INTERNATIONAL STANDARD

ISO 18593

Second edition
2018-06

Microbiology of the food chain — Horizontal methods for surface sampling

Microbiologie de la chaîne alimentaire — Méthodes horizontales pour les prélèvements de surface

Reference number
ISO 18593:2018(E)

COPYRIGHT PROTECTED DOCUMENT

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

Published in Switzerland

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 voluntary nature of standards, 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 18593:2004), which has been technically revised. The main changes are as follows:

— recommendations on sampling procedure have been added, including information on sampling location, area and sampling time;

— examples of neutralizers have been added as Annex A.

Introduction

It can be important to determine the presence of, or the number of microbes on, the surfaces of utensils, work surfaces and other equipment in the food chain environment in order to estimate the level of contamination in the food chain environment.

This document describes horizontal methods for surface sampling.

Microbiology of the food chain — Horizontal methods for surface sampling

1 Scope

This document specifies horizontal methods for sampling techniques using contact plates, stick swabs, sponges and cloths on surfaces in the food chain environment in order to detect and enumerate culturable microorganisms such as pathogenic or non-pathogenic bacteria or yeasts and moulds.

NOTE The term "environment" means any item in contact with the food product or likely to represent a contamination or recontamination source; for example, material, premises or operators.

This document does not apply to the validation of cleaning and disinfection procedures.

This document does not apply to sampling techniques for primary production samples, which are covered by ISO 13307. Sampling techniques for carcasses are covered by ISO 17604. Sampling techniques for analysis of noroviruses and hepatitis A viruses are covered by ISO 15216-1.

This document does not give advice on sampling frequency, the number of sampling points, or the need to rotate sampling points, as these are chosen on a case-by-case basis.

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 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

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

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 16140-2, *Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

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

4 Principle

Sampling schemes aim to evaluate microbial contamination levels of surfaces from the food chain environment in order to implement corrective actions to avoid food contamination by microorganisms. Ineffective sampling programmes or techniques may result in the non-detection of microorganisms when they are present.

This document describes surface sampling methods to detect or enumerate microorganisms from surfaces in the food chain environment. Different sampling techniques are described, including contact plates, stick swabs, cloths and sponges.

This document also gives recommendations on the locations and areas to be sampled and the most appropriate sampling time.

According to the device and the microorganism to be detected or enumerated, determination of the microbial contamination of the surface can be performed by

— sampling of the surface, and

— analysis according to specific standards.

5 Culture media and reagents

Follow current laboratory practice as specified in ISO 7218. Follow performance testing of culture media as specified in ISO 11133.

5.1 Diluent.

In general, the diluent is sterile buffered peptone water, peptone salt as specified in ISO 6887-1, peptone solution at 1 g/l or quarter-strength Ringer's solution as specified in ISO 6887-5, with neutralizer(s) (5.3) added if necessary.

NOTE To extend transport time, an appropriate transport diluent can be added, if properly validated.

5.2 Medium for contact plate.

Plates (6.1) may vary in diameter or area, according to the type of surface to be sampled. The medium is chosen according to the ISO method for the microorganism(s) of concern, with neutralizer(s) (5.3) added if necessary. The medium shall form a convex meniscus with the contact plate.

Alternative media formulations shall be supported by a validation study as specified in ISO 16140-2.

5.3 Neutralizer.

In cases where residues of disinfectants are expected, appropriate neutralizer(s) should be added to the diluent (5.1) or media (5.2) before sampling, to prevent any inhibitory effect of the disinfectants on the growth of microorganisms.

Do not add neutralizer to the diluent when no residual disinfectant is expected[9]. A neutralizer used to quench residual disinfectant can have a slight deleterious impact on bacterial cells and it is likely that such an impact would be greater when cells are stressed.

An appropriate neutralizer for all situations (a "universal neutralizer") cannot be prescribed[9]. A number of neutralizers are recommended in EN 1276, EN 1650, EN 13697 and EN 13704.

See Annex A for examples of neutralizers.

6 Equipment and consumables

Disposable apparatus is an acceptable alternative to reusable glassware if it has similar specifications.

Usual microbiological laboratory equipment in accordance with ISO 7218 and, in particular, the following.

6.1 Contact plate, plastic dish of variable diameter.

NOTE It is also possible to use any other flexible or rigid container which enables contact with the sampled surface.

