BRITISH STANDARD

BS 2646-3: 1993

Autoclaves for sterilization in laboratories —

Part 3: Guide to safe use and operation

Confirmed December 2011

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the Laboratory Apparatus Standards Policy Committee (LBC/-) to Technical Committee LBC/35, upon which the following bodies were represented:

Association of British Health Care Industries
Association of Sterilizer and Disinfector Equipment Manufacturers
British Dental Trade Association
British Stainless Steel Association
Central Sterilising Club
Department of Health
Health and Safety Executive
Infection Control Nurses Association
Institute of Hospital Engineering
Institute of Sterile Services Management
Medical Sterile Products Association
National Blood Transfusion Service
Public Health Laboratory Service
Regional Hospital Boards Engineers' Association
Royal College of Pathologists
Royal Pharmaceutical Society of Great Britain
Society for General Microbiology

The following bodies were also represented in the drafting of the standard, through subcommittees and panels:

Association of Clinical Pathologists
British Glass Manufacturers' Confederation
BLWA Ltd. (The Association of the Laboratory Supply Industry)
Copper Development Association
Institute of Medical Laboratory Sciences
Manufacturing Science Finance
Ministry of Agriculture, Fisheries and Food
Royal Association of British Dairy Farmers
Society for Applied Bacteriology

This British Standard, having been prepared under the direction of the Laboratory Apparatus Standards Policy Committee, was published under the authority of the Standards Board and comes into effect on
15 December 1993

The following BSI references relate to the work on this standard:
Committee reference LBC/35
Draft for comment 92/54443 DC

ISBN 0 580 22511 9

Amendments issued since publication

Amd. No.	Date	Comments

Contents

Foreword

This Part of BS 2646 was prepared under the direction of the Laboratory Apparatus Standards Policy Committee, to provide guidance to laboratory personnel and others concerned with the supervision and management of microbiology laboratories equipped with autoclaves conforming to BS 2646-1. It will also be of value to safety supervisors and should be read in conjunction with *Safety in Health Service Laboratories: Safe working and the prevention of infection in clinical laboratories* [1], produced by the Health and Safety Commission, Health Services Advisory Committee (see note to **3.2**) and *Categorization of pathogens according to hazard and categories of containment,* 2nd edition [2], produced by the Advisory Committee on Dangerous Pathogens (see note to clause **1**); attention is also drawn to the Control of Substances Hazardous to Health Regulations (COSHH) 1988 [3]. BS 2646 comprises several separate Parts. The other Parts of the standard are as follows.

— *Part 1: Specification for design, construction, safety and performance;*

— *Part 2: Guide to planning and installation;*

— *Part 4: Guide to maintenance;*

— *Part 5: Methods of test for function and performance.*

It is anticipated that autoclaves to which this standard applies will be used for the following processes:

a) liquids sterilization;

b) equipment and glassware sterilization;

c) make-safe.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 12, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

Introduction

Many laboratory procedures require the use of sterile materials including glassware, apparatus, instruments, and liquids in a variety of container types.

Because pathogenic micro-organisms and pathological specimens may be examined or stored in microbiology laboratories it is also necessary to ensure that all such material is rendered safe for subsequent handling or before it leaves the laboratory. Acceptable methods are autoclaving and/or incineration.

When selecting an autoclave or an autoclaving process there is a clear need to differentiate between:

a) the sterilization of liquid media and apparatus for use in the laboratory where the presence of viable micro-organisms would spoil the medium or confuse an investigation; and

b) the treatment of discarded, contaminated material so that it may be handled without causing an infection hazard or contaminating the environment.

Three general processes are therefore defined (see BS 2646-1:1993): liquids sterilization; equipment and glassware sterilization; make-safe.

Operating cycles for each process are described in this Part of BS 2646 together with recommended time/temperature conditions, and recommendations on validation and in-use testing. Variations will be adopted within each process for particular load items or for special purposes; the principles which support the conditions recommended in this standard should also form the basis for other requirements a laboratory may have for an autoclave.

"Free-steaming" is not defined as an autoclaving process. Steaming is a means of dissolving constituents of nutrient media and also for reducing numbers of non-sporing and vegetative micro-organisms to acceptable levels in microbiological culture media not able to withstand the higher temperatures of an autoclaving process. It is recommended that steaming is carried out in a purpose-designed, non-pressurized vessel of the traditional "Koch's steamer" type. However, the liquids sterilization process of an autoclave may be used for this purpose if the controlled temperature is reduced to approximately 100 °C.

1 Scope

This Part of BS 2646 gives guidance on the factors that should be taken into account when devising procedures to ensure the safe and effective use of laboratory autoclaves of the types specified in BS 2646-1, i.e. autoclaves for the sterilization of material and equipment including those which may be contaminated with organisms categorized as Hazard Groups 1, 2 or 3 (see note). It does not cover the use of autoclaves for material contaminated with organisms categorized as Hazard Group 4, for which complete containment of condensate is considered to be essential.

The procedures described are designed to minimize hazards to operators and other personnel, and to confirm the ability of the autoclave to carry out effectively each of the processes defined in BS 2646-1.

NOTE The groups of organisms referred to are those listed in *Categorization of pathogens according to hazard and categories of containment,* 2nd edition 1990 [2], produced by the Advisory Committee on Dangerous Pathogens and published by HMSO.

2 References

2.1 Normative references

This Part of BS 2646 incorporates, by reference, provisions from specific editions of other publications. These normative references are cited at the appropriate points in the text and the publications are listed on the inside back cover. Subsequent amendments to, or revisions of, any of these publications apply to this Part of BS 2646 only when incorporated in it by updating or revision.

