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

AMENDMENT 1

iTeh STANDARD PREVIEW

*Microbiologie des aliments — Exigences générales et
recommandations*
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AMENDEMENT 1

ISO 7218:2007/Amd 1:2013

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Foreword

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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, www.iso.org/directives.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received, www.iso.org/patents.

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This corrected version of ISO 7218:2007/Amd.1:2013 incorporates corrected values in Tables C.5, C.6 and C.7, i.e.

- columns four, five, six, seven and eight of Table C.5,
- columns four, five, six, seven, eight and nine of Table C.6, and
- columns four and nine of Table C.7.

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

AMENDMENT 1

Page 1, Clause 2

Delete ISO 8261. This has been superseded by ISO 6887-5 [already included in "ISO 6887 (all parts)"].

Delete the entries numbered "ISO 835 (all parts)", "ISO 8655-1", "ISO/TS 11133 (all parts)", and "ISO 16140" and insert the following.

ISO 835, *Laboratory glassware — Graduated pipettes*

ISO 8655 (all parts), *Piston-operated volumetric apparatus*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 16140-2, *Microbiology of food and animal feed — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

Pages 6 to 30, Clauses 5 and 6

Delete the existing text and insert the following.

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 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/IEC 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 biosafety 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 high-efficiency particulate air (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 biosafety 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 2 and 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

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

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, if they exist, 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, ultraviolet (UV) lamps may be used for disinfection. UV lamps should be regularly cleaned and replaced in accordance with the manufacturer's instructions. If they are used, they should be cleaned regularly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is according to the manufacturer's instructions.

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See Reference [17].

5.2.4 Maintenance and inspection

ISO 7218:2007/Amd.1:2013

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

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. Plate Count Agar) 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). See ISO 6887-1.

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 resolution of the balance should achieve a tolerance of 1 % but shall be sufficient to achieve a maximum tolerance of 5 % of the mass.

EXAMPLE To weigh 10 g, the balance should be capable of being read to 0,1 g.

To weigh 1 g, the balance should be capable of being read to 0,01 g.

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

5.3.3.1 Calibration

Calibration shall be checked across the entire range by a qualified person at a frequency dependent on use.

5.3.3.2 Verification

The performance of the balance system shall be regularly verified during use and after cleaning with check weights in the range of use by a qualified person.

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

5.4 Homogenizers, blenders and mixers

ISO 7218:2007/Amd 1:2013
<https://standards.iteh.ai/catalog/standards/sist/69cd73fc-7220-4631-80c4-2f4c8e512f9a/iso-7218-2007-amd-1-2013>

5.4.1 Description

This equipment is used to prepare the initial suspension from the test sample.

The following apparatus may be used:

— a peristaltic blender with sterile bags, possibly with a device for adjusting speed and time; or

NOTE The Stomacher® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

— a rotary homogenizer (blender), the notional speed of which is between 8 000 r/min and 45 000 r/min inclusive, with sterilizable bowls equipped with covers; or

— a vibrational mixer with sterile bags; or

NOTE The Pulsifier® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

— 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 6887-5).

5.4.2 Use

The usual operating time of a peristaltic homogenizer is 1 min to 3 min (see ISO 6887-2 to 6887-5 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 being read to the nearest 0,01 pH unit, enabling measurements to be made with a tolerance of $\pm 0,1$ pH unit. 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 Calibration and verification

5.5.3.1 Calibration

Calibrate 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 the maximum permissible tolerances for these readings, which shall be more stringent than the tolerance permitted in general use.

The standard solutions should be traceable and 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.

5.5.3.2 Verification

After calibration of the pH meter with the two traceable standard buffer solutions, a third buffer standard solution should be used to perform a check reading (in "read" mode) to demonstrate the functionality of the pH meter.

If readings fall outside the maximum permissible limits adjust the pH meter in accordance with the manufacturer's instructions. This adjustment shall be followed by further calibration and check.

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.

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 sterilization of culture media, the saturated steam in the chamber shall be at a temperature of at least $121\text{ °C} \pm 3\text{ °C}$ or temperature specified by manufacturers or production instructions or specified in the test method.

For the destruction of cultured microorganisms and decontamination of used culture media, the saturated steam in the chamber shall be at a temperature of at least $121\text{ °C} \pm 3\text{ °C}$.

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

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 by calibration and regular checks.

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 $<80\text{ °C}$ 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 them by calibration and regular checks.

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 tolerance 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 a 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 10.2.5).

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 or recording thermocouple used to monitor working temperatures.

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

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.

Check the incubator temperature at least every working day. For this purpose, each incubator shall incorporate at least one working measurement device, which can be immersed in glycerol (or other appropriate heat sink). 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 $(3 \pm 2) ^\circ\text{C}$ (maximum permissible tolerances), except for particular applications. For other uses, the temperature, unless otherwise specified, shall be $(5 \pm 3) ^\circ\text{C}$.

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 $-15 ^\circ\text{C}$, preferably below $-18 ^\circ\text{C}$ 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 $-70\text{ }^{\circ}\text{C}$.

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.

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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 tolerance of $\pm 0,5\text{ }^{\circ}\text{C}$ or better to be achieved. The working temperatures and required maximum permissible tolerance are stipulated in each individual application or standard 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 standard 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

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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.

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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.

NOTE A digital display can 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.