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МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Meat and meat products — Sampling and preparation of test samples —

Part 2: Preparation of test samples for microbiological examination

Viandes et produits à base de viande — Échantillonnage et préparation des échantillons pour essai —

Partie 2: Préparation des échantillons pour essai en vue de l'examen microbiologique

Reference number
ISO 3100-2 : 1988 (E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 3100-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

ISO 3100 will consist of the following parts, under the general title *Meat and meat products — Sampling and preparation of test samples:*

— *Part 1: Sampling*

— *Part 2: Preparation of test samples for microbiological examination*

Meat and meat products — Sampling and preparation of test samples —

Part 2: Preparation of test samples for microbiological examination

1 Scope

1.1 This part of ISO 3100 gives general instructions and specifies procedures to be followed after taking a laboratory sample from meat and meat products, for the purpose of microbiological examination.

1.2 A distinction is made between treatments for the following categories of products:

a) consignments or lots of meat or meat products prepared or packed as individual units of any size (for example sausages, vacuum-packed minced meat, sliced sausages, canned cooked ham), or meat in pieces not exceeding 2 kg in mass;

b) carcasses, cuts of carcasses, or cured meat in pieces exceeding 2 kg in mass, and mechanically separated meat or dried meat.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 3100. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 3100 are encouraged to investigate the possibility of applying the most recent editions of the standards listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3100-1 : —[1], *Meat and meat products — Sampling and preparation of test samples — Part 1: Sampling.*

ISO 6887 : 1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218 : 1985, *Microbiology — General guidance for microbiological examinations.*

3 Principle

Preparation of test samples for microbiological examination. This may require the thawing and/or mincing of "open" meat samples or the pre-incubation, external sterilization, and aseptic opening of products processed or packed in sealed units.

4 Instructions of an administrative character

The sampling report and the label of the laboratory samples received should be checked (see ISO 3100-1). The date of receipt and the condition of the samples, including their temperature, should be noted. It should be clear what type of examination is to follow microbiological examination and whether the same or further samples will be used.

5 Diluents and reagents

5.1 Base components

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluents, dehydrated basic components should be used. Similarly, commercially prepared reagents may also be used. The manufacturer's instructions shall be rigorously followed.

The chemicals used shall be of analytical quality.

The water used shall be distilled or deionized, and shall be free from substances that might inhibit growth of micro-organisms under the test conditions.

pH measurements shall be carried out using a pH meter (6.10), adjusted to a temperature of 25 °C.

If the diluents and reagents are not used immediately, they shall, unless otherwise specified, be kept in the dark at a temperature between 0 °C and +5 °C, and in conditions that prevent any change in their composition. They shall not be kept for longer than 1 month.

1) To be published. (Revision of ISO 3100-1 : 1975.)

5.2 Diluent for cotton swabs.

Composition

peptone	1,0 g
sodium chloride	8,5 g
water	1 000 ml

Preparation and distribution of diluent

Dissolve the components in the water, by heating if necessary. Adjust the pH so that, after sterilization, it is 7,0 at 25 °C. Dispense into tubes or flasks of appropriate capacity in quantities such that, after sterilization, each tube or flask contains 9,0 ml of diluent.

Stopper the tubes or flasks.

Sterilize in the autoclave (6.1) at 121 °C ± 1 °C for 20 min.

5.3 Diluent for alginate swabs.

Composition

sodium chloride	2,25 g
potassium chloride	0,105 g
calcium chloride	0,12 g
sodium hydrogen carbonate ($NaHCO_3$)	0,05 g
sodium hexametaphosphate [mainly $(NaPO_3)_6$]	10 g
water	1 000 ml

Preparation and distribution of diluent

Dissolve the components in the water, or dissolve commercially available tablets of the complete dry medium in 10 ml of water in tubes or flasks. If necessary, adjust the pH so that, after sterilization, it is 7,0 at 25 °C.

Dispense, if tablets have not been used, into stoppered tubes or flasks in such quantities that after sterilization each vessel contains 10 ml.

Sterilize in the autoclave (6.1) at 121 °C ± 1 °C for 20 min.

