SRI LANKA STANDARD 393 PART 4: 2018

(ISO 6887-4:2017)

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CODE OF PRACTICE FOR PREPARATION OF TEST SAMPLES, INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOOD AND ANIMAL FEEDING STUFF

PART 4 – SPECIFIC RULES FOR THE PREPARATION OF MISCELLANEOUS PRODUCTS

(*Second Revision*)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This Standard does not purport to include all the necessary provisions of a contract

Sri Lanka Standard
CODE OF PRACTICE FOR PREPARATION OF TEST SAMPLES, INITIAL SUSPENSION AND DECIMAL DILUTIONS FOR MICROBIOLOGICAL EXAMINATION OF FOOD AND ANIMAL FEEDING STUFFS
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NATIONAL FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2018-08-10.

This Standard was first published in 1976 and revised in 2013 with a view of providing a general guidance with regard to the practice to be followed, precautions to be observed in the sampling of different types of foods and in the handling of the samples for microbiological analysis. However, keeping in view the experience gained during the years and various International Standards brought out by the International Organization for Standardization **(ISO)** on the subject of microbiology of food and animal feeding stuffs, it was decided to revise the Standard with a view of updating the existing rules and by incorporating those not covered earlier.

For different products of food and animal feeding stuffs, it is necessary to take special precautions and in order to accommodate these precautions within the scope of one Standard, this revised Standard is published in several parts.

This part of the Standard is identical with **ISO** 6887-4 : 2017, Microbiology of food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 4 – Specific rules for the preparation of miscellaneous products published by the International Organization for Standardization (**ISO**).

Terminology and Conventions:

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this Standard should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point on the base line as the decimal marker.

c) Wherever page numbers are quoted, they are **ISO** page numbers.

Cross References

International Standard	Corresponding Sri Lanka Standard
ISO 6887-1, Microbiology of 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	SLS 393-Code of Practice for preparation and decimal dilutions for microbiological examination of food and animal feeding stuff- Part1 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.	SLS 1463 - General requirements and guidance for microbiological examination of food and animal feeding stuff.

SLS 393 PART 4: 2018

INTERNATIONAL STANDARD

ISO 6887-4

Second edition
2017-03

Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 4: Specific rules for the preparation of miscellaneous products

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

Partie 4: Règles spécifiques pour la préparation de produits variés

Reference number
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Contents

Page

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.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 6887-4:2003), which has been technically revised.

It also incorporates the Amendment ISO 6887-4:2003/Amd.1:2011 and the Technical Corrigendum ISO 6887-4:2003/Cor.1:2004.

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

INTERNATIONAL STANDARD **ISO 6887-4:2017(E)**

Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 4: Specific rules for the preparation of miscellaneous 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 samples and dilutions for the microbiological examination of specific food products not covered in other parts of ISO 6887, which deal with more general categories. This document covers a wide range of miscellaneous products, but does not include new products brought on to the market after publication.

ISO 6887-1 defines the general rules for the preparation of the initial suspension and dilutions for microbiological examination.

This document excludes preparation of samples for both enumeration and detection test methods when preparation details are specified in the relevant International Standards.

This document is applicable to the following products:

— acidic (low pH) products;

— hard and dry products;

— dehydrated, freeze-dried and other low a_w products (including those with inhibitory properties);

— flours, whole cereal grains, cereal by-products;

— animal feed, cattle cake, kibbles and pet chews;

— gelatine (powdered and leaf);

— margarines, spreads and non-dairy products with added water;

— eggs and egg products;

— bakery goods, pastries and cakes;

— fresh fruit and vegetables;

— fermented products and other products containing viable microorganisms;

— alcoholic and non-alcoholic beverages;

— alternative protein products.

2 Normative references

The following documents are referred to in the 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 https://www.iso.org/obp/

4 Principle

The general principles for sample preparation and subsequent steps are detailed in ISO 6887-1. This document describes specific measures for products not covered in other parts of ISO 6887.

An initial suspension is prepared to obtain as uniform a distribution as possible of the microorganisms contained in the test portion.

