



BSI Standards Publication

Microbiology of the food chain — General requirements and guidance for microbiological examinations

National foreword

This British Standard is the UK implementation of EN ISO 7218:2024. It is identical to ISO 7218:2024. It supersedes BS EN ISO 7218:2007+A1:2013, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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European foreword

This document (EN ISO 7218:2024) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 "Microbiology of the food chain" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2025, and conflicting national standards shall be withdrawn at the latest by January 2025.

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This fourth edition cancels and replaces the third edition (ISO 7218:2007), which has been technically revised. It also incorporates the Amendment ISO 7218:2007/Amd 1:2013.

The main changes are as follows:

- the calculations section has been simplified and two further calculators have been added;
- the equipment section has been reorganized into groups with similar purposes and requirements;
- cross-references have been added to other general microbiology standards such as those for media, validation and verification, and uncertainty to reduce repetition;
- information on laboratory quality control and characterization of control microorganisms has been added.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

When conducting microbiological examinations, it is especially important that:

- only those microorganisms present in the samples are detected and/or enumerated;
- these microorganisms do not contaminate the environment.

To achieve this, good laboratory practices are essential, including personal hygiene and aseptic working techniques which exclude extraneous contamination as far as possible.

Only limited information on the precautions to be taken during microbiological examinations is given in this document, so a thorough knowledge of the microbiological techniques and microorganisms involved is essential. It is important that examinations are conducted safely, correctly and as carefully as possible, including monitoring and recording aspects that can affect results, calculating numbers of microorganisms and assessing the uncertainty of test results.

The most common risks and their control in the microbiological laboratory are given in this document. However, work processes in each laboratory can differ and appropriate risk analysis should be considered to ensure good laboratory practices. Periodic evaluation and control of critical points not only maintains safe and hygienic practices but can also improve reliability of test results.

The purpose of this document is to help to ensure the validity of microbiology examinations in the food chain. In particular, to ensure that general techniques for conducting examinations are the same in all laboratories, to achieve consistent results in different laboratories and to contribute to safety of laboratory personnel by preventing risks of infection.

This document includes the main measures necessary for conducting the wide range of microbiological examinations. Additional information is available from the literature listed in the Bibliography (see References [\[43\]](#) to [\[47\]](#)).

In this document, the following verbal forms are used:

- “shall” indicates a requirement;
- “should” indicates a recommendation;
- “may” indicates a permission;
- “can” indicates a possibility or a capability.

In addition, the imperative mood is used to give instructions or where actions are required.

Microbiology of the food chain — General requirements and guidance for microbiological examinations

1 Scope

This document specifies general requirements and gives guidance on microbiological examinations.

It is applicable to:

- the implementation of specific horizontal or vertical International Standards developed by ISO/TC 34/SC 9 or ISO/TC 34/SC 5 for detection or enumeration of microorganisms, named hereafter “specific standards”;
- good laboratory practices for microbiology laboratories testing samples from the food chain;
- guidance for microbiological laboratories testing samples from the food chain on the technical requirements for conforming to ISO/IEC 17025.

The requirements of this general standard supersede corresponding ones in existing specific standards.

Additional instructions for examinations using the polymerase chain reaction (PCR) are specified in ISO 22174.

This document is applicable to examinations for bacteria, yeasts and moulds and can be used, if supplemented with specific guidance, for parasites and viruses. It does not apply to examinations for toxins or other metabolites (e.g. amines) from microorganisms.

This document is applicable to microbiology of the food chain, from primary production stage to food and animal feed products, including the premises where the food or feed production and handling takes place. It is also applicable to the microbiological examination of water where water is used in food production or is regarded as a food in national legislation.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 food chain

sequence of the stages in the production, processing, distribution, storage and handling of a *food* (3.2) and its ingredients, from primary production to consumption

Note 1 to entry: The food chain includes the environment of primary production, food and feed production, and handling.

Note 2 to entry: The food chain also includes the packaging materials intended to come into contact with food, *feed* (3.3) or raw materials.

[SOURCE: ISO 22000:2018, 3.20, modified — Notes to entry replaced.]

3.2

food

products intended for human consumption

substance (ingredient), whether processed, semi-processed or raw, which is intended for human consumption, and includes beverages and any substance which has been used in the manufacture, preparation or treatment of “food” but does not include cosmetics or tobacco or substances (ingredients) used only as drugs

[SOURCE: CAC/GL 81-2013, 6, modified — Admitted term added. “(ingredient)” added, “beverages” replaced “drink” and “chewing-gum” deleted in the definition.]

3.3

feed

products for feeding animals

single or multiple product(s), whether processed, semi-processed or raw, which is (are) intended to be fed to animals

Note 1 to entry: These products are intended for food-producing animals and non-food producing animals such as pets.

[SOURCE: ISO 22000:2018, 3.16, modified — Admitted term added. “food-producing” deleted in the definition. Note 1 to entry replaced.]

3.4

food or feed production and handling

any operation in the processing (e.g. preparation, cooking, packaging), storage, transport, distribution and service of *food* (3.2) or *feed* (3.3)

[SOURCE: ISO/TS 22002-2:2013, 3.9, modified — “or feed production and” added in the term. “processing (e.g. preparation, cooking, packaging)” replaced “preparation, processing, cooking, packaging”, and “or feed” added in the definition.]