2.2 Informative references

This Part of BS 2646 refers to other publications that provide information or guidance. Editions of these publications current at the time of issue of this standard are listed on the inside back cover, but reference should be made to the latest editions.

3 Definitions

For the purposes of this Part of BS 2646, the definitions given in BS 2646-1:1993 apply, together with the following.

3.1
responsible person

person responsible for the operating policy of autoclaves within the laboratory

3.2
safety supervisor

senior member of the laboratory staff with delegated responsibility for overseeing the management and implementation of safety standards and requirements of local safety rules

NOTE For duties of safety supervisors (safety officers) see *Safety in Health Service Laboratories: Safe working and the prevention of infection in clinical laboratories* [1], produced by the Health Services Advisory Committee of the Health and Safety Commission and published in 1991 by HMSO.

3.3
maintenance/service engineer

person who performs maintenance/service work on the autoclave

NOTE This person may be employed by the laboratory, contracted to the laboratory or employed by the autoclave manufacturer.

4 Operation of autoclaves

4.1 Training and instruction of operators

Autoclaves should be operated only by persons who have been trained and instructed in their use. Operators should receive the necessary training and instruction from the responsible person (see **3.1**) and should be warned of the importance of carrying out only the operation instructions.

4.2 Operation instructions

Operation instructions, including those in the instruction manual, should be provided for the operator by the responsible person (see **4.1** of BS 2646-4:1991).

If the autoclave is provided with keys, switches or codes which can be used to override safety features or manually advance the cycle, the operation instructions should specify the necessary authorization and procedures to be followed before their use.

Operation instructions should include details of action to be taken by the operator in the event of a fault or any abnormality in autoclave performance.

4.3 Autoclave process record

4.3.1 An autoclave process record for each operating cycle should be kept by the operator (see **4.3** of BS 2646-4:1991). This should contain details of each load processed, a chart recording, a record of any fault, and corrective action taken by the operator.

4.3.2 Process records should include results of validation tests and in-use testing (see clauses **13** and **14**).

4.3.3 Process records should be examined by the responsible person at an agreed frequency.

5 Maintenance

5.1 Maintenance schedule

The continuing safe and effective use of the autoclave depends on a programme of planned maintenance throughout its life. Maintenance schedules should therefore follow all of the recommendations in BS 2646-4:1991; guidance on the maintenance log and its use is also given in BS 2646-4. The manufacturer should always be consulted on maintenance intervals.

NOTE Guidance on factors that should be taken into account by the manufacturer when devising a maintenance schedule is given in BS 2646-4.

5.2 Precautions before service or repair of autoclaves

5.2.1 *Permit to work certificate*

The use of permit to work certificates is strongly recommended. Guidance on these and their use is given in BS 2646-4.

5.2.2 *Risk of infection during make-safe*

5.2.2.1 When a fault occurs (see **1.3.30** of BS 2646-1:1993) during a make-safe cycle an assessment of risk should be made and appropriate action taken. The assessment of risk should be carried out in accordance with the safety policy of the laboratory.

It may be necessary to disinfect those chamber attachments on which engineering work is to be carried out. Knowledge of the contents of the load may help in the choice of method and disinfectant for this.

During a make-safe process, chamber condensate should be considered to be contaminated with viable micro-organisms.

5.2.2.2 Exceptionally, some dismantling of the autoclave may be necessary before disinfection. This should be done in the presence of the responsible person and safety supervisor.

Disinfection of the chamber and/or pipework should not involve prolonged contact with disinfectants corrosive to metal.

5.2.2.3 The responsible person should ensure that appropriate protective clothing is supplied to and used by the maintenance/service engineer.

5.2.2.4 A contaminated laboratory autoclave should never be returned to the manufacturer for servicing or repair. Decontamination should be carried out in accordance with local safety rules.

NOTE Health Service Guidelines HSG(93)26 [4] published by the Department of Health gives guidance on the decontamination of equipment prior to servicing.

6 Protective clothing

6.1 Laboratory clothing

A protective laboratory coat of side or back fastening style should be worn in the autoclave loading/unloading area.

6.2 Additional protection

Additional clothing should be available in the loading/unloading area(s) to protect the operator.

The hazards on loading include the following:

a) spills of biohazardous material;

b) broken glass;

c) dropped load contents.

The hazards on unloading include the following:

1) splashes and spillage of hot material from the load;

2) hot condensate;

3) hot equipment;

4) broken glass;

5) dropped load contents;

6) vapour from volatile chemicals (see **7.3.2**).

The additional clothing should include an impervious apron, heat-resistant gauntlet gloves, suitable heavy-duty footwear or overshoes and a full-face visor.

7 Loading the autoclave

7.1 Loading area

7.1.1 Access to the loading area should be limited to personnel aware of the hazards from potentially infective material. The loading position should not be obstructed.

7.1.2 All materials awaiting autoclaving should be positioned so that they cannot be overturned, spilled or damaged.

7.1.3 Discard-containers of infected materials should not be stored in the loading area.

7.1.4 Material in discard-containers should not be handled prior to autoclaving.

7.2 Operator protection

Loading (and unloading) procedures should be designed to avoid health hazards and also injuries to personnel by the elimination of awkward lifting positions and excessively heavy load containers. Heavy loads should not be lifted into (or out of) vertically mounted chambers by staff of unsuitable build or strength.

Consideration should also be given to the provision of mechanical assistance (see section 10 of BS 2646-1:1993).

NOTE Attention is drawn to HSE document *Manual handling — Guidance on regulations,* HMSO 1992 [5], which gives guidance on the Manual Handling Operations Regulations 1992 (in force from 1 January 1993).

7.3 Care on loading

7.3.1 Items should be packed in a way which ensures that steam will penetrate the load.

7.3.2 Before corrosive chemicals or materials and chemicals (including disinfectants) likely to produce harmful vapour are autoclaved, a risk assessment should be made.

