5.2.1).

5.3.1.3 Application

This solution may be used for certain acidic products so that adjustment of the pH can be carried out without the use of a sterile pH probe (see 8.2).

Bromocresol purple is yellow at acidic pH, changing to purple at pH above 6,8.

5.3.2 Peptone solution

5.3.2.1 Composition

Enzymatic digest of casein	1 g
Water	1 000 ml

5.3.2.2 Preparation

Dissolve the components in the water, by heating if necessary.

If necessary, adjust the pH so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

5.3.2.3 Application

This solution may be used for bivalve molluscs, gastropods and other marine shellfish (see [1]).

NOTE Currently available studies do not clearly show that only this diluent may be used for bivalve molluscs, gastropods and other marine shellfish. The diluent for general use, peptone salt solution (5.2.1), may also be used, since it has been shown to give acceptable results for this type of product (see [2] and [3]).

5.4 Distribution and sterilization of the diluent

See ISO 6887-1:1999, 5.4.

6 Apparatus

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

6.1 Homogenizer

6.1.1 **Rotary homogenizer** (blender)

See ISO 7218. If a large test sample is used, the equipment should include a 1 litre bowl.

6.1.2 **Peristaltic homogenizer**

See ISO 7218.

6.2 **Sterile scissors, knives, shellfish picks, scalpels** and **large butcher's knife**

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

6.4 Sterile instruments, used to open shells (special knives, hammer, pliers, adjustable vice, etc.).

6.5 Small stiff brush, for scrubbing shells.

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

7 Preparation of samples

7.1 Frozen products

Products stored frozen should be brought to a consistency that allows sampling; i.e. by storing at 18 °C to 27 °C (laboratory temperature) for a maximum of 3 h, or 2 °C ± 2 °C for a maximum of 24 h. Samples should be tested as quickly as possible after this. See ISO 6887-1:1999, 9.3.

If the product is still frozen when portioning, some diluent at laboratory temperature may be used to facilitate defrosting.

7.2 Hard and dry products

For hard or dry products, do not homogenize in a rotary homogenizer (6.1.1) for more than 2,5 min at a time.

For dry and hard or heterogeneous products, it may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

7.3 Liquid and non-viscous products

Before analysing, the test sample should be taken after having shaken the laboratory sample by hand (e.g. 25 times through an arc of 25 cm; see ISO 8261 for details) or by mechanical means in order to ensure that the microorganisms are uniformly distributed.

7.4 Heterogeneous products

For heterogeneous products (which contain pieces of different foods), sampling should be carried out by taking aliquots of each component representative of their proportions in the initial product.

It is also possible to homogenize the whole laboratory sample to allow the sampling of an homogenized test sample.

It may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

8 General procedures

8.1 General

All preparations and manipulations should be carried out using good aseptic techniques and with sterile equipment to prevent microbial contamination of samples from all external sources. See ISO 7218.

Indicate in the report which procedure is used for analysis if it is different from the procedure described in this part of ISO 6887.

8.2 General case for acidic products

It is important when using a suspension solution of acidic products to ensure that the pH is brought back to neutrality. The use of diluent with an added pH indicator (5.3.1) can avoid the need to use and sterilize pH probes: add sodium hydroxide (NaOH) to bring back the coloration of the suspension until the indicator starts to change.

For use with buffered diluents, the addition of NaOH is often necessary to increase the buffering capacity of the alkaline component. The concentration of added NaOH depends on the product acidity. The most suitable concentration (e.g. 0,1 mol/l or 1 mol/l) is the concentration which is still close to a ratio of 1 to 9 with diluent.

8.3 High fat foods (for example over 20 % of total mass is fat)

The use of a diluent with between 1 g/l and 10 g/l of added sorbitan monooleate (Tween 80), approximately according to fat levels (e.g. at a fat content of 40 %, add 4 g/l) may improve emulsification during suspension.

9 Specific procedures

9.1 Raw fish, crustaceans, molluscs and others

9.1.1 Whole fresh fish

The gills, intestinal area and the anus should be covered with sterile cotton wool, drenched in 70 % alcohol. Take a cube-shaped sample of dorsal muscle, dice and grind it up in the diluent (5.2).

9.1.2 Sliced fish, fillets and steaks

Treat in accordance with ISO 6887-1.