For detection methods, the pre-enrichment or enrichment suspension is prepared in the same way, using the medium recommended in the method of examination concerned. For enumeration methods used for all food types covered in this document, general diluents are documented in the relevant clauses of ISO 6887-1 and specific diluents are included in Clause 5.

If necessary, further dilutions 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 or agar tubes) as stated in each specific standard.

5 Diluents

5.1 Basic materials

Refer to ISO 6887-1.

5.2 Diluents for general use

5.2.1 Peptone salt solution

Refer to ISO 6887-1.

5.2.2 Buffered peptone water

Refer to ISO 6887-1.

5.3 Diluents for special purposes

5.3.1 Double-strength buffered peptone water

5.3.1.1 Composition and preparation

Refer to ISO 6887-1.

5.3.1.2 Application

Use this diluent for highly acidic products of pH ≥ 3,5 to pH < 4,5 to obtain an initial suspension of pH 7,0 ± 0,5.

NOTE Buffered peptone water (5.2.2) is sufficient for products of pH 4,5 and above.

5.3.2 Phosphate buffered diluent

5.3.2.1 Composition

Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 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 pH 7,0 ± 0,2 at 25 °C.

5.3.2.3 Application

Phosphate buffered solution is used as a diluent for gelatine (9.3).

5.4 Distribution and sterilization of the diluent

Refer to ISO 6887-1.

5.5 Performance testing of diluents

Refer to ISO 6887-1.

5.6 Enzyme solutions

5.6.1 Alpha-amylase solution

5.6.1.1 Composition

α-amylase	1,0 g
Water	100 ml

5.6.1.2 Preparation

Dissolve the α-amylase in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to 1 month at 5 °C ± 3 °C or up to 3 months at ≤–20 °C.

Final composition of the α-amylase solution may need to be adjusted depending on the enzymatic activity of the commercial α-amylase used and the thickening properties of the test sample.

5.6.1.3 Application

This enzyme solution is added at the rate of 10 ml to 1 000 ml of diluent (1 % volume fraction) to improve solubility of swelling starch products, cereals and cereal-containing products (9.1.4.3).

5.6.2 Cellulase solution

5.6.2.1 Composition

Cellulase	1,0 g
Water	100 ml

5.6.2.2 Preparation

Dissolve the cellulase in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to two weeks at 5 °C ± 3 °C or up to 1 month at ≤–20 °C.

NOTE Use of fresh solution will ensure maximum enzyme activity.

5.6.2.3 Application

This enzyme solution is added at the rate of 10 ml to 1 000 ml of diluent (1 % volume fraction) to improve solubility of carboxymethyl cellulose, locust beans, carob, guar and cassia gums (9.1.4.3).

5.6.3 Papain solution

5.6.3.1 Composition

Papain	5,0 g
Water	100 ml

5.6.3.2 Preparation

Dissolve the papain in the water and sterilize the solution by passing through a 0,2 µm membrane filter. The enzyme solution can be stored for up to 1 month at 5 °C ± 3 °C.

NOTE Use of fresh solution will ensure maximum enzyme activity.

5.6.3.3 Application

This enzyme solution is added at the rate of 20 ml to 1 000 ml of diluent (2 % volume fraction) to improve solubility of gelatine (9.1.4.3 and 9.3).

6 Apparatus

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

6.1 Homogenizers.

6.1.1 Rotary homogenizer (blender).

Refer to ISO 7218. If a large sample is to be homogenized, the equipment should include a sterile 1 l bowl.

6.1.2 Peristaltic homogenizer.

Refer to ISO 7218. With sterile plastic bags or filter bags to retain particulate material where necessary.

6.2 Domestic grater, sterile.

6.3 Hammer or **other heavy implement**, capable of crushing hard materials.

6.4 Water baths, capable of being maintained at 44 °C to 47 °C or as stated for specific purposes.

6.5 Sterile scissors, **knives**, **scalpels** and **forceps**.

6.6 Sterile spatulas, **spoons** or **scoops**.

6.7 Sterile corers, for taking samples at depth.

6.8 Stirrer, capable of operating with a horizontal motion.

6.9 Sterile wide-necked flasks or **other containers**, of 500 ml capacity.

6.10 Ultrasonic bath, with operating frequency of 35 MHz to 45 MHz.

7 Sampling and sample types

Carry out sampling in accordance with the specific standard appropriate to the product concerned or see ISO/TS 17728. Some guidance on sampling certain products is included in Clause 9 for clarity. If a specific standard is not available, it is recommended that agreement be reached on this subject by the parties concerned.

