
**Microbiology of food and animal feeding
stuffs — Preparation of test samples,
initial suspension and decimal dilutions
for microbiological examination —**

Part 2:

**Specific rules for the preparation of meat
and meat products**

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

*Partie 2: Règles spécifiques pour la préparation des viandes et produits
à base de viande*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6887-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 6887 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*:

- Part 1: *General rules for the preparation of the initial suspension and decimal dilutions*
- Part 2: *Specific rules for the preparation of meat and meat products*
- Part 3: *Specific rules for the preparation of fish and fishery products*
- Part 4: *Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

This first edition of ISO 6887-2 cancels and replaces ISO 3100-2:1988, *Meat and meat products — Sampling and preparation of test samples — Part 2: Preparation of test samples for microbiological examination*.

Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

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

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

1 Scope

This part of ISO 6887 specifies rules for the preparation of meat and meat product samples and their suspension for microbiological examination when the samples require a different preparation from the method described in ISO 6887-1. ISO 6887-1 defines the general rules for the preparation of the initial suspension and decimal dilutions for microbiological examination. This part of ISO 6887 only describes methods of preparation that are applicable to several microorganisms simultaneously. It excludes the preparations that only apply to the detection and/or enumeration of a single microorganism where the method of preparation is described in the relevant International Standard concerning that microorganism.

This part of ISO 6887 is applicable to the following fresh, raw and processed meats and poultry and their products:

- refrigerated or frozen;
- cured or fermented;
- minced or comminuted;
- delicatessen meats;
- pre-cooked meals or poultry based meals;
- dried and smoked meats at various degrees of dehydration;
- concentrated meat extracts.

This part of ISO 6887 is not applicable to the following products whose microbiological examination is described in other International Standards:

- canned products;
- other products (see ISO 6887-4).

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

NOTE 2 The meat from wild animals (from game slaughtering or game shooting) can be analysed following the applicable techniques described here for similar products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1:1999, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of initial suspension and decimal dilutions*

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

ISO 17604, *Microbiology of food and animal feeding stuffs — Carcass sampling for microbiological analysis*¹⁾

3 Terms and definitions

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

3.1

laboratory sample

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

[ISO 7002]

3.2

test portion

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

3.3

initial suspension

primary dilution

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

NOTE For surface samples, the initial dilution should be stated. For example, from a sample (swab or other) from a 25 cm² surface, and diluted in a total volume of 25 ml of diluent, 1 ml of this initial suspension represents 1 cm².

3.4

further decimal dilutions

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

3.5

block

piece

sample whose composition and dimensions (surface and thickness, but thickness in particular) allow a sample at depth to be taken under satisfactory sterile conditions

1) Revision of ISO 3100-1:1991.

3.6**fragment
shaving**

sample from frozen meat resulting from a deep cut of a surface sample or a sample taken deep inside the test piece or by electric drill or a hand-drill fitted with a wood drill bit

3.7**slice**

cut of meat with approximately parallel sides up to several centimetres thick

3.8**carcasses
cuts**

(poultry products, rabbit meat) units similar to units prepared for sale

4 Principle

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

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

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

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

5 Diluents**5.1 Basic materials**

See ISO 6887-1.

5.2 Diluents for general use**5.2.1 Peptone salt solution**

See ISO 6887-1:1999, 5.2.1.

5.2.2 Buffered peptone water

See ISO 6887-1:1999, 5.2.2.

5.3 Diluents for special purposes**5.3.1 Peptone-salt solution with Bromocresol purple****5.3.1.1 Composition**

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

5.3.1.2 Preparation

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

5.3.1.3 Application

This solution may be used in certain acidic products so that adjustment of the pH can be carried out without the use of a sterile pH probe (see 8.4).

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

5.4 Distribution and sterilization of the diluent

See ISO 6887-1:1999, 5.4.

6 Apparatus

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

6.1 Sterile tray, of appropriate dimensions.

6.2 Sterile scissors, forceps or tongs, straight scalpels or knives, and spatulas.

6.3 Mechanical meat mincer, of laboratory size, sterilizable and equipped with a perforated plate, with holes with a maximum diameter of 4 mm.

6.4 Equipment for cauterization of meat surfaces (e.g. portable gas blowtorch).

6.5 Template for surface sampling, metallic frame of appropriate dimensions enabling the delineation of the surface to be sampled, sterilized by immersion in alcohol and burning this off.

