



# **Scottish Health Technical Memorandum 2031**

(Part 1 of 1)

## **Clean steam for sterilization**

NHS in Scotland, HEEU, August, 1999

## Executive summary

The quality of steam supplied to a sterilizer can have a major influence on the efficacy of the sterilization process, the quality of the sterile product and the longevity and serviceability of the sterilizer and its associated equipment. Where concern for steam quality has traditionally focused on its physical characteristics - notably dryness and the presence of non-condensable gases - new European Standards supporting legislation governing the manufacture of medical devices require more comprehensive control of the purity of the sterilizing environment.

This SHTM discusses the nature, effects and sources of chemical and biochemical contaminants in steam, and proposes a readily achievable purity specification for “clean steam” to be used for sterilization. The specification is designed to meet regulatory requirements for medicinal products and medical devices without incurring excessive expenditure.

Clean steam is defined as steam whose condensate meets the purity requirements of Water for Injections BP (including a limit on pyrogens) with additional specifications to protect against corrosion of materials used in the construction of sterilizers and medical devices.

Practical guidance is given on the generation of clean steam from the following sources:

- a. from the existing mains steam supplies commonly used in hospitals;
- b. from dedicated clean-steam generators;
- c. in sterilizers with an internal steam supply, such as transportable sterilizers for unwrapped instruments and utensils.

With minor modifications and adjustments to operating practices, it should be possible to obtain clean steam from the majority of mains steam services currently installed in NHS hospitals. However, the necessary assurance that the supply continues to meet clean-steam specifications will require frequent testing of steam and feed water samples and close supervision of plant normally outside the control of the user of the sterilizer.

In the longer term, a dedicated clean-steam generator, solely supplying one or more sterilizers, is likely to prove a more reliable and economical source of clean steam.

Advice is given on the validation and periodic testing of clean-steam supplies, with guidance on methods of taking steam and water samples for analysis. Confirmation that a supply complies with the clean-steam specification requires a number of laboratory tests, most of which are based on those of the British Pharmacopoeia and are well within the capacity of any hospital pharmacy.

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# 1. Responsibilities

## Introduction

- 1.1 This chapter reviews the roles of the key personnel associated with the operation of a sterilizer and summarises their responsibilities with regard to clean steam.

## Key personnel

- 1.2 The following key personnel are referred to in this SHTM. Further information, including qualifications and general areas of responsibility, can be found in SHTM 2010: Part 1.
- 1.3 **Management** is defined as the person with ultimate management responsibility, including allocation of resources and the appointment of personnel, for the organisation in which the sterilizer is employed.
- 1.4 Depending on the nature of the organisation, this role may be filled by the general manager, chief executive, laboratory director or other person of similar authority. In small, autonomous installations the user may take on this function.
- 1.5 The **User** is defined as the person designated by Management to be responsible for the management of the sterilizer.
- 1.6 In a hospital the user could be a sterile services department manager, laboratory manager or theatre manager; in primary care he or she could be a general practitioner, dentist, or other health professional. Where a sterilizer is used to process medicinal products, the user is normally the Production Manager (see paragraph 1.13) in charge of the entire manufacturing process.
- 1.7 The **Authorised Person (Sterilizers)** is defined as a person designated by Management to provide independent auditing and advice on sterilizers and sterilization and to review and witness documentation on validation. The shorter term "Authorised Person" is used in this SHTM.
- 1.8 The Institute of Healthcare Engineering and Estate Management (formerly the Institute of Hospital Engineering) is the registration authority for Authorised Persons. The address is given in Appendix 1.
- 1.9 The **Test Person (Sterilizers)** is defined as a person designated by Management to carry out validation and periodic testing of sterilizers. The shorter term "Test Person" is used in this SHTM.

- 1.10 The **Maintenance Person (Sterilizers)** is defined as a person designated by Management to carry out maintenance duties on sterilizers. The shorter term “Maintenance Person” is used in this SHTM.
- 1.11 The **Microbiologist (Sterilizers)** is defined as a person designated by Management to be responsible for advising the user on microbiological aspects of the sterilization of non-medicinal products. The shorter term “Microbiologist” is used in this SHTM.
- 1.12 The **Competent Person (Pressure Vessels)** is defined as a person or organization designated by Management to exercise certain legal responsibilities with regard to the written scheme of examination of any pressure vessel associated with a sterilizer described in the Pressure Systems and Transportable Gas Containers Regulations 1989. The shorter term “Competent Person” is used in this SHTM.
- 1.13 The **Production Manager** is defined as a person designated by Management to be responsible for the production of medicinal products.
- 1.14 The **Quality Controller** is defined as a person designated by Management to be responsible for quality control of medicinal products with authority to establish, verify and implement all quality control and quality assurance procedures. (A similar role may be defined for the manufacture of medical devices, but this is rarely the practice in hospitals.)

### Responsibilities regarding clean steam

- 1.15 The **Authorised Person** will be able to advise the user on all aspects of the production and use of clean steam for sterilization.
- 1.16 The **User** will need to:
- appreciate the nature of contaminants in steam supply (especially pyrogens), their possible adverse effects and their sources;
  - understand the requirements of legislation on medicinal products and medical devices as regards sterilization;
  - be familiar with the current and impending standards on steam sterilization and their implications for steam quality;
  - understand the difference between process steam, clean steam and EN 285 steam and the appropriate applications of each;
  - understand the rationale for the clean steam specification;
  - understand the engineering principles required for the delivery of clean steam and how they may be realised for mains steam, dedicated steam generators and sterilizers with internal reservoirs;
  - with appropriate advice, decide whether clean steam is required for any sterilizer unit and if so, what is the best means of achieving it;



- h. after the required engineering work is complete, be satisfied that the chosen system is capable of supplying clean steam;
- i. appoint and liaise with a suitable laboratory for the analysis of steam and feed water samples;
- j. arrange for the steam supply to be formally validated;
- k. on completion of the validation tests, confirm that the sterilizer is fit for use with the steam supply;
- l. arrange for periodic maintenance of any steam generating and distribution plant under the user's control;
- m. arrange for periodic tests of the steam quality at intervals coinciding with periodic tests on the sterilizer.

1.17 The **Test Person** will need to:

- a. understand the operation of the apparatus for taking samples of steam condensate for field analysis (Chapter 6) and be trained in the method of its use;
- b. be aware of the correct procedures for collecting, preserving and handling samples;
- c. be trained in the measurement of electrical conductivity of water samples using a portable meter.

1.18 The **Maintenance Person** will need to:

- a. if maintaining transportable sterilizers, be aware of the guidance on cleaning and rinsing in Chapter 4;
- b. if maintaining clean-steam generators, be suitably trained and aware of the guidance in Appendix 2.

1.19 The **Microbiologist** will be able to advise on all microbiological aspects of clean steam, including avoidance of bacterial contamination and control of pyrogens.

## 2. Contamination in steam supplies

### Introduction

- 2.1 Recent years have seen a growing awareness of the need to improve the quality of steam used for sterilization, spurred on in part by regulatory requirements for medicinal products and medical devices, but also by increasing concern about the harmful effects that even minute quantities of contaminants may have upon patients.
- 2.2 This chapter discusses the adverse effects that impurities in the steam supply may have on patients, equipment and the sterilizer itself, identifies the products most likely to be susceptible to contamination and reviews the means by which various contaminants find their way into steam for sterilization.

### Why does contamination matter?

- 2.3 As will be discussed in Chapter 3, quality assurance in the manufacture of medicinal products and medical devices requires that the quality of the steam used in sterilization be known and controlled. The following sections identify a number of specific contaminants which are known to have adverse effects and whose presence in steam is therefore undesirable.

### Adverse effects on patients

- 2.4 Even small amounts of unwanted substances may be harmful to patients. The danger arises because certain medicinal products and medical devices may introduce contaminants directly into parts of the body that are normally protected by skin or mucous membranes. Water that is safe to drink, for example, may not be safe if injected into the bloodstream. Patients are particularly vulnerable to contaminants carried on sterile instruments precisely because such instruments are used to bypass the body's normal defences.
- 2.5 Several contaminants are known to have adverse effects on patients.
- Metals:** Many of these are toxic (some are cumulative poisons) and therefore their presence is undesirable. Metals of particular concern include cadmium, lead, mercury and other heavy metals.
  - Organic compounds:** Many of these are biologically active and therefore undesirable. The chief compounds of concern are filming amines and other chemicals that may be used in boiler treatment (see paragraph 2.29).
  - Micro-organisms:** Organisms of concern include all pathogens and all Gram-negative bacteria (which are sources of pyrogens).

- d. **Pyrogens:** These are bacterial endotoxins, predominantly derived from Gram-negative bacteria, which can cause severe reactions when administered intravenously (see paragraph 2.6).
- e. **Particulate materia:** Solid particles can lead to a number of adverse effects if injected into the body.

2.6 Pyrogens are of particular concern because, unlike other contaminants, there are no controls on the levels of pyrogens in public water supplies from which steam is generated. Moreover, they are extremely heat-stable and are only destroyed after prolonged exposure to high temperatures (3 hours at 180°C or 30 minutes at 250°C). They are not inactivated by any of the standard sterilization processes employed for medical devices and medicinal products. Control of pyrogens, then, is a priority for steam sterilization. Detailed information about pyrogens may be found in Appendix 3.

### Adverse effects on materials

- 2.7 As well as the obvious risks to patients, contaminants in steam may have a damaging effect on the materials of load items and the sterilizer itself.
- 2.8 Reactive contaminants in the steam may cause corrosion or otherwise impair the longevity or function of the product. Reactions may occur when contaminants interact directly with the product, or indirectly with materials that will subsequently come into contact with the sterilized product.
- 2.9 The steam also comes into direct contact with the internal surfaces of the sterilizer pressure vessel and associated equipment and instrumentation. Contaminants within the steam may react with the materials of construction and cause corrosion of the equipment or otherwise impair its longevity or function.
- 2.10 The reaction of steam with surfaces in contact is affected by its pH. In general steam of a low pH (acidic) will react with and dissolve metals. A pH of approximately 7 (neutral) is ideal and deviations towards alkaline (to e.g. pH 8) is acceptable.
- 2.11 Contaminants of concern include the following.
  - a. **Alkaline earth metals** cause “hardness” which can lead to build-up of lime scale on load items, in the sterilizer chamber and in pipework. Most problems are caused by calcium and magnesium, and to a lesser extent strontium.
  - b. **Iron**, whether in metallic or ionic form, is corrosive to stainless steel.
  - c. **Chlorides** in the presence of oxygen lead to pitting corrosion and (to a lesser extent) crevice corrosion in stainless steel. The effects can be controlled by limiting the amount of oxygen in the feed water (see paragraph 4.48).
  - d. **Phosphates** and silicates act to concentrate chloride ions and so promote their corrosive effects.

- 2.12 Clearly the materials used in the construction of load items and of the sterilizer itself will determine which contaminants are of greatest importance in each case. EN 285, the European Standard on porous-load sterilizers, offers guidance on materials of construction suitable for all steam sterilizers.
- 2.13 Steam sampling systems also must be constructed of materials which will not react with, and hence contaminate, the sample being collected. Suitable equipment is discussed in Chapter 6.

### **Products vulnerable to contamination**

- 2.14 Any product may become contaminated when the steam supplied to sterilizers comes into direct contact with it. Contaminants in the steam are deposited on the product as the steam condenses during the heating-up stage. The amount of steam condensing, and hence the amount of contamination deposited, is proportional to the heat capacity of the load item which in turn is proportional to its mass and the specific heat capacity of the material from which it is made. A massive metal item will therefore receive much more contamination than a light plastic item of similar size and shape heated to the same temperature.
- 2.15 The amount of contamination remaining at the end of the cycle, however, will depend on how much condensate is retained at the surface of the product. Where condensate can drain freely from unwrapped items, a small fraction of the deposited contaminants will be held in a thin film of water and the total amount remaining when the film is evaporated will be proportional to the exposed surface area of the item. Where condensate is trapped in cavities or held in the packaging close to the surface, the amount of contamination retained will be proportionally greater.
- 2.16 To some extent, packaging materials for steam processes (except fluids in sealed containers) have a filtering effect which protects against contamination. Particulate matter is normally trapped on the outer wrapping (giving rise to discoloured packs) but smaller particles and all molecules will pass through with the steam and be transferred to the product as the steam condenses on it. Performance requirements for packaging materials may be found in EN 868.

- 2.17 Whether such contamination has any adverse effect depends upon the nature and intended use of the product. Vulnerable products are:
- those which would permit direct transfer of contaminants to the patient, including:
    - medicinal products;
    - porous goods such as dressings and swabs;
    - surgical instruments and utensils;
  - those which would permit indirect transfer of contaminants to a patient, such as equipment used in pharmaceutical manufacturing (see paragraph 2.18 below);
  - those which would be impaired or inactivated by the presence of one or more of the possible contaminants. These include:
    - certain medicinal products;
    - laboratory products for in-vitro diagnostic use.
- 2.18 Various items of equipment used in the manufacture of sterile pharmaceuticals and medical devices are sterilized before use. It is important that during sterilization these items are not tainted with contaminants which may be transferred to the product being manufactured, whether that product is terminally sterilized or produced aseptically. Such items of equipment may include mixing vessels, filling heads, sterilization grade filters, filling lines, pipes and tubing for material transfer, connectors, and so on.

### Sources of contamination

- 2.19 Contaminants delivered to the sterilizer in steam may arise from a number of sources:
- contaminants present in the public water supply from which the steam is generated;
  - contaminants arising from treatment of the boiler feedwater;
  - contaminants arising in the distribution system carrying steam to the sterilizer.

### Public water supply

- 2.20 While the quality of mains water supplies differs considerably from place to place, it can normally be relied upon to meet the minimum standards set out in The Water Supply (Water Quality) (Scotland) Regulations 1990. These specify more than 50 limits for a wide range of impurities including dissolved minerals, organic compounds and micro-organisms.
- 2.21 There are no controls, however, on the amounts of atmospheric gases dissolved in mains water, all of which will be present in small and varying amounts. Air is the principal non-condensable gas that can impede steam

sterilization, and carbon dioxide and oxygen are important contributors to corrosion in boiler systems (see SHTM 2010: Part 2).