8 Unloading the autoclave

8.1 Conditions to be met before unloading

8.1.1 Temperature and pressure indicators and warning lights should be checked to ensure that the autoclave has successfully completed the operating cycle. If a fault is indicated, attempts to open the autoclave should only be made with the authority of the responsible person.

8.1.2 It is dangerous to attempt to release the autoclave door mechanism before the chamber is vented to atmosphere or whilst the load contents are at high temperature. No attempt should be made by the operator to override door interlocking safety devices.

8.1.3 The temperature of any liquid, whether in the load or lying in the chamber, should not exceed 100 °C when the door is opened. Liquids in bottles within the load should be below 80 °C before the door is opened.

If a temperature indicator shows an abnormal reading, the door should not be opened without the authority of the responsible person, in accordance with the operating instructions (see also **5.2.2.1**).

8.2 Operator protection

8.2.1 In addition to a protective laboratory coat with sleeves, an impervious apron, heat-resistant gauntlet gloves and a visor should be worn (see clause **6**).

8.2.2 The operator should stand clear when opening the door as hot liquid or vapour may escape from the chamber.

8.2.3 After autoclaving, discard-containers should be emptied in a safe manner and their contents disposed of or reclaimed as specified by local laboratory rules.

9 Discard-containers

NOTE See **1.3.21** of BS 2646-1:1993.

9.1 Design

Discard-containers should be easily transported, leak-proof and of robust design with solid sides and bottoms. They should allow adequate steam penetration to the contents (see **11.4**).

If autoclavable plastics bags are used they should be supported in a discard-container whilst in the laboratory and also whilst in the autoclave. The mouth of the bag should remain open during the autoclave cycle so that steam can penetrate its contents. A plastics bag is, in itself, not a discard-container.

9.2 Spillages

9.2.1 Procedures should be established which clearly define actions to be taken in the event of spillage of infective material from a discard-container.

9.2.2 Instructions on dealing with a spillage of infective material should be included with the operation instructions (see **4.2**). These should specify suitable disinfectants and indicate their whereabouts and methods of use.

Disinfectants and cleaning materials should be readily available in the autoclave loading area.

Equipment used to load the autoclave, and protective clothing, should be examined for evidence of spillage or contamination. Contaminated equipment and clothing should be disinfected and the responsible person informed.

10 Potential hazards in the use of laboratory autoclaves

10.1 General

The main hazards associated with the use of autoclaves are:

a) the pressure vessel hazard;

b) the unloading hazard;

c) failure of a make-safe process.

10.2 Pressure vessel hazard

The safe operation of autoclaves depends upon the measures given in items a) to e). Failure to implement any of them may give rise to an explosion.

a) *The provision of appropriate door interlocking safety devices.* Door interlocking safety devices are designed to prevent pressurization of the chamber before the door is secured and to prevent the uncontrolled release of chamber contents whilst it is still under pressure (see section 9 of BS 2646-1:1993).

b) *Examination of the vessel and its fittings.* (See BS 2646-4). Any escape of steam should be reported immediately and appropriate action taken. Any defect during use should be reported to the responsible person and recorded in the maintenance log.

c) *Arrangements for regular systematic inspection and maintenance.* (See clause **5** and BS 2646-4).

d) *Adequate training and supervision of operators.* (See clause **4** and BS 2646-4).

e) *Provision of and adherence to proper operating procedures.* (See clause **4** and BS 2646-4). Clearly printed operating instructions should be displayed beside each autoclave.

10.3 Unloading hazard

During the cooling stage the temperature indicated in the chamber exhaust line may be much lower than the load temperature. Containers of liquid could be pressurized and may explode; volatile liquids may produce harmful vapour; liquids spilled on unloading may cause scalding. The measures given in items a) to e) are designed to minimize the unloading hazard.

a) *Temperature activated door interlocks.* In addition to pressure vessel safety devices, autoclaves designed to be used for liquids sterilization or make-safe are fitted with a safety interlock to prevent the door mechanism being released whilst the temperature of the liquid is too high.

Although it is common practice to segregate make-safe loads into plastics (discard) and glass (reusable) it is not possible to guarantee that every load contains exclusively one or the other. Loads of unknown content should be treated as if they contained liquid.

b) *Timer activated door interlocks.* A "cooling-timer" may be used in addition to a temperature activated device. Its effectiveness should be confirmed during validation tests.

c) *Training and supervision of operators.* The operator and responsible person (also the safety supervisor) should be aware of the temperatures of fluid contents of containers that are to be unloaded and take steps to minimize the hazard to operators, e.g. by the provision of protective clothing.

d) *Protective clothing.* Whilst opening the door or unloading an autoclave, operators should wear clothing additional to their normal laboratory coat (see clause **6**).

e) *Load transfer system*. Reaching into an autoclave, which contains a hot load, can be hazardous. Consideration should be given to the provision of a load transfer system, either sliding shelves or a carriage and trolley.

10.4 Failure of a make-safe process

The effectiveness of the make-safe process can be assured by attention to the following.

a) The contents of discard-containers will vary in type and volume. The operating cycle should take account of the delay in heat penetration caused by entrapped air and melted plastics. The time required for the load to reach sterilizing temperature should be determined during validation tests.

NOTE During the heat-up stage temperatures within the load may be considerably less than the temperature of the autoclave chamber or chamber drain.

b) Typical operating cycle conditions for use in a make-safe process are listed in Table 1. For organisms of exceptional heat resistance the operating cycle may need to be modified (see notes 4 and 5 to **11.4**).