8 Preparation of samples

8.1 General

All preparations and manipulations shall be carried out using aseptic techniques and sterile equipment (see ISO 7218). General sample preparation procedures are given in ISO 6887-1, but additional detail for some categories is given in 8.2 to 8.4.

8.2 Acidic products

It is important to consider the end use of the product when testing acidic samples.

If the product is to be used as an ingredient in a final product of higher pH, then the pH of the initial suspension of the test portion shall be adjusted to pH 7,0 ± 0,5 with the diluents specified at 5.2.2 or 5.3.1 or others with equivalent buffering capacity.

For pH adjustment of moderately acidic samples (pH ≥ 3,5 to pH < 4,5), use double-strength buffered peptone water (5.3.1). See ISO 6887-1.

If highly acidic (pH < 3,5) samples (e.g. low pH fruits and juices or vinegars and pickles) are tested for acid-tolerant and acidophilic spoilage organisms using appropriate media, the pH of such samples shall not be adjusted.

8.3 High-fat foods, excluding margarines and spreads (e.g. over 20 % of total mass as fat)

A diluent with between 1 g/l and 10 g/l of polysorbate 80 [polyoxyethylene (20) sorbitan monooleate] according to the estimated fat content shall be used to improve emulsification during suspension (e.g. for a fat content of 40 %, add 4 g/l).

Alternative surfactants and emulsifiers are available under various trade names, but the proportions to use should be determined by the laboratory.

8.4 Hard and dry products

Do not homogenize hard or dry products in a rotary homogenizer (6.1.1) for more than 2,5 min at a time to avoid excessive heating that may damage the microorganisms present.

Homogenize dry and hard heterogeneous products by mincing or grinding the laboratory sample. Avoid excessive heating during this process by homogenizing for periods of no more than 1 min at a time, with suitable rest intervals applied, depending on the product being processed. Mince or grind until the sample is visibly homogeneous.

Resuscitation at laboratory ambient temperature (18 °C to 27 °C) for up to 1 h is recommended to assist in the recovery of stressed organisms from all hard and dry products.

9 Specific procedures

9.1 Dehydrated and low a_w products

9.1.1 General

The following are regarded as dehydrated products:

— dehydrated meats and vegetables;

— dried soups, bouillon cubes and gravy mixes;

— powdered beverages (tea, cocoa and cocoa-based products, coffee, dehydrated fruit juice);

— raw cellulose, soluble cellulose, dextrin, sorbitol, sugars, glucose, glutamate;

— herbs, spices, flavourings and colourings;

— polysaccharide gelling agents, alginates, gums, etc.;

— coconut, partially dehydrated vegetable/yeast/meat/fish extracts;

— chocolate and confectionery (bars or sweets);

— dehydrated whole egg and dried egg white;

— cereals, flours, animal feeds;

— powdered or pelletized viable microorganisms (e.g. yeasts for bakery).

9.1.2 Apparatus

Plastic bags with filter inserts (6.1.2) are recommended to assist in the pipetting of products with substantial insoluble matter in suspension.

9.1.3 Preparation of samples

Mix powdered products thoroughly in their container using a sterile implement (6.6) and then weigh out using aseptic techniques. Weigh accurately into a pre-dispensed volume of diluent (5.2.2) to minimize osmotic shock to the microflora.

Other products may require breaking or cutting up with sterile tools into small pieces before taking the test portion.

9.1.4 Preparation of initial suspension

9.1.4.1 Powdered products, completely soluble

It is not necessary to homogenize fully soluble products mechanically as mixing by hand is adequate. Prepare the initial suspension in accordance with ISO 6887-1.

9.1.4.2 Other less soluble or non-powdered products

Prepare the initial suspension using a rotary blender (6.1.1) or peristaltic homogenizer (6.1.2).