In Annex A, an example for a frame is shown. Others tools fulfilling the requirements of this subclause can also be used. Some spores may survive the flame. The use of a sterile metallic frame (after oven sterilization) is recommended for the specific case of sporulated microorganisms.

6.6 Equipment for collecting a frozen laboratory sample

6.6.1 Variable speed electric drill, with maximum speed in use of 900 r/min, or **hand-drill**.

6.6.2 Sterile wood bit for electric drill, of 14 mm or 16 mm diameter.

6.6.3 Sterile wood chisel, of 20 mm width.

6.6.4 Hammer or plastic mallet.

6.6.5 Other apparatus that does not cause overheating or contamination of the sample.

7 Preparation of samples

7.1 Frozen products

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

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

7.2 Hard and dry products

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

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

7.3 Liquid and non-viscous products

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

7.4 Heterogeneous products

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

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

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

8 General procedures

8.1 General

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

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

8.2 Types of samples to be sent to the laboratory

Meat and meat-based products are of the following types:

- units of meat or meat-based products, prepared or processed and of different dimensions;
- cuts of meat taken from units with a mass of less than 2 kg;
- cuts of meat samples taken from carcasses or cuts of meat with a mass greater than 2 kg.

In the case of sampling using non-destructive methods (ISO 17604), tissues and swabs (or other, see ISO 17604) should be sent to laboratory.

The physical state of the samples received may vary according to the following factors:

- a) temperature, for products that are
 - refrigerated, or
 - frozen or deep-frozen;

b) water activity (a_w), for products that are

- untreated, or
- partially dehydrated meat products whose reduction in water content inhibits microbial multiplication (reduced a_w).

8.3 Purpose of the analysis

See ISO 7218.

The purpose of the microbiological analysis may be to detect and/or enumerate

- deep microbial flora,
- surface microbial flora, or
- surface and deep microbial flora (total).

The preparation of the test sample shall take account of the aim of the analysis and the nature of the sample.

8.4 General case for acidic products

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

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

8.5 High fat foods (over 20 % of total mass as fat)

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

9 Specific procedures

9.1 Initial preparation of the different types of samples

9.1.1 Laboratory sample with a mass equal to or less than 50 g

Use the entire laboratory sample for the preparation of the initial suspension if the sample mass is equal to or less than 50 g.

NOTE Only the detection or enumeration of surface and deep microbial flora is possible.

9.1.2 Test piece or carcass

Take a sample at depth and/or a surface sample.

For analysis of a surface, a non-destructive technique may be used (using swabs or small cloth). See ISO 17604.

9.1.3 Slice or individual portion of meat or cooked meat

Take a strip from the middle.

9.1.4 Fragments or shavings of frozen products

Homogenize thoroughly.

9.1.5 Meat products in “skins”

If the skin is inedible (synthetic), disinfect the cooked or raw sausages with permeable or non-permeable synthetic (plastic) skins at the point of incision; pull to remove the skin with sterile tongs.

Cut up the chopped slices.

Do not remove the skin from mature raw sausages.

9.1.6 Pre-cooked meals

For packaged pre-cooked meals, open up the packaging in accordance with 9.2.

Take aliquots of each component, taking their proportions into account.

It is possible to homogenize the laboratory sample in its entirety in order to get a homogenized test sample.

9.2 Procedure for manufactured products stored under refrigeration

9.2.1 General

For packaged products, proceed as follows:

- soft packaging: to be removed using scissors or a scalpel (6.2);
- rigid packaging (glass containers, etc.): clean and disinfect the outside surface thoroughly using alcohol; open under sterile conditions.

Clean the surface of rigid or semi-rigid packaging using soap or detergent with water then dry with a clean towel or fresh absorbent paper.

Disinfect the outside of the packaging to avoid contamination when opening. The disinfecting process shall be carried out with great care.

When the packaging or the material acting as packaging is very thin and could be destroyed during the cleaning process (pieces of meat packaged in containers), this operation shall not be carried out.

The cleaning and disinfecting of packaging is not necessary when the contents can be removed without risk of external contamination.

All operations before and after opening shall be carried out to avoid external contamination.

Portions of meat sold on trays shall be opened by peeling off the packaging film from underneath the tray.