9.1.3 Whole and sliced cephalopods

Remove the skin and sucker with forceps (6.3) and a scalpel (6.2). Take cube-shaped samples of dorsal muscles and pieces from the tentacles.

Add diluent (5.2) to make a 1 in 10 dilution. Since the flesh from cephalopods is relatively firm, grind up the laboratory sample in diluent using a rotary homogenizer (6.1.1) or cut it into fine pieces.

9.1.4 Whole crustaceans such as crabs

Use a hammer, pliers (6.4) or forceps (6.3) to break the shell and claws in order to extract the maximum amount of flesh for analysis.

Add diluent (5.2) and preferably grind in a rotary homogenizer (6.1.1). Alternatively, cut into fine pieces and place in a double bag for peristaltic homogenization to avoid leakage when blending during homogenization.

9.1.5 Shelled crustacean flesh

Take the amount of flesh required, and make the initial 1 + 9 suspension in diluent (5.2) and homogenize as in 9.1.4.

9.1.6 Crustaceans such as prawns, crayfish, lobsters or Norway lobsters (whole or tails)

Remove the head and tail fin before analysis of the body flesh.

Except for very small animals, shell the crustacean and cut the flesh into pieces. Blend in a rotary homogenizer (6.1.1).

Add the necessary quantity of diluent (5.2) to give a 1 + 9 dilution.

9.1.7 Live bivalves, gastropods and others

9.1.7.1 General

Upon arrival at the laboratory, store the laboratory sample at a temperature of at 4 °C ± 2 °C. The shellfish should be alive. Discard shellfish with open or damaged shells.

A representative test sample shall contain at least six individuals and shall be about 75 g to 100 g (25 g for small animals, e.g. *Donax* spp.). Analysis of bivalves takes account of both the flesh and intervalvular water. Sufficient shellfish should be opened to yield the amount of flesh and intervalvular fluid specified in the test method.

9.1.7.2 Bivalves

Wash and brush (6.5) each shell under running water of potable quality, especially around the hinge or opening.

Drain the cleaned bivalves and put them on a plate. Cover with an absorbent paper.

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

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

Add 1 part of flesh and intervalvular water to 2 parts of diluent (5.2). Blend with a rotary homogenizer (6.1.1) for approximately 30 s to 2 min depending on the homogenizer used (see ISO 7218).

In this way, a 1 + 2 suspension is obtained to which the diluent (5.2) may be added to obtain a 1 + 9 dilution.

If there are no shell splinters, a peristaltic homogenizer may be used. If there are shell splinters, a peristaltic homogenizer with a double or triple bag may be used.

9.1.7.3 Gastropods (e.g. whelks)

Brush (see 6.5) and wash the shell, clean it with 70 % alcohol then place on a sterile tray (if necessary between two sterile gauze layers). Using a hammer (6.4), smash the shell so that the body of the animal can be extracted.

The shells may also be crushed open using a vice (6.4).

Dice the flesh whilst removing shell debris with forceps (6.3).

Prepare an initial suspension (3.3) of 1 + 2 in diluent (5.2), then complete using an appropriate volume of diluent to obtain a 1 + 9 suspension.

NOTE The analysis of winkles is very difficult, since it is impossible to extract the animal's flesh without contaminating it via the shell.

9.1.8 Sea urchins

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

Hold the sea urchin with forceps or an appropriate glove and cut off a piece of the ventral side with sharp scissors (6.2). Collect the whole flesh and fluid in a sterile container suitable for blending, using a spatula.

Prepare an initial suspension (3.3) of 1 + 2 in diluent (5.2), then dilute to 1 + 9 using the same diluent.

9.1.9 Holothurians and tunicates

Cut these into fine pieces with scissors (6.2) and blend in a rotary homogenizer (6.1.1).

Prepare an initial suspension (3.3) of 1 + 2 in diluent (5.2) as described in 9.1.4, then dilute to 1 + 9 using the same diluent.

9.2 Processed products of fish, crustaceans, molluscs and other products

9.2.1 Salted or pickled products

Take strips of muscle from the test sample to obtain a homogenized preparation. In the case of very salted products, it may be necessary to dilute more than 1 + 9.

9.2.2 Dried fish

Cut pieces from the body of the fish, including the skin, using scissors (6.2).

Prepare an initial suspension (3.3) of 1 + 2 in diluent (5.2) and blend in a rotary homogenizer (6.1.1). Then dilute to 1 + 9 using the same diluent.