3.5

primary production stage

stage of the *food chain* (3.1) at which the production, rearing or growing of primary products, including harvesting, milking and farmed animal production before slaughter, takes place

Note 1 to entry: It also includes hunting, fishing and the harvesting of wild products.

3.6

horizontal method

method for microbiological examination that is broadly applicable to samples within the *food chain* (3.1), excluding any documented limitations

3.7

vertical method

sectorial method

method for microbiological examination that is specifically applicable to samples (e.g. from *primary production stage* (3.5)), a product or a group of products (e.g. milk and milk products, meat and meat products, fish and fishery products, *feed* (3.3))

3.8

specific standard

standardized reference method for the examination (e.g. detection, enumeration, confirmation or identification) of a specific microorganism or group of microorganisms

Note 1 to entry: A specific standard can describe a *horizontal method* (3.6) or a *vertical method* (3.7).

3.9**general standard**

supporting document describing general guidance and requirements necessary for application of *specific standards* ([3.8](#))

3.10**strain**

progeny or subculture of a single isolated colony in pure culture that displays the phenotypic characteristics or possesses the molecular attributes/properties as identified with being associated within the classification of the species of that microorganism

3.11**target strain**

strain ([3.10](#)), defined according to the scope of the method

[SOURCE: ISO 16140-1:2016, 2.74, modified — “method” replaced “reference method that is expected to be detected or enumerated by the alternative method”.]

3.12**target organism**

microorganism that is the designated analyte for a microbiological examination

[SOURCE: ISO 22117:2019, 3.1, modified — “microbiological examination” replaced “proficiency testing sample”.]

3.13**laboratory strain**

microorganism that is defined to at least the genus and species level, and characterized biochemically, and/or serologically and/or with molecular testing, and preferably originating from the *food chain* ([3.1](#))

3.14**reference strain**

microorganism obtained directly from an official culture collection or reference laboratory and defined to at least the genus and species level, catalogued and described according to its characteristics and preferably originating from *food* ([3.2](#)), food production areas, *primary production stages* ([3.5](#)), animals or water, as applicable

[SOURCE: ISO 22117:2019, 3.4]

3.15**natural background microorganism**

microorganism that is naturally present or can be introduced to compete with or mimic the target microorganism

[SOURCE: ISO 22117:2019, 3.2, modified — “natural background microorganism” replaced “background flora” as the term. “included in a proficiency testing sample” deleted in the definition.]

3.16**matrix**

all the components of the sample

[SOURCE: ISO 16140-1:2016, 2.38, modified — “(product)” deleted in the term.]

3.17**biological resource centre****BRC**

service provider or repository of the living cells, genomes of organisms and information relating to heredity and the functions of biological systems

Note 1 to entry: BRCs contain collections of culturable organisms (e.g. microorganisms), replicable parts of these (e.g. genomes, plasmids, viruses, cDNAs), viable but not yet culturable organisms, cells and tissues, as well as databases containing molecular, physiological and structural information relevant to these collections and related informatics

[SOURCE: OECD, 2007^[44]]

3.18

microbial (sub)type

group of closely related microorganisms (within a species) distinguished by their shared specific characteristics as determined by, for example, serological testing (serotype) or molecular testing (genotype)

[SOURCE: ISO 16140-6:2019, 3.5]

3.19

challenge testing

study of the growth or inactivation of microorganism(s) artificially inoculated in *food* (3.2)

[SOURCE: ISO 20976-1:2019, 3.5]

3.20

accuracy

accuracy of measurement

measurement accuracy

closeness of agreement between a measured quantity value and a true quantity value of a measurand

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — “accuracy” replaced “measurement accuracy” as the preferred term. Notes to entry deleted.]

3.21

resolution

smallest change in a quantity being measured that causes a perceptible change in the corresponding indication

[SOURCE: ISO/IEC Guide 99:2007, 4.14, modified — Notes to entry deleted.]

3.22

uncertainty

uncertainty of measurement

measurement uncertainty

non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used

[SOURCE: ISO/IEC Guide 99:2007, 2.26, modified — “uncertainty” replaced “measurement uncertainty” as the preferred term. Notes to entry deleted.]

3.23

calibration

set of operations that establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values of a reference standard

3.24

verification

provision of objective evidence that a given item fulfils specified requirements

[SOURCE: ISO/IEC Guide 99:2007, 2.44, modified — Examples and notes to entry deleted.]

3.25

contamination

undesirable presence of microorganisms in the environment, on surfaces (including human skin) or in (or on) laboratory samples

3.26**cross-contamination**

unintentional transfer of microorganisms or their constituents, such as DNA, or metabolites from one area or article to another

Note 1 to entry: This can include, but is not limited to, transfer from:

- the laboratory environment to laboratory samples;
- laboratory personnel to laboratory samples;
- one laboratory sample to other laboratory samples;
- one laboratory area to another;
- the laboratory area to adjacent production areas.