5.4 Ethanol, 95 % to 96 % (*V*/*V*).

5.5 Disinfectant mixture.

Composition

ethanol (5.4)	60 ml
hydrochloric acid (ϱ = 1,19 g/ml)	10 ml
water	30 ml

6 Apparatus and glassware

NOTE — Disposable apparatus is an acceptable alternative to glassware if it has appropriate specifications.

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

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave).

Apparatus that will enter into contact with the diluents or the sample, except for apparatus that is supplied sterile (particularly plastics apparatus), shall be sterilized either

— by being kept at 170 °C to 175 °C for not less than 1 h in the oven (6.1), or

— by being kept at 121 °C ± 1 °C for not less than 20 min in the autoclave (6.1).

6.2 Blending equipment.

One of the following shall be used:

a) mechanical meat mincer, laboratory size, capable of being sterilized, fitted with a plate with holes of diameter not exceeding 4 mm;

b) peristaltic-type blender (Stomacher), with sterile plastic bags.

6.3 Incubators, for maintaining cans at a prescribed temperature for the detection of defective cans and for the rapid thawing of frozen samples.

6.4 Refrigerator, capable of being maintained at 2 °C, and a **freezer** capable of being maintained at or below −24 °C, for the storage of samples.

6.5 Instruments (sterilizable), for opening packages of meat and cutting up samples, for example can-openers, scissors, knives and forceps.

6.6 Swabs, made of cotton or alginate.

6.7 Tubes or **flasks,** with glass beads, in which swabs can be shaken.

6.8 Flasks, for drips from samples.

6.9 Pipettes or **syringes,** for removing drips from thawed or packaged meat samples.

6.10 pH meter, accurate to 0,1 pH unit at 25 °C.

7 Storage and receipt

7.1 General

Samples shall be stored at the prescribed temperature, protected from direct sunlight or other sources of heat.

Contamination shall be prevented (see also ISO 3100-1).

Start the examination as soon as possible after receipt of the samples, and in any case, within the limits given in 7.2 and 7.3.

7.2 Meat and meat products prepared or packed as individual units of any size, and meat in pieces not exceeding 2 kg in mass

7.2.1 Fresh meat

Store the samples in the refrigerator (6.4) on receipt and examine them within 24 h.

If a longer storage period is absolutely unavoidable, freeze them in the freezer (6.4) as soon as possible.

If a sample has been frozen, indicate this in the test report and state the temperature and duration of frozen storage.

7.2.2 Frozen meat

The samples shall reach the laboratory in a frozen condition and at the temperature laid down by any legislation in force or, in any case, at a temperature of −24 °C or lower. Store the samples in the freezer (6.4).

7.2.3 Semi-preserved products

The samples shall be stored in the refrigerator (6.4).

Defective samples shall be placed in sealed containers (for example plastic bags), so as to avoid environmental contamination.

7.2.4 Shelf-stable packaged or non-packaged products

Apparently normal samples shall be stored, protected from direct sunlight or other sources of heat, at a temperature not exceeding 25 °C. Visibly defective samples shall be placed in sealed containers (for example plastic bags), so as to avoid environmental contamination, and shall be stored in the refrigerator (6.4).

Dried meat shall be stored in an airtight container.

Examination shall take place within 3 days.

In case of doubt, treat as in 7.2.1.

7.3 Carcasses, cuts of carcasses or meat in pieces exceeding 2 kg in mass and mechanically separated meat or dried meat

7.3.1 Fresh meat

See 7.2.1.

7.3.2 Frozen meat

See 7.2.2.

7.3.3 Dried meat

See 7.2.4.

7.3.4 Drips

Store the samples in the refrigerator (6.4).

The samples shall be examined as soon as possible, but in any case on the day of receipt.

7.3.5 Swabs

Store the cotton or alginate swabs in the refrigerator (6.4) on receipt.

The samples shall be examined as soon as possible, but in any case on the day of receipt.

8 Treatment of laboratory samples

8.1 General

Handle samples so as to avoid any risk of contamination, taking the following precautions:

a) ensure that the working area is clean and draught-free; do not subject samples to direct sunlight;

b) clean the work surface with a suitable disinfectant (5.5) both before and after testing;

c) sterilize containers, trays, apparatus, etc., and instruments for handling and opening packs or cans in advance.

If a period of incubation is desired or required (for example, for cans), proceed as specified in 8.2.