6.2 Sterile stick swab, stick with cotton or synthetic material (such as alginate or rayon) contained in a tube or envelope. The material used shall be documented to be free of inhibitory substances.

NOTE For some types of surface, the cotton residues can contaminate the internal parts of these surfaces after sampling.

6.3 Sterile cloth (or wipe), free from inhibitory substances.

6.4 Sterile sponge, with or without stick/handle, free from inhibitory substances.

6.5 Containers, such as bottles, tubes or flasks, suitable for the sterilization and storage of culture media.

6.6 Cool box, cooler, insulated box containing ice packs, capable of maintaining the samples at low temperature during transportation to the laboratory.

6.7 Mixer, for mixing liquids in culture tubes, e.g. a vortex mixer.

6.8 Peristaltic blender, with sterile plastic bags to prepare initial suspensions by peristaltic movement.

6.9 Petri dishes, made of plastic or glass.

6.10 Sterile disposable or reusable template, enclosing a specified area.

7 Sampling procedure

7.1 General

Sampling locations and areas, sampling times and sampling techniques should be selected according to risk-based principles and should relate to the higher probability of detecting contaminated surfaces during food processing, when measuring the hygiene of specific production steps or the entire process as appropriate. Always keep the same sampling procedure for a specific routine to allow trending of data.

7.2 Sampling location

Microorganisms can be found on visually clean surfaces but are most frequently found on wet and soiled places where the bacteria are able to grow and persist. Hard to reach places such as holes or crevices in fibrous, porous, difficult-to-clean equipment, rusting and hollow materials, are potential harbourage sites that should be sampled. It can be difficult to sample unreachable areas where food debris can collect. Dismantling may be necessary to sample unreachable locations.

The choice of sampling location shall be defined according to historical data linked to each site and after step-by-step examination of the process.

A non-exhaustive list of potential sampling locations is given below.

— Non-food contact surfaces: drains, floors, pools of water on the floor, cleaning tools, wash areas, in-floor weighing equipment, hoses, hollow rollers for conveyances, conveyors, equipment framework, internal panels of equipment, condensate drip pans, forklifts, hand trucks, trolleys, trolley wheels, trash cans, freezers, ice makers, cooling fans in condensers, aprons, walls, ceilings, cold spots where water condenses, wet insulation in walls or around pipes, cooling units, rubber seals around doors (especially in coolers), vacuum cleaners, door handles and taps.

— Food contact surfaces: conveyor belts, slicers, cutting boards, dicers, hoppers, shredders, blenders, peelers, assembly machines, filling and packaging equipment, containers, other utensils, gloves and hands.

7.3 Sampling area

A specified area of the surface to be examined shall be identified. It is important that the laboratory receives a sample that is representative of the surface sampled. The area is not always defined by a numerical size.

If the area is not defined by a numerical size, the area sampled shall be clearly described.

If the area is defined by a numerical size, follow the instructions below.

For the detection of microorganisms, when the areas are accessible, the total sampled area should be as large as possible to increase the probability of detecting microorganisms. In this regard, it is recommended to sample between 1 000 cm^2 and 3 000 cm^2 (i.e. 0,1 m^2 to 0,3 m^2) when possible.

For the enumeration of microorganisms, the area does not need to be so large, e.g. ≤ 100 cm^2.

7.4 Time of sampling and frequency

Sampling can be performed either during/after production or after cleaning and disinfection. The sampling time shall be specified in the sampling procedure of each producer, depending on the objective of sampling.

The detection of certain microorganisms can be difficult if samples are taken immediately or soon after cleaning and disinfection. Cells can still be alive but non-culturable, as a result of injury caused by the chemical agents used for cleaning and disinfection, and may not be easily detectable. To increase the probability of detecting these microorganisms, sampling should be performed either during production: after at least two hours of production or at the end of production runs (i.e. before cleaning and disinfection).

When sampling is not performed daily, it should not always be performed on the same day(s) of the week. It may be appropriate to take surface samples following repairs to equipment, or construction and increases in production capacity as these can increase the risk of microbial contamination.

Sampling should be done frequently in areas where the food product is exposed to contamination, but it may be interesting to also sample, less frequently, in areas where it is not (storage areas).