3.27**process control**

internal quality control (IQC) system that is used to confirm acceptable conditions of the entire process have been achieved

Note 1 to entry: This includes the use of a target organism that is tested in accordance with the method from start to finish with each batch of samples, as well as positive, negative and blank samples that are used to confirm the acceptability of media/reagents and consumable supplies, and of the incubation environment (temperature/equipment), and to confirm the competence of personnel.

4 Premises**4.1 General**

This clause gives general requirements, including the principles of design and organization, for the layout of a microbiological laboratory testing samples from the food chain.

NOTE Guidelines referring to PCR in this document apply equally to other nucleic acid amplification methods. Further specifications for laboratories using PCR are given in ISO 22174.

4.2 Biosafety considerations

The laboratory design shall comply with the relevant biosafety requirements for the type of microorganism and potential for causing human illness.

The current four biosafety levels and laboratory design requirements for each are fully described in the WHO Laboratory Biosafety Manual^[47] or the OIE Terrestrial Manual^[45].

NOTE Regional or national regulations can differ in definitions of biosafety levels or risks from microorganisms.

4.3 Laboratory design

The guidelines for laboratory layout described in this clause cover examinations for the detection and enumeration of microorganisms belonging to Biosafety Levels 1 and 2, as only these are routinely handled in food microbiology laboratories. Further details can be found in the WHO Laboratory Biosafety Manual^[47].

NOTE National and regional legislation can require different and/or additional safety measures.

4.4 Laboratory areas**4.4.1 General**

The laboratory comprises separate areas associated with samples and testing (see [4.4.2](#)) and other general areas (see [4.4.3](#)).

4.4.2 Areas associated with samples and testing

It is good practice to allocate separate rooms or clearly designated areas, with dedicated equipment where necessary, for the following activities:

- receipt and storage of laboratory samples before and after testing;
- preparation of laboratory samples and test portions (separate powders and those likely or known to contain high numbers of microorganisms to reduce the risk of cross-contamination, and also commercially sterile foodstuffs to prevent contamination by other samples);
- examination of test portions from the initial suspension, including all dilution, plating, incubation and counting steps of enumeration tests and detection tests up to isolation of presumptive pathogens;
- manipulation of presumptive pathogens, including those from proficiency testing schemes or laboratory spiked samples deliberately contaminated with pathogens;
- handling and storage of reference cultures and other laboratory strains;
- preparation and testing of samples by PCR nucleic acid amplification methods (see ISO 22174 for full details);
- preparation and sterilization of culture media, reagents and necessary equipment;
- storage of culture media and reagents;
- decontamination and disposal of biohazard waste;
- cleaning of glassware and other equipment;
- storage of hazardous chemicals, preferably in specially designated cabinets, cupboards, rooms or buildings.

The areas can be interconnected provided hygiene recommendations (see [5.4](#)) are met and, if space is limited, activities can be separated by time.

4.4.3 General areas

Separate areas are set aside for the following:

- entrances, corridors, stairways and lifts;
- cloakrooms, toilets and staff rooms;
- administration (e.g. secretarial, offices, document archives);
- storage of general laboratory supplies.

4.5 Layout and fittings of the premises

4.5.1 Objectives

The primary objective is to ensure that the environment in which microbiological examinations are carried out is safe and does not adversely affect the validity of test results.

Arrange the premises to avoid risk of cross-contamination, for example:

- construct the laboratory according to the pattern and flow of work (the “no way back” principle);
- carry out procedures in a sequential manner using appropriate precautions to ensure test and sample integrity (e.g. use of sealed containers);
- separate activities by space or time (see [4.4.2](#)).

Avoid conditions such as extremes of temperature outside the accepted range (18 °C to 27 °C), draughts, dust, humidity, steam, noise, vibration, etc., all of which can affect the validity of microbiological test results.

Locate air flows into and out of the laboratory to minimize the risk of contaminating samples under test or any adjacent food processing facilities.

Sufficient space should be available so work areas can be kept clean and tidy. The space required should be proportional to the number of samples and tests handled, the number of staff and the overall internal organization of the laboratory.

4.5.2 Fittings

The test premises should be constructed and equipped as follows to reduce the risk of contamination by dust and therefore by microorganisms:

- The walls, ceilings and floors should be smooth, easy to clean, and resistant to detergents and disinfectants used in laboratories.
- Floors should be slip-resistant.
- Overhead pipes should not cross the premises unless they are sealed or enclosed. Any other overhead structures should be covered or readily accessible for regular cleaning.
- Windows and doors should be closed when testing to minimize draughts and potential airborne contamination. They should be designed to avoid formation of dust traps and to facilitate cleaning.
- The ambient temperature (18 °C to 27 °C) and air quality (microorganism content, dust spreading rate, etc.) should be compatible with testing. Air-conditioning systems should supply filtered air and be regularly cleaned and serviced or maintained.
- Adequate measures, such as special workstations, should be taken to minimize exposure to and cross-contamination from dust when handling dehydrated culture media and dusty or powdered samples.
- When tests are to be conducted in a low-contamination atmosphere, the room should be equipped with a clean laminar airflow and/or safety cabinet.
- If necessary, the laboratory environment should be protected from the harmful effects of solar radiation by the use of shutters or suitably treated window glass. Internally installed blinds are not suitable as they can be difficult to clean and can become a source of dust.

