

Australian Standard[®]

AS 5013.14.1—2010

Food microbiology

Method 14.1: Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations (ISO 7218:2007, MOD)

PREFACE

This Standard was prepared by the Standards Australia Committee, FT-024, Food Products and Subcommittee FT-024-01, Food Microbiology (Constituted), to supersede AS 5013.14, *Food microbiology, Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examinations*.

This Standard is an adoption with national modifications and has been reproduced from ISO 7218:2007, *Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations*.

The objective of this revision is to adopt the current edition of ISO 7218. However, inconsistencies exist between ISO 7218 and other ISO Standards with regard to specifying one plate versus specifying duplicate plates, when performing quantitative counts. Therefore, Australian technical variations have been made to the following Clauses of ISO 7218:

- (a) Clause 10.2.2, Number of Petri dishes per dilution.
- (b) Clause 10.3.2, Expression of results.
- (c) Clause 10.5.6.2.2, Tables for multiple-dilution systems: three successive dilutions.

These variations, which are necessary for Australian conditions, are listed in Appendix ZZ, which is added at the end of the source text.

As this Standard is reproduced from an International Standard, the following applies:

- (i) In the source text ‘this International Standard’ should read ‘this Australian Standard’.
- (ii) A full point substitutes for a comma when referring to a decimal marker.
- (iii) Substitute ‘mL’ for ‘ml’ wherever it appears.

In Clause 8.2, Transport, the sample temperatures specified are guidelines. The temperature of the sample at the time of receipt, and the time of sampling should be recorded. This information should be taken into account when deciding on the suitability of the sample for testing.

The laboratory should have a clearly defined quality control system to ensure the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

References to International Standards should be replaced by references to Australian Standards, as follows:

<i>Reference to International Standard</i>		<i>Australian Standard</i>	
ISO		AS	
6887	Microbiology—General guidance—Preparation of dilutions for microbiological examination (series)	5013 5013.11	Food microbiology Method 11.1: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination (series)

Other International Standards referenced in the source document have not been adopted as Australian Standards.

The terms ‘normative’ and ‘informative’ have been used in this Standard to define the application of the annex/appendix to which they apply. A ‘normative’ annex/appendix is an integral part of a Standard, whereas an ‘informative’ annex/appendix is only for information and guidance.

CONTENTS

1	Scope	1
2	Normative references	1
3	Premises	2
3.1	General.....	2
3.2	Safety considerations.....	2
3.3	Laboratory design.....	2
3.4	Laboratory areas	3
3.5	Layout and fittings of the premises	4
3.6	Cleaning and disinfection	5
4	Staff	5
4.1	General.....	5
4.2	Competence	6
4.3	Verification of on-going staff competence.....	6
4.4	Hygiene	6
5	Apparatus and equipment	6
5.1	General.....	6
5.2	Protective cabinets	7
5.3	Balances and gravimetric diluters	8
5.4	Homogenizers, blenders and mixers	9
5.5	pH meter	10
5.6	Autoclave.....	11
5.7	Media preparator.....	12
5.8	Incubator.....	12
5.9	Refrigerator, cold-storage room.....	13
5.10	Freezer and deep freezer	14
5.11	Thermostatically controlled bath	15
5.12	Steamers, including boiling-water baths.....	16
5.13	Sterilizing oven	17
5.14	Microwave oven	17
5.15	Glass washer.....	18
5.16	Optical microscope.....	19
5.17	Gas burner or wire incinerator	19
5.18	Dispenser for culture media and reagents.....	20
5.19	Vortex mixer	20
5.20	Colony-counting device	21
5.21	Equipment for culture in a modified atmosphere.....	22
5.22	Centrifuge	22
5.23	Hotplate and heating mantle.....	23
5.24	Spiral plater	23
5.25	Stills, deionizers and reverse-osmosis units.....	24
5.26	Timers and timing devices.....	24
5.27	Pipettes and pipettors	25
5.28	Thermometers and temperature-monitoring devices, including automatic recorders	26
5.29	Immunomagnetic separator.....	27
5.30	Filtration system	27
5.31	Other equipment and software	28