11 Operating cycles

11.1 Functional design

The design and establishment of each operating cycle should ensure that the likelihood of microbial survival is appropriate for the purpose intended (see Annex A). This is achieved by selecting a particular operating cycle, each stage of which is designed to ensure that the necessary process conditions are met. The method of selecting the operating cycle depends on whether the autoclave has pre-set temperature and/or pressure controls or controls which can be varied by the operator before each cycle.

11.2 Cycle stages

Operating cycles generally comprise 3 or 4 stages as follows.

Stage 1 Heat-up

Stage 1 prepares the load for stage 2.

Sufficient air is removed to ensure the attainment of stage 2.

Stage 2 Sterilizing

All parts of the load are heated to, and maintained at, sterilizing temperature for the pre-set time, i.e. equilibration time plus holding time.

Stage 3 Cooling

The chamber pressure falls to a safe level and the contents to a safe temperature before it is possible to vent the chamber and open the door.

NOTE 1 Cooling may be assisted.

Stage 4 Drying

NOTE 2 Stage 4 is applicable to equipment and glassware sterilization only.

Steam is allowed to escape from the chamber or is mechanically extracted sufficiently to permit the evaporation of condensate from the load.

11.3 Operating cycles for liquids sterilization

NOTE 1 See **12.1** of BS 2646-1:1993.

The operating cycle should ensure that sterilization is achieved with minimum damage to the liquid. Microbiological culture media are particularly heat sensitive; the degree of deterioration is related to the length of time the medium is maintained at sterilizing temperature; the heat-up and cooling stages also contribute significantly to this deterioration.

Heat-up. Heat-up times should be as short as possible, achieved by uniformly filling the chamber with steam at sterilizing temperature. Large volumes of fluids will heat up slowly, therefore volumes of liquid should be kept small; a maximum container volume of 500 ml is recommended, larger volumes taking considerably longer to heat up (and cool down).

Sterilizing. This stage comprises the equilibration time, followed by the holding time, during which latter the sterilizing temperature is maintained.

NOTE 2 Combinations of time and temperature other than those given in Table 1 may be required by the user. Such combinations should deliver an adequate probability of sterility for the purpose (see Annex A).

Cooling. Cooling loads quickly helps to protect heat-sensitive constituents and also shortens operating cycle times. Air at high pressure may be admitted to ballast the chamber, minimize boiling and prevent bottles exploding.

NOTE 3 Any air admitted to the chamber should be filtered to remove oil-mist and micro-organisms (see **6.4** of BS 2646-2:1990).

Containers should be loosely capped unless they are specifically designed for sealing. However, sealing bottles can increase the likelihood of explosion during autoclaving and slows cooling.

A fault (see **1.3.30** of BS 2646-1:1993) may result in contaminated or over-heated culture media. After a fault, careful assessment should be made before the batch of medium is reprocessed or discarded.

Table 1 — Typical operating cycle conditions

Process	**Sterilizing temperature** (see **1.3.24** of BS 2646-1:1993)		**Holding time** (see **1.3.23** of BS 2646-1:1993)	
	Minimum °C	**Maximum** °C	**Minimum** min	**Maximum** min
Liquids sterilization	121	124	—	15
	115	118	—	30
Equipment and glassware sterilization	121	124	15	—
	126	129	10	—
	134	138	3[a]	—
Make-safe[b]	121	125[b]	15	—
	126	130[b]	10	—
	134	138	3[a]	—

[a] Loads which comprise a variety of items and containers do not heat uniformly. Short holding times are therefore subject to large proportionate variations and should be avoided if possible.

[b] The maximum temperature is greater for some make-safe processes than for corresponding equipment and glassware processes, to permit easier attainment of sterilizing conditions throughout the load.

11.4 Operating cycles for make-safe

NOTE 1 See **12.2** of BS 2646-1:1993.

Discarded material should be autoclaved in such a way that an adequate margin of safety is incorporated in the procedure (see Annex A).

NOTE 2 Material will include single-use items to be discarded, e.g. plastics specimen tubes and culture plates, and/or items for cleaning and re-use, e.g. glass containers and filter assemblies.

Heat-up. Sufficient air is removed or displaced from the chamber and load to achieve the conditions required in the sterilizing stage.

NOTE 3 When maximum air removal is accomplished before the load reaches 100 °C, deformation of any polystyrene items present will be delayed with a shortening of the equilibration time.

Sterilizing. This stage comprises the equilibration time, followed by the holding time, during which latter the sterilizing temperature is maintained.

NOTE 4 Combinations of time and temperature other than those given in Table 1 for make-safe may be required by the user. It is essential that any alternative process is validated to deliver a level of assurance of lethality not less than that given by the conditions listed in Table 1 (see also Annex A).

NOTE 5 The discovery of non-sporing infective agents with an increased resistance to chemical and heat treatment (e.g. "slow viruses") has led to the need for increased temperatures and holding times for treatment of material from a suspected case of infection by these agents. Advice is contained in the DHSS Letter DA(84)16, *Management of patients with spongiform encephalopathy (Creutzfeld-Jakob disease, CJD),* 1984 [6].

Cooling. Cooling loads quickly shortens operating cycle times. Air at high pressure may be admitted to ballast the chamber, minimize boiling and prevent bottles exploding.

NOTE 6 When a rapid cooling process commences whilst fluids are above 100 °C bottles may explode or their contents boil over unless the chamber is pressurized with air.

11.5 Operating cycles for equipment and glassware sterilization

NOTE 1 See **12.3** of BS 2646-1:1993.

Items are to be dry after the completion of this sterilizing process.

Heat-up. Air may be displaced by steam or removed mechanically from the chamber and load.

NOTE 2 Some microbiological filter membranes are damaged by rapid, large variations in pressure.

Sterilizing. This stage comprises the equilibration time, followed by the holding time, during which latter the sterilizing temperature is maintained.

