

Title:
Sampling techniques for microbiological analysis of food and feed samples

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Note: Write as in ISO 7218 :Two kinds of writing to separate standard (normative) parts and guidelines (informative) parts.

Introduction

Some information given in this standard is intended as guidance (Times typeface) only; other parts are mandatory (usual typeface).

For some aspects of sampling it is necessary to have agreement and/or contracts with customers to ensure the method and extent of sampling meets their requirements. (*in relation to ISO 17025 “contracts”*).

1 Scope

This standard gives general requirements for sampling techniques outside the laboratory to obtain samples for subsequent bacteriological analysis and to transport them to the laboratory.

This standard concerns all food and feed products, including blocks of frozen products, carcasses or meat (but excluding surface sampling of carcasses) and bulk products.

The following samples types are outside the scope of the present standard:

- Milk and dairy products (See EN ISO 707 Milk and milk products: guidance on sampling;
- Surface sampling of carcasses (See ISO 17604: Microbiology of food and animal feeding stuffs: Carcass sampling for microbiological analysis),
- Samples from environmental surfaces (See ISO 18593: Microbiology of food and animal feeding stuffs: Horizontal methods for sampling techniques from surfaces using contact plates and swabs),
- Samples from the primary production stage (See pr ISO 13307).

(The packaging of samples may or may not be sterile: in some cases, the food product can be packed or wrapped by the food manufacturer. Do we have to separate the sampling technique and taking of samples prepared by the manufacturer in the scope of the standard? In the second case, there is no sampling, but the requirements for transport and receipt and storage are the same; are the requirement in ISO 7218 sufficient? Write a note?)

2 Normative references

EN ISO 7218

3 Definitions

3.1 Sampling

Sampling: The procedure used to draw and constitute a sample (ISO 7002).

Sampling plan: The predetermined procedure for the selection, withdrawal and preparation of samples from a lot to yield the required information so that a decision can be made regarding the acceptance of the lot (ISO 7002).

Sampling technique: *The procedure used to take the sample. (No definition in ISO 7002).*

Cluster sampling: A method of sampling in which the lot is divided into aggregates (or clusters) of items bound together in some manner. A sample of these clusters is taken at random and all the items within a cluster are included in the sample (ISO 7002).

Sample compositing: Action to permit analyse of a number of samples in one test using the same analysis. (This step is not a sampling step but a laboratory step).

- Dry compositing: Mixing of tests portions of different samples;

- Wet compositing: Mixing of portions of enrichment broth, after the first enrichment stage (No definition in ISO 7002).

Multi-stage sampling or multiple sampling: A type of sampling in which a sample is selected by stages, the sampling units at each stage being sub sampled from larger units chosen at the previous stage (ISO 7002).

Batch or Lot: An identified quantity of some commodity, manufactured or produced under conditions that are presumed uniform (ISO 7002).

Lot size: The number of items or quantity of material constituting the lot (ISO 7002).

3.2 Samples

Item; individual; unit: An actual or conventional object (a defined quantity of material) on which a set of observations may be made

An observed value, either qualitative or quantitative (ISO 7002).

Sample (general term): One or more items (or a proportion of material) selected in some manner from a population (or from a larger quantity of material). It is intended to provide information representative of the population, and possibly to serve as a basis for a decision on the population or on the process which had produced it (ISO 7002).

(Note: in food microbiology, each unit or item is often referred to as a sample, as each unit is analysed separately. In this standard, the units are referred to as laboratory samples); once prepared, (following the ISO 6887 series standard) the laboratory sample becomes the sample for analysis).

Laboratory sample: (or sample): The units (in the case of separate products) or the amount of product that arrives in the laboratory to be analysed (ISO 6887)

Sample for analysis: The laboratory sample, or sample, after any preparation necessary to permit the analysis has been carried out. (In some cases no preparation is needed; the sample for analysis is the laboratory sample (or sample).

Test sample: A sample prepared from the laboratory sample according to the procedure specified in the method of test and from which test portions will be taken (ISO 7002). (Use this accepted definition in preference to "sample for analysis")

Representative sample: A sample drawn so as to reflect as accurately as possible the properties of interest of the lot (the bias of the sample should be a minimum against the lot) from which it is taken (ISO 7002).

Sample size: The number of items or quantity of material constituting the sample (ISO 7002).