6	Preparation of glassware and other laboratory materials	28
6.1	Preparation	28
6.2	Sterilization/decontamination	28
6.3	Disposable equipment and materials	28
6.4	Storage of clean glassware and materials	28
6.5	Management of sterile glassware and materials	29
6.6	Use of decontamination and disinfection.....	29
6.7	Waste management	29
6.8	Washing	30
7	Preparation and sterilization of culture media.....	30
8	Laboratory samples	30
8.1	Sampling	30
8.2	Transport.....	31
8.3	Receipt	31
8.4	Storage	32
8.5	Test portion.....	32
9	Examination	32
9.1	Hygienic precautions during analysis	32
9.2	Preparation of initial suspension and dilutions.....	34
10	Enumeration	34
10.1	General	34
10.2	Enumeration using a solid medium	35
10.3	Calculation and expression of results obtained with solid media.....	37
10.4	Enumeration of yeasts and moulds	43
10.5	Enumeration using a liquid medium	44
11	Detection method (qualitative method)	49
11.1	General	49
11.2	Principle	50
11.3	Measurement of uncertainty	50
12	Confirmation methods.....	50
12.1	General	50
12.2	Preparation of a pure culture.....	50
12.3	Gram's stain (modified Hucker technique).....	50
12.4	Use of biochemical galleries for identification	52
12.5	Use of nucleic probes for identification	52
12.6	Serological methods	53
13	Test report.....	53
14	Validation of microbiological methods	54
14.1	Validation of reference methods	54
14.2	Validation of alternative methods.....	54
14.3	Validation of in-house methods.....	54
15	Quality assurance of results/quality control of performance.....	54
15.1	Internal quality control	54
15.2	Reference strains	54
15.3	External quality assessment (proficiency testing)	55
	Annex A (informative) Properties of some disinfectants.....	56
	Annex B (normative) Determination of most probable number (MPN)	57
	Bibliography	64

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).

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 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.

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).

A food microbiology laboratory shall be equipped with balances of the required range and measurement uncertainty for the different products to be weighed.

Unless otherwise stated, the maximum permissible errors should be 1 % or better when weighing out test samples.

Place the equipment on a stable horizontal surface, adjusted as necessary to ensure that it is level and protected from vibration and draughts.

5.3.2 Cleaning and disinfection

Equipment should be cleaned and disinfected after use or following spillage during weighing with an appropriate and non-corrosive disinfectant.

5.3.3 Performance verification and calibration

The performance of the balance system shall be regularly verified during use and after cleaning with check weights by a trained person. Calibration shall be checked across the entire range by a qualified person at a frequency dependent on use.

Check weights may also be verified immediately after calibration of the balance.

5.4 Homogenizers, blenders and mixers

5.4.1 Description

This equipment is used to prepare the initial suspension from the test sample of non-liquid products.

The following apparatus may be used:

- a peristaltic blender (stomacher) with sterile bags, possibly with a device for adjusting speed and time; or
- a rotary homogenizer (blender), the notional speed of which is between 8 000 r/min and 45 000 r/min inclusive, with sterilizable glass or metals bowls equipped with covers; or
- a vibrational mixer (pulsifier) with sterile bags; or
- another homogenizing system with equivalent efficiency.

In certain cases, manual mixing may be carried out using sterile glass beads having an appropriate diameter (approximately 6 mm; see ISO 6887-2 to ISO 6887-4 and ISO 8261).

5.4.2 Use

The usual operating time of a peristaltic homogenizer is 1 min to 3 min (see ISO 6887-2 to ISO 6887-4 and ISO 8261 for specific foods).

Do not use this type of apparatus for certain foodstuffs, such as:

- products which risk puncturing the bag (presence of sharp, hard or dry particles);
- products which are difficult to homogenize because of their texture (e.g. salami-type sausage).