- 2.22 While mains water contains negligible numbers of pathogens and faecal contaminants (such as *Escherichia coli*) it may contain low numbers of other micro-organisms. Most water authorities use chlorine as a means of microbiological control. The disinfection effect of the chlorine may be largely lost, however, by the time the water reaches the point of use.
- 2.23 Water taken from the mains and subsequently kept in storage tanks before use may have significantly higher counts than the original mains water. Although bacteria tend to settle out on prolonged storage in reservoirs or lagoons, the intermittent throughput in storage tanks maintains their buoyancy and can cause counts to rise rapidly. Particularly in the summer months counts as high as  $10^5$ – $10^6$  ml<sup>-1</sup> may not be uncommon. This is of particular concern for sterilization since some 98% of the bacteria found in water supplies are reported to be Gram-negative bacteria, which are the predominant source of pyrogens (see Appendix 3).
- 2.24 There are no requirements for suppliers to measure or control the level of pyrogens in mains water.

#### **Boiler feedwater treatment**

- 2.25 Further contaminants may be introduced either deliberately or inadvertently as a result of treatments applied to mains water before it can be used as boiler feedwater.
- 2.26 Dealkalisation treatments can raise the levels of dissolved air and carbon dioxide.
- 2.27 Base-exchange water softeners remove calcium and magnesium ions from the water and replace them with sodium ions (see paragraph 4.43). Sodium levels will therefore be raised in mains water softened by this method. The use of brine to regenerate the ion-exchange beds may temporarily raise the level of chloride.
- 2.28 Bacterial growth may occur in water softening, deionisation or reverse osmosis plant unless the manufacturer's operating and maintenance procedures are strictly adhered to. While bacteria will not survive the steam generating process, the pyrogens they produce could be delivered to the sterilizer.
- 2.29 Any chemicals added to the boiler water may be carried into the steam as contaminants either in droplets of water entrained in the steam during the evaporative process or as volatile components present as gases. Filming amines (such as hydrazine), commonly used to protect condensate return systems, are toxic and should not be used where the steam is to be used for sterilization.

## Steam distribution system

- 2.30 Steam is chemically aggressive; the purer the steam the more reactive it is. Reaction with pipework and valves can lead to contamination of the steam with corrosion products such as magnetite ( $\text{Fe}_3\text{O}_4$ ). Often in the form of fine particulates, these products are not readily removed by the strainers normally installed in steam services. Users of old installations may have occasionally noted black or reddish brown discoloration of packaging material by particles of magnetite shed from the walls of the steam pipes.
- 2.31 The hydrogen liberated by the formation of magnetite (400 ml for each gram of iron) may contribute appreciably to the amount of non-condensable gases in the steam delivered to the sterilizer, especially in new installations with long pipe runs.
- 2.32 Contamination is also likely to arise at points where water can collect, such as dead-legs, gauges and poorly maintained traps. Trapped water can result in rust, which can be shed into the steam as particles, and bacterial growth, which can lead to the formation of bio-films which periodically generate high levels of contamination as they slough off.
- 2.33 Guidance on avoiding contamination from mains steam distribution systems may be found in paragraphs 4.20–4.24.



### 3. Steam quality requirements

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#### Introduction

- 3.1 This chapter discusses the purity requirements for steam to be used in sterilization, with special emphasis on the grade of “clean steam” recommended for general use within the NHS.

#### Regulatory requirements

- 3.2 The move towards higher quality steam for sterilization has been brought about, in the main, by regulatory requirements for the manufacture of medicinal products and, more recently, sterile medical devices (see SHTM 2010: Parts 1 and 4 for a summary of the relevant legislation). In both cases there is a clear principle that products should not be adulterated with unwanted or unspecified compounds during sterilization, or any other stage in processing. Such an objective can only be attained if the physical, chemical and biological properties of steam coming into contact with the product are known and controlled.

#### Medicinal products

- 3.3 Annex 1 of the ‘The Rules governing medicinal products in the European Community: Volume IV: Good manufacturing practice for medicinal products’ states “*Care should be taken to ensure that steam used for sterilization is of suitable quality and does not contain additives at a level which could cause contamination of product or equipment.*”
- 3.4 The steam quality need not be very high where the product does not come into direct contact with the steam. This is the case for aqueous products processed in fluid sterilizers, provided that the method of sealing the containers has been validated and shown to have a quantified risk of failure and that failed containers can be readily identified and removed (see SHTM 2010: Part 4 for details). However, such assurance normally requires a degree of testing and monitoring of containers that may not be justified in smaller hospital pharmacies. It may be more cost-effective to ensure that the steam is of sufficient quality that a failure of a seal will not have adverse effects on the product.
- 3.5 Further guidance on legislation governing medicinal products may be obtained from the Medicines Control Agency whose address may be found in Appendix 1.



## Medical devices

- 3.6 Annex I of the Medical Devices Directive, implemented by the Medical Devices Regulations 1994, lists a number of “essential requirements” for the manufacture of medical devices. Section 7.2 requires that devices are *“designed, manufactured and packaged in such a way as to minimise the risk posed by contaminants and residues to the persons involved in the transport, storage and use of the devices and to the patients, taking account of the intended purpose of the product.”* This has clear implications for the quality of steam used in sterilization processes.
- 3.7 The Directive is supported by the European Standard on validation and monitoring of moist heat sterilization (EN 554) which requires that the *“purity of the sterilizing environment in contact with the medical device shall not affect the safety of the product.”*
- 3.8 In practically all steam sterilization processes, the medical device comes into direct contact with the steam and therefore the quality of the steam must be known and controlled. Steam quality is also of concern in ethylene oxide sterilizers in which steam is used for humidification and therefore, again, comes into direct contact with the medical devices.
- 3.9 Further guidance on legislation governing medical devices may be obtained from the Medical Devices Agency. The address may be found in Appendix 1.

## Requirements of SHTM 2010

- 3.10 SHTM 2010 is the NHS in Scotland Estates Environment Forum's guide to sterilization in Scotland. Part 3 describes steam quality tests for determining the non-condensable gas content, dryness and superheat values of steam supplied to porous load sterilizers, LTSF and LTS disinfectors. The steam quality specified is as follows:
- the volume of non-condensable gases should not exceed 3.5 ml for every 100 ml of displaced water when measured by the method given in SHTM 2010: Part 3 (this is not equivalent to a fraction of 3.5% by volume of the steam, as incorrectly implied in EN 285 and elsewhere);
  - the superheat measured on expansion of the steam to atmospheric pressure should not exceed 25°C when measured by the method given in SHTM 2010: Part 3;
  - the dryness value should be not less than 0.9 (or, if only metal loads are to be processed, not less than 0.95) when measured by the method given in SHTM 2010: Part 3.

- 3.11 This specification, which complies with both EN 285 and BS 3970: Part 1, addresses the basic requirements for assurance that the sterilization process is carried out under moist heat conditions, without excessive moisture, and without random, localised, impairment of the sterilization conditions caused by excessive amounts of non-condensable gases. The condensate from the steam should be clear, colourless and free from oil and particulates. To meet this specification steam should be generated by boiler plant which is designed, operated and maintained in accordance both with the recommendations of SHTM 2010 and of the manufacturer. Experience shows that these requirements are readily met in the majority of hospitals.
- 3.12 Saturated steam, which is clean and substantially free from moisture and non-condensable gases, is the minimum standard required for all sterilization processes.
- 3.13 The SHTM 2010 requirements, however, say little about the purity of steam for sterilization. From the discussion of the adverse effects of contaminants (see Chapter 2) it is apparent that different minimum specifications could be devised for each of the possible applications and for each of the available sterilization processes. Ideally one would review the nature and intended use of the process, together with any constraints imposed by the materials of which the distribution system and sterilizer are constructed, and select a specification appropriate to the particular circumstances. For specialised products it may be necessary to specify limits for a particular contaminant not considered in this general guidance. Such procedures would be grossly impractical, however, for the wide range of products processed in hospitals.
- 3.14 Although steam of the highest possible purity may be suitable for all applications it is significantly more expensive to produce than steam of a lower standard. Chemically pure steam is also highly corrosive.
- 3.15 There is a clear need for a steam purity specification that would meet regulatory requirements and which could be attained in hospitals without excessive expenditure. The rest of this chapter discusses three proposed grades of steam: process steam, EN 285 steam and clean steam. They are summarised in Table 1.

**Table 1: Classification of steam quality**

Steam quality	Description	Sterilizer applications
Process steam	All-purpose steam supply, not optimised for sterilization.	Fluid sterilizers with validated closure systems Laboratory sterilizers (make safe cycles).
Clean steam	Steam whose condensation meets the specification for clean steam in Table 2.	Porous load, unwrapped instruments, LTS and LTSF sterilizers processing medical devices.  Fluid sterilizers without validated closure systems  Ethylene oxide sterilizers processing medical devices (steam for humidification)  Laboratory sterilizers (loads vulnerable to contamination)
EN 285 steam	Steam whose condensate meets the specification for EN 285 steam in Table 3.	Not required in the NHS

*Note: Clean steam may be used for all sterilizer applications*

## Process steam

- 3.16 “Process steam” is defined here as general-purpose steam whose quality has not been optimised for sterilization.
- 3.17 Where it is not intended to be in direct contact with medical devices, medicinal or culinary products, no specific physical, chemical or biological contamination limits are set. The steam may contain various volatile additives (such as those intended to inhibit corrosion in condensate return pipes) which are unacceptable for topical, enteral or parenteral administration to human beings.
- 3.18 Process steam intended for use as a heating medium in culinary applications, where it is in direct contact with food products or food contact surfaces, is sometimes known as “potable steam”. The condensate from such steam should then meet the purity requirements of drinking water.
- 3.19 The recommendation of this SHTM is that process steam, as defined above, is not acceptable for sterilizers in which medicinal products and medical devices are in contact with steam and therefore vulnerable to contamination. It may also be unacceptable for certain loads processed in laboratory sterilizers, but may be used where discard loads only are to be processed (see Table 1).

## EN 285 steam

- 3.20 EN 285 is the draft European Standard on large steam sterilizers (essentially porous load machines). When EN 285 was being developed it was considered desirable to include recommendations on the quality of steam with which a sterilizer should be designed to operate. The result was a specification both for steam condensate and feedwater that would ensure that the steam environment in the chamber would not “impair the sterilization process or harm the sterilizer or sterilized load.” Identical recommendations are likely to appear in a future standard on sterilizers for unwrapped instruments and utensils. EN 1422, which sets out requirements for EO sterilizers, also recommends limits on impurities in steam used for humidification, although the permitted levels are generally higher than those of EN 285.
- 3.21 “EN 285 steam” is defined here as steam whose condensate complies with the specification recommended in EN 285 and reproduced in Table 3. It should be emphasised that steam of this quality is a recommendation and not a requirement. Sterilizer plant may conform fully to EN 285 without meeting the recommended specification for steam purity.
- 3.22 While EN 285 steam is appropriate for its intended use, it was not designed to meet the requirements of the legislation and standards on medicinal products and medical devices. It is not regarded as suitable for use in NHS hospitals for the following reasons:
- a. EN 285 steam is designed primarily to protect materials, not patients; it does not, for example, set limits on pyrogens;
  - b. steam of this purity is chemically aggressive and will attack many materials, including iron, steel and copper, commonly found in existing steam distribution systems, sterilizers and sterilizer loads;
  - c. it is unlikely that steam of this purity can be generated and delivered with the steam systems currently used in NHS hospitals without excessive engineering costs.
- 3.23 There appear to be few, if any, sterilizer applications in which EN 285 steam would be preferable to “clean steam” as discussed below. The recommendation of this SHTM is that EN 285 steam is unnecessary for sterilizers in use in the NHS.

## Clean steam

- 3.24 The concept of “clean steam” has been developed to meet all regulatory requirements while meeting a reasonable standard of purity that can be readily attained in hospitals without excessive expenditure.
- 3.25 The recommendation of this SHTM is that clean steam should be provided for all clinical sterilizers where the steam may come into direct contact with

medical devices, medicinal products or equipment intended for use in the manufacture of medicinal products or medical devices. It may also be required for use with laboratory sterilizers where the product is sensitive to contamination. It is expected that clean steam will in due course become the norm for all sterilization applications in the NHS.

- 3.26 Clean steam is defined as steam whose condensate meets the specification given in Table 2. This specification is compared with those for drinking water and EN 285 steam in Table 3.

**Table 2: Specification for clean steam.**

Determinand	Value	Recommended test for compliance
<i>Based on Sterilized Water for injections BP:</i>		
Acidity or alkalinity	NQ	BP test. Tests for pH are not an acceptable substitute
Ammonium	0.2 mg litre <sup>-1</sup>	BP test or other suitable method
Oxidisable substances	NQ	BP test
Calcium and magnesium	NQ	BP test. Tests for hardness are not an acceptable substitute
Heavy metals substitute	0.1 mg litre <sup>-1</sup>	BP test. Tests for individual elements are not an acceptable substitute.
Chloride	0.5 mg litre <sup>-1</sup>	BP test or other suitable method.
Nitrate	0.2 mg litre <sup>-1</sup>	BP test or other suitable method.
Sulphate	NQ	BP test.
Residue on evaporation	30 mg litre <sup>-1</sup>	BP test. Conductively measurement is not an acceptable substitute.
Pyrogens	0.25 EU ml <sup>-1</sup>	BP test.
<i>Based on EN 285:</i>		
Phosphate	0.1 mg litre <sup>-1</sup>	Any suitable method.
Silicate	0.1 mg litre <sup>-1</sup>	Any suitable method.
<i>Routine monitoring only:</i>		
Electrical conductivity at 25°C	35 µS cm <sup>-1</sup>	See Appendix 4 and Chapter 7.

NQ=not quantified; BP=British Pharmacopoeia; EU=endotoxin unit.

- 3.27 The purity requirements are defined in terms of physical, chemical and biochemical properties and are independent of any engineering measures that may be needed to attain them. The quality specified is to be determined at the point of delivery to the sterilizer. Provided that a suitable quality can be attained and sustained, and that the process has been validated, the source of steam can be selected on economic grounds.
- 3.28 Test schedules to demonstrate compliance of a condensate sample are discussed in Chapter 5, with sampling methods in Chapter 6 and methods of analysis in Chapter 7.
- 3.29 The rationale for the clean steam specification is discussed below under the headings of health and safety, sterilizer protection and routine monitoring.

### **Health and safety**

- 3.30 Rather than make detailed assessments of the health and safety implications of all possible steam contaminants and determine safe levels for each, this SHTM adopts Water for Injections BP (identical to Water for Injections PhEur) as a suitable standard that clean steam condensate should meet. WFI has been in use throughout Europe and elsewhere for many years as the basis for pharmaceutical preparations to be administered intravenously. Steam condensate meeting the requirements of WFI can therefore be regarded as free of harmful contaminants.
- 3.31 The British Pharmacopoeia (BP) defines two grades of WFI: Water for Injections in Bulk (for use in the manufacture of medicinal products) and Sterilized Water for Injections (essentially WFI in Bulk that has been bottled and sterilized, and intended for dilution of medicinal products for injection). Clean steam is based upon the requirements for Sterilized WFI; experimental measurements with WFI in Bulk show that it is too acidic for the materials used in sterilizers.
- 3.32 The BP defines WFI both in terms of its means of production and in terms of a number of tests for specified contaminants.