4.5.3 Other arrangements for laboratory premises

Consider general arrangements such as the following:

- availability of water supply, of suitable quality for the intended use;
- availability of electricity;
- availability of gas (piped or bottled);
- sufficient lighting for tasks undertaken in each area of the laboratory;
- laboratory bench tops and furniture manufactured in smooth, impermeable material that is easily cleaned and disinfected;
- laboratory furniture that is movable or designed to allow access for cleaning floors and equipment;
- no furniture, documents or other items than those strictly necessary for testing activities to be kept in the testing areas;
- separate storage for test records and other necessary documents away from samples and test materials;

- provision of handwashing facilities (or special solutions or gels containing alcohol) in each testing room, preferably near the door;
- provision of designated handwashing facilities, preferably with hands-free operation, at the entrance/exit to pathogen testing areas;
- provision for hanging used laboratory clothing when exiting laboratory areas;
- availability of an autoclave for destruction of contaminated waste materials and culture media, unless another appropriate system such as removal and incineration is in place (see [6.2.2](#));
- provision of safety systems for fire and electrical emergencies;
- provision of safety showers and eyewash facilities, where necessary;
- provision of first aid facilities.

4.5.4 Cleaning and disinfection

Consider methods, frequency and controls for cleaning and disinfection according to risk and check the following points:

- The floors, walls, ceilings, laboratory bench tops, furniture and junctions between these should be regularly maintained and repaired to avoid cracks which can harbour contamination.
- Carry out regular cleaning and disinfection to keep the premises in a condition suitable for conducting tests. Contaminated or potentially contaminated surfaces should be decontaminated using a disinfectant known to be bactericidal and fungicidal (see [Annex A](#)).
- Use dedicated cleaning equipment for all microbiology laboratories, with separate equipment for high-risk areas such as pathogen laboratories.

NOTE Rooms and equipment can be decontaminated by fumigation with formaldehyde vapour if appropriate.

- Maintain the ventilation systems and filters and change the filters when necessary.
- Monitor the microbiological contamination level of laboratory work surfaces, staff contact surfaces and the air regularly (at a frequency depending on previous monitoring results and risk to validity of test results).
- Surface contamination may be estimated by applying a contact plate, containing suitable neutralizing agents against sanitizers (e.g. lecithin, sodium thiosulfate) or by swabbing (see ISO 18593).
- Contamination level of laboratory air may be monitored by exposing (e.g. for 15 min to 30 min) an open Petri dish containing a non-selective agar medium, such as plate count agar, or a selective agar appropriate for the target microorganisms sought (e.g. moulds). Active air sampling equipment may also be used.

5 Personnel

5.1 General

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

5.2 Competence

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

Competence can be assessed by internal quality control (IQC) or proficiency testing (PT) (see [Clause 16](#)).

NOTE A method of investigating the cause of poor performance (pipetting, poor homogeneity of the initial suspension, dilution preparation, colony counting, etc.) for enumeration by counting colonies is given in ISO 14461-1.

5.3 Verification of ongoing staff competence

Verification of ongoing staff competence should be carried out at a documented frequency against objective parameters. This includes participation in regular IQC, PT (see ISO 22117), use of (certified) reference materials or by self-assessment tests for enumeration of microorganisms as described in ISO 14461-2.

Any deviations from expected results or unsatisfactory results should be investigated and personnel retrained.

5.4 Hygiene

Take the following personal hygiene precautions to avoid contaminating samples, culture media and/or reagents, and to avoid the risk of infecting laboratory personnel or those in adjacent areas:

- Wear properly fastened laboratory clothing that is clean and in good condition. Use clothing made from fabric which limits the risks of flammability where open flames are in use. Consider dedicated, fully fastening laboratory coats, preferably with long sleeves and elasticated cuffs, and laboratory shoes for designated pathogen handling areas. Do not wear laboratory clothing outside the laboratory work areas (and changing rooms).
- Store outside clothing and personal possessions, such as phones, headphones and jewellery, before entering the laboratory as these can be a source of contamination.
- Wear protection for hair and beards, if required and necessary.
- Keep fingernails clean and preferably short.
- Wash hands thoroughly under running water, preferably delivered hands free, before and after microbiological examinations and immediately after visiting the toilets. Use liquid, foam or powdered soap, preferably delivered by an automatic dispenser. Dry hands with single-use paper or cloth towels and discard these into an adjacent sealed bin. Hot-air hand dryers are not suitable for use in testing areas of microbiology laboratories but can be used in other areas. Use of a hand sanitiser after drying is recommended. These precautions are applicable both to laboratory staff and visitors.
- When working with exposed samples, cultures, culture media, reagents, etc. and when manipulating tests, avoid speaking, coughing, etc.
- Staff with skin infections or other illnesses likely to spread microorganisms which can contaminate samples and invalidate test results shall take the necessary precautions, such as wearing disposable gloves or covering affected areas.
- Do not eat or drink in the laboratory and do not put food for personal consumption in laboratory refrigerators, freezers or microwaves.
- Mouth pipetting is strictly prohibited.

