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

*Microbiologie des aliments — Exigences générales et
recommandations*

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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 7218 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with CEN Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*.

This third edition cancels and replaces the second edition (ISO 7218:1996), which has been technically revised. It also incorporates the Amendment ISO 7218:1996/Amd.1:2001.

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Introduction

When conducting microbiological examinations, it is especially important that

- only those microorganisms which are present in the samples are isolated and enumerated;
- the microorganisms do not contaminate the environment.

In order to achieve this, it is necessary to pay attention to personal hygiene and to use working techniques which ensure, as far as possible, exclusion of extraneous contamination.

Since, in this International Standard, it is possible to give only a few examples of the precautions to be taken during microbiological examinations, a thorough knowledge of the microbiological techniques and of the microorganisms involved is essential. It is important that the examinations are conducted as accurately as possible, including monitoring and recording aspects that may affect results and calculation of the number of microorganisms and the uncertainty of the results.

Ultimately, it is the responsibility of the head of the laboratory to judge whether the manipulations are safe and can be considered to be good laboratory practice.

A large number of manipulations can, for example, unintentionally lead to cross-contamination, and the analyst should always verify the accuracy of the results given by his or her technique.

In order to conduct the examinations correctly, it is necessary to take certain precautions when constructing and equipping the laboratory.

Certain precautions must be taken, not only for reasons of hygiene, but also to ensure good reproducibility of the results. It is not possible to specify all the precautions to be taken in all circumstances, but this International Standard at least provides the main measures to be taken when preparing, sterilizing, storing the media, and using the equipment.

If the guidance given in this International Standard is followed, this will also contribute towards maintaining the health and safety of personnel. Additional information on this subject is to be found in the literature listed in the Bibliography.

In order to distinguish the guidance in this International Standard, it has been printed in a different typeface (Times New Roman).

Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

1 Scope

This International Standard gives general requirements and guidance/options intended for three main uses:

- implementation of ISO/TC 34/SC 9 or ISO/TC 34/SC 5 standards for detection or enumeration of microorganisms, named hereafter “specific standards”;
- good laboratory practice for food microbiological laboratories (the purpose is not to detail them in this International Standard, manuals are available for that purpose);
- guidance for accreditation of food microbiological laboratories (this International Standard describes the technical requirements according to Annex B of ISO/IEC 17025:2005 for the accreditation of a microbiological laboratory by national organizations).

The requirements of this International Standard supersede the corresponding ones of existing specific standards.

Additional instructions in the field of molecular biology examinations are specified in ISO 22174.

This International Standard covers examination for bacteria, yeasts and moulds and can be used if supplemented with specific guidance for prions, parasites and viruses. It does not cover the examination for toxins or other metabolites (e.g. amines) from microorganisms.

This International Standard applies to the microbiology of food, animal feeding stuffs, the food production environment and the primary production environment.

The purpose of this International Standard is to help to ensure the validity of food microbiology examinations, to assist in ensuring that the general techniques used for conducting these examinations are the same in all laboratories, to help achieve homogeneous results in different laboratories, and to contribute towards the safety of the laboratory personnel by preventing risks of infection.

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 835 (all parts), *Laboratory glassware — Graduated pipettes*

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO 7218:2007(E)

ISO 8655-1, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

ISO/TS 11133 (all parts), *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media*

ISO 16140, *Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods*

ISO/TS 19036, *Microbiology of food and animal feeding stuffs — Guidelines for the estimation of measurement uncertainty for quantitative determinations*

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Premises

3.1 General

This clause gives general requirements, e.g. the principles of design and organization, for the layout of a microbiological laboratory.

Examination of primary production stage samples (especially for sample reception and sample preparation) shall be separated from examination of other samples to reduce the risks of cross-contamination.

3.2 Safety considerations

The laboratory design shall comply with safety requirements which will depend on the type of microorganism. To this end, microorganisms are classified in four risk categories:

- **Risk category 1** (no or very low risk to the individual and to the community).

A microorganism that is unlikely to cause human or animal disease.

- **Risk category 2** (moderate risk to the individual, low risk to the community).

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community or the environment. Laboratory exposures may cause serious human infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

- **Risk category 3** (high risk to the individual, low risk to the community).

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

- **Risk category 4** (high risk to the individual and to the community).

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

WARNING — Refer to national regulations which will define, in particular, the risk category of the microorganisms encountered within the boundaries of the country concerned.