**Table 3: Comparison of clean steam with drinking water and EN 285 steam**

Determinand and unit	Maximum permitted values		
	Drinking water (a)	Clean steam condensate (b)	EN 285 steam condensate (c)
Acidity or alkalinity	-	NQ	-
<i>Degree of acidity(pH)</i>	<i>5.5 -9.5</i>	-	<i>5 - 7</i>
Ammonium, NH <sub>4</sub> (mg litre <sup>-1</sup> )	0.5	0.2	-
Calcium & magnesium (mg litre <sup>-1</sup> )	300	NQ	-
<i>Total hardness, CaCO<sub>3</sub> (mg litre<sup>-1</sup>)</i>	<i>&gt; 150 (d)</i>	-	<i>2.0 (e)</i>
Heavy metals (mg litre <sup>-1</sup> )		0.1 (f)	
<i>Iron, Fe (mg litre<sup>-1</sup>)</i>	<i>0.2</i>	-	<i>0.1</i>
<i>Cadmium, Cd (mg litre<sup>-1</sup>)</i>	<i>0.005</i>	-	<i>0.005</i>
<i>Lead, Pb (mg litre<sup>-1</sup>)</i>	<i>0.05</i>	-	<i>0.05</i>
<i>Heavy metals (mg litre<sup>-1</sup>) other than Fe, Cd, Pb</i>		-	<i>0.1 (g)</i>
Chloride, Cl (mg litre <sup>-1</sup> )	400 (h)	0.5	0.1
Nitrate, NO <sub>3</sub> (mg litre <sup>-1</sup> )	50	0.2	-
Sulphate, SO <sub>4</sub> (mg litre <sup>-1</sup> )	250	NQ	-
Oxidisable substances	-	NQ	-
Residue on evaporation (mg litre <sup>-1</sup> )	1500 (h)	30	1
Silicate, SiO <sub>2</sub> (mg litre <sup>-1</sup> )	-	0.1	0.1
Phosphate, P <sub>2</sub> O <sub>5</sub> (mg litre <sup>-1</sup> )	10 (i)	0.1	0.1
Conductivity at 20°C (µS cm <sup>-1</sup> )	1500 (h)	35 (j)	3
Bacterial endotoxins (EU ml <sup>-1</sup> )	-	0.25	-
Appearance		Clear, colourless	Colourless, clean without sediment

*Entries in italics are not applicable to clean steam.*

NQ. not quantified

- Source: The Water Supply (Water Quality) (Scotland) Regulations 1990
- See paragraphs 3.24 onward
- Source: EN 285 (draft)
- Expressed in EN 285 as 0.02 mmol litre<sup>-1</sup>
- Expressed in the Regulations as > 60 mg litre<sup>-1</sup> Ca
- See paragraph 3.38
- Identity of heavy metals not specified
- 12-month average.
- Expressed in the Regulations as 2.2 mg litre<sup>-1</sup> P
- At 25°C



- 3.33 First, WFI “is obtained by distilling potable water or Purified Water from a neutral glass, quartz or suitable metal still fitted with an effective device for preventing the entertainment of droplets; the apparatus must be correctly maintained to ensure the production of apyrogenic water.” A high degree of purity is clearly implicit in this statement. For example, a sample of tap water treated chemically to remove only the impurities specified in the BP would not be WFI. For the purposes of this SHTM, the guidance on clean steam generation in Chapter 4 is deemed to meet the distillation requirement for WFI.
- 3.34 Second, Sterilized WFI is required to comply with a number of tests designed to confirm that a given water sample contains less than a certain amount of a specified contaminant. Test procedures are given in the BP and reproduced here in Appendix 4.
- 3.35 It has to be said that these tests are not entirely satisfactory as a specification for clean steam. They employ traditional reagent methods which rely upon the observation of colour changes, and are poor at determining the extent to which a sample deviates from specification. Moreover, limits are not always quantified: concentrations are quoted for some contaminants but not others. For this reason it has not been possible to set numerical limits for all the contaminants in Table 2. On the other hand, the tests can be carried out in any hospital pharmacy and do not require the facilities of a specialised analytical laboratory.
- 3.36 Where no concentration is quoted (*acidity or alkalinity, calcium and magnesium, oxidisable substances and sulphate*), the only safe way of ensuring that a sample meets the WFI specification is to conduct the test described in the BP; there are no generally accepted equivalent concentrations.
- 3.37 Where the BP does quote an equivalent concentration, however (*ammonium, nitrate, chloride and residue on evaporation*), the way is open to employing a variety of modern analytical techniques to demonstrate compliance (see Chapter 7), though the stated concentrations are not precise and should be treated with caution.
- 3.38 While the BP quotes a concentration for *heavy metals* (expressed as Pb), the test responds to different metals in varying degrees and it is not possible to express the BP limit as a simple sum of individual elements. For this reason the BP test alone should be used to ascertain compliance; alternative methods are not recommended (see paragraphs 7.18–7.20 for more details).
- 3.39 The limit for *pyrogens* is 0.25 endotoxin units (EU) ml<sup>-1</sup> (see Appendix 3 for a discussion on the meaning of the endotoxin unit). The BP test for pyrogens (bacterial endotoxins) is the LAL test described in Appendix 4.
- 3.40 It is likely that these traditional tests will be replaced by more precise quantitative tests in the future. If so, the requirements for clean steam will be modified accordingly. *Clean steam should always comply with the current pharmacopoeial specification for Sterilized Water for Injections.*



### Sterilizer protection

- 3.41 The WFI specification is designed to ensure that water can be administered safely by injection and is therefore regarded as a suitable minimum standard for health and safety purposes. It is not, however, concerned with effects on materials, and so additional specifications have been added to lessen the corrosion problems discussed in Chapter 1.
- 3.42 The levels at which *phosphate* and *silicate* begin to contribute to corrosion are poorly understood and little experimental work has been done. The levels in Table 2 have therefore been taken from the EN 285 specification without modification.
- 3.43 The BP test for *chloride* is considered adequate to limit its corrosive effects on stainless steel.

### Routine monitoring

- 3.44 For the reasons explained in Chapter 7, *electrical conductivity* is a convenient diagnostic tool for routine monitoring of steam quality once the system has been validated. The BP does not specify a conductivity for Sterilized WFI. While it is possible to determine a corresponding value experimentally, experience shows that the evaporative residue in steam samples is considerably lower than the BP value of 30 mg litre<sup>-1</sup> and therefore a correspondingly lower conductivity would be appropriate for routine monitoring. A figure of 35 µS cm<sup>-1</sup> has been adopted as a reasonable upper limit for contaminants that may be found in the steam supply. Conductivity is to be measured only during field testing of the steam supply and is not specified where samples are subject to a full laboratory analysis.
- 3.45 The drinking water and EN 285 conductivities are specified at 20° C, which is below room temperature in many sterilizer installations. A standard temperature of 25° C has been chosen for clean steam because it can normally be attained without the need for refrigeration.

## 4. Clean steam in practice

### Introduction

- 4.1 This chapter discusses the principles by which steam conforming to the clean-steam specification of Chapter 3 may be generated. It offers practical guidance on how to achieve clean-steam standards for sterilizers supplied by mains steam, sterilizers supplied by a dedicated clean-steam generator and for sterilizers (such as transportables) which generate steam from an internal reservoir.
- 4.2 Full costings should be obtained when the relative merits of different steam supplies are being assessed. The cost of the testing required to demonstrate that a mains steam system can consistently produce clean steam may amount to a considerable fraction of the capital cost of a dedicated clean-steam generator.

### How steam is made

- 4.3 At first sight it may be surprising that there should be any contaminants in steam at all. Steam is generated by boiling, in which liquid water is converted into a gas. One might expect that any impurities in the water would be left behind, as in distillation, while pure steam in the form of H<sub>2</sub>O molecules was delivered to the sterilizer.
- 4.4 Boiling occurs at a temperature where evaporated water vapour has sufficient pressure to displace the water immediately below the surface to form bubbles of steam. (At lower temperatures evaporation occurs only from the surface.) The bursting of bubbles from the surface of the boiling water is accompanied by the ejection of small droplets of water. *These droplets contain the same dissolved and suspended solids that are present in the water in the boiler.* They are readily entrained in the flow of steam and thus carry contaminants to the sterilizer. Even if the water droplets subsequently evaporate, the contaminants will still be present in the form of solid particles.
- 4.5 “Priming” is a related phenomenon where significant quantities of the boiler water can sporadically be carried over into the steam. This is often as a result of a sudden increase in the demand for steam, which reduces the pressure above the water and effectively lowers the boiling point, so increasing the violence of bubbling. A too-high level of water in the boiler can also lead to priming. Priming can be reduced by standard good operating practice, such as running the boiler at or near its maximum permissible pressure, using pressure-reducing valves where the demand causes a reduction in pressure in the distribution system, and maintaining correct water levels.

- 4.6 High concentrations of impurities in the boiler water also promote carry-over. They reduce the surface tension and so increase the agitation of the water surface. They can also cause the formation of a stable foam above the water surface leading to severe carry-over. Slugs of water are intermittently discharged from the boiler along with the steam, severely prejudicing the quality of the steam.
- 4.7 A crucial aspect of boiler design, therefore, is to ensure the best possible separation and removal of such entrained moisture.

### **Summary of requirements for clean steam**

- 4.8 From the above considerations and the discussions in Chapter 1, the requirements for generating clean steam can be summarised as follows:
- a. feedwater should be as free as possible of contaminants, especially those specified for clean steam in Table 2;
  - b. the boiler should be designed to prevent water droplets being carried over into the steam;
  - c. the boiler should be operated to prevent foaming and priming;
  - d. the distribution system carrying steam from the boiler to the sterilizer should be resistant to corrosion.
- 4.9 It is apparent that a boiler system designed and operated to provide minimal carry-over of entrained water droplets will be able to maintain a low level of contaminants in the steam even where the quality of feedwater is poor. Feedwater treatment, then, may not be the decisive factor in the ability of a system to deliver clean steam. However, if the feedwater is of low quality, even small deviations from optimum operating conditions may result in large amounts of contaminants being carried over and delivered to the sterilizer. The designer of a robust clean-steam supply will therefore ensure that all the above requirements are met.
- 4.10 A suggested process for assessing how a clean steam supply may be achieved is illustrated in Figure 1.

### **Clean steam from the mains steam supply**

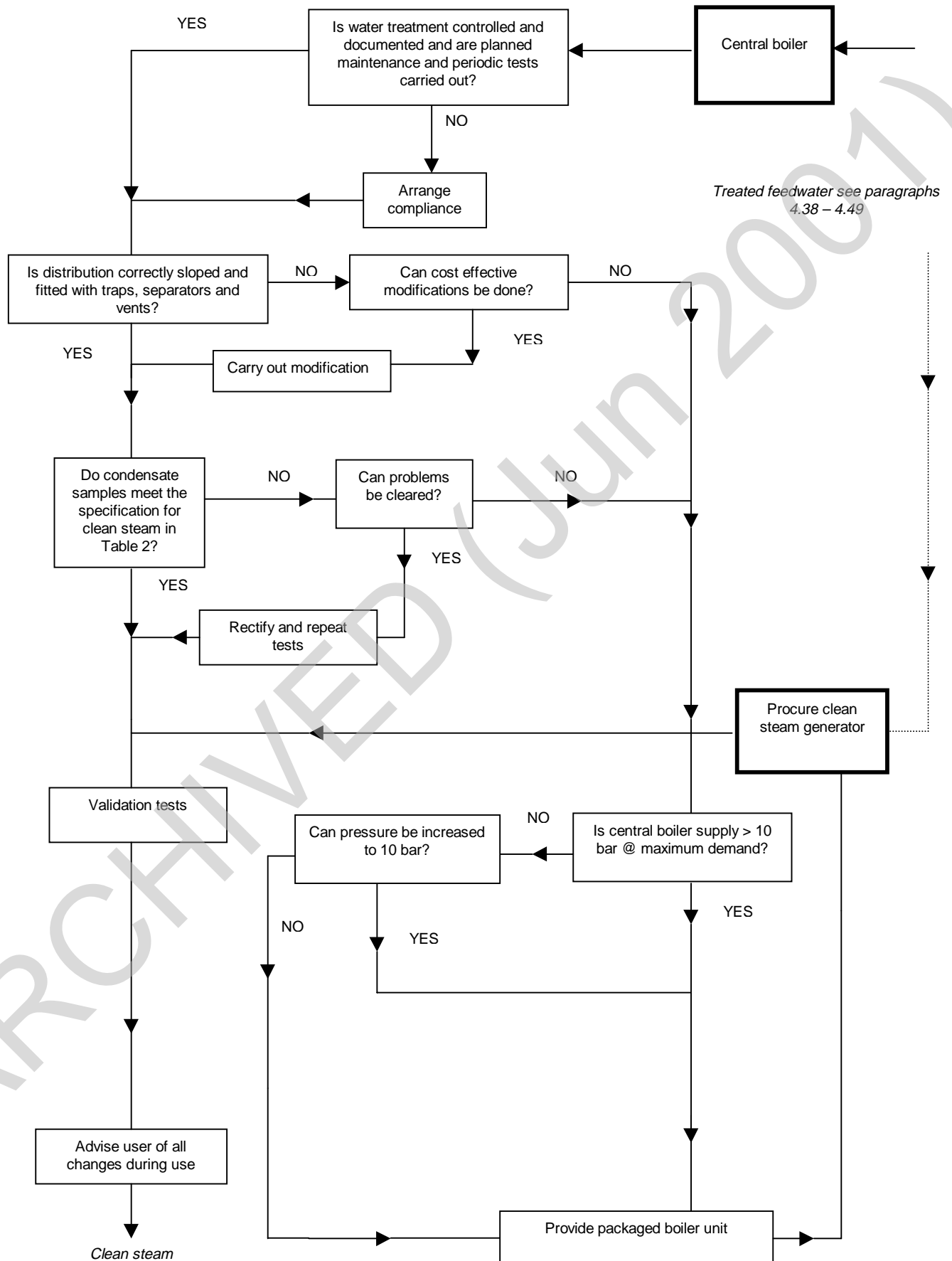
- 4.11 Recent tests have shown that clean steam can be obtained from well-designed, constructed and operated conventional boilers and distribution systems of the type found in most NHS hospitals. If steam from this source is chosen, it is essential to demonstrate compliance and identify maintenance and boiler treatment regimes necessary for reproducibility.
- 4.12 Where a central supply does not deliver steam of acceptable standard, it is possible that the quality may be sufficiently improved by changes in operating practice and relatively minor engineering modifications. However, it is unlikely to be economical to embark on extensive remedial works such as the

introduction of new feedwater treatment plant or the replacement of distribution pipework. It may be more cost-effective to install a dedicated clean-steam generator solely to supply sterilizers (see paragraph 4.26 onwards).