7.5 Sampling techniques

7.5.1 General

The contact plate method is only applicable to flat surfaces, whereas the other methods can be used for all types of surfaces.

For the sampling of hard-to-reach, small areas (≤ 100 cm^2), sterile stick swabs should be used to sample.

For the sampling of large surfaces (> 100 cm^2), sterile cloths or sponges should be used.

After sampling, the surface is cleaned and/or disinfected, if necessary, to avoid traces of nutrients, moisture, chemical or physical elements resulting from the sampling procedure remaining on the sampled surface. This can be done with sterile wipes, moistened with alcohol.

7.5.2 Contact plate method

Press the agar surface of the contact plate firmly and without any lateral movement against the test surface. Consistency of time (e.g. 10 s) and pressure (e.g. with a mass of 500 g) allows better reproducibility of results. Close the contact plate immediately after sampling and put it back in the transport container.

Do not use the contact plate method for qualitative methods.

7.5.3 Stick swab method

7.5.3.1 General

Stick swabs should be used to sample hard-to-reach small locations (e.g. inside hollow rollers or motor housing). It is recommended to use sterile disposable templates to prevent the transfer of contamination and/or disinfectant compounds. The size of the sampled area shall be approximately known and/or the location well described.

In the event that the area to sample is wet, a dry swab may be used unless neutralizers are needed. In the event that the area to sample is dry, a moistened swab shall be used, except if moisture cannot be removed from the processing area. In order to increase the recovery of microorganisms, it is better to use moistened swabs.

7.5.3.2 Moistened stick swab

To use a moistened swab, remove a stick swab from the sterile wrapping and moisten the tip by immersing it in a tube containing the diluent/neutralizer. Press the tip of the swab against the wall of the tube to remove excess diluent/neutralizer. Place the tip of the swab on the surface to be examined and streak an estimated area of, e.g. ≤ 100 cm^2, while rotating the stick swab between thumb and forefinger. For flat surfaces, the sampling should be performed horizontally and vertically, e.g. 10 times in each direction. For hard-to-reach small surfaces, make sure to sample the entire described location including crevices, gaps, surface connections, etc. Return the stick swab in the tube with the diluent/neutralizer. Make sure the tube is closed so that the swab stays moist until the analysis.

7.5.3.3 Dry stick swab

To use a dry swab, remove a stick swab from the sterile wrapping and place the tip of the swab on the surface to be examined and streak an estimated area of, e.g. ≤ 100 cm^2, while rotating the stick swab between thumb and forefinger. For flat surfaces, the sampling should be performed horizontally and vertically, e.g. 10 times in each direction. For hard-to-reach small surfaces, make sure to sample the entire described location including crevices, gaps, surface connections, etc. Return the stick swab in the tube. Make sure the tube is closed until the analysis.

7.5.4 Sponge/cloth method

7.5.4.1 General

A sponge or cloth should be used to sample large areas. In contrast with stick swabs they can be rubbed more vigorously over surfaces and are highly absorptive. The sponge/cloth should have been moistened with a sufficient quantity of diluent/neutralizer (without excess). In the event that the area to sample is wet, a dry sponge/cloth may be used, unless neutralizers are needed. In the event that the area to sample is dry, a moistened sponge/cloth shall be used, except if moisture cannot be removed from the processing area. In order to increase the recovery of microorganisms, it is better to use moistened sponge/cloth.

7.5.4.2 Moistened sponge/cloth

Open the plastic bag or container containing the sponge/cloth. Remove aseptically the sponge/cloth, e.g. with sterile forceps and/or a sterile gloved hand, or grab the sponge/cloth through the bag and pull the reversed bag over the hand. Sample the chosen surface horizontally and vertically using even and firm pressure, changing the face of the sponge/cloth and ensuring the whole area is sampled. Return the sponge/cloth to the plastic bag or container and make sure that it remains moistened until the time of analysis. If neutralizers are used, right after sampling squeeze the sponge, let it soak in the diluent with neutralizers to fully react with the sanitizer. Close the plastic bag or container in a manner that will ensure neither leakage nor cross-contamination.