NOTE 3 Combinations of time and temperature other than those given in Table 1 may be required by the user. Such combinations should deliver an adequate probability of sterility for the purpose (see Annex A).

Drying. Any air admitted to the chamber should be filtered (see **6.4** of BS 2646-2:1990) to remove oil-mist and micro-organisms.

12 Autoclave performance

The stages of testing which comprise the testing programme are described in Annex A of BS 2646-1:1993. Attention is there drawn to the limitations of the performance tests and the need for validation and in-use testing.

13 Validation

NOTE See **1.3.29** and **A.5** of BS 2646-1:1993.

13.1 General

Validation should be carried out after commissioning or recommissioning before the autoclave is put into service.

Knowledge is required of the nature of loads for which each autoclaving process is suitable and for which the autoclave is to be used, and an example load should be defined and tested for each load type likely to be processed. Guidance in selecting example loads for each process is given in **13.2**, **13.3** and **13.4**.

Validation will determine autoclave control settings for the example loads tested. These settings should then be used only for loads of similar size and type.

13.2 Validation for liquids sterilization

13.2.1 Good laboratory practice should ensure that, so far as is practicable, a uniform size and type of liquids container is used and the number of containers comprising each load is constant, thus reducing the number of example loads necessary. However, the need may arise to process containers and liquids of unusual nature; these will require validation.

Factors which affect the rate of heat-up and cooling of loads and temperature distribution in containers within each load should be considered when example loads are defined. These factors include:

a) container size;

b) container material and contents;

c) variations in container size and material;

d) load size;

e) load distribution;

f) type of container holder.

13.2.2 When tested as described in Annex B, with each example load defined (see **13.2.1**), the autoclave should satisfy the following criteria.

a) The stages of the operating cycle are completed in the correct sequence.

b) A fault has not been indicated.

c) The holding time is within the prescribed limits.

d) During the holding time all sensors are at sterilizing temperature.

e) The temperature of any water, or other liquid, present in the chamber at the end of the operating cycle does not exceed its boiling point.

f) The temperature of all monitored bottles is not more than 80 °C and not less than 65 °C when the door securing mechanism releases.

NOTE It may be necessary to adjust the control settings and/or the setting of the thermal door interlock to achieve this performance throughout the load.

13.3 Validation for make-safe

13.3.1 The rate of heat-up and cooling of containers and material in each discard-container should be considered when example loads are defined.

Factors which affect the rate of heat-up and cooling of make-safe loads include:

a) container size;

b) load size;

c) variations in size and material of items and containers in each discard-container;

d) the distribution of items within discard-containers;

e) the size and material of the discard-containers themselves.

A uniform size and type of discard-container will simplify validation and allow the more efficient use of chamber space. However, the need may arise to process unusual items or containers of large size requiring larger discard-containers. The frequency with which these items require autoclaving will determine whether the autoclave controls and thermal interlock are set for them or whether special arrangements can be made when the need arises. If the latter, controls may be set for the discard-containers in regular use.

13.3.2 When tested as described in Annex B, with each example load defined (see **13.3.1**), the autoclave should satisfy the following criteria.

a) The stages of the operating cycle are completed in the correct sequence.

b) A fault has not been indicated.

c) The holding time is within the prescribed limits.

d) During the holding time all sensors indicate the prescribed sterilizing temperature.

e) The temperature of any water, or other liquid, present in the chamber at the end of the operating cycle does not exceed its boiling point.

f) The temperature of liquid in any monitored bottles within the load is not more than 80 °C when the door securing mechanism releases.

NOTE It may be necessary to adjust the control settings and/or the setting of the thermal door interlock to ensure these requirements throughout the load.

13.4 Validation for equipment and glassware sterilization

13.4.1 Similar factors to those which affect the heat-up of material for make-safe apply to equipment and glassware. These include:

a) variations in size and material of the items to be sterilized;

b) size and material of vessels or holders which contain the items;

c) load distribution in the chamber.

13.4.2 When tested as described in Annex B, with each example load defined (see **13.4.1**), the autoclave should satisfy the following criteria.

a) The stages of the operating cycle are completed in the correct sequence.

b) A fault has not been indicated.

c) The holding time is within the prescribed limits.

d) During the holding time all sensors indicate the prescribed sterilizing temperature.

e) The temperature of any water, or other liquid, present in the chamber at the end of the operating cycle does not exceed its boiling point.

f) There is no visible water in any container within the load at the end of the operating cycle.

NOTE It may be necessary to adjust the control settings and/or the setting of the thermal door interlock to ensure these requirements throughout the load.

14 In-use testing

NOTE See **A.6** of BS 2646-1:1993.

14.1 General

14.1.1 In-use tests monitor the operation of the autoclave during routine use and also confirm that the control settings, determined during validation, and the door interlock controls are effective.

If biological or chemical indicators are employed, their use should be additional to the tests described in **14.2**, **14.3** and **14.4**.

14.1.2 After each operating cycle chart recordings should be studied and kept as part of the autoclave process record (see **4.3**).

14.1.3 Thermometric tests should be carried out at agreed intervals using test recorders such as those specified in **1.4.3** of BS 2646-5:1993.

Recorders with up to three leads and sensors, or even the repeated use of a single load temperature probe, may provide sufficient assurance of effectiveness if records of validation tests are consulted to determine their most effective position within the load.

14.1.4 If a load temperature probe is fitted, it may be used for in-use testing. The probe should be placed in a container by the operator for monitoring a liquids sterilization or equipment and glassware sterilization process.

NOTE Use of the probe for monitoring a make-safe cycle is for in-use test purposes only by the responsible person. For day to day processing of infective material, the probe should be in a holder or on the load support.