3.3 Laboratory design

The guidelines for laboratory layout described below cover examinations for the detection of microorganisms belonging to risk category 1, 2 and 3 for food microbiology.

It should be noted that additional safety measures may be necessary depending on local legislation.

3.4 Laboratory areas

3.4.1 General

The laboratory comprises areas associated with samples and testing (see 3.4.2) and general areas (see 3.4.3). These shall be separated.

3.4.2 Areas associated with samples and testing

It is considered good practice to have separate locations, or clearly designated areas, for the following:

- receipt and storage of samples;
- preparation of samples, particularly in the case of raw materials (e.g. powdered products containing a high number of microorganisms);
- examination of samples (from the initial suspension), including incubation of microorganisms;
- manipulation of presumptive pathogens;
- storage of reference and other strains;
- preparation and sterilization of culture media and equipment;
- storage of culture media and reagents;
- examination of foodstuffs for sterility;
- decontamination;
- cleaning of glassware and other equipment;
- storage of hazardous chemicals, preferably kept in specially designated cabinets, cupboards, rooms or buildings.

3.4.3 General areas

Separate areas should be considered for the following:

- entrances, corridors, stairways, lifts;
- administrative areas (e.g. secretarial, offices, documentation rooms, etc.);
- cloakrooms and toilets;
- archive rooms;
- stores;
- rest rooms.

3.5 Layout and fittings of the premises

3.5.1 Objectives

The objective is to ensure that the environment within which the microbiological examinations are carried out does not affect the reliability of the test results.

Arrange the premises so as to avoid risk of cross-contamination. Ways to achieve this objective are, for example:

- a) to construct the laboratory according to the “no way back” layout principle;
- b) to carry out procedures in a sequential manner using appropriate precautions to ensure test and sample integrity (e.g. use of sealed containers);
- c) to separate activities in time or space.

Avoid extreme conditions such as excess temperature, dust, humidity, steam, noise, vibration, etc.

Space should be sufficient to allow work areas to be kept clean and tidy. The space required should be commensurate with the volume of analyses handled and the overall internal organization of the laboratory. The space should be as required by national regulations, when such exist.

3.5.2 Fittings

The test premises should be constructed and equipped in the following ways in order to reduce the risk of contamination by dust and therefore by microorganisms (for risk category 3 microorganisms, refer to national regulations).

- a) The walls, ceilings and floors should be smooth, easy to clean and resistant to detergents and disinfectants used in laboratories.
- b) Floors should be slip-resistant.
- c) Overhead pipes conveying fluids should not cross the premises unless they are hermetically enclosed. Any other overhead structures should be covered or readily accessible for regular cleaning.
- d) Windows and doors should be able to be closed when conducting the tests in order to minimize draughts. Furthermore, they should be designed so as to avoid the formation of dust traps and thus facilitate their cleaning. The ambient temperature (18 °C to 27 °C) and air quality (microorganism content, dust spreading rate, etc.) should be compatible with carrying out the tests. A filter ventilation system for incoming air and for outgoing air is recommended for this purpose.
- e) An adequate extraction system should be installed to prevent exposure to dust arising from handling of dehydrated culture media, and dusty or powdered samples.
- f) When tests are to be conducted in a low-contamination atmosphere, the room should be specially equipped with a clean laminar airflow cabinet and/or a safety cabinet.
- g) If necessary, the laboratory environment should be protected from the harmful effects of solar radiation by use of shutters or suitably treated glass panels. Internally installed blinds are not suitable as they may be difficult to clean and could become a source of dust.

3.5.3 Other points

The following points should be considered:

- availability of water supply, of suitable quality for the intended use;

- availability of electricity;
- availability of gas (piped or bottled);
- adequate light in every section of the laboratory;
- laboratory bench tops and furniture manufactured in smooth, impermeable material that is easy to clean and disinfect;
- laboratory furniture designed so as to facilitate cleaning the floors (e.g. movable furniture);
- no furniture, documents or other items other than those strictly necessary for testing activities kept in the testing areas;
- availability of storage facilities for storing documents used when manipulating the samples, culture media, reagents, etc.;
- provision of hand wash-basins in each testing room and, if needed, in general areas, preferably near the door;
- availability of an autoclave for destruction of contaminated waste materials and culture media, unless an appropriate system for removal of contaminated waste for incineration is in place;
- provision of safety systems to cover fire, electrical emergency and emergency shower and eyewash facilities;
- provision of first aid facilities.