### **Boiler design and operation**

- 4.13 The first step in assessing whether clean steam can be supplied from the mains is to examine the design and operation of the boiler plant.
- 4.14 An important consideration is the proportion of boiler feedwater that is fresh "make-up" water rather than steam condensate returned from the distribution system. In most large hospitals where steam is supplied centrally only a small fraction of the steam demand is due to sterilizers (which discharge most of their condensate to waste) and therefore the bulk of the condensate is returned to the boiler. This makes it more feasible to control the level of contaminants in the boiler. While the nature of the feedwater treatment is also of importance, the requirements for clean steam are unlikely to be achieved if the proportion of make-up feedwater exceeds 15%.
- 4.15 The level of total dissolved solids (TDS) in the boiler water is an important factor both in the prevention of foaming (see paragraph 4.6) and for the contaminants that may be present in the entrained water droplets. Acceptable TDS levels if clean steam is to be produced are typically below 2000 ppm. While some control of TDS concentration can be exercised by appropriate feedwater treatments, the boiler usually has a "blow-down" facility to allow accumulated sludge to be expelled from the bottom of the vessel. The water level gauge and TDS sensor element should also be blown down at regular intervals.

**Figure 1: How to provide clean steam**



- 4.16 Filming amines, which are often added to feedwater to prevent corrosion of condensate return pipes, are toxic and are not acceptable for boilers supplying clean steam for sterilizers. If it is not possible for the boiler to be operated without filming amines, then another source of steam must be found.
- 4.17 While the boiler is unlikely to have been designed with the requirements of clean steam in mind, it should nonetheless have some means of preventing water being carried over into the steam. The chief precaution against carry-over is good practice in operating the boiler so that foaming and priming do not occur (see paragraph 4.5). Discussion with boiler-room staff will ascertain the degree to which operating procedures are successful in this regard.
- 4.18 Steam sampling points on the boiler, as discussed in Chapter 5, are desirable and should be installed if they are not already fitted.
- 4.19 As the operational management of the steam supply will normally be outside the user's control, the user will also need to assess whether the boiler-room management are aware of the principles of clean steam and whether the necessary cooperation will be forthcoming (see paragraph 4.25d). Well-trained and knowledgeable boiler personnel, and clean and tidy working conditions, are all good signs.

### **Distribution system**

- 4.20 The distribution system also influences the quality of steam delivered to the sterilizer. The design of distribution systems suitable for the delivery of dry, saturated steam is considered in SHTM 2010; Part 2.
- 4.21 A purpose-built distribution system for clean steam would normally be constructed of stainless steel. However, when a large conventional installation has been in use for a number of months, a hard protective layer of oxide (magnetite) may have formed on the inside of the steam pipes (see paragraph 2.30). Providing the steam condensate is neutral or alkaline, this coat will remain intact and permit the use of the pipework for the distribution of clean steam. Acidic condensate in the presence of moist air, however, can break down the layer leading to corrosion which may then be shed as contaminating particles.
- 4.22 As a precaution, final steam filters capable of removing all particles down to 5  $\mu\text{m}$  in size should be installed on all distribution systems.
- 4.23 It is important that the distribution system is free of dead-legs and other places where condensate may become trapped. During periods when the steam supply is off, such accumulations may become a focus of microbial growth. The trapped water may then be swept up into the steam when the supply is restored. Although the micro-organisms may be killed by the steam, pyrogens will not be inactivated at the temperature of the steam and may be delivered to the sterilizer.
- 4.24 Other key points for a distribution system suitable for clean steam include:

- a. correctly sized automatic air vents throughout the pipework distribution system to minimise the amount of air and other non-condensable gases delivered to the sterilizer;
- b. properly sized and selected steam traps to remove condensate;
- c. steam pipeline velocities kept below  $25 \text{ m s}^{-1}$  to allow steam traps to remove entrained moisture effectively and to prevent condensate being drawn out of them;
- d. steam separators near the steam take-off on boiler plant prone to generating wet steam;
- e. strainers to protect control valves, steam traps, etc.

### Quality assurance

4.25 Where a mains steam supply is found to be capable of meeting the clean-steam specification, users should assess whether the steam quality can be maintained under all operating conditions. There are several points to consider.

- a. Frequent testing of the steam at the sterilizer will be required to provide assurance that the clean-steam specification is consistently met.
- b. Competing demands on the steam service from other units in the hospital may degrade the steam quality at the sterilizer.
- c. Steam quality is apt to vary through the year as the boiler room responds to changing seasonal demands.
- d. An otherwise effective clean steam supply may quickly deteriorate if appropriate periodic maintenance is not carried out.
- e. Arrangements should be made for the user to be warned of imminent engineering modifications, maintenance and changes in steam generation, distribution and operating practice. If changes are likely to be made without the user's knowledge, the supply cannot be considered a reliable source of clean steam.

### Clean steam from a clean-steam generator

4.26 A dedicated clean-steam generator, whether supplying one or several sterilizers, is the recommended solution where clean steam cannot be reliably obtained from the mains supply. Since the bulk of the condensate from sterilizers is discharged to waste and not returned to the boiler, such generators may have to run on practically 100% make-up feedwater.



- 4.27 A dedicated system must therefore:
- minimise the amount of non-condensable gases and other contaminants in the boiler feedwater;
  - prevent liquid water leaving the boiler and being delivered in the steam;
  - prevent microbial growth in any storage tank or pipework;
  - be constructed from materials resistant to corrosion and particle shedding, such as low-carbon stainless steel (type 316L).
- 4.28 The capacity of the generator should be sufficient to meet both maximum and minimum demands while still maintaining the requirements for dryness and non-condensable gases specified in SHTM 2010: Part 3 (see paragraph 3.10).

### **Moisture separation**

- 4.29 An essential component of a clean-steam generator is a means of separating entrained water droplets from the steam before it is delivered to the sterilizer. The baffles used in some conventional boilers are not normally adequate for this purpose, but good results have been obtained on experimental machines using cyclonic separators which essentially spin-dry the steam by causing it to rotate at high speeds.
- 4.30 The manufacturer will have measured the efficiency of moisture removal by spiking the feedwater with high levels of endotoxin (at least  $10^3$  EU ml<sup>-1</sup>) and testing samples of the steam for endotoxin levels by means of the LAL test (see Appendix 4). (This work should be undertaken only by personnel with appropriate training and experience.) Tests on an experimental clean-steam generator have shown that reduction factors greater than 105 can be consistently achieved.
- 4.31 Adequate moisture removal should be maintained over the entire range of steam demand, typically up to 200 kg h<sup>-1</sup> for each sterilizer.

### **Heating**

- 4.32 A single 500-litre porous-load sterilizer requires a steam generator capable of converting energy at a rate of up to 50 kW. A group of sterilizers will require a proportionately higher heating power.
- 4.33 Where existing sterilizers are supplied from a central boiler the ideal solution is to install a generator heated by mains steam. The steam generator is then effectively a steam-to-steam calorifier, in which the mains steam is used only to heat the feedwater and does not come into contact with the clean steam for the sterilizer. Primary steam requirements for this type of calorifier will normally be 300 kg h<sup>-1</sup> for each sterilizer at a minimum pressure of 10 bar and operating on 100% condensate return. Where mains steam is not available, a small packaged boiler may be a convenient source of steam for heating, but should not itself be regarded as a source of clean steam.



- 4.34 Generators may be heated by electricity, but size for size, an electrically heated generator cannot match a steam-to-steam generator for heating power. Experience shows that the pressure in the boiler cannot be maintained at a high enough level to ensure adequate removal of droplets by the cyclonic method described above. Gas-fired heating is not recommended for stainless-steel boilers.

### **Materials**

- 4.35 The boiler and other parts of the generator that come into contact with feedwater or steam should be constructed of corrosion-resistant stainless steel (such as low-carbon 316L grade).
- 4.36 Pipework connecting the clean-steam generator to the sterilizer should be also constructed in stainless steel. Since the generator can be sited close to the sterilizer, it is a false economy to re-use existing sections of the steam supply system.
- 4.37 While existing sterilizers should not be harmed by a carefully-designed clean-steam system, steam-contact surfaces of iron, mild steel or copper should be avoided in new machines. In most cases this will require contact surfaces to be fabricated in stainless steel as specified in EN 285.

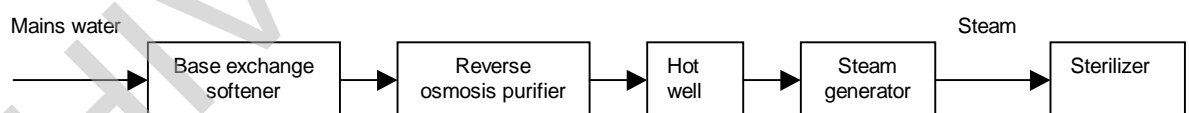
### **Feedwater treatment**

- 4.38 Since there is no return of chamber condensate from the sterilizer, the quality of feedwater is crucial to the performance of a clean-steam generator. It is especially critical for those generators that operate on a straight-through principle and have no reservoir of water within the boiler.
- 4.39 Water drawn from the public supply may be hard, that is containing significant concentrations of the salts of the alkaline earth metals (chiefly calcium and magnesium), and may also have traces of other contaminants which need to be removed. To assess the need for water treatment, users are recommended to obtain an analysis of the mains water from the supply company. Under The Water Supply (Water Quality) (Scotland) Regulations 1990 such an analysis must be supplied to customers on request and free of charge.
- 4.40 Although the stated water quality may be relied on most of the time, gross contamination of water supplies may occasionally occur due to engineering works and treatment failures. Users should take adequate precautions to protect any installed equipment from damage in such circumstances.

- 4.41 Full water treatment consists of three stages:
- softening (to remove scale-forming contaminants which may harm the boiler);
  - purification (to remove other undesirable contaminants);
  - degassing (to remove corrosive and non-condensable gases).
- 4.42 The need for softening treatment will depend on the hardness of the local water supply. Where the water is soft it may be possible to achieve the clean steam requirements without further treatment. In such cases users should be aware that the quality of the steam will vary with the quality of the water supply, and that frequent monitoring will be required to ensure that the clean steam specification is maintained.
- 4.43 In hard-water areas a base-exchange softening plant will normally be required. In this process calcium and magnesium ions are exchanged for sodium ions in a zeolite column (permutite process). The columns are periodically regenerated by flushing with brine (sodium chloride). It is important that the flushing is carried out in accordance with the manufacturer's instructions to prevent chloride ions being introduced into the softened water.
- 4.44 Microbial growth may occur in the columns unless the equipment is correctly operated and scrupulously maintained. Although mains water should be free of micro-organisms, a recirculating system should be fitted to maintain a flow of water through the columns at times of low demand. The softened water should be monitored regularly for microbial content. Periodic sanitising of the columns may be required and in-flow filters and regular decontamination may be needed to prevent colonisation. Although the brine flushing process should destroy most micro-organisms, bacteria such as *Bacillus* species and *Staphylococcus aureus* are tolerant of high salt concentrations.
- 4.45 Steam generators that are highly efficient at removing water droplets may be able to attain clean-steam standards without the need for further purification of the feedwater, but this can only be determined by experiment. Until clean-steam technology has been further developed and proven, users are recommended to consider installing feedwater purification plant.

- 4.46 Purification may be achieved either by reverse osmosis or deionisation. In reverse osmosis (RO), water is forced through a semi-permeable membrane which filters out contaminants to a high degree of efficiency. In deionisation (DI), ions and charged particles are removed either by electric fields or by ion exchange in resin beds. Although RO cannot normally attain the degree of purity possible with DI methods, it is more than adequate for feedwater intended for purpose-built clean-steam generators. Moreover:
- RO is cheaper to install and to run than DI;
  - RO removes particulate matter, organic molecules and pyrogens that DI cannot;
  - RO water is less corrosive to steel and copper than DI water;
  - maintenance requirements are less demanding than for DI units.
- 4.47 When seeking quotations for the supply of water purification plant, the user should ensure that the manufacturer is aware of the intended use of the purified water and establish that it will not be corrosive to the materials of the clean-steam generator.
- 4.48 Further treatment of the feedwater to remove dissolved gases will be necessary. This is usually achieved by pre-heating the water in a “hot well” maintained at temperatures of 80–190°C (at atmospheric pressure) to drive dissolved gases out of solution. The hot well is often provided by the manufacturer of the steam generator as an integral part of the unit.
- 4.49 A schematic illustration of a complete water treatment system is shown in Figure 2.

**Figure 2: Typical feedwater treatment for a clean steam generator**



### Internally generated clean steam

- 4.50 A large number of sterilizers in use in the NHS generate steam from a reservoir of water within the machine. Examples of such machines include:
- transportable sterilizers (bench-top) for unwrapped instruments and utensils;
  - small EO sterilizers in which water is used to generate steam for humidification;
  - certain laboratory sterilizers with internal reservoirs.
- 4.51 These machines can be readily converted to clean steam, although demonstrating compliance poses severe difficulties. While it may be possible to modify a sterilizer so that steam samples may be taken from the chamber,

the amount of steam generated in each cycle is so small that the volume of condensate obtained is insufficient for the required laboratory tests.

- 4.52 The problem is compounded since manufacturers have traditionally provided neither steam sampling points nor drainage valves on transportable sterilizers. Users should consider specifying such features when procuring new sterilizers. For the foreseeable future, however, assurance of clean steam conditions in the chamber must rely on good operating practice rather than the testing of samples.