9.1.4.3 Products which swell in water

For all products that swell in water (e.g. polysaccharides and gum gelling agents, dehydrated parsley or chives), make further dilutions (1 in 20, 1 in 50 or 1 in 100, as appropriate) until a suitable suspension is obtained.

Record the use of additional diluent to ensure the correct calculation of enumeration test results.

Where greater dilutions are made, the number of inoculated plates for enumeration tests shall be increased to ensure a minimum of 0,1 g of the test portion is distributed between all plates when low counts are expected.

The solubility of some substances is improved by the addition of a specific enzyme solution to the initial suspension in buffered peptone water (5.2.2). Some examples of suitable enzymes are the following:

— 1 % (volume fraction) alpha-amylase (5.6.1) for swelling starch products, cereals and cereal-containing products;

— 1 % (volume fraction) cellulase (5.6.2) for carboxymethyl cellulose, locust beans, carob, guar and cassia gums;

— 2 % (volume fraction) papain (5.6.3) for gelatine.

9.1.4.4 Inhibitory food materials

For food materials that contain inhibitory substances (e.g. onion powder, garlic, oregano, peppers, certain teas and coffees, vitamin premixes and highly salted products), it is necessary to decrease the antimicrobial activity before testing by using special preparation procedures such as the following:

— use of greater dilutions (e.g. 1 in 100 for cinnamon and oregano and 1 in 1 000 for cloves);

— addition of potassium sulphite (K_2SO_3) to the buffered peptone water (5.2.2) to achieve a final concentration of 0,5 % (w/v);

— use of diluent (5.2.2) at 37 °C ± 1 °C, to aid dissolution, and higher dilutions (e.g. 1 in 50) for vitamin premixes;

— use of higher dilutions for products containing more than 10 % (mass fraction) salt (sodium chloride) to ensure the total concentration in the initial suspension (not including any salt content of the diluent or enrichment broth) does not exceed 1 % (w/v).

If any of these techniques is used, spiked sample process controls shall be included at first use to verify the effectiveness of the neutralization process chosen.

9.1.4.5 Cocoa and cocoa-containing products

Use either UHT milk or reconstituted non-fat dry milk powder (100 g/l water; sterilized after reconstitution) as the pre-enrichment broth for detection of the significant pathogens *Salmonella* spp. and STEC. BPW may be used as a general diluent for other tests.

NOTE Milk is used to neutralize the bactericidal effect of cocoa or cocoa-containing products. The probable inhibitory factor in cocoa is anthocyanin.[9],[11],[13]

Preheat the diluent to 37 °C to 40 °C.

Weigh the test portion (e.g. 25 g) into a plastic bag (6.1.2), add the warmed diluent (e.g. 225 ml) to achieve a 1 in 10 initial suspension and mix by hand immediately.

Leave the suspension at laboratory ambient temperature (18 °C to 27 °C) for 20 min to 30 min to melt. Then, mix completely in a peristaltic homogenizer for 60 s ± 5 s.

For cocoa powder and any other samples which may be highly contaminated with Gram-positive bacteria as a result of inadequate thermal processing, addition of 0,45 ml 1 % (w/v) aqueous brilliant green solution (1 g/100 ml water) to the initial suspension of 250 ml can reduce inhibition of low levels of Gram-negative target organisms during non-selective pre-enrichment.[8],[12] Whether this is used depends on the type of sample and laboratory experience with such samples.

When large portions of solid chocolate or other cocoa-containing materials which cannot be broken up easily are tested, it may be necessary to melt the chocolate at a temperature between 42 °C and 47 °C, for no longer than necessary, before taking the test portion.

For chocolate products containing >20 % fat, unless the products already contain sufficient emulsifier, add sufficient polysorbate 80 [polyoxyethylene (20) sorbitan monooleate] or other emulsifier to the diluent (see 8.3 and ISO 6887-1).

9.1.4.6 Confectionery (bars or sweets)

Pre-heat the diluent to 37 °C to 40 °C.

Weigh out the test portion in a plastic bag (6.1.2) and add the warmed diluent. Mix immediately by hand to distribute the test portion. Very hard sweets or candies may also be partially crushed with a heavy object, such as a hammer (6.3), to aid dispersion.