Composite sample: A sample consisting of portions from each unit, taken in proportion to the quantity of product in each unit selected (ISO 7002).

Bulk sample:

1. A collection of increments or groups thereof intended for separate investigation (**raw bulk sample**)
2. A composite of the increments taken from a bulk lot (**bulk sample** in a proper sense).
3. A combined aggregation of the items or portions of items taken from a lot of pre-packed products (**bulked sample**) (ISO 7002).

Primary sample: A sample taken from a lot during the first stage of multi-stage sampling (ISO 7002).

Primary sample made by incrementation?

(Add a chart to explain the different kinds and steps of samples: see ISO 7002 to harmonise the terminology and codex guidelines on sampling) one chart for bulk and one for packaged products.)

3.3 Products

Bulk products: Products that are not separated into individual items or units.

(Sealed or Wrapped) Packaged product: Products separated into units or items wrapped by the manufacturer.

Open products: Products separated into unpackaged units.

Collection, picking up: The action of collecting units or samples prepared, for example by the owner of the product, without knowledge about the way the sampling was taken.

Transport: The care and handling of the sample from when it was taken until arrival at the laboratory.

Cold chain, refrigeration: Maintenance of the samples or products at a cold temperature, to minimise change in the microbial load.

Receipt: Actions taken at the laboratory when the samples arrive.

Acceptance criteria: Characteristics of samples necessary before they can be accepted for analysis on arrival at the laboratory (e.g. size, weight, integrity of wrapping, temperature correct for physical state etc.)

4 Principles and general requirements

The sample shall be representative of the batch to be controlled.

Sample handling procedures including transport shall not affect the microbiological quality of samples in any way. In all cases it is important to retain the microbiological quality of the product; the sampling technique shall not modify the flora.

It is important to cause minimum disruption at the sampling site and follow any security instructions.

Before starting sampling, the minimum quantity required for analysis and any instructions on pooling sub-samples on site shall be agreed with the client.

Before sampling, other details should be agreed with the client, to ensure correct interpretation of the results of analysis. For example, it is important to decide:

- what kind of product and which batches are to be sampled;

- what is the purpose of the analyse of the product (survey or analyse of a batch, to know the microbial quality of the product itself or to know the quality of the product as given to the consumer);
- how samplers must be dressed (according to local rules in the factory for example);
- whether sterile or non- sterile tools will be used.,

Acceptance criteria and permitted deviations for samples on receipt at the laboratory shall be defined (in accordance with client requirements).

Unique identification of samples and labelling requirements shall be defined.

Sufficient information shall be recorded in the sampling report, to give full traceability of the samples and allow interpretation of the results of analysis.

5 Sampling plan:

The sampling plan to be used is excluded from the scope of this standard. Information about sampling plans is available in the ISO 2859 series (*put the reference numbers in bibliography*).

In the case of bulk products, locations for sub-sampling (*and the sampling techniques*) shall be included in the sampling plan.

All interested parties shall agree upon the sampling plan to be used.

6 Personnel

6.1 General arrangements (*from EN ISO WD 13307*)

The parties concerned or their representatives shall be given the opportunity to be present when sampling is performed.

Whenever special requirements are given for the sampling and/or arise from a specific analysis to be performed, these requirements shall be followed.

6.2 Sampling personnel (samplers) (*from EN ISO WD 13307*)

Sampling for microbiological examination shall always be undertaken by personnel trained and experienced in the techniques of sampling for microbiological purposes.

Personnel shall have training in aseptic techniques and experience of the types of products being sampled as well as the requirement to minimize change in the normal microflora of the products.

7 Sampling techniques

7.1 Reagents and media

Disinfectants for decontamination of packaging, instruments and surfaces of certain samples:

- Disinfectants (*which types?*)
- Ethanol 70% v/v
- (Alcohol) wipes or pads

7.2 Apparatus and equipment

- Bags
- Boxes
- Bottles, tubes

- Thermometers (different kinds of thermometers?), electronic and surface probes, *infra red probes*.
- Labelling systems (labels, permanent ink pens, etc.)
- Spoons, forceps, knives, scalpels, dip samplers
(*Sterile or not, or disinfected? Client contract: utilisation of client equipment? +/- client explanation for the results interpretation? Different purposes of analyses*)
- Electric or hand drill with suitable bits or corer, for frozen products, band saw or core sampler for soft products (cheese and meat for example)

(*Add the clothing for the operator (+/-coat, +/-hat, +/-shoes, +/-gloves? Is it more to have in the customer contract? Is it depending of the factory?*)

- sampling form or sampling report

7.3 Sampling techniques protocol

Note: Sampling some large products may be done at the factory (see further to the protocol).