### **Feedwater quality**

- 4.53 The first consideration is the quality of the feedwater. Because there is normally nothing in these sterilizers to prevent entrained moisture or carried-over water reaching the load from the reservoir, the purity of the steam must be assumed to differ little from that of the water in the reservoir. If the feedwater itself complies with the clean steam specification then, provided that the sterilizer chamber and reservoir are known to be clean and free of corrosion, the steam generated from it can also be presumed to be clean. *The quality of feedwater, then, is critical to the attainment of clean-steam conditions in the chamber.*
- 4.54 It follows from the definition of clean steam (see paragraphs 3.24–3.45) that the feedwater for these sterilizers should meet the purity requirements of Sterilized Water for Injections BP.
- 4.55 The situation is complicated, however, as soon as production loads are introduced into the chamber. Most sterilizers with an internal reservoir are designed to hold sufficient water for several operating cycles. After each cycle the condensate, together with any contaminants introduced with the load items, will be drained down into the reservoir. After a few cycles the level of contaminants in the feedwater may be so high that the steam generated from it no longer meets clean-steam specifications.
- 4.56 The possibility of pyrogens accumulating in the reservoir is of particular concern. Some pyrogens will be washed down from load items, while others may arise from bacterial growth, especially where the sterilizer is unused for long periods between refills. Even if such bacteria are subsequently killed by the sterilization process, pyrogens will not be inactivated and will be deposited on the next load. The level of pyrogens in the steam may exceed the permitted maximum for clean steam even though Sterilized WFI was used as the original feedwater.

### **A practical approach**

- 4.57 By following good operational practice and using Sterilized WFI it is possible to meet in full the requirements for clean steam. Whilst good operational practice should also be employed in the maintenance and cleaning of the reservoir and chamber, the use of Sterilized WFI may not always be justified.

- 4.58 Small transportable (benchtop) steam sterilizers are used in various healthcare premises, ranging from chiropody clinics to primary care premises in which, increasingly, minor surgical procedures are performed. The procedures for which sterilized instruments from a small steam sterilizer are used, therefore, vary widely. In some circumstances, the user may decide that Sterile Water for Irrigation may be a suitable alternative to Sterilized WFI. Sterile Water for Irrigation is sterilized nonpyrogenic distilled water, intended to be used for cleaning and irrigating body surfaces, wounds and body cavities. It differs from Sterilized WFI primarily in having a higher maximum endotoxin limit (0.5EU per ml compared with 0.25EU per ml for WFI). It is readily available in 1 litre, or larger, packs and at a similar price to the retail price for distilled water.
- 4.59 At the end of each working day the reservoir and chamber should be drained and left dry. The contents of part-used containers of sterilized feedwater should be discarded.
- 4.60 Small ethylene oxide sterilizers using steam for humidification are more likely to process products for complicated procedures, and Sterilized WFI is recommended.
- 4.61 Similarly, laboratory sterilizers with internal reservoirs used to process products vulnerable to contamination, or sterilizers processing medicinal products with unproven closure systems, should utilise Sterilized WFI.

#### **Good operating practice**

- 4.62 In view of the above considerations, it is clear that the key to achieving clean steam in this type of sterilizer lies in appropriate operating procedures, adhered to rigorously.
- 4.63 Sterile Water for Irrigation should be used during validation tests of a new sterilizer, at the time of all periodic or revalidation tests.
- 4.64 The following procedure should be used during validation tests of a new sterilizer, at the time of the yearly or revalidation tests, and where a sterilizer has not previously been used to generate clean steam:
- where practicable, examine all internal surfaces (reservoir, chamber, connecting pipework and other surfaces in contact with steam or feedwater) for signs of dirt, obstructions, scaling and corrosion; if present, consult the manufacturer for advice on cleaning and repair and remedy accordingly;
  - rinse all internal surfaces several times with Sterile Water for Irrigation, checking that the discarded water is clear, uncoloured and free of particulates;
  - fill the reservoir with Sterile Water for Irrigation to the level recommended by the manufacturer and run an operating cycle with an empty chamber; drain the reservoir;

- d. if the sterilizer is to be used immediately, refill the reservoir with Sterile Water for Irrigation to the level recommended by the manufacturer; otherwise rinse all internal surfaces twice with Sterile Water for Irrigation and leave dry.

4.65 In routine operation the following procedures should be observed:

- a. ensure that all load items are scrupulously clean and dry before being placed in the chamber;
- b. when the reservoir is to be replenished, drain the contents, rinse all internal surfaces twice with distilled water and once with Sterile Water for Irrigation; refill the reservoir with Sterile Water for Irrigation to the level recommended by the manufacturer;
- c. at the end of the working day, or whenever the sterilizer is to be unused for several hours, drain the reservoir, rinse all internal surfaces once with distilled water and once with Sterile Water for Irrigation and leave dry;
- d. when the sterilizer is to be used again, rinse all internal surfaces once with Sterile Water for Irrigation and refill the reservoir with Sterile Water for Irrigation to the level recommended by the manufacturer.

4.66 A guiding principle is that water is not allowed to remain standing in the reservoir for more than a few hours. If water has inadvertently been allowed to stand for a long period, or is suspected to have become contaminated, drain the reservoir and repeat the rinsing procedure in paragraph 4.64b.



## 5. Testing for compliance

### Introduction

- 5.1 This chapter discusses the testing regimes necessary for the initial validation of a clean-steam supply and for subsequent periodic testing. Methods for taking samples are given in Chapter 6 and their analysis is discussed in Chapter 7.

### Where to take samples

- 5.2 For a thorough assessment of the quality of the steam supply, samples of water and steam should ideally be taken throughout the steam-generating and distribution system from incoming water to steam at the sterilizer, though such extensive sampling will rarely be needed in practice. Examples of points at which water and steam samples may be taken include:
- mains water*, which after suitable treatment will be used as feedwater to the boiler;
  - treated water*, which may include one or more distinct treatment stages. Samples should be taken from the inlet and outlet pipes as close as possible to the treatment plant. To monitor the various stages of water treatment samples should be taken after each stage.
  - feedwater*, the water admitted to the boiler from the hot well, but without any dosing treatments admitted simultaneously or separately to the boiler;
  - boiler water*, the water in the boiler prior to blow-down;
  - boiler steam*, the steam leaving the boiler;
  - steam for use in sterilizer*, the steam delivered to the sterilizer, sampled at the steam service pipe.
- 5.3 The sampling points should be chosen so that the samples obtained will allow, when required, the identification and quantification of significant changes which may occur in contamination levels at each stage in the process. For example, sampling before and after a base-exchange water softener may reveal an increase in bacterial endotoxin levels from a contaminated ion-exchange column. A full set of sampling points at strategic locations will allow such problems to be investigated with a minimum of disruption, even though most of them will rarely be used in routine operation. Guidance on the design and use of sampling points is given in Chapter 6.
- 5.4 The design and construction of the system will determine how many sampling points would be of value. For a mains system supplying a large hospital, all the above points may be desirable. For a sterilizer with an adjacent,

dedicated clean-steam generator supplied from a simple treatment plant, fewer would be needed.

## Validation and periodic testing

- 5.5 Validation tests should normally be carried out on the following occasions:
- on initial validation of the steam-raising and distribution plant;
  - on initial validation of the sterilizers served by the steam plant, if not the same occasion;
  - on yearly testing or revalidation of the sterilizers;
  - where there is operational evidence that the steam quality may have deteriorated;
  - after any significant modification of the steam plant or its operation which might adversely affect the quality of the steam.
- 5.6 Periodic tests should be carried out on quarterly testing of the sterilizers.
- 5.7 As a minimum, samples for validation should always include both the feedwater and the steam for use in the sterilizer. Testing the steam without testing the water from which it is raised can lead to a false sense of security. For example, high levels of pyrogens in the feedwater will not necessarily produce contamination in the steam when the boiler is operating under loads which do not induce carry-over or priming. But during normal operation this could occur and therefore the contamination in the feedwater would require urgent investigation and remedial action.
- 5.8 Once a clean-steam supply has been validated, periodic testing of steam quality will be necessary for assurance that the clean-steam specification continues to be met. Quarterly testing of electrical conductivity is recommended here (see paragraphs A4.39–A4.48), but the frequency will depend upon the particular application and the consistency of control established from historical data. Other tests may be desirable if one or more of the possible contaminants is critical for the process or product.

## Mains steam supply

- 5.9 Formal validation should be carried out once the user is satisfied that the chosen system is capable of supplying clean steam and boiler operating procedures have been established. Much exploratory testing may be required before this point is reached.



## Validation test

- 5.10 The user should consult boiler room records to establish how the demand on the boiler varies through a typical working day (in a large hospital sterilizers themselves are likely to contribute only a small fraction of this load). The object is to ensure that times of highest and lowest demand can be reliably identified so that representative steam samples can be taken.
- 5.11 Because of the large amount of steam contained within a mains distribution system, it may take several minutes for steam produced in the boiler to arrive at the sterilizer. The quality of the steam at the sterilizer, then, may not be representative of the quality at the boiler. In particular, the steam in the pipes may have been generated under more favourable conditions at a time of less extreme demand and therefore be of higher quality, so invalidating the tests. On the other hand, steam that has been standing in the pipes is more likely to have received contamination from the distribution system. For these reasons users should take care to ensure that the steam sample was indeed generated when the boiler was operating at the presumed demand. This may require the pipework feeding the plant room manifold to be flushed with fresh steam immediately before samples are taken. In practice the samples should be satisfactory if the boiler demand has been steady for several minutes and remains steady while the flushing takes place and the samples are taken.
- 5.12 Two samples each of both feedwater and steam at the sterilizer should then be taken:
- at a time of highest demand;
  - at a time of lowest demand.
- 5.13 Samples should consist of:
- a full set of duplicate samples for laboratory analysis as described in paragraphs 6.18–6.25;
  - a field sample as described in paragraphs 6.8–6.17.
- 5.14 Where more than one sterilizer is supplied from the same steam manifold, the steam samples should be taken at the sterilizer furthest downstream from the boiler. It is not necessary to sample the steam at each sterilizer.
- 5.15 Samples should be subject to a full laboratory analysis as described in Chapter 7. The field sample should be tested for electrical conductivity on site as described in Appendix 4.
- 5.16 If the steam samples do not conform, the feedwater analysis should be examined to determine whether the failure could be remedied by a simple adjustment of the treatment regime. If not, further samples may need to be taken at other points in paragraph 5.2 to establish where the problems are arising.

- 5.17 When validation has been completed successfully, the mains supply may be used as a source of clean steam for sterilization. Users, however, should proceed with caution until sufficient experience has been gained to build confidence in the system. During the first year of clean-steam operation, the validation tests should be repeated at intervals chosen to coincide with the peak variations in seasonal demand. Such additional tests will provide further assurance that the system is capable of meeting the clean-steam specification under all normal operating conditions. If any tests fail during this period corrective action should be taken and the tests repeated.

### **Periodic tests**

- 5.18 Periodic testing of the steam supply (testing of feedwater is unnecessary) should be carried out quarterly to coincide with the quarterly tests scheduled for the sterilizer. The test should consist of a conductivity measurement of a field sample (see Appendix 4). Provided that the conductivity value remains below the limit established during validation, the steam supply may be regarded as continuing to meet the clean-steam requirements. Failure of the periodic test requires further investigation, however, normally by a full laboratory analysis of both feedwater and steam.
- 5.19 Revalidation should be carried out once a year, to coincide with the yearly testing of the sterilizer.

### **Dedicated clean-steam generator**

- 5.20 A dedicated clean-steam generator supplying one or more sterilizers does not suffer competing demands from other equipment and is more likely to be within the user's control. Consistency of steam quality can therefore be demonstrated more readily than for a mains steam supply.

### **Validation test**

- 5.21 Validation can normally be carried out as soon as the contractor has installed the equipment and completed his own installation tests.
- 5.22 The user should first establish the conditions under which the steam generator will be subject to the highest and lowest demand. Depending on the design of the steam plant, it is possible for either to constitute the worst-case conditions for carry-over of moisture. For example, a large plant designed to supply several sterilizers and relying on a cyclonic separator for removal of entrained water droplets may be inefficient at the lower velocities generated by a single sterilizer on light load. The other extreme requires the generator to operate at the lowest pressure and at the highest demand rate which would be expected under normal use.

- 5.23 The highest demand on the boiler usually occurs when all sterilizers are operating simultaneously. However the period of peak demand is brief (steam admission into the chamber) and it is difficult to synchronise the operating cycles so that the peaks coincide for long enough to allow a sample to be taken.
- 5.24 An alternative method is to vent steam from the relief valve on the plant room manifold. Users should first ensure that the steam will be discharged to a safe position outside the building (see SHTM 2010: Part 2 for guidance). By its very nature, the relief valve is designed to limit pressure in the system under all conditions and therefore creates a demand on the boiler that is greater than the maximum demand of the sterilizers. If steam samples collected under these conditions comply with clean-steam specification then the User can be confident that the generator will cope with the demand of the sterilizers. If not, then the generator may still comply if loaded at the lesser demand of the sterilizers. Further testing will be required.
- 5.25 A third possibility is to install a discharge valve on the steam manifold designed to simulate the peak demand of all sterilizers operating at the same time.
- 5.26 Lowest demand in normal operation typically occurs when a single sterilizer is on stand-by, with steam only being used to heat the jacket. However, since that steam will not come into contact with load items, its quality is not critical and it matters little whether it is clean or not. It may be better to regard the lowest demand as occurring during the holding time of a single sterilizer.
- 5.27 Unlike the mains systems discussed in paragraph 5.10, the amount of steam contained within the distribution system will be small, the steam produced in the boiler will arrive at the sterilizer almost instantly, and the steam sample collected can be assumed to be representative of that created in the boiler.
- 5.28 Two samples each of both feedwater and steam at the sterilizer should then be taken:
- under conditions of highest demand;
  - under conditions of lowest demand.
- 5.29 Samples should consist of:
- a full set of duplicate samples for laboratory analysis as described in paragraphs 6.18–6.25;
  - a field sample as described in paragraphs 6.8–6.17.
- 5.30 Where more than one sterilizer is supplied from the same steam generator, the steam samples should be taken at the sterilizer furthest downstream. It is not necessary to sample the steam at each sterilizer.

- 5.31 Samples should be subject to a full laboratory analysis as described in Chapter 7. The field sample should be tested for electrical conductivity on site as described in Appendix 4.

#### **Periodic tests**

- 5.32 Periodic testing of the steam supply (testing of feedwater is unnecessary) should be carried out quarterly to coincide with the quarterly tests scheduled for the sterilizer. The test should consist of a conductivity measurement of a field sample (see Appendix 4). Provided that the conductivity value remains below the limit established during validation, the steam supply may be regarded as continuing to meet the clean-steam requirements. Failure of the periodic test requires further investigation, however, normally followed by a full laboratory analysis of both feedwater and steam.
- 5.33 Revalidation should be carried out once a year, to coincide with the yearly testing of the sterilizer.

#### **Internally generated clean steam**

- 5.34 As explained in paragraph 4.50, transportable sterilizers pose problems in demonstrating compliance with clean steam due to the difficulty of obtaining adequate steam samples. For this reason, no validation or periodic tests are specified for these sterilizers.
- 5.35 Users should follow the good practice guidance given in paragraphs 4.63–4.67. In particular, the cleaning and rinsing procedure described in paragraph 4.65 should be carried out on validation, revalidation and yearly testing of the sterilizer.