6 Equipment and consumables

6.1 General

In accordance with good laboratory practice, keep all apparatus and equipment clean and in good working condition. Equipment shall be constructed and installed to facilitate operation and to allow for ease of maintenance, cleaning, decontamination and calibration. Dedicated equipment, such as trolleys, pipettors, etc., should be allocated to pathogen testing areas.

Check that all equipment is suitable for the intended purpose and conforms to specified requirements before use. Ensure equipment used for measurements is capable of achieving the accuracy and/or uncertainty required to produce a valid result.

Calibrate critical equipment that directly affects the validity of test results and/or associated uncertainty in a metrologically traceable manner. For non-critical measurement equipment, verification is sufficient.

NOTE The decision chart in ISO 20836:2021, Figure 1, includes guidance on whether a metrological traceable calibration is necessary or not.

To maintain confidence in the status of the calibration, establish a documented calibration schedule to minimize invalid test results.

Maintain equipment regularly to ensure safety and suitability for the intended use and perform intermediate checks when necessary to give confidence in the ongoing performance of the equipment. The frequency of calibration and verification checks of each item of equipment is, in most cases, not specified in this document, since it is determined by each individual laboratory using a risk-based approach. The frequency depends on many factors, including the type of equipment and the level of laboratory activity, and is in accordance with the manufacturer's instructions. In a limited number of cases, a frequency has been specified because it is considered essential.

Throughout this clause, requirements for accuracy and/or uncertainty of measuring equipment are given. These are based on the practical tolerance required to demonstrate suitable control of equipment in routine use. The tolerance stated is related to the metrological uncertainty of the device (see ISO/IEC Guide 99). Any performance criteria given in this clause apply to the equipment concerned and not to the whole method of analysis.

6.2 Sterilization and other heating equipment

6.2.1 General

This equipment includes autoclaves (see [6.2.2](#)), culture media preparators (see [6.2.3](#)), steamers, including boiling-water baths (see [6.2.4](#)), sterilizing ovens (see [6.2.5](#)), microwave ovens (see [6.2.6](#)), hotplates, induction cookers and heating mantles (see [6.2.7](#)), and gas (Bunsen) burners or wire incinerators (see [6.2.8](#)).

6.2.2 Autoclave

6.2.2.1 Description

An autoclave enables a saturated steam temperature under pressure to be attained in the chamber.

The autoclave should be equipped with:

- at least one safety valve and a safety lock;
- a drain cock;
- a regulation device allowing the temperature in the chamber to be maintained to within ± 3 °C of the target temperature (to take into account the uncertainty associated with the monitoring thermocouple);
- a temperature probe or a recording thermocouple;
- a timer and temperature recorder.

6.2.2.2 Use

With steam sterilization, all air is expelled before the pressure can build up. If the autoclave is not fitted with an automatic evacuation device, it is necessary to expel the air until a continuous jet of steam is emitted. Load the autoclave to ensure a free flow of steam to replace the air.

For the sterilization of culture media, the saturated steam in the chamber shall be at a temperature of $121\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, or other temperatures specified by the manufacturers in the preparation instructions or in the test method.

For the destruction of cultured microorganisms and for decontaminating equipment and/or culture media after use, the saturated steam in the chamber shall be at a temperature of at least $121\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$.

Do not autoclave unsuitable materials and media, especially those that can pose a safety risk, e.g. due to decomposition or build-up or corrosive or volatile compounds.

To avoid cross-contamination, use separate sterilization cycles to sterilize clean equipment and/or culture media and to decontaminate used equipment and/or used culture media. It is preferable to use separate autoclaves, which can be located in separate areas, for these two processes.

After autoclaving, allow all materials and equipment to cool within the autoclave before removal.

For safety reasons, do not remove the contents until the chamber temperature has dropped below $80\text{ }^{\circ}\text{C}$.

Sterilization cycles for large loads (such as those used by commercial manufacturers) may be evaluated using F_0 values, taking into account the heat treatment during heating and cooling. The heat treatment should be defined for each particular load to be treated to ensure suitable conditions irrespective of the container type or placement in the autoclave (see ISO 17665).

6.2.2.3 Maintenance

Clean the chamber, and drain the filter and door seals regularly. Check the door seals for integrity. Carry out draining and descaling, if necessary, at regular intervals. Follow the manufacturer's instructions.

6.2.2.4 Calibration and verification

Keep the autoclave in good operating condition and schedule regular inspections by qualified personnel in accordance with the manufacturer's instructions.

Keep the monitoring instruments in good working order and verify them by calibration and regular checks.

Initial commissioning should include performance studies for each operating cycle and each load configuration used in practice. This process should be repeated after significant repair or modification. Sufficient temperature sensors should be positioned within the load to demonstrate adequate heat penetration at all locations. Validation and revalidation should consider the suitability of heat-up and cool-down times as well as the sterilization temperature.

For each load, as a minimum, a process indicator (e.g. steam chemical integrator or biological indicator) should be included at the centre of the load to show that the target temperature was reached where a traceable record of process efficiency is not available.

6.2.3 Culture media preparator

6.2.3.1 Description

A culture media preparator is principally designed for sterilization of large volumes of media ($>1\text{ l}$). It consists of a heating vessel, water jacket and continuous stirring device, and is also fitted with a temperature gauge, pressure gauge, timer and safety valve.