Sometimes, these large products may be received in the laboratory; the protocol used to prepare the test portion may be the same, but see the ISO 6887 series for details.

In some cases, an additional sample may be taken for temperature recording during transport or on receipt at the laboratory.

7.3.1 Bulk product (liquids, solids, powder, granules, etc.)

Description

- Liquid products
- Powdered or granular products (flour) (*seeds?*)

Specific equipment

Spoons, spatulas, ladles, specific drillers, bags or boxes for solid products.
Syringes, pipettes, probes and bottles for liquid products.

Specific procedure

With an appropriate tool (spoon, spatula, ladle, etc) take a portion of the product and put it in a bag or a box (solid product) or in a bottle (liquid product) and close the container "hermetically". Label the container.

Put the container in an icebox, refrigerator or isothermal box as appropriate to the state of the sample.

NOTE: For some bulk products, the requirements for taking sub-samples may be given in regulations or specific standards.

7.3.2 Packaged or wrapped products (refrigerated, frozen or ambient)

Description

The product is wrapped or packaged in the place where the sampling is done (eg. factory, store, restaurant, etc.)

Specific equipment

No specific equipment.

Specific procedure

Take the wrapped product without damage the wrapping, put it in a bag or a box, label the container and put the container in an icebox, refrigerator or isothermal box as appropriate to the state of the sample.

7.3.3 Refrigerated

Description

Specific equipment

Specific procedure

Nothing is specific to refrigerated products apart from taking the samples quickly to avoid temperature increases.

Wrapped products: see 7.3.2.

Non-wrapped (open) products: see 7.3.1 “bulk products” (eg. cooked product in a pan).

7.3.4 Frozen products (separated frozen products)

Description

Specific equipment

Specific procedure

Nothing is specific to frozen products apart from taking the samples quickly to avoid temperature increases.

See 7.3.2 “wrapped products”.

7.3.5 Blocks of frozen products (e.g. meat or fish)

Description

Large blocks of frozen product are an intermediate case, between packaged unit products and bulk product.

The blocks may be samples in the factory or brought to the laboratory.

Specific equipment

Electric or hand drill with suitable bits or corer

Spatula or spoon for the dust

Bags or boxes

Specific procedure (*Copy here the ISO 6887-2 text (9.3.2.2) and add the drawings*):

“Using the electric drill equipped with the appropriate bit or any other apparatus, or failing this, the hand-drill, make holes at the specified points. For this operation, set the speed of the drill or apparatus (about 900r/min) to avoid fusion and dispersion of the shavings.

Using the spatula, collect the resultant shavings and place them in the container or bag.”

7.3.6 Ambient products

Description

Specific equipment

Specific procedure

Nothing is specific.

(Proposal: delete 7.3.3, 7.3.4 and 7.3.6, and add the specific sentences in 7.3).

7.3.7 Hot products

Description

Prepared products just before they are given to the consumer (e.g. cooked products in the kitchen, in the pan, prepared hamburger, heated ready-to-eat products, etc.)

Specific equipment

All tools (spoons, ladle) and containers (boxes or bags) shall be made of heat-resistant material.

Specific procedure

Product in the kitchen: see bulk product 7.3.1

Wrapped product: see wrapped product 7.3.2

Customer portion: see 7.3.8

Hot products and cold products shall not be included in the same transport container.

7.3.8 Customer portions in restaurants

Description

Prepared products just before service; the portions are usually not wrapped.

Specific equipment

Tools as spoons, spatulas, ladles.

Bags or boxes.

Specific procedure

With an appropriate tool (spoon, spatula, ladle, etc) take a portion of the product and put it in a bag or a box (solid product) or in a bottle (liquid product) and close the container "hermetically". Label the container.

Put the container in an icebox, refrigerator or isothermal box as appropriate to the state of the sample.

For customer portions served on a plate, slide the product into the sample bag. Use an appropriate tool or the wall of the bag if necessary to gather the entire portion.

In some situations, the sample bag may be used to take the product like a glove by inverting it over the hand and then returning the bag with the product inside.