A load temperature probe should not be used as an alternative to any of the safety devices specified in **9.4** of BS 2646-1:1993.

14.2 In-use tests for liquids sterilization

14.2.1 Tests should be carried out to confirm that temperatures in containers of liquid are between 65 °C and 80 °C and that any fluid in the chamber or load is below 100 °C at the end of the operating cycle when the door securing mechanism releases.

14.2.2 Thermometric testing of typical loads will also be a useful quality assurance check. Placing the load temperature probe in a container of each load will provide information, which, over a number of operating cycles, will help to confirm the optimal container sizes and loading patterns.

14.2.3 After sterilization quality assurance checks should be carried out on each batch of liquids.

Quality assurance checks on microbiological culture media should identify:

a) volume loss;

b) pH change;

c) darkening of media with alteration of chemical structure;

d) poor gelling;

e) loss of growth supporting and/or inhibiting properties;

f) failure to sterilize (microbial survival).

14.3 In-use tests for make-safe

14.3.1 Thermometric checks should be carried out monthly on autoclaves used for make-safe.

Extreme care should be exercised when placing sensors into discard-containers of infective material. The operation should be carried out under the direct supervision of the responsible person or safety supervisor.

14.3.2 If a load temperature probe is fitted, it should be placed in material within a discard container, in a position determined during validation to be the slowest to heat up.

NOTE This position of the probe is for in-use test purposes only. For the day to day processing of infective material the probe should be in a holder or on the load support (see also **14.1.3**).

14.3.3 Heat-up time, sterilizing time and temperatures should be measured and compared with the prescribed values.

14.3.4 Tests should be carried out to confirm that temperatures in containers of liquid are below 80 °C and that any fluid in the chamber or load is below 100 °C at the end of the operating cycle, when the door securing mechanism releases.

14.4 In-use tests for equipment and glassware sterilization

14.4.1 Thermocouple testing of items in typical loads will provide assurance that sterilization temperatures and holding times have been achieved. Placing the load temperature probe in an item of each load will provide helpful information, which, over a number of operating cycles, gives confirmation of the optimal loading patterns.

14.4.2 Tests should be carried out to confirm that the temperature of any fluid in the chamber is below 100 °C.

Annex A (informative) Concept of sterility

A.1 General

Although the term "sterile" has an absolute and unqualified meaning it is not possible to assure absolute sterility.

Microbial populations subjected to lethal temperatures assume a logarithmic death rate; the law of first-order kinetics can be applied and the rate of reduction in the number of viable micro-organisms expressed. The higher the initial numbers of micro-organisms, the greater the probability of survival.

It is not possible to demonstrate unequivocally that sterility has been achieved; it is only possible to calculate the probability that a single cell may have survived. This is determined by the extrapolation of data, presented graphically, into areas which cannot be investigated experimentally and where it can only be assumed that the exponential rate of death continues. Heat resistance data are conventionally analysed by plotting survival curves of the logarithm of number of survivors as a function of heating time. In a homogeneous suspension of micro-organisms subjected to constant, uniform heat the resulting experimental survivor curve should therefore be a straight line.

The impracticability of demonstrating the presence of viable micro-organisms in such small numbers has led to alternative methods of assessing the "level of sterility" by probability.

Reliance on such methods depends on knowledge of:

a) the likely numbers of microbial cells prior to heating;

b) the heat resistance of the micro-organisms and their spores; and

c) acceptance of a level of probability of survival for a particular purpose.

A.2 Concentration of microbial cells

The effect of the initial cell population on survivors after heating reinforces the need to reduce numbers by cleaning equipment and glassware prior to sterilization. In microbiology laboratories it is possible, with good laboratory practice and using dehydrated microbiological culture media from reputable manufacturers, to ensure that there are minimal numbers of contaminating micro-organisms in prepared media prior to autoclaving. However, in discard-containers to be subjected to a make-safe process in a laboratory autoclave, the numbers of micro-organisms present are inevitably several orders of magnitude greater and no pre-treatment is possible to reduce the concentration of what may be very heat resistant spores.

A.3 Heat resistance

The resistance of micro-organisms to heat may be expressed in terms of the Decimal Reduction Time (*D*-value) which is the time in minutes required to reduce the number of viable cells by 90 % (1 log cycle) at a specified temperature. The term "*D*-value" is employed with a subscript which denotes the temperature to which it refers, e.g. D_{121}-value is the Decimal Reduction Time at 121 °C. *D*-values may be applied to spores, to non-sporing bacteria and to viruses and are calculated from experimental data.

For a particular micro-organism the determination of *D*-values at different temperatures may be used to calculate its *z*-value, which is a measure of the influence of temperature change on heat resistance. The *z*-value is defined as the change in temperature required to change the *D*-value by 90 %. It has been found that the change in *D*-value with temperature is large (about 10-fold for a 10 K change). The holding times quoted in Table 1 are derived from knowledge of *D*-values of the common, most resistant organisms and experience. It is, however, important to note that the suspected presence of particularly resistant organisms, e.g. thermophilic spores, may require an increase in holding time. (See also note 5 to **11.4**.)

A.4 Autoclaving conditions and probability of survival

British Pharmacopoeia requirements for aqueous preparations and surgical materials are that a theoretical level of not more than one micro-organism survives in 106 containers (see British Pharmacopoeia 1988 VII, Appendix XVIII Annex 2 [7]). There is no universally accepted probability of survival for laboratory purposes. In laboratory practice, for make-safe loads, the high initial concentration is considered to be balanced by a higher acceptable probability of survival than in pharmaceutical preparations. This has allowed the time/temperature conditions adopted in the pharmaceutical field to be used for laboratory make-safe loads. The same time/temperature conditions are also used for sterilizing liquids or equipment and glassware; for these loads (but not for make-safe loads), times and temperatures may be reduced if necessary to minimize deterioration of the product. Account should also be taken of the contributory effect of high temperatures during the heat-up and cooling stages, to the degradation of culture media constituents.