A temperature sensor is positioned in contact with the culture media to demonstrate adequate heat treatment according to the temperature tolerances established for each medium.

In addition, the unit should have a safety lock to prevent opening until a temperature of $< 80\text{ }^{\circ}\text{C}$ is reached.

6.2.3.2 Use

Follow the manufacturer's instructions at all times.

The entire production process takes place within the preparator. After the addition of all the ingredients, they are dissolved by stirring and heating. This is followed by sterilization.

6.2.3.3 Maintenance

Wash the preparator and rinse thoroughly with purified water (see 6.7.1) between each culture media batch.

NOTE Rinsing with a volume fraction of 70 % ethanol can be useful to remove staining from strongly coloured indicators present in certain culture media, followed by thorough rinsing with purified water.

Maintain the culture media preparator in good operating condition and schedule regular inspections by qualified personnel in accordance with the manufacturer's instructions.

6.2.3.4 Calibration and verification

Keep the monitoring instruments in good working order and verify them by calibration and regular checks.

Initial commissioning should include performance studies for each operating cycle and each load configuration used in practice. Repeat this process after significant repair or modification.

For each load, keep a traceable record of process efficiency or, as a minimum, place a temperature sensor in contact with the culture media produced to verify the heating process.

6.2.4 Steamers, including boiling-water baths

6.2.4.1 Description

Steamers and boiling-water baths consist of a heating element surrounded by water in a vessel with a close-fitting lid. In a steamer, this creates steam at atmospheric pressure. In a boiling-water bath, this heats the water to a temperature at or close to the boiling point, with or without the production of steam.

6.2.4.2 Use

The main uses are as follows:

- melting solid media;
- preparation of heat-labile culture media;
- reduction of contamination of small items of equipment between use.

A safe and adequate level of water shall be present in the vessel to ensure that the heating elements are covered at all times.

An autoclave with a free-steaming facility that is without pressure may also be used.

6.2.4.3 Maintenance

Keep steamers and boiling-water baths clean.

If necessary, regular descaling should be performed at a frequency dependent on local water hardness.

6.2.5 Sterilizing oven

6.2.5.1 Description

A sterilizing oven is a chamber capable of maintaining a temperature of 160 °C to 180 °C for the destruction of microorganisms by dry heat.

6.2.5.2 Use

Only robust equipment such as glass and metalware shall be sterilized in the sterilizing oven. Do not use it for plastic and rubber items or for sterilizing unsuitable materials (see [6.2.2.2](#)).

Clean all glassware and metal items before sterilization in the oven.

If volumetric glassware is sterilized in the sterilizing oven, verify the accuracy of marked volumes regularly.

The temperature shall be uniform throughout the chamber. The oven shall be equipped with a thermostat and a thermometer or temperature-recording device of suitable resolution.

It should be equipped with a duration indicator, programmer or timer.

Once the operating temperature is reached, the sterilizing procedure shall last for at least 1 h at $170\text{ °C} \pm 10\text{ °C}$.

After sterilization, allow glassware to cool in the oven before removal to prevent cracking.

6.2.5.3 Maintenance

Clean internal surfaces when required.

6.2.5.4 Calibration and verification

Check the stability and homogeneity of the temperature throughout the oven before initial use and after any repair or modification which can have an effect on temperature control.

The oven shall be fitted with a calibrated temperature-monitoring device or thermocouple of suitable accuracy which is independent of the automatic temperature-regulation system. The monitoring device shall have a resolution of 1 °C or better at the oven temperature used.

For each load, as a minimum, a process indicator (e.g. steam chemical integrator or biological indicator) should be included at the centre of the load to show that the target temperature was reached where a traceable record of process efficiency is not available. Monitor the temperature of the oven and record during each use.

6.2.6 Microwave oven

6.2.6.1 Description

A microwave oven is a device that allows heating of items by microwave energy at atmospheric pressure.

The oven shall be capable of heating liquids and culture media in a controlled manner via a microwave emission cycle. The distribution of microwaves shall be homogeneous to avoid zones of overheating. Ovens fitted with a turntable or a stirrer give better heat distribution.

Heating for longer periods at lower power ratings can give better heat distribution.

6.2.6.2 Use

It is advisable to use the equipment to heat liquids or melt agar media only.

Do not heat culture media containing heat-sensitive components in a microwave unless it has been verified that this method of heating has no effect on medium performance.

Do not use metal equipment, including metal closures. Loosen bottle caps or stoppers before heating.

When melting solid media, the use of a low power setting (e.g. defrost cycle) and placing the bottle in a water heat sink (e.g. 50 ml to 100 ml of water in a microwaveable beaker) are recommended to aid control of the heating process.

WARNING — Handle heated items with care. Contents can become super-heated and boil over or bottles can explode.

A standing time of at least 5 min is recommended after the heating process before removal from the microwave oven.

6.2.6.3 Maintenance

Clean the oven immediately when any spillage occurs, as well as at regular intervals dependent on usage.

Inspect the oven door seals and check for any other damage which can lead to radiation leakage at regular intervals according to the manufacturer's instructions. Checks with a radiation meter by competent personnel or servicing agent are recommended.