7.3.9 Specific products

Live shellfish:

Description

Specific equipment

Specific procedure

Finfish:

Description

Specific equipment

Specific procedure

Fruits and vegetables:

(Raw and cooked? in bulk or wrapped?)

Spices and herbs, coffee, tea etc.

(In bulk or wrapped?)

Whole eggs

Take the eggs into suitable egg boxes if possible to avoid breakage.

Pet foods and animal feeds

Description

Specific equipment

Specific procedure

7.3.10 Special cases, e.g. neck skin of poultry or carcass rinses

See EN ISO 6887-2(?).

In the slaughter house, samples are taken for different purposes.

If the purpose is to show the health status of animals, the analysis belongs to the primary stage (see ISO 6887-6).

If the purpose is food hygiene, then analysis belongs to food microbiology standards

Description

Specific equipment

Specific procedure

7.3.11 Spoiled samples

Description

The purpose of sampling and analysis is to find the cause of the spoilage.

Samples may be of all kinds of products but for all types it is important to maintain the integrity of the spoiled product until arrival at the laboratory for examination.

Specific equipment

No specific equipment is required.

Take care to wrap or package the samples well to prevent leakage.

Specific procedure

No specific procedure is needed, other than the need to prevent cross-contamination of other samples and potential hazards to personnel.

7.3.12 Sampling with automatic apparatus

Description

In some production situations, samples may be taken directly from the production line automatically and at specified time intervals. Such samples shall be collected, contained and labelled in a suitable manner.

7.4 Packing and labeling of samples (from EN ISO WD 13307)

Samples shall be packed in order to avoid cross-contamination and to prevent leakage or loss of moisture, and they must be clearly identified.

The minimum details that shall accompany the samples are:

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- the nature of the product;
- the identification code;
- the name or initials of the person responsible for taking the samples;
- the date, time and place of sampling.

All this information shall be recorded on the sampling form. One form may be used for several samples, provided each has a unique identifying code and the samples are accompanied by a list of the sample details with their unique identifying codes.

7.5 Preparation of a sampling form (sampling report) (from EN ISO WD 13307)

Samples shall be accompanied by a report, ideally completed on a standard form provided by the laboratory, signed or initialed by the sampling personnel. The report shall give the following particulars:

- a) Place, date and time of sampling;
- b) Names of the sampling personnel;
- c) Nature, number, and identity of samples constituting the consignment;
- d) Purpose of sampling and the microorganisms to be sought.

When appropriate, the report shall also include any relevant conditions or circumstances, and any special information relating to the product being sampled, for example difficulty in achieving representative samples.

Any additives such as diluents, transport media or neutralizing agents used must be specified.

Whatever format is used for the sampling report, the following details shall be recorded and accompany the samples to the laboratory:

- Type and name of product
- Description of the sample, number of units
- Name of the owner
- Place of sampling or collection
- Number of batch
- Date and time of sampling
- Name of the sampling officer
- Temperature of the sample and storage
- Purpose of analysis.

8 Transport

Some regulations for contaminated or hazardous products exist but these do not generally apply to food samples, only for cultures or heavily contaminated materials likely to be hazardous.

Transport time to the laboratory shall be as short as possible, and shall be in temperature conditions ensuring good preservation of the samples. All necessary steps shall be taken to avoid change in the microbial flora. These shall be documented.

8.1 Reagents and media

No specific needs for food samples; only needs for specific microorganisms as documented in the appropriate standards.

8.2 Apparatus and equipment

Refrigerator, freezers, ice boxes, boxes or containers

- **Vehicle refrigerator:**
Portable refrigerator made to be used in a vehicle to keep food at + 4,5°C +/- 3,5°C. The refrigerator is equipped with a battery.
- **Vehicle freezer :**
Portable freezer made to be used in a vehicle to keep food at – 18°C. The freezer is equipped with a battery.
- **Ice box:**
Isothermic container equipped with cold packs.
The ice box shall maintain the temperature at – 15°C or less for frozen products, or at + 4,5°C +/- 3,5°C for refrigerated products.

Thermometer, temperature probes, temperature recorders

Integrated temperature recorder: This recorder works with a battery and associated software. The software is necessary for the preparation of the recorder (programming) and interpretation of the records.

The device should be able to record temperatures from - 20°C to + 10°C, with a measurement uncertainty of +/- 0,5°C. It shall be waterproof to avoid damage by samples.