Rehydrate the sample by soaking, if necessary, for 60 min at 18 °C to 27 °C (laboratory temperature).

9.2.3 Salted dried fish

Take a sample in the same way as for dried fish (9.2.2), and as for salted products (9.2.1) when making the suspension.

Rehydrate the sample by soaking, if necessary, for 60 min at 18 °C to 27 °C (laboratory temperature).

9.2.4 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.

The sample is taken from the dorsal area and the flesh cut, diced and homogenized in diluent (5.2).

9.2.5 Smoked fish fillets and slices, with or without skin

Take pieces of the fillet and dice them, under sterile conditions, without removing the skin.

9.2.6 Marinated products

Treat as low pH/acidified product (8.2).

9.2.7 Breaded fish, surimi; fish, crustacean and mollusc delicatessen

See ISO 6887-1.

9.2.8 Fish and crustacean and mollusc-based cooked dishes

Take aliquot parts of each component, taking their proportions into account.

The whole test sample may be homogenized for laboratory use, so that a homogeneous test sample may be obtained.

9.2.9 Whole or shelled cooked crustaceans and molluscs, crustacean and mollusc flesh

See 9.1.5, 9.1.6, and 9.1.7.

9.2.10 Cooked gastropods

Remove the operculum with a scalpel (6.2) then extract the animal's body using forceps (6.3), a winkle picker or a fine metal rod shaped into a hook at one end.

9.2.11 Cooked or precooked shelled bivalves

See ISO 6887-1.

9.3 Frozen fish, crustaceans, molluscs and other products

9.3.1 Shelled prawns frozen in blocks

Defrost slightly in order to be able to break the block, then separate the prawns and take pieces of flesh with forceps (6.3).

9.3.2 Whole prawns frozen in blocks

Leave to defrost for 1 h at 18 °C to 27 °C (laboratory temperature) so that the block can be broken into.

Extract the prawns with forceps (6.3) and shell them with the forceps on a sterile tray.

Blend the pieces in a rotary homogenizer (6.1.1).

9.3.3 Flaked crab flesh frozen in blocks

Either take a sample from the frozen block using a drill (6.6),or leave it to defrost at 18 °C to 27 °C (laboratory temperature) for approximately 1 h and remove chunks with pliers (6.4) or forceps (6.3).

9.3.4 Whole cephalopods frozen in blocks

Defrost slightly (for approximately 1 h for large blocks) at 18 °C to 27 °C (laboratory temperature). Cut off pieces with scissors or a large well-sharpened knife (6.2).

9.3.5 Precooked shelled snails, molluscs frozen in blocks

See crab flesh (9.1.5).

9.3.6 Fish fillets frozen in blocks

Either take a sample from the frozen block using a drill (6.6), or leave it to defrost at 18 °C to 27 °C (laboratory temperature) for approximately 1 h, then remove chunks with pliers (6.4) or forceps (6.3).

Leave to defrost until soft enough to cut, for 1 h and not more than 3 h, at 18 °C to 27 °C (laboratory temperature) and remove pieces from the block with a large knife and forceps (6.3).

9.3.7 Large fish pieces (e.g. tuna fillets) frozen in blocks

Leave to defrost until soft enough to cut, for 1 h and not more than 3 h, at 18 °C to 27 °C (laboratory temperature). Cut a slice from the block, in the middle, with a large well-sharpened knife (6.2).

9.3.8 Frozen small parts and single portions

Leave to defrost until soft enough to cut, for 1 h and not more than 3 h, at 18 °C to 27 °C (laboratory temperature).

Treat the samples as a fresh product.

9.3.9 **Whole and large portion frozen fish** (salmon, tuna, etc.)

9.3.9.1 Whole frozen tuna

Samples are normally taken on commercial premises.

If the tuna is defrosted, take a piece of the muscle from under the skin using a knife.

If the tuna is not defrosted, use a drill (6.6).

9.3.9.2 Frozen fish and frozen fish pieces

If the product mass means that defrosting will take more than 3 h at 18 °C to 27 °C (laboratory temperature), either defrost in a refrigerator, at 0 °C to 4 °C, for a maximum of 48 h, or take samples using the drill (6.6), avoiding the bones if possible.

10 Further decimal dilutions

See ISO 6887-1.

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ICS 07.100.30

Price based on 11 pages