## 6. Sampling

### Introduction

- 6.1 This chapter discusses methods for taking water and steam samples for both field and laboratory analysis.
- 6.2 Field samples will normally be taken and analysed by the Test Person in the course of testing the sterilizer. Laboratory samples may be taken either by personnel from the receiving laboratory or by the Test Person if qualified to do so.

### Sampling points

- 6.3 As discussed in Chapter 5, sampling is required in each part of the system where the composition of the water or steam may need to be confirmed, or where changes in composition may need to be determined. Sampling points should be designed and constructed to ensure that:
- a. the sample taken is as nearly as possible representative of the water or steam being sampled in that section of the system;
  - b. the sample can be taken without contaminating it;
  - c. the sample can be taken safely.
- 6.4 When possible, samples should be taken from flowing rather than static parts of the system. For example, for sampling a tank the samples are best taken from the inflow or outflow pipes but not from the static reservoir in the tank.
- 6.5 Where boiler water is to be sampled the position of the sampling point must be chosen with care. The composition of water at various locations in the boiler may show considerable variation. For boilers with forced circulation the sampling point is best located on the discharge side of the pump.
- 6.6 It is good practice to install coolers to ensure that representative samples of the boiler water may be taken safely.
- 6.7 Guidance on the design and construction of sampling points is given in BS 6068: Section 6.7.

## Sampling for field analysis

- 6.8 This method is suitable for taking steam and water samples to be tested for electrical conductivity during periodic tests. It should not be used for samples intended for laboratory analysis.

### Apparatus

- 6.9 Figure 3 shows the apparatus connected to a pitot tube identical to the one specified for the steam quality tests in SHTM 2010: Part 3. The pitot is fitted to the steam supply pipe near the sterilizer. This standard pitot is not suitable for the system for laboratory samples described below (see paragraph 6.18) so Figure 4 shows an alternative pitot which may be used for all steam testing. If this pitot is used for field samples or the tests in SHTM 2010: Part 3, the ball valve, nipple and socket should be removed.
- 6.10 Steam is led through a length of polypropylene tubing and is condensed as it passes through a bath of cold or iced water.
- 6.11 This apparatus is suitable for use for samples which are to be analysed immediately, such as for periodic tests for electrical conductivity. It is not suitable for samples intended for more sensitive analysis in the laboratory since the polypropylene is contra-indicated for several of the determinands of interest. It is also unsuitable where samples are to be taken for pyrogen testing since the polypropylene tubing cannot withstand the extended exposure to high temperatures needed to ensure that all components of the sampling system are free from pyrogens (see paragraph 6.22).

### Method

- 6.12 Clean the polypropylene sample bottle and the polypropylene tube with dilute hydrochloric acid and rinse several times with distilled water. Detergents should not be used. Leave them to dry.
- 6.13 If the pitot is not already fitted, isolate the steam supply and vent the pipe of pressure. Fit the pitot tube into the pipe and secure the polypropylene tube to it with a clip.
- 6.14 Restore the steam supply and allowed steam to vent through the polypropylene tube for at least 5 minutes to restore the steam service to its stable operating temperature. Ensure that the condensate drains freely. Close the steam valve.
- 6.15 Coil part of the polypropylene tube into sufficient number of coils to ensure condensation of steam, place it in the 8-litre container and retain it in place. Fill the container with enough cold water (ice may be added if required) to immerse the coils.



- 6.16 Open the steam valve. The steam will condense in the coils and condensate will emerge from the end of the tube. Allow the first 50 ml of condensate to discharge to waste and then collect approximately 250 ml in the sample bottle.
- 6.17 Seal and label the bottle. The electrical conductivity should be measured promptly as described in Appendix 4.

### **Sampling for laboratory analysis**

- 6.18 This method is suitable for taking all required samples, including those to be subjected to full laboratory analysis including the test for pyrogens.

#### **Apparatus**

- 6.19 The apparatus is shown in Figure 5. All components, including the condenser and valves, are constructed in stainless steel. The tubing is made in short sections which are connected by compression joints to form the required length and configuration. The sections are short enough to allow each element to be thoroughly cleaned, sterilized and depyrogenated before use.
- 6.20 The standard pitot used with the field sampling apparatus described above is not designed to take compression fittings and so cannot be used with this apparatus. It should be replaced with the modified pitot and ball valve shown in Figure 4.
- 6.21 The apparatus is suitable for taking samples for all the determinands of interest. It may be used for steam condensate or water samples throughout the steam-raising system. In theory there is a risk of some contamination of the sample from metals which could be extracted from the stainless steel. However the grade of steel chosen is no more reactive than those used in the construction of steam pipes and equipment. If, for whatever reason, the steam reacts with the sampling apparatus it will also have reacted with the installed system.

#### **Method**

- 6.22 All the stainless steel components should be depyrogenated by processing in a dry-heat sterilizer at a sterilization temperature of 180°C for 3 hours. If a suitable oven is available they may alternatively be baked at 250°C for 30 minutes (dry-heat sterilizers cannot attain this temperature).
- 6.23 Clean and prepare sample bottles according to the instructions from the receiving laboratory. Normally, two sets will be used for steam samples and one for control samples. Ensure that the bottles are labelled as described in paragraph 6.36.



**Figure 3: Steam sampling system for field analysis**

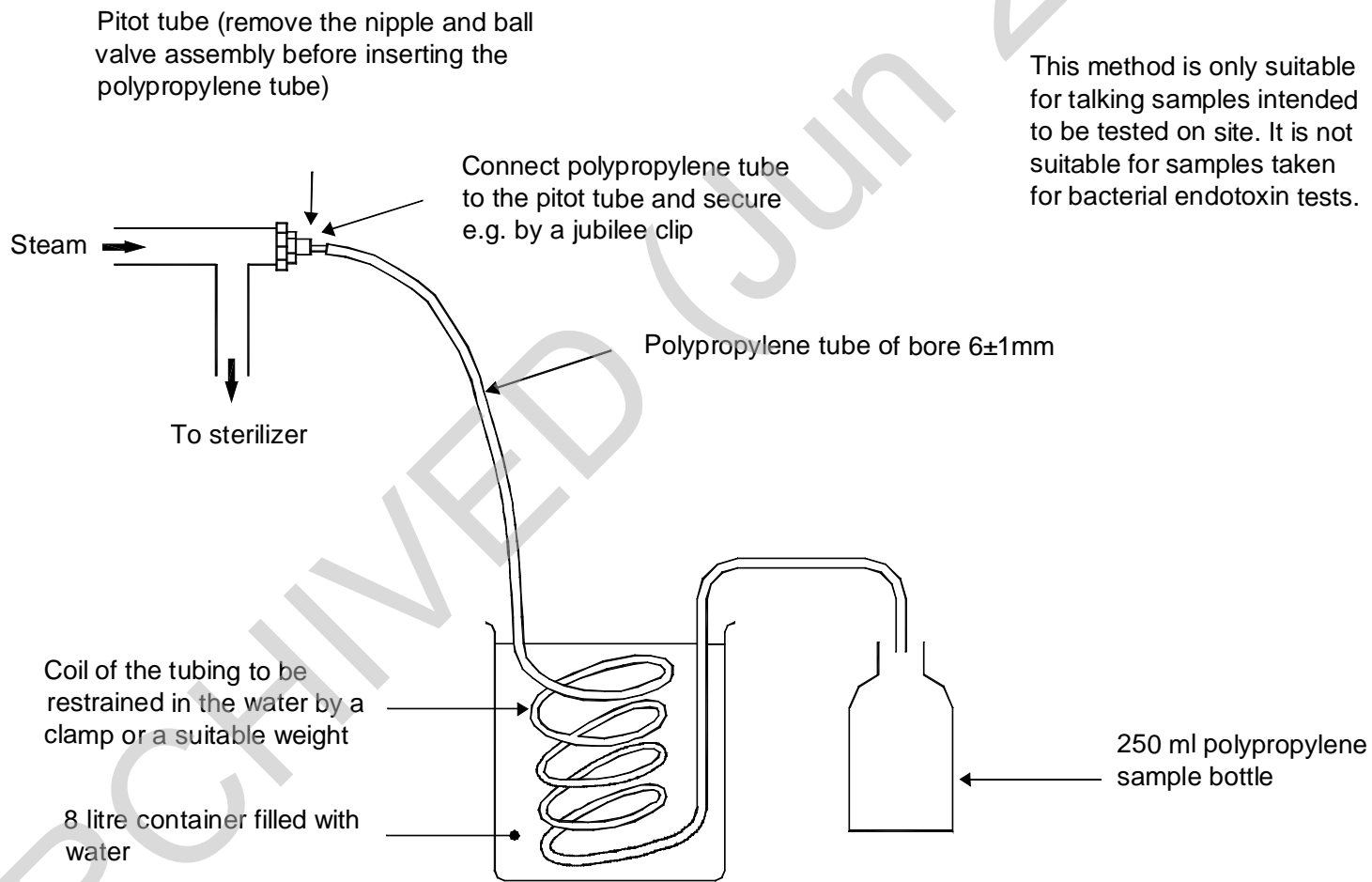
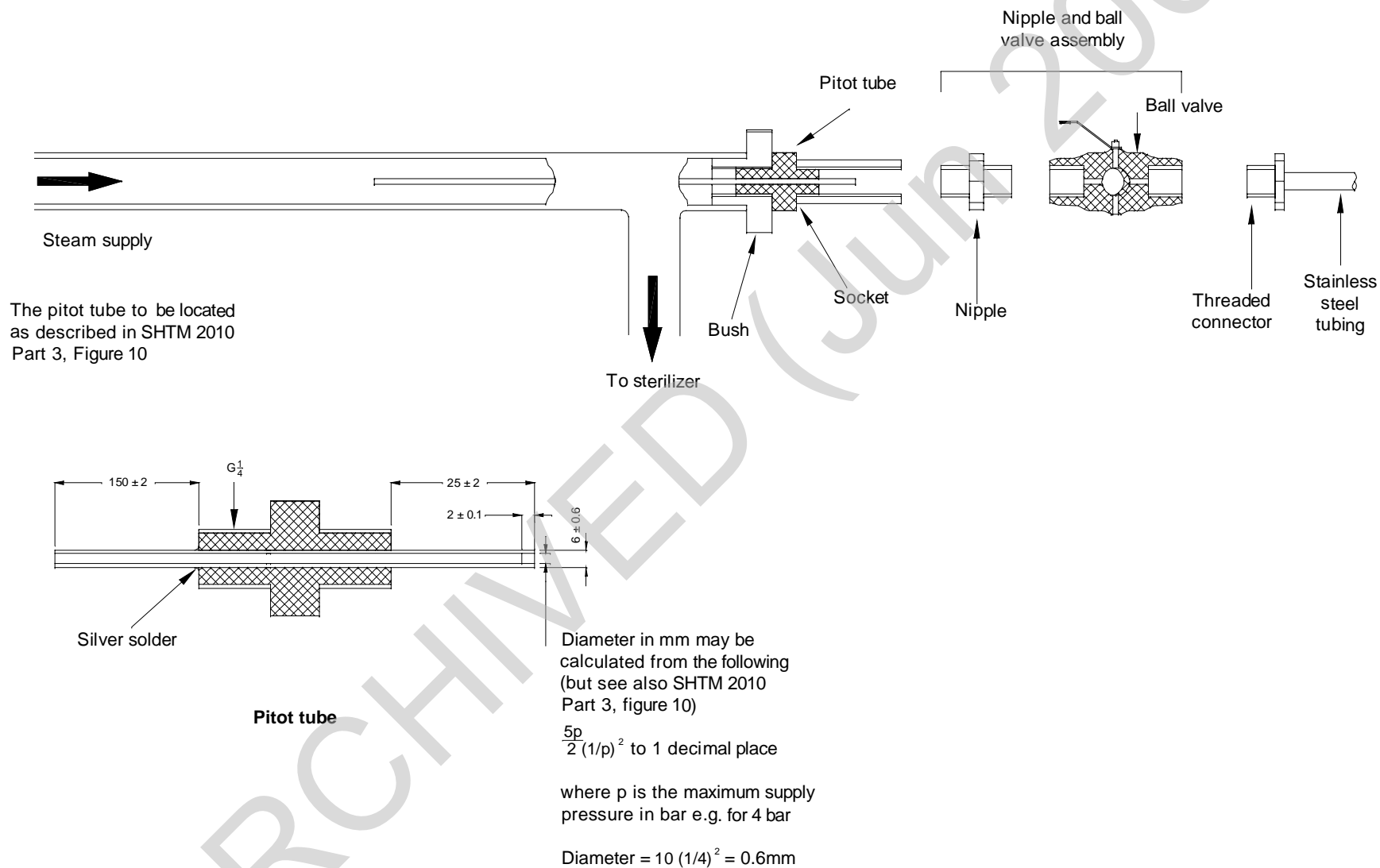




Figure 4: Typical pitot sampling tube assembly



- 6.24 Open the valve on the pitot. The steam will condense in the coil and condensate will emerge from the end of the tube. Allow the first 50 ml of condensate to discharge to waste and then collect samples in the first two sets of bottles.
- 6.25 The third set of bottles should be filled with distilled water of known quality, which should be preserved and analysed in the same manner as the two sets of steam samples. These negative control samples provide evidence that the choice of container, cleaning system and preservative is appropriate.