The rotary homogenizer shall operate for a duration such that the total number of revolutions is between 15 000 r/min and 20 000 r/min inclusive. Even with the slowest homogenizer, this time shall not exceed 2,5 min.

The vibrational mixer may be used for most foodstuffs, including hard or dry products. The usual operating time is 0,5 min to 1 min. If microorganisms are likely to be encountered deep inside cohesive structures, the sample should be cut into small pieces prior to processing.

Glass beads can be used for the preparation, by shaking, of the initial suspensions of certain viscous or thick products, in particular certain dairy products (see specific standards).

5.4.3 Cleaning and disinfection

Clean and disinfect peristaltic homogenizers and vibrational mixers regularly and after any bag spillage or leakage.

For rotary homogenizers, clean and sterilize the glass or metal bowl after each use.

5.4.4 Maintenance

Inspect and maintain equipment in accordance with the manufacturer's instructions.

5.5 pH meter

5.5.1 Description

A pH meter is used to measure the potential difference, at a determined temperature, between a measuring electrode and a reference one, both electrodes being introduced into the product. It shall be capable of measuring to an accuracy of $\pm 0,05$ pH units and its resolution shall be 0,01 pH units. The pH meter shall be equipped with either manual or automatic temperature compensation.

NOTE The measuring electrode and the reference electrode are usually grouped together in a combined electrode system.

5.5.2 Use

A pH meter is used to measure the pH value of culture media and reagents to check if adjustment is needed during preparation and as a quality check after sterilization.

It may also be used to measure the pH value of samples and sample suspensions. The use of a pH meter is discussed in the standard specific to the product to be analysed, in which the conditions for the determination of the pH value and for adjustment of the pH value are specified.

Adjust the pH meter as indicated in the manufacturer's manual to measure the pH value at a standardized temperature, e.g. 25 °C. Read the pH value after stabilization has been reached. Record the value to two decimal places.

NOTE The reading may be considered stable when the pH value measured over a period of 5 s varies by not more than 0,02 pH units. Using electrodes in good condition, equilibrium is normally achieved within 30 s.

5.5.3 Verification and gauging

Verify the pH meter in accordance with the manufacturer's instructions, using at least two, and preferably three, standard buffer solutions at least daily before use. Define maximum permissible errors for this verification, depending on the use.

The standard solutions shall have pH values specified to two decimal places at the measurement temperature (in general, pH 7,00 and pH 4,00 and/or pH 9,00 at 25 °C, in accordance with the manufacturer's instructions). The standards used shall encompass the pH value to be measured.

After the verification of the pH meter with the two traceable standard buffer solutions, the pH should be checked by the use of a third buffer, namely a control buffer, e.g. pH 5 or 8.

Gauge the pH meter when the verifications give a result falling outside the maximum permissible errors and in accordance with the manufacturer's instructions.

This gauging may be followed by a calibration which would allow the measurement uncertainty of the pH meter to be estimated.

5.5.4 Maintenance

Check and maintain the electrodes in accordance with the manufacturer's instructions. It is necessary, in particular, to monitor regularly

- the condition of the electrodes with respect to ageing and soiling, and
- the response time and stability.

Rinse the electrodes with distilled or deionized water after each use. In order to take into account the soiling and ageing of the electrodes, regularly clean them more thoroughly in accordance with the manufacturer's instructions.

Store the electrodes in accordance with the manufacturer's instructions.

5.6 Autoclave

5.6.1 Description

An autoclave enables a saturated steam temperature to be attained in the chamber, and is used for the destruction of microorganisms.

The autoclave should be equipped with

- at least one safety valve,
- 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 measurement uncertainty associated with the measuring thermocouple), and
- a temperature probe or a recording thermocouple.

It should also be equipped with a timer and temperature recorder.