For meats packaged in a controlled atmosphere and vacuum-packed meats, open the sealed packaging using a sterile knife, scissors or forceps.

9.2.2 Sampling from deep within the test material

This sample is to analyse only the depth of the meat sample and sampling is carried out after cauterization of the surface. Use scalpels and tongs (6.2) to remove the appropriate area of skin from cuts of meat from wild animals (when presented with skin).

If packaged, remove the sample using an aseptic technique and sterile knife or scalpel, and place on a sterile tray. Using a blowtorch (6.4), remove a surface layer 2 mm thick from the upper surface to expose an area 5 cm by 5 cm. Cauterize this exposed surface until charring occurs. Using a fresh sterile knife or scalpel (6.2), remove a layer about 4 cm by 4 cm and 1 cm deep from below the charred area. Using sterile forceps and scalpel, remove the required sample from the exposed area and place it in a sterile container or plastic bag.

Weigh the sample and add 9 times this mass of diluent (5.2).

9.2.3 Sampling from the surface of meat

Samples are taken *without prior cauterization*.

If packaged, remove the meat using an aseptic technique with scissors and scalpel and place it on a sterile tray (6.1) with the test surface uppermost. Use a sterilized or disinfected template (6.5) and apply to the designated area (see Annex A).

Using a sterile scalpel, cut along the inside edges of the grid. Then, using forceps to lift the sample, cut along the inside edges of the grid. Using forceps to lift the sample, cut across the whole area to a depth of 2 mm to 3 mm. Place the pieces in a sterile container or plastic bag to be used when homogenizing the sample in diluent.

Weigh the sample and add 9 times this mass of diluent (5.2).

To correlate easily the sampled area with the total volume of the initial suspension, adjust if possible the quantity of added diluent (e.g. area of 25 cm² minus the volume of initial suspension of 250 ml).

9.2.4 Sampling from individual sliced portions

Samples are taken *without prior cauterization*.

If packaged, remove the meat using an aseptic technique with scissors and scalpel (6.2) and place it on a sterile tray (6.1). Lay it out flat.

Using a sterile scalpel and forceps, cut a strip 1 cm wide along the centre of its greatest length. Cut the strip into small pieces and place them in a sterile container or plastic bag to be used when homogenizing the sample in diluent (5.2).

Weigh the sample and add 9 times this mass of diluent (5.2).

9.2.5 Sampling of poultry carcasses

9.2.5.1 General

Samples may be taken as follows:

- from the depth of the pectoral muscle;
- as a surface sample from the skin;
- by rinsing the whole carcass in diluent (non-destructive sampling).

9.2.5.2 Sampling the pectoral muscle

This sample shall be taken from within the depth of the pectoral muscle after cauterization of the surface. Pluck the appropriate area for game birds (when presented with feathers).

If packaged, use an aseptic technique with scissors and scalpel (6.2) to remove the packaging and place the carcass on a sterile tray (6.1) in the dorsal position.

Using the blowtorch (6.4), cauterize the part of skin covering the pectoral muscles.

Use the scalpel and tongs (6.2) to remove the cauterized skin.

Using the blowtorch (6.4), cauterize the exposed part of the pectoral muscle.

Using the tongs and the scalpel, take a sample at depth (without touching the lower part of the muscle).

Place it in the sterile container or the plastic bag to be used for homogenizing.

Weigh and add 9 times this mass of diluent (5.2).

9.2.5.3 Sample taken of neck flap skin

Samples are taken *without prior cauterization*.

If packaged, remove the carcass using an aseptic technique with scissors and scalpel (6.2), and place it on a sterile tray (6.1) so that the sampling areas are uppermost.

Using a sterile forceps and scalpel or scissors (6.2), cut 5 g to 10 g of skin from the neck flap. If the trachea or oesophagus is still present cut them away and remove excess fat from the inner surface of the skin. Place the sample in a weighed container or plastic bag used for homogenization.

Weigh and add 9 times this mass of diluent (5.2).

9.2.5.4 Sampling whole carcass by the wash method

Samples are taken *without prior cauterization*.

If packaged, remove the carcass using an aseptic technique with scissors and scalpel (6.2), and place it in a large plastic bag so that it can be shaken.

