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Microbiology of food and animal feeding stuffs — Horizontal methods for sampling techniques from surfaces using contact plates and swabs

Microbiologie des aliments — Méthodes horizontales pour les techniques de prélèvement sur des surfaces, au moyen de boîtes de contact et d'écouvillons

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Foreword

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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.

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Introduction

It can be important to determine the presence of, or the number of, viable microbes on the surfaces of utensils, work surfaces and other equipment in contact with food to estimate the level of contamination during production, or the effectiveness of cleaning and disinfecting protocols.

The horizontal methods described in this International Standard include a surface contact method using contact plates and a swab method. The contact plate method is only applicable to flat surfaces, whereas the swab method can be used for all types of surfaces. For the sampling of large surfaces ($> 100\ cm^2$), sterile cloths or sponges can be used. This alternative method is useful for the estimation of the microbial load of surfaces. Results are often presented as hygiene scores based on the number of colony-forming units (CFU) per square centimetre present on a test surface.

Microbiology of food and animal feeding stuffs — Horizontal methods for sampling techniques from surfaces using contact plates and swabs

1 Scope

This International Standard specifies horizontal methods for sampling techniques using contact plates or swabs on surfaces in the food industry environment (and food processing plants), with a view of detecting or enumerating viable microorganisms.

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, operators.

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, *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 Principle

3.1 Because these methods are not quantitatively reliable or reproducible, results should only be used in a “trend analysis”.

3.2 A contact plate (or dipslide) filled with a suitable agar medium is pressed against the surface to be tested. After incubation, an estimate of the surface contamination is obtained by counting the number of developed colonies.

3.3 Using the swab method, a specified area of the surface to be examined is marked (e.g. using a template) and then wiped. The swab sticks are broken into a tube or bottle containing a sterile dilution fluid or neutralizing fluid and mixed by hand.

If the surface is wiped with a sterile (damp) cloth or sponge, the sampling device is stored in a known volume of dilution liquid (e.g. 100 ml for 100 cm^2).

In the laboratory the initial suspension and, if necessary, further decimal dilutions are used to determine the number of microorganisms using the procedures described in the methods for the enumeration of the (groups of) microorganisms to be investigated.

NOTE The incubation time and temperature depend on the type of microorganisms to be detected.

3.4 For selective media, appropriate confirmatory tests can be performed. The number of colony-forming units of specific microorganisms per square centimetre or per swab is calculated from the number of (confirmed) colonies.

3.5 After sampling, the surface is cleaned and disinfected, if necessary, to avoid traces of nutrients resulting from the sampling procedure remaining on the sampled surface.

4 Culture media and dilution fluid

NOTE For further information, see the relevant International Standards for the target microorganisms to be detected or enumerated.

4.1 Neutralizing liquid

In general, the base for neutralizing liquid is buffered peptone water, or peptone salt, or any other appropriate diluent (such as quarter-stength Ringer's solution, phosphate buffer at pH 7,5, peptone solution at 1 g/l).

In cases where residues of disinfectants are expected, appropriate neutralizers should be added to the dilution fluid and to the media used on the contact plates to prevent any inhibitory effect of the disinfectants on the growth of microorganisms.

5 Apparatus and glassware

For general requirements, see ISO 7218.

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

Usual microbiological laboratory apparatus and, in particular, the following.

5.1 Contact plate, plastic dish with diameter 65 mm, filled with a controlled volume of agar medium (chosen according to the target microrganisms), especially made for the sampling of surfaces.

Dishes vary in diameter or area, according to the type of surface to be sampled. The agar shall form a convex meniscus with the dish.

NOTE It is also possible to use any other device (nutritive medium in a flexible or rigid container) which enables contact with the surface to be sampled, such as a dipslide (5.2).

5.2 Dipslide, synthetic slide (7 cm^2 to 10 cm^2), one or both sides of which are covered with a layer of a solid growth medium (chosen according to the target microorganisms).

NOTE Various growth media are available according to the microorganism(s) sought.