Leave the suspension at laboratory ambient temperature (18 °C to 27 °C) for 20 min to 30 min to dissolve. Then, mix completely using the peristaltic homogenizer (6.1.2).

9.1.5 Resuscitation

In general, leave the initial suspension of low-moisture products requiring resuscitation for up to 1 h at laboratory ambient temperature (18 °C to 27 °C) before preparing any further dilutions. Some specific cases are detailed in the relevant International Standards.

9.1.6 Water activity

Table 1 gives examples of food types and typical ranges of water activity for guidance when test methods specify different procedures to be used depending on the water activity (e.g. ISO 21527-1 and ISO 21527-2).

Table 1 — Water activity of different products (adapted from Reference [10])

Water activity[5] a_w	Examples of product
≥0,95	Highly perishable foods, fresh and canned fruits, vegetables, meats, fish, milk, cooked sausages, breads
≥0,91	Cheeses (e.g. cheddar, Swiss, muenster, provolone), cured meats, some fruit juice concentrates, foods containing 55 % (mass fraction) sucrose or 12 % (mass fraction) salt
≥0,87	Fermented sausages, sponge cakes, dry cheeses, margarine, foods containing 65 % (mass fraction) sucrose or 15 % (mass fraction) salt
≥0,80	Most fruit juice concentrates, sweetened condensed milk, maple and fruit syrups, flour, rice, pulses (15 % to 17 % mass fraction moisture), fruit cake, country-style ham, fondants, high-sugar cakes
≥0,75	Jam, marmalade, marzipan, glace fruits, some marshmallows
≥0,65	Rolled oats (≅10 % mass fraction moisture), jelly, fudge, molasses, some dried fruits, nuts
≥0,60	Dried fruits (15 % to 20 % mass fraction moisture), toffees, caramels, honey, cereal bars, pet chews, granulated foods, cereals, cereal products and grains
≥0,50	Noodles (12 % mass fraction moisture), spices (10 % mass fraction moisture)
≥0,40	Nougat, whole egg powder (5 % mass fraction moisture), chocolate
≥0,30	Biscuits and cookies, crackers, dehydrated sauce powders
≥0,03	Whole milk powders, instant coffee, dehydrated soups

9.2 Flours, cereal grains and by-products and animal feeds

Mix dry powders well in the sample container, using a sterile implement (6.6), before weighing out the test portion.

Weigh the test portion accurately and add it to the required volume of peptone salt solution (5.2.1) to minimize osmotic shock to the microflora. This is the initial suspension at a 1 in 10 dilution.

For flours, take a proportion of the required volume of diluent and add the test portion. Mix well by hand and then add the remainder of the diluent to obtain a 1 in 10 dilution.

Before homogenization, leave to stand for 20 min to 30 min at laboratory ambient temperature (18 °C to 27 °C) to assist resuscitation of damaged organisms.

If the viscosity of the suspension increases so that it becomes too thick or viscous to mix well or to pipette, add a further equal volume of peptone salt solution to produce a 1 in 20 initial suspension and record this to ensure correct calculation of enumeration test results.

Mix for 60 s ± 5 s using a peristaltic homogenizer (6.1.2) for flours or a rotary blender (6.1.1) for cereal grains or animal feeds.

A test portion of 50 g is required when testing cereals and other heterogeneous products to improve the reliability of test results. In this case, use a 1 in 5 suspension, homogenize and then make a further 1 in 2 dilution to obtain the initial 1 in 10 suspension.

NOTE Hard materials (e.g. grains and seeds) can puncture plastic bags (6.1.2); double- or triple-bagging can help to prevent leakage and subsequent contamination. Test portions can also be partially crushed with a heavy object, such as a hammer (6.3), before homogenizing.

Animal feeds are presented for testing in a variety of forms, but the general rules for cereal products above apply to many.

For pet chews, kibble (extruded product) and similar hard products, immerse in diluent at a 1 in 10 dilution, then leave to soak for approximately 1 min before massaging by hand for 30 s ± 5 s. Mix

when the products have softened using a peristaltic homogenizer (6.1.2) or rotary blender (6.1.1) for 60 s ± 5 s, if necessary.