Add 500 ml of diluent and shake for about 30 s so that all parts of the carcass are rinsed. Pour the diluent into a sterile container.

9.2.6 Samples from cuts of poultry meat

Follow the procedures described in 9.2.2 or 9.2.3 as appropriate.

9.2.7 Sampling rabbit carcasses

For animals presented with hair, use scalpels and tongs (6.2) to remove the appropriate area of skin from cuts of meat from ground game.

Follow the procedures in 9.2.3 and 9.2.5.4 *but with cauterization* to take a deep sample within the thigh muscle.

To take a surface sample, without cauterisation, use sterile scalpel and forceps to take samples from designated surfaces of the carcass and place in a sterile container for further preparation.

Weigh and add 9 times this mass of diluent.

9.3 Procedure for frozen meats

9.3.1 General

Consider the following two cases (9.3.2 or 9.3.3).

9.3.2 Large pieces or blocks from which samples are taken without prior defrosting

9.3.2.1 General

Separate the sample from its packaging using the scissors or scalpel (6.2), and place it on the tray (6.1), with its flat side facing upwards.

Consider the following three cases (9.3.2.2, 9.3.2.3, 9.3.2.4).

9.3.2.2 Total sample (surface and deep parts)

Using the electric drill (6.6.1) equipped with the appropriate bit (6.6.2) or any other apparatus (6.6.5), or failing this, the hand-drill (6.6.1), make holes in the specified points (see Annex B). For this operation, set the speed of the drill (6.6.1) or apparatus (6.6.5) (about 900 r/min) to avoid fusion or dispersion of the shavings.

Using the spatula (6.2), collect the resultant shavings and place them in the fared container or the plastic bag to be used for homogenization. If their mass is greater than 50 g, mix them in the plastic bag to obtain a sample for homogeneous testing.

This operation shall not cause a notable increase in the temperature of the sample.

9.3.2.3 Sample at depth

Using the wood chisel (6.6.3) and the hammer (6.6.4), remove a surface strip with a thickness of around 3 mm from an area of about 6 cm by 6 cm. Caulterize it using the blowtorch (6.4) until the previously cleared surface is carbonized. Then proceed according to 9.2.2 by making perforations in the cauterized areas without touching the lower side of the block.

9.3.2.4 Surface sample

Sterilize the template (6.5) and the wood chisel (6.6.3) by dipping in alcohol and burning. Whilst the template is still hot, apply it to the surface of the frozen meat.

Using the sterile chisel (6.6.3) and hammer (6.6.4), chip off the upper layer of meat within the template to a depth of about 2 mm. Collect the pieces aseptically in a flask or stomacher bag.

Weigh and add 9 times this mass of diluent (5.2).

9.3.3 Small samples likely to be defrosted

Such samples include packaged small cuts of (diced) meat and joints from poultry and rabbits.

With the sample still in its package, defrost it at ambient temperature until the interior of the sample is barely thawed and thaw water is still absent. This operation should not exceed 2 h to 3 h.

If defrosting is to last more than 3 h, defrost slowly in an enclosed chamber with a temperature of $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a maximum of 18 h.

Proceed as described in 9.2.4, 9.2.5, 9.2.6 or 9.2.7 depending on the case.

Defrosting in a temperature-controlled water bath is not recommended because of the risk of contamination to the sample in the event of the packaging not being properly watertight.

NOTE For poultry and rabbit carcasses, it is normal practice to defrost slowly in a cold room with a positive temperature (between 0 °C and 2 °C) for 15 h to 16 h. It is noted that the poultry carcasses cooled down by immersion often lead to a more noticeable exudation during the defrosting stage.

9.4 Procedure for partially dehydrated meat extracts

Open the packaging (see 9.2).

Using a spatula (6.2), take a sample of a certain product mass and operate using the same method as for refrigerated products (see 9.3).

For dehydrated meats, refer to ISO 6887-4.

9.5 Surface samples (swabs and small cloths)

For sampling, refer to ISO 17604.

Mix the swabs in the same diluent as the one used to saturate them, in order to disperse the microorganisms that adhere to the swabs.

In order to do this, break the handles of the swabs so that the swabs themselves can be shaken in the small flasks containing a defined quantity of diluent and some glass balls.

The resulting solution may be diluted afterwards in a decimal manner.

10 Further decimal dilutions

See ISO 6887-1.