6.2.6.4 Verification

Suitable heating times and power settings shall be established at initial commissioning for the different volumes of liquids and culture media routinely heated, to ensure optimum performance and avoid overheating of sensitive products.

6.2.7 Hotplate, induction cooker and heating mantle

6.2.7.1 Description

Hotplates, induction cookers and heating mantles are thermostatically controlled heating devices, some of which incorporate magnetic stirring systems.

6.2.7.2 Use

Hotplates, induction cookers and heating mantles equipped with magnetic stirring systems are used for heating relatively large volumes of liquid such as during preparation of culture media.

Do not use hotplates, induction cookers and heating mantles without stirring systems for preparation of culture media.

6.2.7.3 Maintenance

Clean up any spillages as soon as the unit has cooled.

6.2.8 Gas burner or wire incinerator

6.2.8.1 Description

Gas (Bunsen) burners produce a narrow open flame from either mains or bottled gas. Varying the amount of air mixed with the gas controls the degree of heat produced.

Wire incinerators (electronic) include a narrow ceramic chamber designed to contain any aerosols generated when decontaminating at high temperatures.

6.2.8.2 Use

Gas burners and wire incinerators are mainly used for sterilizing metal loops and straight wires by bringing them to red heat and for flame-sterilizing other small durable items of equipment.

Wire incinerators are preferred when handling pathogenic bacteria to prevent splatter and avoid risk of cross-contamination.

The use of gas burners should be avoided in protective cabinets for safety reasons and because they can interfere unacceptably with the laminar airflow. Sterile disposable equipment is recommended as an alternative.

Gas burners also produce much heat and air turbulence in the laboratory and aseptic techniques can be achieved by using disposable equipment instead.

6.2.8.3 Maintenance

Clean and disinfect burners and covers on wire incinerators regularly, particularly if any microbial culture has been spilled on the devices.

6.3 Temperature controlled equipment and monitoring devices

6.3.1 General

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

6.3.2 Incubator

6.3.2.1 Description

An incubator consists of an insulated chamber which enables the temperature to be kept stable and uniformly distributed to within the maximum permissible temperature tolerance specified in the test method. Heat distribution may be by convection or fan-assisted forced air.

Refrigerated incubators which cycle between incubation and refrigeration when the incubation period has been completed are also available.

6.3.2.2 Use

Incubators are primarily used to incubate inoculated culture media at a constant temperature. However, they can also be used for other purposes. These include melting laboratory samples (e.g. chocolate), prewarming pre-enrichment broths before inoculation (see [12.1](#)) or tempering sterile molten culture media before use, if the time taken for the culture media to reach the correct temperature has been evaluated as appropriate.

All incubators shall be equipped with a regulation system that allows the temperature or other parameters to be kept even and stable over their entire working volume. If the correct temperature is not achieved throughout, mark the areas which cannot be used for incubation.

If the ambient temperature is close to or higher than that required for incubation, it is necessary to use an incubator fitted with a cooling system.

Protect incubators from direct sunlight to minimize heat gain.

When loading incubators, ensure free air circulation (see [11.2.5](#)).

If possible, incubators should not be completely filled in a single operation as this extends the time for the contents to reach temperature equilibrium in all types of incubator.

Avoid opening incubator doors frequently and leaving them open for long periods.

6.3.2.3 Maintenance

Clean the inner and outer walls of the incubator regularly. If applicable, remove dust from the ventilation system.

Ensure that the cleaning and disinfection techniques will not affect test results, particularly by leaving inhibitory residues.

6.3.2.4 Verification

Use a temperature-monitoring device of appropriate resolution. The instrument display may be used for checks during use if it has been shown to be accurate.

Check the temperature stability and homogeneity of the temperature distribution at the working temperature throughout the usable volume of the incubator with a number of temperature-monitoring devices of known accuracy and uncertainty and appropriate temperature range. More monitoring devices are required for large incubators and incubation rooms, depending on the chamber volumes.

Use the information from this temperature mapping/profiling to define the acceptable operating range of the incubator and the optimum position of the monitoring device or recording thermocouple used to monitor working temperatures.

For example, to achieve a target temperature of $37\text{ °C} \pm 1\text{ °C}$ when the profiling data/temperature mapping shows a range of $36,8\text{ °C}$ to $37,2\text{ °C}$ across the incubator, take the observed temperature variation of $\pm 0,2\text{ °C}$ into account to define the operating limits of the incubator. Amend the lower operating range to $36,2\text{ °C}$ and the upper limit to $37,8\text{ °C}$ to ensure all areas of the incubator achieve the target temperature.

When re-defining the operating range, the uncertainty of the monitoring device should also be taken into account, although this is usually minimal.

If the monitoring device is fixed elsewhere than at the centre of the temperature range, adjust the incubator temperature so that the device reads the target temperature and amend the permitted operating range accordingly.

Temperature mapping is necessary before initial use and at a defined frequency to show any deviation in use. It is also necessary after each significant repair or modification.

In addition, temperature profiling of (larger) enrichment broths shall be checked before implementing a method or changing the test portion size, to ensure the time taken to reach target incubation temperature is considered with the incubation times stated in the specific standard (see [12.2](#)). Use a separate incubator for pre-warming broths if necessary, so that the recovery time cannot affect any other test results.