Cold pack

8.3 Transport protocol

Samples may be transported by laboratory personnel with laboratory equipment, or by a specific transport organization (sub-contractor). In all cases, the transport conditions shall be the responsibility of the laboratory.

In the transport protocol, specific attention shall be paid to critical factors such as:

- Duration of the journey;
- Temperature and method of recording the temperature (for example, before and after or throughout transportation);
- Wrapping and secondary wrapping to avoid sample damage;
- Arrangement in the transport boxes or equipment to avoid mixing frozen, refrigerated and hot products.

To allow temperature recording during transportation or on receipt, an additional sample may be taken for this purpose.

8.3.1 Transportation by the laboratory

Immediately after collection, samples bottles, bags or boxes shall be placed in a protective container such as an ice box containing cold packs.

If the laboratory vehicle is refrigerated or it is equipped with a portable refrigeration unit, the samples may be transferred from the container used for sampling.

NOTE: the refrigeration unit shall be switched on before the use and run for long enough to ensure the required temperature is maintained.

If the vehicle is not refrigerated, an ice box should be available in the vehicle and shaded from direct sunlight to minimize heat gain during transport.

The temperature range permitted during transportation shall be documented in the client contract and should depend on the food type (ambient, refrigerated, frozen or hot product) and the sample size.

The vehicle refrigerator or ice box should contain a thermometer or a temperature recorder. If this is not the case, the temperature of the products / ice box should be recorded each time the ice box is opened and on final receipt at the laboratory.

The maximum duration of transportation should be documented in the client contract and should depend on the food type (ambient, refrigerated, frozen or hot product).

If a temperature recorder is used it should be used placed in the same conditions as the samples (e.g. in a bottle or box) in the middle of the samples being transported). Ideally an extra sample shall be taken for temperature monitoring.

Some ambient-stable products do not require refrigerated transport (e.g. powdered products, cans, etc), however, in summer it may be useful to record the vehicle and/or container temperature to check whether excessively high temperatures (e.g. > 40°C) which could affect the samples have been reached.

8.3.2 Transportation by a contractor or courier

Where laboratory transport cannot be used (e.g. because of the distance involved) a haulage contractor or courier, preferably using refrigerated vehicles, may have to be engaged. Conditions for transporting the samples shall be carefully documented and agreed before the contract is placed.

Put the samples in a container (strong box or preferably an ice box). A temperature recorder should be used if possible in the way described in 8.3.1. If this is not possible, record the temperature inside the container just before closing, and ensure it is recorded again just after arrival at the laboratory). This information will enable calculation of the average temperature during transportation.

Record also the time the container is given to the contractor and ensure the time of arrival in the laboratory will also be recorded.

The permitted temperature range should be related to the transportation duration (for example: >1°C to < 8°C temperature range for long transport duration, or >8°C to < 10°C temperature range for short transport duration, etc.)

In all cases when the average transport temperature was not in accordance with the client contract, the client shall be informed, and if analysis is still required, the transport temperature shall be recorded on the test report.

9 Reception at the laboratory

See ISO 7218

9.2 Apparatus, equipment and reagents

- Disinfectant, ethanol 70% v/v, (alcohol) wipes or pads as required
- Labelling systems (labels, permanent ink pens, etc.)
- Thermometers, digital probes, surface probes, infra red probes etc.

9.2 Reception protocol

Acceptance criteria shall have been agreed with the client (temperature, size and packaging of samples).

On arrival, the sample temperature (or container temperature) shall be recorded or the temperature history downloaded if a recorder has been used.

Accordance of the samples with the requirements of the client contract shall be verified.

The samples shall be labelled and stored appropriately before analysis.

10 Storage before analysis

(See ISO 7218).

Condition and duration of storage before analysis shall be defined in the client contract.

Annexes

Flow chart of sampling

Figure to explain means of sampling a frozen piece or block (see annex B in ISO 6887-2)

Bibliography

- EN ISO 707: "milk and milk products: guidance on sampling",
- ISO 17604: "Microbiology of food and animal feeding stuffs: Carcass sampling for microbiological analysis",
- ISO 18593: "Microbiology of food and animal feeding stuff: Horizontal methods for sampling techniques from surfaces using contact plates and swabs",
- Pr EN ISO 13307: "Samples from primary production stage".
- ISO 6887-1 to - 6: "Microbiology of food and animal feeding stuff: Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – part 1 to part 6".