9.3 Gelatine (powdered and leaf)

9.3.1 Preparation of samples

Take a test portion of 20 g of the laboratory sample using aseptic techniques.

9.3.2 Preparation of initial suspension

Transfer this test portion to a 500 ml sterile flask (6.9). Add 180 ml of phosphate buffered diluent (5.3.2) and mix to disperse the granules in the liquid.

Leave the gelatine to adsorb the diluent for 60 min at laboratory ambient temperature (18 °C to 27 °C).

Place the flask in a water bath (6.4) at 44 °C to 47 °C for a maximum of 30 min; mix frequently to dissolve the gelatine to obtain the initial 1 in 10 suspension.

Alternatively, papain may be used to dissolve the gelatine (see 9.1.4.3).

9.4 Margarine and spreads

9.4.1 Sampling

9.4.1.1 General

Samples may be taken from within the bulk product or from within and/or on the surface of packaged items ready for sale, using aseptic techniques throughout.

9.4.1.2 Bulk or pre-wrapped products of ≥1 kg

To determine the microbiological quality of bulk product, examine only core samples. First, remove a slice of 3 mm to 5 mm thickness from the outer layer with a sterilized spatula (6.6) or knife (6.5). Push a sterile metal corer (6.7) into the product diagonally without going all the way through. Turn the corer in a full circle, then remove it with the cylindrical sample.

Transfer a portion of this core sample to a sterile container or plastic bag (6.1.2), using a spatula (6.6) or knife (6.5), but retain the upper 25 mm to plug the hole made by the corer.

Take one or more core samples to obtain an adequate laboratory sample.

NOTE Any other sampling method (such as taking one mass of at least 500 g) is permitted if the product is regarded as homogeneous.

9.4.1.3 Pre-wrapped product of ≤1 kg

The laboratory sample shall be made up of one or more pre-wrapped, intact items.

Remove the wrapping and take a representative test sample from one or more packs using aseptic techniques. Remove the outer 5 mm section before sampling if the packs are ≥500 g. Take the test sample using a sterile instrument or use a sterile corer (6.7) to take a cylindrical portion through the laboratory sample.

If the customer requests testing of the product surface only, remove the test sample by scraping sufficient material from the surface with a sterile implement.

9.4.2 Preparation of test sample

9.4.2.1 General

Weigh 50 g from the laboratory sample containing a volume-to-mass ratio of water of *W* % into a sterilized flask or other container (6.9).

9.4.2.2 Preparation of the aqueous phase (primary dilution)

Pre-warm a volume of [50 − (50 · *W*/100)] ml of diluent (5.2) in a water bath (6.4) at 44 °C to 47 °C and add it to the test sample in the container. In these circumstances, 1 ml of the aqueous phase is equivalent to 1 g of the margarine or spread in the test sample (see ISO 6887-1).

EXAMPLE For a 50 g test sample of margarine with an 84 % fat content and therefore a volume-to-mass ratio of about 16 %, the aqueous phase represents 8 ml of water. Add [50 − (50 × 16/100)] = 42 ml of diluent. This can also be calculated by taking the sample weight of 50 g and multiplying by the fat content of 84 % (i.e. 50 × 84 / 100 = 42 ml).

Place the container in the water bath (6.4) at 44 °C to 47 °C until the product has completely melted. The time taken shall not exceed 20 min.

Mix in a peristaltic homogenizer (6.1.2) for 60 s ± 5 s and then for further 30 s increments until an emulsion is produced. Leave the container at laboratory ambient temperature (18 °C to 27 °C) so that the fatty (upper) layer and the aqueous (lower) layer are separated fully.

Use the aqueous layer to take the test portion (1 ml corresponds to 1 g of the test portion) and prepare the initial suspension in accordance with ISO 6887-1.

9.4.2.3 Preparation of an enrichment or pre-enrichment suspension

See the relevant International Standard for the microorganism to be detected or enumerated.

If the method requires enrichment or pre-enrichment, the sample may be an entire portion of the product rather than the aqueous layer (9.4.2.2), but this shall be recorded.

9.5 Eggs and egg products

9.5.1 Fresh whole eggs

9.5.1.1 General

Eggs used for routine microbiological examination shall not have any visible cracks in the shells.