5.3 Swab, breakable stick with cotton or synthetic material (such as alginate or rayon) swab contained in a tube or envelope.

The swab shall be individually wrapped and sterilized. The material used shall be documented free of inhibiting substances.

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

5.4 Cloth, damp, sterile woven material, free from antimicrobial substances, packed individually in sterile plastic bags, used for the sampling of large surfaces ($\geqslant$ 100 cm^2).

5.5 Sponge, damp, sterile square of flat sponge, free from antimicrobial substances, packed individually in sterile plastic bags, used for the sampling of large surfaces ($\geqslant$ 100 cm^2).

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

5.7 Cool box, insulated, capable of maintaining the samples at low temperature during transportation to the laboratory.

5.8 Graduated pipettes, having wide openings and a nominal capacity of 1 ml, graduated in 0,1 ml divisions, or **automated pipettes** delivering 100 µl and 1 000 µl.

5.9 Mixer, for mixing liquids in culture tubes.

5.10 Peristaltic homogenizer or **homogenizer using horizontal shaking**, with sterile plastic bags to prepare initial suspensions by peristaltic movement (peristaltic homogenizer) or vibration movement (homogenizer using horizontal shaking).

5.11 Petri dishes, made of plastic, of diameter 65 mm.

5.12 pH-meter, capable of being read to the nearest 0,01 pH unit at 25 °C ± 1 °C, enabling measurements to be made which are accurate to ± 0,1 pH unit.

5.13 Template, made of a corrosion-resistant material (e.g. a frame of stainless steel enclosing an area of 20 cm^2 to 100 cm^2), which is easy to clean and can be sterilized.

6 Sampling techniques

6.1 General

It is important that the laboratory receive a sample which is representative of the surface tested and has not been changed during transport and storage, or by residues of disinfectants.

Disinfectants are generally formulated for a disinfection contact time of 5 min to 15 min. Wait for a period in accordance with the disinfectant specification before investigating the surface with swabs or contact plates to assess the performance of the cleaning and disinfection programme (or otherwise according to the disinfectant specification).

An appropriate neutralizer for all situations cannot be prescribed. Generally, sorbitan monooleate (30 g/l) and lecithin (3 g/l) are useful to neutralize residues of absorbed disinfectants (e.g. quaternary ammonium compounds, amphotericides). Sodium thiosulfate (5 g/l) is a good neutralizer for halogen-based products. In the case of peroxide-based disinfectants, catalase or peroxidase may be used as neutralizer. One unit of these enzymes catalyses the decomposition of 1 µmol of hydrogen peroxide per minute at 25 °C and at pH 7,0 ± 0,2. A number of disinfectant neutralizers are recommended in EN 1276 [1], EN 1650 [2], EN 13697 [3] and EN 13704 [4].

The components of a neutralizer which may be used in most situations is given in Table 1. Make up in a solution of peptone (1 g/l) and sodium chloride (8,5 g/l), distribute in tubes or bottles, and sterilize for 15 min at 121 °C.

Table 1 — Neutralizer which can be used in most situations

Component	Conc.
Sorbitan monooleate (Polysorbate 80)	30 g/l
Lecithin	3 g/l
Sodium thiosulfate	5 g/l
L-Histidine	1 g/l
Saponin	30 g/l

6.2 Contact plate method

After removal from the transport containers, press the agar surface of the contact plate or the dipslide firmly and without any lateral movement against the test surface. From the literature it is known that optimal results for contact plates are obtained with a contact time of 10 s and a pressure obtained with a mass of 500 g. Close the contact plates or dipslides immediately after inoculation and put them back in the transport container.

6.3 Swab or cloth/sponge method

6.3.1 Swab method

Remove a swab from the sterile wrapping and moisten the tip by immersing it in a tube containing dilution liquid. Press the tip of the swab against the wall of the tube to remove excess water. Place the tip of the swab on the surface to be investigated and streak an estimated area of 20 cm^2 to 100 cm^2, whilst rotating the swab between thumb and forefinger in two directions at right angles to each other.

Put the swab in the tube with dilution liquid and aseptically break or cut off the stick.