A.5 Temperature/time integration

Methods have been described to calculate the lethal effects of heat-up and cooling of loads which, by reference to *z*-value curves, can be related to a standard reference process temperature. When the standard reference process temperature is 121.1 °C and the *z*-value is 10 K, the overall heat treatment delivered can be calculated in terms of values of F_o.

NOTE 1 F_o is a measure of the lethality obtained at any temperature referred to the time in minutes at 121.1 °C.

This method is used to control the process more precisely and minimize overheating, but its value is dependent on accurately monitoring the temperature within a load of containers so that the heat delivered to the monitored container(s) is representative of that throughout the load. It is therefore essential that the load has uniform heating characteristics.

NOTE 2 For further information see:

a) *Introduction to Sterilization and Disinfection and Infection Control* [8], by J F Gardner and M M Peel;

b) *Principles and Practice of Disinfection, Preservation and Sterilization* [9], by A D Russell, W B Hugo and G A J Ayliffe.

Annex B (normative) Validation

B.1 Validation test for liquids sterilization

B.1.1 *General*

The test assesses the effectiveness of the autoclave when processing each example load containing liquids.

Effectiveness is tested in respect of:

a) temperature distribution within the load;

b) ability to maintain the prescribed temperature for the set time throughout the load;

c) overall time taken to complete the operating cycle;

d) the effectiveness of the thermal door interlocks.

B.1.2 *Apparatus and materials*

B.1.2.1 *Test recorder(s)*, conforming to **1.4.3** of BS 2646-5:1993, with six input channels each connected to a sensor conforming to **1.4.6** of BS 2646-5:1993.

B.1.2.2 *Example load,* comprising containers of liquids selected with regard to the factors outlined in **13.2.1**.

B.1.3 *Preparation for the test*

B.1.3.1 Set the temperature activated door interlock (and timer activated door interlock) so that the largest container of liquid in regular use is cooled to between 65 °C and 80 °C before the autoclave door is released at the end of the operating cycle.

B.1.3.2 Position the sensors as follows:

a) in the chamber drain (or vent) within 100 mm of its connection to the chamber;

b) in the chamber free space;

c) in containers known to be the slowest:

1) to attain sterilizing temperature; and

2) to cool to 80 °C;

d) in the container known to be the fastest to attain sterilizing temperature;

e) in the chamber within 10 mm of, but not touching, the chamber base.

NOTE 1 In order to establish the positions for the containers mentioned in items c) and d) it is necessary to consult the recorded results of commissioning tests for liquids sterilization; some preliminary validation tests may also be required.

NOTE 2 The sensor mentioned in item e) is to measure the temperature of any liquid present in the chamber at the completion of the operating cycle.

Sensors positioned as in c) or d) can be inserted using a purpose-made or modified closure and entry gland or between the threaded neck and closure.

NOTE 3 A piece of glass tubing or heat-resistant plastic rod may be used to support the sensor where it will not be in contact with any surface but suspended in the liquid.

If a load temperature probe is fitted, place it in a container in the centre of the load and use it to support one of the sensors c) or d).

B.1.3.3 Place the load in the autoclave chamber on load supports or shelves.

B.1.3.4 Set the autoclave controller to produce the prescribed sterilizing temperature.

B.1.4 *Test procedure*

Operate the autoclave in accordance with the manufacturer's instructions for liquids sterilization. During each stage of the operating cycle:

a) monitor the temperatures recorded by the test recorder;

b) monitor and record the autoclave instrument readings;

c) measure, independently of the autoclave controller, and record:

1) the duration of the holding time;

2) the operating cycle time.

When the door securing mechanism releases at the end of the operating cycle, note the temperatures recorded by all of the test sensors.

If these temperatures do not exceed 80 °C, open the autoclave and remove the load.

B.2 Validation test for make-safe

B.2.1 *General*

The test assesses the effectiveness of the autoclave when processing each example load of laboratory discard material in respect of:

a) ability to maintain the prescribed temperature for the set time throughout the load;

b) overall time taken to complete the operating cycle;

c) the effectiveness of the thermal door interlocks.

B.2.2 *Apparatus and materials*

B.2.2.1 *Test recorder(s)*, conforming to **1.4.3** of BS 2646-5:1993, with six input channels each connected to a sensor conforming to **1.4.6** of BS 2646-5:1993.

B.2.2.2 *Example load,* comprising sufficient discard-containers to fill the usable chamber space, each of which is filled with containers and material selected with regard to the factors outlined in **13.3.1**. The load should include discard-containers chosen to present the most difficult challenge to heat penetration of any material likely to be processed, and also the largest container of liquid in regular use.

NOTE Typical difficult challenges are large quantities of disposable plastics items which melt, thus preventing air removal and heat penetration, and large numbers of small items, which hinder steam penetration and have a high thermal capacity.

B.2.3 *Preparation for the test*

B.2.3.1 The temperature activated door interlock (and timer activated door interlock) is set so that the largest container of liquid in regular use is cooled to 80 °C or below before the autoclave door is released at the end of the operating cycle.

B.2.3.2 Position the sensors as follows:

a) in the chamber drain (or vent) within 100 mm of its connection to the chamber;

b) in the chamber free space;

c) inside discarded items placed in discard-containers. The discard-containers are then placed within the load, in positions known to be the slowest:

1) to attain sterilizing temperature; and

2) to cool to 80 °C;

d) in the chamber within 10 mm of, but not touching, the chamber base.