5.6.2 Use

With steam sterilization, all air is expelled prior to the pressure build-up. If the autoclave is not fitted with an automatic evacuation device, it is necessary to remove the air until a continuous jet of steam is emitted.

For the destruction of microorganisms, the saturated steam in the chamber shall be at a temperature of at least 121 °C.

During the same sterilization cycle, do not use the autoclave to sterilize clean equipment (and/or culture media) and at the same time to decontaminate used equipment (and/or used culture media).

It is preferable to use separate autoclaves for these two processes. After autoclaving, all materials and equipment should be allowed to cool within the autoclave before removal.

For safety reasons, do not remove the contents until the temperature has dropped below approximately 80 °C.

5.6.3 Maintenance

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

5.6.4 Verification and calibration

The autoclave shall be kept in good operating condition and shall be regularly inspected by competent qualified personnel in accordance with the manufacturer's instructions.

Keep the monitoring instruments in good working order and verify them regularly.

Initial validation 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 should be included at the centre of the load to verify the heating process where a traceable record of process efficiency is not available.

5.7 Media preparator

5.7.1 Description

A media preparator is principally designed for the sterilization of large volumes of media (> 1 l). It consists of a heating vessel, water jacket and continuous stirring device. The equipment shall also be fitted with a temperature gauge, pressure gauge, timer and safety valve.

In addition, the unit should have a safety lock to prevent opening until a temperature of < 80 °C is reached.

5.7.2 Use

Follow the manufacturer's instructions at all times.

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

5.7.3 Maintenance

Wash the preparator and rinse thoroughly with purified water between each media batch.

5.7.4 Verification

The preparator shall be kept in good working condition and inspected regularly by competent qualified personnel in accordance with the manufacturer's instructions.

Keep the monitoring instruments in good working order and verify their performance regularly.

Initial validation should include performance studies for each operating cycle and each load size used in practice. This process should be repeated after significant repair or modification. Two temperature probes, one adjacent to the control probe and another remote from it, may be used to demonstrate uniform heating.

The temperature and duration of each cycle should be checked.

5.8 Incubator

5.8.1 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 error specified in the test method.

5.8.2 Use

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. Define the working volume to ensure that this is achieved.

If the ambient temperature is close to or higher than that of the incubator, it is necessary to fit a cooling system to the chamber.

The walls of incubators should be protected from sunlight.

If possible, incubators should not be completely filled in one single operation because the culture media will take a long time to come to temperature equilibrium, whatever type of incubator is used (forced-air convection or otherwise). Refrain from leaving the incubator door open for long periods.

When loading incubators, attention should be paid to air circulation (see 10.2.4).

5.8.3 Cleaning and sanitization

Clean and sanitize regularly the inner and outer walls of the incubator and, if appropriate, remove dust from the ventilation system.

5.8.4 Verification

Check the temperature stability and the homogeneity of the temperature distribution at the working temperature(s) throughout the working volume of the incubator through simultaneous use of a number of thermometers or thermocouples of known accuracy and appropriate temperature range.

Use the information to define the acceptable operating range of the incubator and the optimum position of the thermometer used to monitor working temperatures.

For example, to achieve a target temperature of $37\text{ °C} \pm 1\text{ °C}$ when the profiling data shows a range of $36,8\text{ °C}$ to $37,3\text{ °C}$ across the incubator, then the operating range should be reduced to $36,2\text{ °C}$ to $37,7\text{ °C}$ in order to ensure all parts of the incubator achieve the target temperature of 37 °C .

This process should be repeated after each significant repair or modification.

The temperature of operation should be checked with one or more maximum and minimum thermometers or recording thermocouples, for example.

The thermometer or recording thermocouple used for routine monitoring of the incubator shall be fixed in a position defined from the profiling data as achieving the target temperature.

Check the incubator temperature at least every working day. For this purpose, each incubator shall incorporate at least one working measurement device, whose bulb can be immersed in glycerol (or other appropriate heat sink) contained in a sealed bottle.