### **Handling of samples for laboratory analysis**

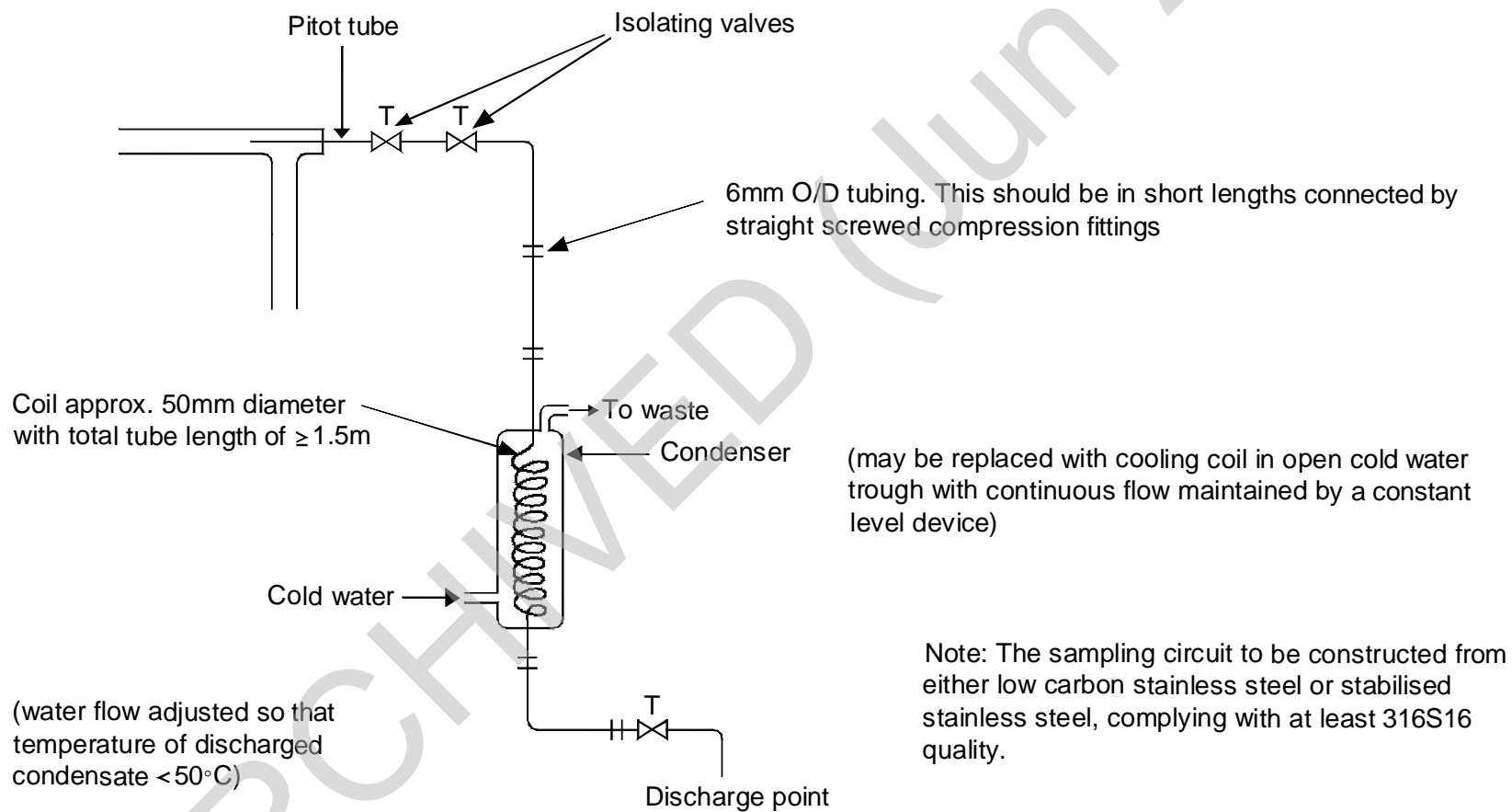
- 6.26 As soon as a steam or water sample is taken, it is important that its physical, chemical and biological properties remain stable until it arrives at the laboratory for analysis. The conditions in which the sample should be kept are determined by the contaminants for which the water is to be tested. The material of the sample container is also important since it may interact with substances in the water; plastic is suitable for some parameters, glass for others.
- 6.27 General guidance on these points is given below; more specific advice may be found in BS 6068: Section 6.3. The laboratory carrying out the analysis will normally provide all the necessary containers, preservatives and labels with full instructions for their use.

#### **Containers**

- 6.28 There is no one material suitable for all contaminants of interest. Containers may be made variously from polyethylene, polystyrene, polypropylene, glass or borosilicate glass. The receiving laboratory should supply the appropriate containers with full instructions for their use.
- 6.29 Each type of container requires a different cleaning procedure to ensure that samples are not contaminated by residues. Again, the instructions of the receiving laboratory should be followed.
- 6.30 Observe the laboratory's instructions on filling and closing the bottles. Most bottles should be filled to the brim and then stoppered or capped to ensure that as little air as possible remains above the sample. A small air space should be left above samples to be frozen.

#### **Sample preservation**

- 6.31 The purpose of preservation is to transfer the sample to the laboratory in a manner which, as far as may be practicable, maintains the concentration and state of the contaminant of interest unchanged from the moment the sample was taken.



**Figure 5: Steam sampling system for laboratory analysis**



- 6.32 There are many possible interactions which can occur that will adversely affect the sample. The contaminant of interest may:
- polymerise or, if already a polymer, depolymerise;
  - react with other constituents of the sample;
  - react with atmospheric oxygen or carbon dioxide becoming dissolved in the sample;
  - be consumed, modified or be produced in higher concentrations by micro-organisms growing in the sample;
  - react with, or be adsorbed or absorbed by, the material of which the container is constructed.
- 6.33 The extent to which these and other reactions will modify the sample is a function of several factors. The sample itself, and the extent and nature of any contaminants present, will determine which reactions and changes may occur. The more contaminated a sample is the more likely it is that changes will occur. The temperature during transport and storage, the exposure to light, the material of which the container is made and any special precautions used in the preparation of the container, and the elapsed time before analysis will all have a significant effect.
- 6.34 While it is desirable for all samples to be cooled (normally at 2–5°C) some will require the addition of an acid preservative and others will need to be frozen. The receiving laboratory will specify the preservative treatment for each container and supply suitable reagents where necessary.
- 6.35 Few preservative treatments for the contaminants specified for clean steam are valid for more than 24 hours and some for a much shorter time. Prompt despatch and analysis are therefore essential.

#### **Identification of samples**

- 6.36 Each container must be legibly and unambiguously labelled with a water-resistant label at the time of sampling. The laboratory will supply suitable labels and instructions. The information to be noted will normally include:
- the establishment at which the sample was taken;
  - the date and time at which the sample was taken;
  - the name of the person taking the sample;
  - clear identification of hazardous materials present (e.g. acids used as a preservative);
- and either
- a reference number, which unambiguously relates to contemporaneous notes of the following information;
- or

- f. the sampling point;
- g. the nature of the sample (e.g. condensed steam);
- h. the determinand(s) for which the sample is to be analysed;
- i. any preservative treatment;
- j. notes on any observations pertinent to the analysis, such as an event not in accordance with the sampling procedure which may affect the analysis.

### **Packaging and transport**

- 6.37 The samples should be packaged securely in containers providing suitable protection from breakage or external contamination during transport. The containers should be kept as cool as possible during transport. For transporting small quantities of samples, domestic cool boxes provide suitable protection and cooling.
- 6.38 The transport container should be accompanied by a list of the samples being sent, and a duplicate retained. The list should be sufficiently comprehensive to allow confirmation of the identity of each sample in the consignment.

## 7. Analysis of samples

### Introduction

- 7.1 This chapter discusses the means by which a sample of steam condensate may be analysed for compliance with the clean-steam specification. The tests are equally suitable for testing samples of steam or water from elsewhere in the steam supply system, provided the limitations of the pharmacopoeial tests are understood (see paragraph 3.35).
- 7.2 The methods of collecting samples are discussed in Chapter 6.

### Testing of samples

- 7.3 The quality of a water sample cannot be assessed merely by visual inspection. To determine whether a steam sample conforms with the requirements for clean steam it is necessary to carry out tests for all the determinands listed in Table 2.
- 7.4 Appendix 4 describes all the tests, with the exception of phosphate and silicate (see paragraph 7.23) required to analyse a sample for compliance. These tests are taken from the British Pharmacopoeia and should be well within the capacity of any hospital pharmacy. Although they do not require expensive analytical equipment, they are intended to be used by trained personnel in a properly equipped laboratory and are not suitable for on-site determinations under field conditions.
- 7.5 Laboratories invited to carry out these tests should be accredited to a recognised standard.
- 7.6 The field test for electrical conductivity is also described in Appendix 4. Note that it is required to be preceded by the BP test for acidity or alkalinity, which may also be carried out in the field.

### Reporting of results

- 7.7 The report obtained from the laboratory in respect of each test should contain the following information:
- the exact identity of the water sample;
  - the date and time the sample was received;
  - the date and time at which the test was commenced;
  - the storage conditions if (b) and (c) are not the same date;
  - the determinand for which the sample was analysed;



- f. for non-quantitative tests, a statement as to whether the result complies with specification;
- g. for quantitative tests:
  - (i) the numerical value expressed in the unit specified (see paragraph 7.11) for each of the duplicate determinations;
  - (ii) the mean of the results of the duplicate determinations and the uncertainty which may be associated with the final result;
- h. a description of any sample pre-treatment;
- i. a description of the method used, including reference to specific items of equipment, calibration standards, etc;
- j. any deviations from the method or other facts which might reasonably be expected to influence the result obtained; and should be signed both by the analyst responsible for carrying out the determinations and the analyst or quality controller responsible for checking the report.

### Alternative methods

- 7.8 Where numerical values are given in Table 2, laboratories may offer alternatives to the BP tests of equivalent or greater accuracy and sensitivity if these are methods which they routinely use. (Users should note that such methods will generally be more expensive than the BP tests.) Experienced analysts with appropriately equipped laboratories may favour the use of one of the many instrumental analytical techniques available. Instrumental methods which provide the same or better precision than the BP tests are suitable. See paragraph 7.23 for guidance on phosphate and silicate.
- 7.9 For any given determinand there will usually be several methods which are suitable and cover the range of concentrations of interest. The choice of method will be determined by a number of factors including availability of equipment, previous experience with the method, cost, sensitivity to interfering substances which may be present in the sample, etc. Significance should be given to:
- a. the limit of detection, which must be lower than the specified limit for the contaminant;
  - b. the accuracy of the method, which will be of particular importance in observing changes in quality;
  - c. the likely presence of interfering substances in the samples to be tested.
- 7.10 For further guidance see *General principles of sampling waters and associated materials*, 2nd edition, in the series, *Methods for the examination of waters and associated materials*.

## Comments on the tests

- 7.11 Since there are several ways in which numerical results from any given analysis may be presented, the user should specify that the results are quoted in the units used in the clean-steam specification in Table 2 so that the sample can readily be compared with the specification.
- 7.12 The following sections give background information on interpreting the results of some of the clean-steam tests and explains the relationships between them.

### Concentrations; residue on evaporation

- 7.13 The levels of some of the impurities in Table 2 are expressed as mass concentrations in units of milligrams per litre ( $\text{mg litre}^{-1}$ ). An alternative unit seen occasionally is milligrams per kilogram ( $\text{mg kg}^{-1}$ ) which is identical to parts per million by mass (ppm). Since one litre of pure water has a mass of almost exactly one kilogram, these units may be taken to be numerically equivalent for steam condensate. Hence:
- $$1 \text{ mg litre}^{-1} = 1 \text{ mg kg}^{-1} = 1 \text{ ppm} = 0.0001\% \text{ by mass.}$$
- 7.14 Alternatively, concentrations may be expressed in moles or millimoles per litre ( $\text{mol litre}^{-1}$ ,  $\text{mmol litre}^{-1}$ ), where one mole is equal to Avogadro's number of entities (atoms, molecules or ions). A concentration of one mole per litre is known as a "molar" (M) solution. To convert to a mass concentration, the relative molecular mass (RMM, formerly known as "molecular weight") of the entity is required. Thus:
- $$(\text{Mass concentration} / \text{mg litre}^{-1}) = (\text{RMM} / \text{g mol}^{-1}) \times (\text{molar concentration} / \text{mmol litre}^{-1}).$$
- 7.15 It is important to understand precisely what the reported concentration represents, since the same units are often used in different ways to express the results of the same analytical procedure. For example in the determination of phosphate the results may be reported as  $\text{mg litre}^{-1}$  of P,  $\text{P}_2\text{O}_5$ , or  $\text{PO}_4$  (see paragraph 7.24). Although the three values will be different, they represent the same experimental result.
- 7.16 The sum of the concentrations of individual ionic species must always be less than the concentration of total dissolved solids (measured as residue on evaporation). Unfortunately the BP tests are not sufficiently quantitative to allow this check to be made. However, the residue figure should be consistent with the electrical conductivity as described in paragraph 7.28 onwards.

## Acidity and alkalinity

- 7.17 The test for WFI in Bulk corresponds approximately to a pH in the range 4.2 to 7.0. Since a pH of 7.0 represents a neutral solution, the test requires the sample to be acidic. This is unacceptable for steam condensate, since acidic conditions promote corrosion of materials. For this reason the clean-steam specification adopts Sterilized WFI as a purity standard; the acidity-alkalinity test then corresponds to a much more acceptable pH in the approximate range 6.8 to 8.4.

## Heavy metals

- 7.18 In the BP test for heavy metals the sample is concentrated by a factor of 10 by evaporation and then calibrated against a standard solution containing 1 mg litre<sup>-1</sup> of lead ions. The test fails if the sample contains a sufficient concentration of heavy metals to produce a more intense brown colour than the standard solution subjected to the same test. The colour is not easy to discern and so the test should be carried out in conditions of good controlled lighting.
- 7.19 In normal circumstances the test will react to metals which form acid-insoluble sulphides, but the BP gives no indication of which metals will be detected. Table 4 shows the result of experimental work to determine the sensitivity of the test to various metals (Healthcare Science Ltd 1996). This shows that only lead, copper and silver can be detected at the 0.1 mg litre<sup>-1</sup> limit, mercury must be present at 1.5 mg litre<sup>-1</sup> before it is detected. Cadmium and zinc give a pale yellow colour (but not brown) at 0.6 mg litre<sup>-1</sup> and zinc gives a pale white opalescence at 1.2 mg litre<sup>-1</sup>. The test is insensitive to antimony, iron, nickel, cobalt, manganese and tin.

**Table 4: Sensitivity of the BP test for heavy metals.**

Metal	Concentration in sample (mg litre <sup>-1</sup> )
Lead	0.1
Copper	0.1
Silver	0.1
Bismuth	0.6
Mercury	1.5

*The table gives the concentration of each metal that will cause the same reaction as 0.1 mg litre<sup>-1</sup> of lead.*

- 7.20 It is therefore not possible to express the 0.1 mg litre<sup>-1</sup> figure as an equivalent sum of concentrations of individual metals. For this reason the test cannot be replaced by more precise quantitative tests for individual elements.

## Pyrogens

- 7.21 In the BP test for pyrogens, the water sample is incubated with a reagent known as LAL (Limulus amoebocyte lysate) derived from the horseshoe crab, *Limulus polyphemus*. If a clot forms, the amount of endotoxin in the sample may be estimated from the known sensitivity of the lysate. The limit of detection is 0.03 EU ml<sup>-1</sup>.
- 7.22 The test should not be confused with the alternative “test for pyrogens”, also described in the BP, which is carried out on live rabbits.