Eggs may be examined singly or in batches according to the purpose of the testing. Where specified by the customer, carry out pooling of the contents of several eggs according to ISO 6887-1.

Examination of whole eggs may be carried out with or without cleaning/disinfecting of the eggshell as required by the customer. To examine only the contents, always disinfect the eggs before opening. For detection of pathogens (which may also be found on the outside of the egg), disinfection of the shell may not be required, but agreement on the procedure to be used shall be reached between the parties.

9.5.1.2 Disinfecting the shell

Remove any dirt or faeces with a damp tissue and blot dry.

Wearing sterile gloves and using a clean gauze or wipe soaked in a solution of either 70 % (volume fraction) ethanol or isopropanol to water, wipe the entire shell surface. This reduces the risk of contamination of the egg yolk and albumen when the egg is broken open to remove the contents.

Allow to dry completely without re-contaminating the shells before breaking the egg to take the test portion.

9.5.2 Microflora of whole egg shell

9.5.2.1 Method by rinsing the whole egg shell

Place the whole intact egg in a peristaltic homogenizer bag (6.1.2) or other sterile container and add a known volume of the diluent or culture medium required in the test method. Then, massage or rotate the egg carefully in the liquid. Remove the egg and use the liquid as the initial suspension to continue with the test method.

9.5.2.2 Friction method

Use sterile gauze (or other equivalent fabric/paper) soaked in diluent or the required culture medium. Hold the gauze with sterile forceps and rub over the entire eggshell.

Place the pieces of gauze in the volume of diluent or culture medium required by the test method.

9.5.2.3 Soaking method

Break the egg aseptically and discard the contents into a bowl or beaker.

Retain the shell and place it in a peristaltic homogenizer bag (6.1.2) with the required volume of diluent or culture medium.

Massage and crush the shell in the bag by hand and use this as the initial suspension.

9.5.3 Internal microflora

Using fresh sterile gloves for each egg, break the egg open aseptically into a sterile container. If the yolk and white are to be examined separately, separate them and place each in a different sterile container.

Add peptone salt solution (5.2.1) to give a 1 in 10 dilution for the yolk and 1 in 40 dilution for the white to overcome inhibition by the naturally occurring lysozyme.

To examine the whole egg contents, place all of the yolk and white (of approximately 20 ml) in a sterile container with 180 ml of buffered peptone water (5.2.2) or into the appropriate diluent or enrichment broth required by the specific International Standard and use this as the initial 1 in 10 suspension.

9.5.4 Bulk whole liquid egg, egg white and egg yolk

These bulk products may or may not be pasteurized.

For bulk whole liquid egg or egg yolks, dilute 1 in 10 with buffered peptone water (5.2.2).

For bulk liquid egg whites, use a 1 in 40 suspension in buffered peptone water (5.2.2) to overcome inhibition by the naturally occurring lysozyme.

9.5.5 Dehydrated whole egg and dried egg white

Treat as dehydrated products (see 9.1).

9.5.6 Whole egg microflora (shell plus yolk plus white)

Using aseptic techniques, break the egg and place the shell and contents in a sterile plastic bag (6.1.2) or other container.

Crush and shake the mixture to homogenize it by hand.

Take the required test portion to make the initial suspension.

9.6 Bakery goods, pastry and cakes

9.6.1 General

Bakery goods, including sweet pastries and cakes, are made from flour, butter, eggs and other ingredients and some also include dairy or fruit products. As such, they should be treated in the same way as other multi-component products (see ISO 6887-1).

The test portion of relatively homogeneous products, such as loaves of bread, rolls and other finished items made from plain dough, should be taken according to the purpose of the testing. For example, surface samples may be required for investigations of mould spoilage.

9.6.2 Preparation of samples

For packaged pre-cooked products, open the packaging aseptically.

Take pieces of each component in proportion to the amounts in the whole product.

Alternatively, homogenize the entire laboratory sample to reflect the microflora of the whole item and take a representative test portion.

Treat biscuits or cookies in the same way as dehydrated products if they are hard and low in moisture (9.1).