7.5.4.3 Dry sponge/cloth

Open the plastic bag or container containing the sponge/cloth. Remove aseptically the sponge/cloth for example with sterile forceps and/or a sterile gloved hand, or grab the sponge/cloth through the bag and pull the reversed bag over the hand. Sample the chosen surface horizontally and vertically using even and firm pressure, changing the face of the sponge/cloth and ensuring the whole area is sampled. Return the sponge/cloth to the plastic bag or container. Close the plastic bag or container in a manner that will ensure no cross-contamination.

8 Storage and transport

8.1 Contact plate

The delay between sampling and testing should be as short as possible. Right after sampling, the samples are put into insulated transport containers at 1 °C to 8 °C, in a way that no contamination can occur, and then transported at 1 °C to 8 °C. Samples should be incubated within 48 h from sampling.

Alternatively, the samples can be transported in insulated transport containers in order to maintain consistent temperature, in a way that no contamination can occur. Samples should be incubated within 48 h from sampling taking into account the time from sampling to incubation as part of the incubation time if appropriate.

8.2 Stick swab, sponge/cloth

The delay between sampling and testing should be as short as possible. The samples should preferably be cooled before being put into insulated transport containers, and transported at 1 °C to 8 °C. Samples should preferably be examined within 24 h from sampling.

If testing is delayed after receipt in the laboratory, the samples shall be stored at 3 °C ± 2 °C for a maximum of 48 h from sampling.

9 Microbiological analysis of samples

9.1 Contact plate method

Incubate the contact plates according to the type of microorganisms to be enumerated using the appropriate International Standard. After incubation, an estimate of the surface contamination is obtained by counting the number of developed colonies.

9.2 Stick swab/cloth/sponge method

Add enough diluent/enrichment broth to cover the device. The exact volume shall be known. Examples of volumes to be used for dilution are 9 ml to 10 ml for swabs, 90 ml to 100 ml for sponges and 225 ml for cloths. Thoroughly homogenize the contents by hand or mechanical massaging the sponge/cloth or vortexing the stick swab.

For enumeration, this represents the initial suspension.

For enumeration of the (groups of) microorganisms to be analysed in the laboratory, the initial suspension and, if necessary, further decimal dilutions can be used to determine the number of microorganisms following the procedures described in the ISO 6887 series, depending on the food chain environment. According to ISO 7218, the maximum number of colonies that can be reliably counted is proportional to the diameter of the plate.

For detection of microorganisms, follow the appropriate International Standard. After the pre-enrichment, follow the instructions according to the microorganism(s) sought.

10 Expression of results and calculation

10.1 General

For the expression of results and calculation refer to ISO 7218.

10.2 Contact plate method

Divide the number of characteristic colonies by the surface area of the plate. Report the count as the number of colony-forming units (cfu) per sampled surface.

10.3 Method using stick swab, cloth or sponge

10.3.1 Calculate the number of cfu per sampled surface when the surface is not measurable or per square centimetre, N_S, using Formula (1):

$$N_S = \frac{N \times F}{A} \times D \tag{1}$$

where

N is the number of cfu in 1 ml of dilution liquid (or neutralizing liquid);

F is the amount of dilution fluid (or neutralizing liquid) in the tube or homogenizer bag, in millilitres;

A is the sampled surface, e.g. in square centimetres (A is equal to 1 when surface is not measurable);

D is the reciprocal of the dilution used.

10.3.2 In the case of qualitative methods, report the target microorganism as detected or not detected on the sampled surface, or per device if the area is not known.

11 Test report

The test report shall specify the following:

— the test method used, with reference to this document, i.e. ISO 18593;

— the sampling device used, date, hour and identification of the sampling location, date of start of analysis;

— all operating conditions not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— any deviation in the media or the incubation conditions used;

— all information necessary for the complete identification of the sample;

— the results obtained expressed according to the surface sampled (size or name).

Annex A
(informative)

Neutralizers

WARNING — Neutralizers of the residual antimicrobial activity of chemical disinfectants and antiseptics shall be validated according to the prescriptions of the appropriate standard (for example EN 13697).

The list shown in Table A.1 is intended to provide examples and is not exhaustive (EN 1276:2009, Annex B).

Other neutralizer mixtures may be required for products containing more than one antimicrobial agent.

The concentrations of the various neutralizing compounds or of the neutralizer as such may not be adequate to neutralize high concentrations of the products.