3.6 Cleaning and disinfection

The following points should be checked.

- a) The floors, walls, ceilings, laboratory bench tops, furniture, and junctions between these should be subjected to regular maintenance and repair in order to avoid cracks which may act as a source of contamination.
- b) Regular cleaning and disinfection should be carried out in order to keep the premises in a condition suitable for conducting tests. Contaminated or potentially contaminated surfaces should be decontaminated using disinfectant known to be bactericidal and fungicidal.

NOTE 1 Rooms and equipment can be decontaminated by fumigation with formaldehyde vapour, if allowed by national regulations.

- c) The ventilation systems and their filters should be regularly maintained and filters changed when necessary.
- d) The microbiological quality of laboratory working surfaces, staff contact surfaces, and air should be monitored regularly (the frequency depends on the results of previous testing).
- e) Surface contamination may be estimated by directly applying to the surface a contact plate containing suitable neutralizing agents against sanitizers (e.g. lecithin, sodium thiosulfate). The air quality may be examined by exposing for 15 min an open Petri dish containing a non-selective agar medium (e.g. plate count agar — PCA) or a selective agar appropriate for the target microorganism sought (e.g. mould).

NOTE 2 Other methods can also be used in order to estimate contamination of surfaces and the air. See ISO 18593.

4 Staff

4.1 General

General requirements on the competence of staff can be found in ISO/IEC 17025.

4.2 Competence

For each method or technique, objective criteria shall be defined for assessment of appropriate competence, both initially and on an ongoing basis.

The competence may be established within the laboratory by internal quality control (see 15.1.2).

NOTE One of the means of investigating the cause of poor performance (pipetting, poor homogeneity of the initial suspension, counting, etc.) in the case of enumerations by counting colonies is given in ISO 14461-1.

4.3 Verification of on-going staff competence

Verification of on-going staff competence should be evaluated regularly against objective parameters. This includes participation in internal quality assurance programmes, proficiency tests (see ISO/IEC Guide 43-1), the use of reference materials or by self-assessment tests for enumeration of microorganisms as described in ISO 14461-2.

4.4 Hygiene

The following personal hygiene precautions shall be taken in order to avoid contaminating the samples and culture media and to avoid the risk of infection of personnel.

- a) Wear properly fastened laboratory clothing that is clean and in good condition, manufactured from a fabric which limits the risks of flammability. This clothing shall not be worn outside the work areas and, possibly, cloakrooms.
- b) Wear protection for the hair and beard, if necessary for the integrity of the sample.
- c) Keep nails clean and preferably short.
- d) Wash hands thoroughly in lukewarm water, preferably delivered by a non-manually operated tap, before and after microbiological examinations and immediately after visiting the toilets. Use liquid or powder soap or, possibly a sanitizer, delivered preferably by a dispenser maintained in clean condition. For drying hands, use single-use paper or single-use cloth towels. These precautions are applicable both to laboratory staff and visitors.
- e) When working with exposed samples, cultures, media, and when inoculating, avoid speaking, coughing, etc.
- f) Persons having skin infections or illnesses shall take precautions where microorganisms from these are likely to contaminate samples and may invalidate results.
- g) Do not eat or drink in the laboratory and do not put food for personal consumption in the laboratory refrigerators or freezers.
- h) Mouth pipetting is prohibited.

5 Apparatus and equipment

5.1 General

In accordance with good laboratory practice, all apparatus and equipment should be kept clean and in good working condition. Before use, equipment should be verified as fit for the intended purpose and its performance monitored during use, where appropriate.

Where necessary, equipment and monitoring devices should be calibrated to traceable national standards, and recalibration and any necessary intermediate checks performed, and procedures and results documented.

Equipment should be regularly checked and maintained to ensure safety and fitness for use. Equipment should be monitored according to the working conditions and the accuracy demanded for the results.

The frequency of calibration and verification checks of each item of equipment is, in most cases, not specified in this International Standard, since it shall be determined by each laboratory, depending on the type of equipment and on the laboratory's level of activity, and in accordance with the manufacturer's instructions. In a limited number of cases, a frequency has been specified since it was considered to be essential.

Apparatus and equipment shall be constructed and installed to facilitate operation and to allow for ease of maintenance, cleaning, decontamination and calibration.

Any measurement uncertainties given in this clause relate to the apparatus and equipment concerned and not to the whole method of analysis.