Other checking systems of equivalent performance may be used.

5.9 Refrigerator, cold-storage room

5.9.1 Description

These are chambers which allow maintenance of cold storage. For the conservation of food samples for analysis, the temperature shall be $3\text{ °C} \pm 2\text{ °C}$ (maximum permissible errors), except for particular applications. For other uses, the temperature, unless otherwise specified, shall be $5\text{ °C} \pm 3\text{ °C}$.

5.9.2 Use

In order to avoid cross-contamination, use different chambers, or at least different containers, to achieve physical separation, for the storage of

- uninoculated culture media and reagents,
- test samples, and
- microorganism cultures and incubated media.

Load refrigerators, chillers and cold-storage rooms in such a way that appropriate air circulation is maintained and the potential for cross-contamination is minimized.

5.9.3 Verification

Check the temperature of each chamber each working day using a thermometer or a permanently installed probe. The accuracy required of the temperature-monitoring device is dependent on the purpose for which the unit is used.

5.9.4 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;
- cleaning and sanitization of the inside of the chambers.

5.10 Freezer and deep freezer

5.10.1 Description

A freezer is a chamber which allows frozen storage to be guaranteed. The temperature, unless otherwise specified, shall be below -15°C , preferably below -18°C for food samples.

A deep freezer is a chamber which allows deep-frozen storage to be guaranteed. The temperature, unless otherwise specified, shall be below -70°C .

5.10.2 Use

5.10.2.1 Freezer

Different chambers, or at least different containers, shall be available to achieve physical separation for the storage of

- uninoculated reagents,
- samples for analysis, and
- microorganism cultures.

Load the freezer in such a way that a sufficiently low temperature is maintained, in particular when unfrozen products are introduced.

5.10.2.2 Deep freezer

The principle use is storage of microorganisms, reference and/or working cultures, and reagents.

Load the freezer in such a way that a sufficiently low temperature is maintained and cross-contamination between microorganisms and reagents is prevented.

5.10.3 Verification

Check the temperature of each chamber regularly using a suitable temperature-monitoring device.

5.10.4 Maintenance

Carry out regularly the following maintenance operations:

- removal of dust from the motor blades and from the external heat-exchange plates (if accessible);
- defrosting;
- cleaning and sanitization of the inside of the chambers.

5.11 Thermostatically controlled bath

5.11.1 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 maximum permissible errors of $\pm 0,5^{\circ}\text{C}$ or better to be achieved. The working temperatures and required maximum permissible errors are stipulated in each individual application or method. A cooling system is necessary to maintain a temperature near or below ambient temperature.

5.11.2 Use

The main uses are as follows:

- incubation at a constant temperature of inoculated culture media;
- maintenance of sterile molten agar media during media preparation;
- tempering of sterile molten agar media for use in specific methods;
- preparation of initial sample suspensions or solutions at a controlled temperature;
- heat treatment of initial sample suspensions at a controlled temperature (e.g. pasteurization).

Where precise temperature control is required, the bath shall be equipped with a circulating-water pump and an automatic temperature-regulation system. 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 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 the incubation.

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

The depth of immersion shall preclude entry of water through the closure.

Devices to maintain stability of the containers may be required, for example racks.

All containers should be dried after removal from the bath and before further use.

5.11.3 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 the temperature control.

Monitor each bath with a thermometer, thermocouple or temperature-recording device of suitable minimum measurement uncertainty (see 5.28.2), and independent of the automatic temperature-regulation system.

A digital display may also be used, provided that its accuracy and resolution are verified.

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

5.11.4 Maintenance

Baths should be filled with liquid as recommended by the manufacturer. For incubation of cultures, distilled or deionized water should preferably be used.

Check regularly the level of the liquid 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.

5.12 Steamers, including boiling-water baths

5.12.1 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.

5.12.2 Use

The main uses are as follows:

- melting of agar media;
- preparation of heat-labile 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 may also be used.