Visibly defective samples shall never be incubated.

In the case of frozen products that are still frozen (see 7.2.2), or samples frozen after sampling (see 7.2.1), proceed according to 8.3. In all other cases proceed directly according to clause 9.

8.2 Incubation

Incubate at the required temperature for the required period as arranged or prescribed by legislation.

Carry out daily controls to check whether samples have become defective (for example swelling, extrusion of moisture). If this is the case, terminate the incubation. Register the period of time and proceed according to clause 9.

Shake or invert samples containing a liquid phase every 2 days.

After incubation proceed according to clause 9.

8.3 Thawing in the refrigerator

Thaw the unopened samples in the refrigerator (6.4) until thawing is complete, but for not longer than 24 h. When samples need more than 24 h to thaw, other sampling methods should be used.

9 Opening of package

9.1 General

Clean rigid or semi-rigid packages externally with soap or detergent and water and dry them with a clean towel. Then dry them with clean, single-use absorbent paper.

Disinfect the packages over such a part of the exterior that contamination is avoided on opening. When, however, the packaging or wrapping material is very thin and could be damaged by the cleaning process (wrapped portions of meat on trays) this procedure shall be omitted. Disinfection should be carried out very carefully.

When the packaging can be removed without any risk of contamination, cleaning and disinfection are not necessary.

All operations during and after opening shall be carried out under aseptic conditions preferably without interruptions; if interruption is unavoidable, it shall be as short as possible.

During the whole of any interruption, the product shall be stored in the refrigerator (6.4).

Apparently normal samples and defective samples shall be treated differently. Proceed according to 9.2 and 9.3 as appropriate.

9.2 Apparently normal samples

Carry out disinfection by flaming (with or without ethanol and avoiding overheating), or by applying the disinfectant mixture (5.5) and allowing to dry but not by applying heat.

Open wrapped portions of meat on trays by removing the packaging film starting beneath the tray.

Open gas-packed meat packages using a sterile knife, scissors or forceps, after disinfecting the sealed cover with disinfectant mixture. Open vacuum-packed sliced meat products according to the same procedure.

Disinfect cooked or raw sausages in permeable or non-permeable synthetic casings at the point of incision; remove the casing by stripping.

Do not remove the casing of raw ripened sausage.

Open cans, after cleaning and disinfecting by flaming, using a sterile can-opener; when secondary samples (for example from centre and surface) have to be obtained, open the can at both ends and push the meat product(s) out onto a sterile tray. Do not damage seams as it may be necessary to examine them.

Open glass jars with an appropriate opener which cuts a circular opening in the lid.

9.3 Defective units

Open defective units in a special room which is never used for sterility control.

Disinfect by swabbing with the disinfectant mixture (5.5) and allowing to dry but never by applying heat.

Puncture cans with great care and open them with a sterile can-opener (6.5).

During opening, avoid contamination of the operator and surroundings.

Proceed according to clause 10 or clause 11.

10 Taking secondary samples

If necessary, secondary samples may be taken, for example drips, swabs, or separate portions from different parts of each sample (centre, surface).

For (primary or secondary) samples that need comminution, and for swabs, proceed according to clause 11.

11 Final preparation before the examination if necessary

11.1 Comminution

11.1.1 General

When by its nature the material under investigation is expected to cause difficulties if homogenization is carried out directly, dice it beforehand. Proceed according to 11.1.2 and/or 11.1.3.

11.1.2 Dicing

Place the material on a sterile surface and cut it under aseptic conditions into dice of approximately 1 cm^3. Proceed according to 11.1.3.

11.1.3 Homogenization by mincing

Put the material (whether or not diced) into the blending equipment (6.2) under aseptic conditions.

Mix and homogenize twice in the blending equipment, putting any drips back before the second blending, and proceed as indicated in ISO 6887.

11.2 Treatment of swabs

Shake the swabs in the diluent (5.2 for cotton swabs, 5.3 for alginate swabs) to disperse the adhering micro-organisms into the fluid.

To achieve this, break wooden swab applicators so that the swabs themselves can be shaken in small flasks containing a specified amount of fluid together with some glass beads.

The dispersion obtained can be further diluted decimally.

12 Subsequent treatment

For the subsequent treatment of the products to be tested, refer to existing International Standards.

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