9.7 Fresh fruit and vegetables (pre-packed)

9.7.1 Sample preparation of multi-component products

For multi-component products (those containing pieces of different fruit or vegetables), take pieces of each component in proportion to the amounts in the whole product to provide the test portion.

Alternatively, homogenize the entire laboratory sample to reflect the microflora of the whole item and take a representative test portion.

Dilute the test portion 1 in 10 with buffered peptone water (5.2.2).

Homogenize using a peristaltic homogenizer (6.1.2) until a suitable initial suspension is obtained.

9.7.2 Pre-packed products of one type of fruit or vegetable

Weigh the test portion and dilute 1 in 10 with buffered peptone water (5.2.2).

Homogenize using a peristaltic homogenizer (6.1.2) until a suitable initial suspension is obtained.

9.8 Fermented products or other products containing viable microorganisms

9.8.1 General

Such products are examined for contamination by microorganisms other than those used as starter cultures for fermentations or as the active constituent microflora of probiotic products.

To test these products for contaminants, use suitable inhibitors to suppress growth of the starter culture or probiotic organisms.

9.8.2 Diluent

Use buffered peptone water (5.2.2) routinely or at double-strength (5.3.1) if the sample is highly acidic (pH < 4,5).

In the case of yeast cultures or fermentations, add an anti-fungal agent (e.g. cycloheximide or nystatin at a concentration of 50 mg/kg or amphotericin at 10 mg/kg) to the counting medium to reduce overgrowth by unwanted yeasts and moulds.

For other products, use an antibiotic active against the microflora of the product being examined (see ISO 27205 for probiotics[6]).

Record the use and concentration of the antibiotic and add these details to the test report.

9.9 Beverages (alcoholic and non-alcoholic drinks and bottled waters, still or carbonated)

9.9.1 General

To detect contamination of these products, use membrane filtration of specified volumes through 0,45 µm sterile membranes (see ISO 8199).

For carbonated beverages, preliminary de-gassing is required to ensure accurate volumes are filtered or pipetted.

9.9.2 De-gassing by inversion and mixing

Invert the laboratory sample by hand (through an arc of 25 cm five times) and then loosen the cap carefully to release evolved carbon dioxide. Tighten the cap again and repeat the process until no more gas is evolved. Take the test portion by pipetting or filtering accurately. Use aseptic technique throughout.

9.9.3 De-gassing using ultrasound

Ultrasound may also be used to de-gas carbonated beverages.

Invert the container (through an arc of 25 cm five times) to mix and aseptically decant 10 % of the contents into a sterile container.

Replace the lid of the container loosely and place it in an ultrasonic bath (6.10) for 120 s ± 5 s. Check for any remaining gas and, if necessary, repeat the ultrasound treatment. Do not repeat this procedure more than twice to minimize potential damage to microorganisms in the sample.

Take the test portion by pipetting or filtering accurately. Use aseptic technique throughout.

9.10 Alternative protein products (cooked insects, textured vegetable protein or mycoprotein)

9.10.1 General

Many of these products may be handled using the general preparation procedures given in ISO 6887-1. Some additional details are given in 9.10.2 and 9.10.3 for particular products.

9.10.2 Cooked insects

Weigh the test portion into a plastic bag (6.1.2) and dilute 1 in 10 with buffered peptone water (5.2.2).

Homogenize in a peristaltic homogenizer until a suitable initial suspension is obtained.

Some insects with a chitinous cuticle exoskeleton, such as locusts, may puncture the bag so use double- or triple-bagging to prevent perforation and leakage. Test portions may also be partially crushed with a heavy object, such as a hammer (6.3), before homogenizing.

9.10.3 Textured vegetable protein and mycoprotein

Weigh the test portion into a plastic bag (6.1.2) and dilute 1 in 10 with buffered peptone water (5.2.2).

Homogenize in a peristaltic homogenizer until a suitable initial suspension is obtained.

Use of a plastic bag with filter (6.1.2) is recommended to reduce carry-over of particulate residues.

10 Further dilutions

Prepare further dilutions in accordance with ISO 6887-1.

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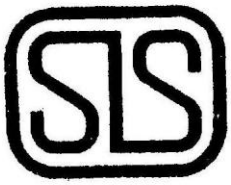

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