5.12.3 Maintenance

Keep steamers and boiling water baths clean.

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

5.13 Sterilizing oven

5.13.1 Description

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

5.13.2 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.

Before sterilization, clean all glassware and metalware to be sterilized in the oven.

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

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 accuracy.

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 °C or an equivalent time/temperature combination.

After sterilization, to prevent cracking, glassware should be allowed to cool in the oven before removal.

5.13.3 Verification

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

The oven shall be fitted with a calibrated thermometer, thermocouple or temperature-recording device 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.

The temperature of the oven should be monitored and recorded during each use.

5.13.4 Maintenance

Clean internal surfaces when required.

5.14 Microwave oven

5.14.1 Description

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

5.14.2 Use

Use the equipment currently available only to heat liquids or melt agar culture media.

WARNING — Do not heat media containing heat-sensitive components in a microwave unless it has been verified that this way of heating has no effect on medium performance. No assessment has yet been made of the efficiency of microwaves for sterilizing culture media and microwave ovens shall not be used for this purpose.

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 for the microwaves give better heat distribution.

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

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

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

When melting agar media, a low power setting (e.g. defrost cycle) and 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.

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

5.14.3 Verification

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

5.14.4 Maintenance

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

Ovens door seals should be inspected for integrity and the oven checked for radiation leakage at regular intervals.

5.15 Glass washer

5.15.1 Description

Laboratory glass washers are electronically controlled machines for washing general laboratory glassware, which can be programmed for different washing cycles and rinses (e.g. distilled or deionized water or acid).

Devices for washing glass pipettes are special glass washers designed to clean the narrow bores of pipettes.

5.15.2 Use

Many types of glass washer are available, and these shall generally be installed and used following the manufacturer's instructions.

5.15.3 Verification

Check the effectiveness of cleaning by visual inspection and, in critical applications, carry out tests to ensure that glassware is free from inhibitory substances.

Alkaline or acidic residues may be checked for by using a pH indicator solution; a pH within the range 6,5 to 7,3 should be achieved.

5.15.4 Maintenance

Programme regular maintenance as specified by the manufacturer at a suitable frequency.

More frequent servicing may be required for heavily used equipment or in hard-water areas.

5.16 Optical microscope

5.16.1 Description

There are several different types of microscope: monocular, biocular, with a VDU, a camera or fluorescence equipment, etc., and with an internal or external light source. For bacteriological examinations, objectives with magnifications from $\times 10$ (dry lens) to about $\times 100$ (oil immersion with spring-loaded turret) are used to obtain an overall magnification of $\times 100$ to $\times 1\,000$. Phase contrast microscopy is also invaluable for examination of "wet preparations".

5.16.2 Use

Set up the optics of the microscope in accordance with the manufacturer's instructions. The optical axis of the light from the high-intensity light bulb shall pass through the centre of the substage condenser, the slide and the object lens to the eyepiece so that spherical and chromatic aberrations do not occur.

5.16.3 Maintenance

Follow the manufacturer's instructions concerning storage, cleaning and servicing. Prevent condensation occurring where humidity is high as this may lead to deterioration of lens quality.

Each day or after use, remove oil from the immersion lenses and related parts using lens tissue. Use a solvent recommended by the manufacturer. Regularly remove grease caused by eyelashes from the eyepiece lens.

The optical systems can be easily damaged, and servicing, preferably by the manufacturer, is therefore desirable.

5.17 Gas burner or wire incinerator

5.17.1 Description

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

Wire incinerators use gas or electricity to achieve red heat without a flame for sterilizing loops and straight wires used for manipulating cultures.

5.17.2 Use

A gas burner is 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.

The wire incinerator is used for sterilizing metal loops and straight wires and is preferred when handling pathogenic bacteria as it prevents splatter and avoids risk of cross-contamination.

Gas burners can produce much heat and air turbulence in the laboratory.

Aseptic techniques can be achieved without a gas burner by using disposable materials.