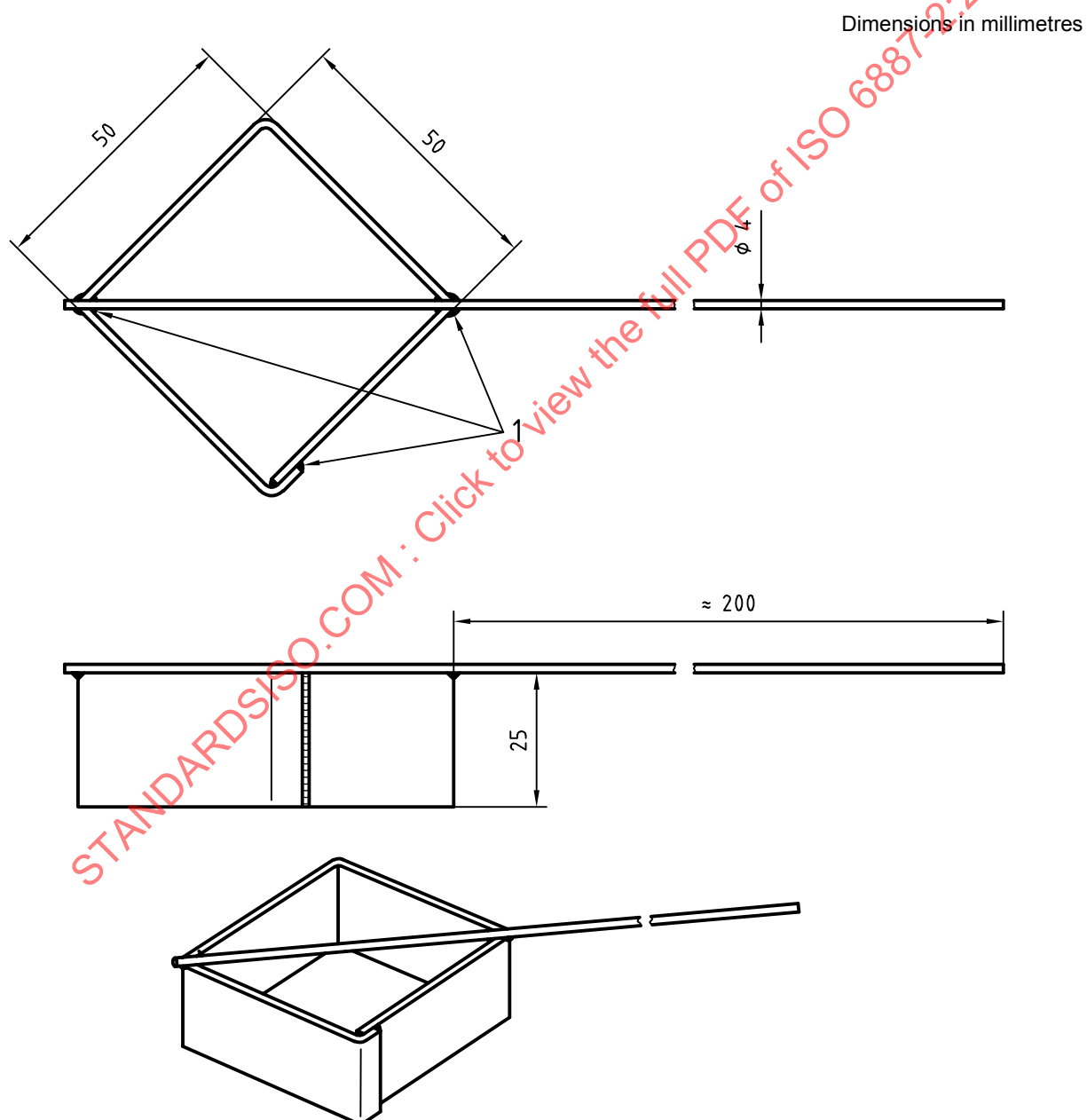
Annex A (informative)

Frame for the delineation of a surface sample area

Composition materials may be as follows:

- frame: stainless-steel sheet with a thickness of 3/10 mm;
- handle: stainless-steel cylindrical stem with a diameter of 4 mm.

An example of a suitable frame is shown in Figure A.1.



Key

- 1 brazing points

Figure A.1