Table A.1 — Examples of neutralizers

Antimicrobial agent	Chemical compounds able to neutralize residual antimicrobial activity	Examples of suitable neutralizers[a]
Quaternary ammonium compounds and fatty amines	Lecithin, Saponin, Polysorbate 80, Sodium dodecyl sulphate, Ethylene oxide condensate of fatty alcohol (non-ionic surfactants)[b]	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l. — Polysorbate 80, 30 g/l + sodium dodecyl sulphate, 4 g/l + lecithin, 3 g/l. — Ethylene oxide condensate of fatty alcohol, 3 g/l + lecithin, 20 g/l + polysorbate 80, 5 g/l.
Biguanides and similar compounds	Lecithin[c], Saponin, Polysorbate 80	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l.
Oxidizing compounds (Chlorine, iodine, hydrogen peroxide, peracetic acid, hypochlorites, etc.)	Sodium thiosulphate[d] Catalase or peroxidase [for hydrogen peroxide or products releasing hydrogen peroxide][e]	— Sodium thiosulphate, 3 g/l to 20 g/l + polysorbate 80, 30 g/l + lecithin, 3 g/l. — Polysorbate 80, 50 g/l + catalase 0,25 g/l + lecithin 10 g/l.
Aldehydes	L — histidine or glycine	— Polysorbate 80, 30 g/l + lecithin, 3 g/l + L-histidine, 1 g/l (or + glycine, 1 g/l). — Polysorbate 80, 30 g/l + saponin, 30 g/l + L-histidine, 1 g/l (or + glycine, 1 g/l).
Phenolic and related compounds: orthophenylphenol, phenoxyethanol, triclosan, phenylethanol, etc. Anilides	Lecithin, Polysorbate 80, Ethylene oxide condensate of fatty alcohol[b]	— Polysorbate 80, 30 g/l + lecithin, 3 g/l. — Ethylene oxide condensate of fatty alcohol, 7 g/l + lecithin, 20 g/l, + polysorbate 80, 4 g/l.

Table A.1 *(continued)*

Antimicrobial agent	Chemical compounds able to neutralize residual antimicrobial activity	Examples of suitable neutralizers[a]
Alcohols	Lecithin, Saponin, Polysorbate 80[f]	— Polysorbate 80, 30 g/l + saponin, 30 g/l + lecithin, 3 g/l.
Mercurials	Sodium thioglycolate	— Sodium thioglycolate at 0,5 g/l to 5 g/l

[a] According to the pH of the tested product, the pH of the neutralizer or the rinsing liquid may be adjusted at a suitable value or prepared in phosphate buffer [ex: phosphate buffer 0,25 mol/l: potassium dihydrogen phosphate (KH2PO4) 34 g; distilled water (500 ml); adjusted to pH 7,2 ± 0,2 with sodium hydroxide (NaOH) 1 mol/l; distilled water up to 1 000 ml].

[b] The carbon chain-length varies from C_{12} to C_{18} carbon atoms.

[c] Egg and soya; egg is preferable.

[d] The toxic effect of sodium thiosulphate differs from one test organism to another.

[e] One unit of these enzymes catalyses the decomposition of 1 µmol of hydrogen peroxide per min at 25 °C and at pH 7.

[f] For the neutralization of short chain alcohols (less than C_5), simple dilution may be appropriate. Care should be taken if the alcohol-based-products contain additional antimicrobial agents.

Bibliography

[1] ISO 6887 (series), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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

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

[4] ISO 17604, *Microbiology of the food chain — Carcass sampling for microbiological analysis*

[5] EN 1276:2009, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas — Test method and requirements (phase 2, step 1)*

[6] EN 1650, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal and yeasticidal activity of chemical disinfectants and antiseptics in food, industrial, domestic, and institutional areas — Test method and requirements (phase 2, step 1)*

[7] EN 13697, *Chemical disinfectants and antiseptics — Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas — Test method and requirements (phase 2, step 2)*

[8] EN 13704, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas — Test method and requirements (phase 2, step 1)*

[9] Sutton S.V., Proud D.W., Rachui S., Brannan D.K. Validation of Microbial Recovery From Disinfectants. *J of Pharmaceutical Science and Technology*, 2002, 56 (5), pp. 255–266

ICS 07.100.30

Price based on 11 pages