Check and record the incubator temperature to confirm it is stable and within the maximum permissible tolerance during the incubation period. Alternatively, dataloggers or electronic monitoring systems may be used.

6.3.3 Thermostatically controlled bath

6.3.3.1 Description

A thermostatically controlled bath, filled with a liquid (water, ethylene glycol, etc.), with or without a fitted lid or other device to limit evaporation, is required to maintain a specified temperature.

Temperature control is often more precise than an air incubator, enabling a stability of $\pm 0,5\text{ °C}$ or better to be achieved. The working temperatures and required maximum permissible tolerance are stipulated in each individual application or specific standard.

A cooling system is necessary to maintain a temperature near or below ambient temperature.

6.3.3.2 Use

The main uses are as follows:

- incubation of inoculated culture media at a constant temperature;
- maintenance of sterile molten culture media during media preparation;
- tempering of sterile molten culture media for use in specific standards;
- preparation of initial sample suspensions or solutions at a controlled temperature;

- heat treatment of initial sample suspensions at a controlled temperature (e.g. pasteurization or for spore counts).

Where precise temperature control is required, the bath shall be equipped with a circulating-water pump and an automatic temperature-regulation system (including a cut-out when it is running dry). Any agitation of the liquid shall not cause droplet dispersal.

Lidded baths are preferable for precise or high-temperature usage. Sloping lids that allow condensate to drain should be used.

For incubation of inoculated culture media, maintain the liquid level so that the top of the test medium is at least 2 cm below the liquid level in the bath throughout incubation.

Other containers should be placed within baths such that the level of their contents is below that of the liquid.

Maintain the depth of immersion to prevent entry of water through the closure.

Devices made of non-corroding materials to maintain stability of the containers can be required (e.g. racks or weight rings).

Dry all containers after removal from the bath and before further use.

6.3.3.3 Maintenance

Baths should be filled with liquid as recommended by the manufacturer. For incubation of cultures, distilled or deionized water should be used and a suitable microbial growth inhibitor can be added.

Check the level of the liquid regularly to ensure the correct functioning of the bath and satisfactory immersion of items in the bath. The liquid level shall always cover the heating elements.

Baths should be emptied, cleaned, sanitized and refilled regularly and at a frequency depending on usage, or after a spillage occurs.

6.3.3.4 Verification

Check the stability and homogeneity of the temperature throughout the bath before initial use and after any repair or modification having an effect on temperature control. For usage where temperature is critical (e.g. tempering agar for pour plates or incubation of cultures), use the information to define the acceptable operating range (see [6.3.2.4](#)).

Use a temperature-monitoring device (see [6.3.7](#)) of suitable resolution (see [6.3.2.4](#)) independent of the automatic temperature-regulation system.

The digital display can also be used, provided that the accuracy (uncertainty) and resolution are verified.

Monitor and record the temperature of the bath during each use and at least daily for periods of extended incubation.

6.3.4 Heating blocks

6.3.4.1 Description

Heating blocks are solid metal or ceramic blocks with wells, which deliver consistent heat to small vessels, such as microtubes or cuvettes, and their contents. The operating temperature can be precisely set across the block.

6.3.4.2 Use

Heating blocks can be used to melt or boil contents, to control and optimize enzyme reactions, and to incubate or inactivate microorganisms. Monitor and record the temperature at each use.

6.3.4.3 Maintenance

Clean the block and wells regularly and after any spillage.

6.3.4.4 Verification

Verify the temperature of the heating block across the working area in a selected number of wells and use the information to modify the acceptable operating range if necessary (see [6.3.2.4](#)).

6.3.5 Refrigerators and cold-storage rooms

6.3.5.1 Description

These are chambers which allow maintenance of cold storage.

For general laboratory purposes, these maintain a temperature of $5\text{ °C} \pm 3\text{ °C}$.

For storage of samples from the food chain before testing, these should maintain a temperature of $5\text{ °C} \pm 3\text{ °C}$ unless otherwise required in specific standards (see [Clause 9](#)).

6.3.5.2 Use

To avoid cross-contamination, use different chambers, or at least different containers, to achieve physical separation for storage of:

- uninoculated culture media and reagents;
- test samples;
- cultures of microorganisms;
- cultures retained after incubation.

Load refrigerators and cold-storage rooms so that appropriate air circulation is maintained and the potential for cross-contamination is minimized.

6.3.5.3 Maintenance and cleaning

Carry out the following maintenance operations at regular intervals to ensure proper operation:

- removal of dust from the motor blades or from the external heat-exchange plates;
- defrosting, if not automatic;
- cleaning and sanitization of the inside of the chambers;
- cleaning and sanitizing of door seals and checking their integrity.

6.3.5.4 Verification

Check and record the temperature of each chamber to confirm it is stable and within the maximum permissible tolerance, using a thermometer or a permanently installed probe.

Alternatively, maximum and minimum thermometers, dataloggers or electronic monitoring may be used.

The resolution and accuracy (uncertainty) required for the temperature-monitoring device are dependent on the purpose for which the unit is used and the specified temperature tolerances (see [6.3.2.4](#) for guidance).