NOTE 1 In order to establish the positions for discard-containers mentioned in item c), it will be necessary to consult the recorded results of commissioning tests for make-safe; some preliminary validation tests may also be required.

NOTE 2 The sensor mentioned in item d) is to measure the temperature of any liquid present in the chamber at the completion of the operating cycle.

If a load temperature probe is fitted, place it in the largest container of liquid likely to be included in a make-safe load, which is then placed in a discard-container, and use it to support a sensor.

NOTE 3 This position of the probe is for validation only. For the routine processing of infective material the probe should not be placed, by the operator, in a discard-container but in its holder or on the load support.

B.2.3.3 Place the example load in the autoclave chamber on load supports or shelves.

B.2.3.4 Set the autoclave controller to produce the prescribed sterilizing temperature.

B.2.4 *Test procedure*

B.2.4.1 Operate the autoclave in accordance with the manufacturer's instructions for make-safe.

B.2.4.2 During each stage of the operating cycle:

a) monitor the temperatures recorded by the test recorder;

b) monitor and record the autoclave instrument readings;

c) measure, independently of the autoclave controller, and record:

1) the duration of the holding time;

2) the operating cycle time.

When the door securing mechanism releases at the end of the operating cycle, note the temperatures recorded by all of the test sensors.

If these temperatures do not exceed 80 °C, open the autoclave and remove the load.

B.3 Validation test for equipment and glassware sterilization

B.3.1 *General*

The test assesses the effectiveness of the autoclave when processing each example load of laboratory equipment and/or glassware in respect of:

a) ability to maintain the prescribed temperature for the set time throughout the load;

b) overall time taken to complete the operating cycle;

c) the effectiveness of the thermal door interlock;

d) ability to effectively dry the load.

NOTE The thermal door interlock mentioned in item c) is the device set to the temperature of liquid in the chamber described in **9.5.1.1** of BS 2646-1:1993.

B.3.2 *Apparatus and materials*

B.3.2.1 *Test recorder(s)* conforming to **1.4.3** of BS 2646-5:1993, with four (or more) input channels each connected to a sensor conforming to **1.4.6** of BS 2646-5:1993.

B.3.2.2 *Example load,* comprising apparatus and glassware of the type and quantity expected to comprise autoclave loads. Items should be held or contained by a method which will preserve their sterility after the process, selected with regard to the factors outlined in **13.4.1**.

B.3.3 ***Preparation for the test***

B.3.3.1 Position the sensors as follows:

a) in the chamber drain (or vent) within 100 mm of its connection to the chamber;

b) in the chamber free space;

c) inside pieces of apparatus and glassware, placed on the load support in positions known to be:

1) the slowest; and

2) the fastest to attain sterilizing temperature.

If a load temperature probe is fitted, place it in its holder or on a load support.

Place the load in the autoclave chamber on load supports or shelves.

B.3.3.2 Set the controller to produce the prescribed sterilizing temperature.

B.3.4 ***Test procedure***

Operate the autoclave in accordance with the manufacturer's instructions for equipment and glassware sterilization.

During each stage of the operating cycle:

a) monitor the temperatures recorded by the test recorder;

b) monitor and record the autoclave instrument readings;

c) measure, independently of the autoclave controller, and record:

1) the duration of the holding time;

2) the operating cycle time.

When the door securing mechanism releases at the end of the operating cycle, note the temperatures recorded by all of the test sensors.

If these temperatures do not exceed 100 °C, open the door immediately after the completion of the operating cycle and observe all the items within the load for the presence of condensate.

List of references (see clause 2)

Normative references

BSI standards publications

BRITISH STANDARDS INSTITUTION, London

BS 2646, *Autoclaves for sterilization in laboratories.*

BS 2646-1:1993, *Specification for design, construction, safety and performance.*

BS 2646-4:1991, *Guide to maintenance.*

BS 2646-5:1993, *Methods of test for function and performance.*

Informative references

BSI standards publications

BRITISH STANDARDS INSTITUTION, London

BS 2646, *Autoclaves for sterilization in laboratories.*

BS 2646-2:1990, *Guide to planning and installation.*

Other references

[1] *Safety in Health Service Laboratories: Safe working and the prevention of infection in clinical laboratories,* 1991. London: HMSO.

[2] *Categorization of pathogens according to hazard and categories of containment,* 2nd edition 1990. London: HMSO.

[3] GREAT BRITAIN. Control of Substances Hazardous to Health Regulations (COSHH) (SI 1988 No. 1657, as amended by SI 1990 No. 2026, SI 1991 No. 2431 and SI 1992 No. 2382). London: HMSO.

[4] Health Notice HSG(93)26 *Decontamination of equipment prior to inspection, service or repair,* 1987. London: DoH.

[5] *Manual handling — Guidance on regulations,* Health and Safety Executive, 1992. London: HMSO.

[6] DHSS Letter DA(84)16, *Management of patients with spongiform encephalopathy (Creutzfeld-Jakob disease, CJD)*, 1984. Department of Health.

[7] *British Pharmacopoeia* 1988 VII, Appendix XVIII Annex 2.

[8] GARDNER J F and M M PEEL, *Introduction to Sterilization, Disinfection and Infection Control,* 2nd edition, Churchill-Livingstone, 1991.

[9] RUSSELL A D, W B HUGO and G A J AYLIFFE, *Principles and Practice of Disinfection, Preservation and Sterilization,* 2nd edition, Blackwell Scientific Publications, 1992.

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: 020 8996 9000. Fax: 020 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: 020 8996 9001. Fax: 020 8996 7001.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: 020 8996 7111. Fax: 020 8996 7048.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: 020 8996 7002. Fax: 020 8996 7001.

Copyright

BSI
389 Chiswick High Road
London
W4 4AL