6.3.2 Sponge/cloth method

Open the plastic bag or container containing the cloth or sponge.

Remove aseptically the cloth or sponge with sterile forceps or a sterile gloved hand. Moisten the cloth or sponge with sufficient quantity of diluent (without excess). In the case of humid surfaces, this is not necessary.

Return the cloth or sponge to the plastic bag and close it in a manner that will ensure no leakage.

Sample the chosen surface in two perpendicular directions, changing the face of the cloth or sponge. Place the cloth or sponge in the sterile container, add the diluent and close. Add a known and sufficient volume of diluent, so that the cloth or sponge is still moist at the analysis.

Alternatively, open the plastic bag containing the cloth or sponge. Gripping the sponge through the bag, pull the reversed bag over the hand. Use the sponge to collect the sample as described above and transfer the cloth or sponge to a sterile plastic bag. Close the bag in a manner that will ensure no leakage.

7 Transport

Transport the samples obtained with the swab method, preferably within 4 h, to a cool box set at 1 °C to 4 °C. Examine in the laboratory as soon as possible and not later than 24 h, as described in Clause 8.

Transport the contact plates and/or dipslides, preferably within 4 h, in a way that no contamination can occur.

8 Procedure

8.1 Contact plate method

Incubate the contact plates or dipslides according to the type of microorganisms to be enumerated (see Note to Clause 4).

The contact plate method shall be not used for the specific detection of pathogenic microorganisms.

8.2 Swab method (including cloth and sponge)

Thoroughly mix the contents of tubes containing swabs using a mixer (5.9) for 30 s, adjusting the speed so that the wall of the tube is wetted up to a height of 2 cm to 3 cm below the top.

Add to the plastic bags containing cloths or sponges an amount of dilution fluid or neutralizing fluid (4.1) depending on the size of the area investigated (e.g. 100 ml for 100 cm^2). Then treat the contents of the bags in a peristaltic homogenizer (5.10) for 1 min, or a homogenizer using horizontal shaking (5.10) for 30 s. This represents the initial suspension.

If high numbers of microorganisms are expected, prepare further decimal dilutions in peptone water diluent to obtain countable number of colonies (see ISO 6887-1).

According to the enumeration methods used (see appropriate International Standards), inoculate duplicate plates of media with initial suspension, using 1 ml of inoculum for pour plates and 0,1 ml of inoculum for spread-plate techniques. Treat any further dilutions in the same manner. Invert the dishes and incubate the plates for the appropriate time and temperature for each medium.

As an alternative to the plating method described above, it is possible to use the drop-plate method. Starting with the highest dilution, use sterile pipettes to transfer aliquots of 0,05 ml of the initial suspensions (swabs, cloths or sponges) and of the further dilutions to the appropriate sectors of the culture medium marked on the bottom of the agar plate (in duplicate, using the same dilutions for different agar plates). Each predried agar plate may be used for drops of not more than six different dilutions. Use the reverse pipetting technique by depressing the plunger completely when drawing up the aliquot of 0,05 ml, and inoculate the plates by depressing the plunger to the first stop only. Keep the agar plates horizontal (lid uppermost) until the surface is dry.

If the swab method is used to demonstrate the presence of specific microorganisms (e.g. *Listeria monocytogenes* or *Salmonella* spp.), the area investigated should be at least 100 cm^2 and preferably about 1 000 cm^2. Transfer the swab, cloth or sponge to the appropriate enrichment broth and mix well.

8.3 Counting and detection of colonies

8.3.1 Contact plate method

Count the number of typical colonies on the contact plates or dipslides directly after the specified incubation period and confirm these, if necessary, according to the microorganism(s) sought.

8.3.2 Swab method (including cloth or sponge)

Count the colonies in each dish and confirm these, if necessary, according to the microorganism(s) sought.

8.3.3 Drop plate method

Count the number of (target) colonies at the dilutions yielding 5 to 50 colonies.

8.3.4 Enrichment procedures

After the pre-enrichment, follow the instructions according to the microorganism(s) sought.