Throughout this clause, requirements for accuracy of measuring of measuring equipment are given. These are based on the practical tolerance required to demonstrate suitable control of equipment in routine use. The accuracy stated is related to the metrological uncertainty of the device (see ISO Guide 99).

For temperature control equipment, check the stability and homogeneity of the temperature before initial use and after any repair or modification which might have an effect on the temperature control.

5.2 Protective cabinets

5.2.1 Description

A protective cabinet is a work station with horizontal or vertical laminar airflow to remove dust and other particles, such as microbes, from the air.

The maximum tolerable number of particles per cubic metre with a size greater than or equal to 0,5 µm represents the dust-spreading class of a safety cabinet. For cabinets used in food microbiology, the number of particles shall not exceed 4 000 per cubic metre.

Cabinets for use in food microbiology laboratories are of four types.

- a) Class I safety cabinets are open-fronted exhaust-protective cabinets that are intended to protect the operator and the environment but will not protect the product from extraneous contamination. Potentially infected aerosols will be contained within the cabinet and trapped by impaction on the filter. The filtered air is normally discharged to the atmosphere; if this is not done, the air shall pass through two HEPA filters mounted in series. They are not recommended for work with risk category 3 pathogens because of the difficulties in maintaining and ensuring appropriate operator protection.
- b) Class II safety cabinets protect the product, the operator and the environment. They recirculate some filtered air, exhaust some to the atmosphere and take in replacement air through the working aperture, thereby providing operator protection. They are suitable for work with risk category 3 pathogens.
- c) Horizontal laminar outflow cabinets protect the work from contamination, but blow any aerosols generated into the operator's face. Therefore they are not suitable for handling inoculated cultures or preparation of tissue culture.
- d) Vertical laminar airflow cabinets protect the product by the use of vertical laminar flow of HEPA-filtered air. They also protect the operator by the use of internally recirculated air. They are particularly suitable for providing an aseptic environment for handling sterile products and for protecting the operator when handling powders.

Use protective cabinets for all work involving the handling of pathogens and contaminated powders, if required by national regulations.

The use of a gas burner or wire incinerator is not recommended in protective cabinets. If it is necessary, the gas burner should have a small flame so that the airflow is not disturbed. The use of disposable equipment (loops, pipettes, etc.) is a suitable alternative.

5.2.2 Use

Cabinets should be kept as free of equipment as possible.

Where practicable, place everything needed inside the cabinet before starting work to minimize the number of arm movements into and out of the working aperture. Position equipment and materials so as to minimize disturbance to the airflow at the working aperture.

Operators should be adequately trained in the correct use of cabinets to ensure their safety and the integrity of the product or culture.

5.2.3 Cleaning and disinfection

Clean and disinfect the working area after use with appropriate and non-corrosive disinfectant in accordance with the manufacturer's instructions. Regularly examine wire grids protecting prefilters and wipe clean with a disinfectant-soaked cloth.

For laminar flow cabinets, the filter face should be vacuum cleaned regularly, taking care not to damage the filter medium.

Safety cabinets should be fumigated before filter changing or servicing.

After cleaning of the cabinets, UV lamps may be used for disinfection. UV lamps should be regularly cleaned and replaced in accordance with the manufacturer's instructions.

5.2.4 Maintenance and inspection

Use protective cabinets that are appropriate for the intended application and environmental conditions in the laboratory.

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The efficiency of a protective cabinet shall be checked by a qualified person on receipt and thereafter at regular intervals as recommended by the manufacturer, as well as after any repair or modification.

Periodic verification of freedom from any microbial contamination should be carried out by a check of the working surface and walls of the cabinet.

A periodic verification of the number of airborne microorganisms present should be carried out during operation of the filters using the usual equipment. For example, expose several open Petri dishes containing a non-selective agar culture medium (e.g. PCA) in each cabinet for 30 min. Other methods may be used.

5.3 Balances and gravimetric diluters

5.3.1 Use and measurement uncertainty

Balances are mainly used for weighing the test portion of the sample to be examined and the components of the culture media and reagents. In addition, they may be used for carrying out measurements of dilution fluid volumes by mass.

Gravimetric diluters are electronic instruments consisting of a balance and programmable liquid dispenser and are used during the preparation of initial sample suspensions; they function by adding diluent to a subsample at a set ratio. The subsample is then weighed to the tolerance specified in the application, and the diluter set to dispense sufficient diluent for the ratio required (e.g. 9 to 1 for decimal dilutions).