## Phosphate and silicate

- 7.23 These contaminants differ from the others in that they are not listed in the pharmacopoeial specification for Water for Injections. Consequently there are no simple BP tests that can be used to demonstrate compliance. A suitable analytical method for phosphate may be found in BS 6068: Section 2.28 (ammonium molybdate spectroscopic method) and for phosphate and silicate in *Phosphorous and silicon in waters, effluents and sludges* 1992 in the series *Methods for the examination of waters and associated materials*.
- 7.24 Conversion factors for different expressions of phosphate are as follows:  
 $1.00 \text{ mg litre}^{-1} \text{ P} = 3.07 \text{ mg litre}^{-1} \text{ PO}_4 = 4.58 \text{ mg litre}^{-1} \text{ P}_2\text{O}_5$ .

## Electrical conductivity

- 7.25 Pure water, which contains no ions except H<sup>+</sup> and OH<sup>-</sup> (formed by the dissociation of H<sub>2</sub>O) is a poor conductor of electricity. Any dissolved ionic species will raise the conductivity of the water sample. Measurement of the conductivity therefore provides a simple means of measuring the concentration of ionic species. That is why conductivity is so useful in monitoring steam quality.
- 7.26 The SI unit of conductance (reciprocal of resistance) is the *siemens* (S) which has the same dimensions and magnitude as the older unit, the *mho* (or reciprocal ohm). The SI unit of conductivity is the *siemens per metre* (S m<sup>-1</sup>) but the practical unit for aqueous solutions (and the unit used in this SHTM) is the *microsiemens per centimetre* (μS cm<sup>-1</sup>). This gives a numerical value of conductivity which is the same order of magnitude as the concentration of dissolved ionic species expressed in milligrams per litre.  
 $1 \text{ mS m}^{-1} = 10 \text{ μS cm}^{-1}$ .
- 7.27 A number of factors affect the measurement of conductivity. These include:
- the ionic species present (the particular ions, and the extent to which they become hydrated);
  - polarisation; gases produced at the surface of the electrodes will increase the electrical resistance and rapidly reduce the current to near zero. This

can be avoided by the use of an alternating voltage which prevents the build-up of gases at the electrodes;

- c. temperature; for which the relationship with conductivity is non-linear. Temperature compensation is therefore essential.

- 7.28 When a water sample contains predominantly ionisable solids in solution, and the composition of the various constituents is reasonably constant, the conductivity is proportional to the concentration of total dissolved solids (TDS) for concentrations up to 10 000 mg litre<sup>-1</sup>. A measured conductivity is multiplied by a suitable conversion factor to give an estimate of the TDS in mg litre<sup>-1</sup>. The conversion factor can be derived experimentally for waters of consistent ionic composition by making direct comparison of the measured mass of total dissolved solids and the electrical conductivity. It should be emphasised that TDS values estimated this way are not as reliable as direct measurements by gravimetric methods and reported as *residue on evaporation*.
- 7.29 Conductivity meters calibrated directly in TDS mg litre<sup>-1</sup> are available, but readings should not be taken at face value. The conversion factor being used must be established and shown to be appropriate.

## Appendix 1: Useful addresses

### Scotland

Healthcare Engineering and Environment Unit,  
Room 8:51 Graham Hills Building, 50 George Street, Glasgow G1 1QE  
Tel: 0141 548 3446  
Fax: 0141 553 4109

Scottish Centre for Infection and Environmental Health,  
Clifton House, Clifton Place, Glasgow G3 7LN  
Tel: 0141 300 1100  
Fax: 0141 300 1170

Scottish Healthcare Supplies,  
Trinity Park House, South Trinity Road, Edinburgh EH5  
Tel: 0131 552 6255  
Fax: 0131 552 6535

Health and Safety Executive (East),  
Belford House, 59 Belford Road, Edinburgh EH4 3UE  
Tel: 0131 247 2000  
Fax: 0131 247 2121

Health and Safety Executive (West),  
375 West George Street, Glasgow  
Tel: 0141 275 3000  
Fax: 0141 275 3100  
Health and Safety Executive Information Line Tel: 0541 545500

### UK health agencies

NHS Estates,  
1 Trevelyan Square, Boar Lane, Leeds LS1 6AE  
Tel: 0113 254 7000

Medicines Control Agency,  
Market Towers,  
1 Nine Elms Lane, London SW8 5NQ  
Tel: 0171 273 3000

Medical Devices Agency,  
Hannibal House,  
Elephant and Castle, London SE1 6TQ  
Tel: 0171 972 8000  
Internet address: [mda\\_mail@mda.win-uk.net](mailto:mda_mail@mda.win-uk.net)

Public Health Laboratory Service,  
Central Public Health Laboratory,  
61 Colindale Avenue, London NW9 5HT  
Tel: 0181 200 4400



## **Other organisations**

Institute of Healthcare Engineering and Estate Management,  
2 Abingdon House, Cumberland Business Centre, Northumberland Road,  
Portsmouth PO5 1DS  
Tel: (01705) 823186

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## Appendix 2: Operation and maintenance of clean-steam generators

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### Introduction

- A2.1 Clean-steam generators are steam boilers and are subject to the Pressure Systems and Transportable Gas Containers Regulations 1989.
- A2.2 Users should ensure that operation and maintenance of the generator is carried out correctly, both to ensure safety and also to maintain the quality of the steam.
- A2.3 Steam generators are subject to a written scheme of examination for pressure vessels.
- A2.4 Guidance on the design, maintenance, testing and operation of steam generators may be found in HSE Guidance Note PM 5, *Automatically controlled steam and hot water boilers*.
- A2.5 The advice of the boiler manufacturer about water supply, water treatment, blowing down and other operational practices should be strictly observed.
- A2.6 Failure to provide adequate supervision, with consequential inadequate control of water quality and insufficient blow-down, has resulted in such severe corrosion of steam generators that in some cases internal parts have collapsed and operators have been put in danger.

### Operation

- A2.7 A risk assessment should be undertaken to establish the level of supervision required. While it is not acceptable for steam generators to be left continuously unattended, it is not necessary for an operator to be present at all times. The amount and frequency of attention necessary in each case will depend largely on the nature of the water supply, water treatment arrangements and the intensity of use. The operator, who may also be the sterilizer operator, should be adequately trained.

### Maintenance

- A2.8 Because there is little condensate return to these steam generators, their feedwater is usually almost 100% make-up, and as a result the concentrations of dissolved and suspended solids in the boiler water quickly build up to very high levels. Such boilers are provided with a “blow-down” facility to expel deposits of sludge from the bottom of the boiler. It is essential

that an effective blow-down regime is established and adhered to. There are three possibilities:

- a. continuous blow-down - sludge is expelled continuously;
- b. automatic intermittent blow-down - sludge is expelled automatically under the control of a conductivity device;
- c. manual intermittent blow-down - sludge is expelled manually under the control of the operator.

- A2.9 With manual blow-down there is a risk of affecting the steam quality if this is undertaken at a time when there is a high demand for steam. For this reason manual blow-down should be undertaken at times of light load, preferably when none of the sterilizers are operating. Continuous and automatic blow-down systems need to be carefully managed to ensure they do not affect steam quality.
- A2.10 Guidance on blow-down may be found in HSE Guidance Note PM 60, *Steam boiler blow-down systems* (PM 60).
- A2.11 Generator vessels constructed from stainless steel will be subject to the same risk of stress corrosion cracking encountered in stainless steel sterilizer chambers (see SHTM 2010: Part 4) To minimise the risk, the manufacturer's guidance on feedwater quality should be followed.
- A2.12 A record of all tests and maintenance should be kept in the machine's plant history file.

## Appendix 3: Pyrogens

### Bacterial endotoxins

- A3.1 Bacterial endotoxins are a group of compounds, derived predominantly from Gram-negative bacteria, which give rise to high temperatures and fever-like reactions when injected into man and other mammals. This febrile reaction is referred to as *pyrexia* and compounds which can cause this reaction when injected are known as *pyrogens*. Bacterial endotoxins are not the only pyrogenic compounds but they are by far the most common and are also of the greatest significance in sterile product manufacture.
- A3.2 The majority of bacterial endotoxins causing a pyrogenic reaction are lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria. They consist of a lipid A molecule with long polysaccharide side chains. The toxicity resides in the lipid portion of the molecule. The lipid moiety is hydrophobic and on its own would be insoluble in water but it is rendered soluble by the polysaccharide side chains. (The polysaccharide side chains are the molecules in the bacterial membrane which provide the surface antigens used to characterize individual strains of bacteria.)
- A3.3 Organisms other than Gram-negative bacteria may give rise to endotoxins. For example fragments of the cell wall peptidoglycan from  $\beta$  haemolytic *Streptococci* produce a similar pyrogenic reaction.
- A3.4 The relative molecular mass (RMM) of the LPS is typically in the range 3 000–25 000 daltons. However, there is usually significant aggregation of endotoxin molecules in aqueous media; a number of molecules group together with the hydrophobic lipid moieties to the centre and the polysaccharide side chains to the outside. This effective increase in the size of the endotoxin explains why ultrafilters with cut-offs within the range 20 000–100 000 daltons can be used to effect almost complete removal of bacterial endotoxins from solution.
- A3.5 Bacterial endotoxins are extremely heat-stable and are only destroyed after prolonged exposure to high temperatures (3 hours at 180°C or 30 minutes at 250°C). They are not destroyed by any of the sterilization processes commonly employed for medical devices and medicinal products.

## Clinical significance

- A3.6 In small doses the injection of endotoxins causes pyrexia (fever), transient leukopenia followed by leukocytosis, hyperglycaemia, haemorrhagic necrosis of certain tumours, abortion, altered resistance to bacterial infection, various circulatory disturbances and vascular hyperreactivity to adrenergic drugs. When injected in larger amounts, endotoxins cause shock, usually accompanied by severe diarrhoea; absorption of endotoxin from the bowel is a major cause of terminal irreversibility in haemorrhagic shock.
- A3.7 Endotoxins appear to cause pyrexia, not directly but through an endogenous pyrogen released from polymorphonuclear leukocytes.
- A3.8 Endotoxins are generally assumed to play a large role in the vascular, metabolic, pyrogenic and haematalogic alterations which occur in severe Gram-negative infections but the evidence is indirect since, unlike most bacterial exotoxins, no specific protective antibody is available.
- A3.9 Subcutaneous injection of microgram quantities of endotoxins produces a mild inflammatory reaction but, when the injection is repeated with the same or a different endotoxin 24 hours later, the originally injected site becomes haemorrhagic within a few hours. This reaction (the Shwartzman reaction) is accentuated by the presence of cortisone. A similar programme of injections given intravenously to rabbits causes bilateral cortical necrosis of the kidneys and death.
- A3.10 Many sterile medical devices are intended for use on wounds where the dermis may have been breached. The sterile product may thus come into direct contact with the vascular system and if endotoxins are present may cause a pyrogenic reaction.

## Detection and measurement

- A3.11 The classic method of detection of pyrogens in pharmaceutical products is by measurement of the temperature rise in rabbits to which the substance has been administered. This method does not readily permit assay of the amount of endotoxin present. However it is sensitive to all pyrogenic substances, whether or not they are bacterial endotoxins.
- A3.12 In-vitro assay, which depends on the gelation of extracts of lysed blood cells of the horseshoe crab *Limulus polyphemus*, can be used quantitatively and will detect picogram quantities of lipopolysaccharide (endotoxin) in the so-called LAL test (Limulus amoebocyte lysate). A modification of the LAL test to provide a chromogenic test has been made, which allows reading of the endotoxin concentration by spectrophotometry. A turbidimetric method, which requires dedicated capital equipment, is also available as a quantitative method. Sensitivities as low as  $0.001 \text{ EU ml}^{-1}$  are available.

- A3.13 There is considerable variability in endotoxins derived from different bacterial species and it is difficult to set limits of permissible amount in terms of mass per unit volume. The US Food and Drugs Administration devised a unit of potency, the *endotoxin unit* (EU), to overcome this problem. The units are related to the endotoxin derived from *Escherichia coli* assigned by comparison with a USP reference endotoxin. The 1st International Standard for Endotoxin, established in 1986, consists of lyophilised endotoxin from *E. coli* 0113:H10:K(-)ve with trehalose (normally supplied in ampoules containing 14 000 EU). This, or another suitable preparation (such as the European Pharmacopoeia Biological Reference Preparation) the activity of which has been determined in relation to the International Standard using a gelation method, permits standardisation of the sensitivity of the lysate.

### Generation of bacterial endotoxin

- A3.14 Endotoxins arise, almost without exception, from the cell wall of Gram-negative bacteria. This is present both on the surface of the living bacteria and as persistent fragments of dead bacteria. As previously noted the endotoxins are thermally very stable.
- A3.15 Gram-negative bacteria include a wide range of organisms, for example:
- the sheathed bacteria e.g. *Sphaerotilus spp* which are large rods in a mucilaginous sheath found anchored to the substrate in running water (also called sewage fungus);
  - some 17 genera of budding or stalked bacteria such as *Caulobacter*;
  - the aerobic rods and cocci which include:
    - Pseudomonas spp*, which are ubiquitous;
    - Xanthomonas spp*, common plant pathogens;
    - Halobacterium spp*, which live in saturated brine;
    - Brucella spp*, etc;
  - the facultative anaerobes:
    - Escherichia*, indicator of faecal contamination;
    - Salmonella*, *Shigella*, intestinal pathogens;
    - Erwinia*, plant pathogen;
    - Enterobacter*, *Serratia*, *Proteus*, soil and aquatic;
    - Vibrio*, commonly marine aquatic;
  - the obligate anaerobes of the family *Bacteroidaceae*, *Bacteroides*, *Fusobacterium*.
- A3.16 These, or any other Gram-negative species, will inevitably give rise to endotoxins. However there are other organisms, such as  $\beta$  haemolytic *Streptococci*, where the cell wall peptidoglycan produces the same reaction as endotoxins from Gram-negative bacteria.

- A3.17 The quantity of endotoxin produced per cell varies from about 4 femtograms (fg) in bacteria growing in very pure water to as much as 16 fg for those grown under nutrient-rich conditions. For *E. coli*, 0.03 EU ml<sup>-1</sup> corresponds to approximately 0.003 ng per ml of endotoxin. Allowing that each cell produces approximately 6 fg of endotoxin then 500 bacteria per ml would give rise to 0.03 EU ml<sup>-1</sup>.
- A3.18 None of the sterilization processes used routinely for the preparation of pharmaceuticals, medical devices or surgical instruments will destroy or remove endotoxins once they are present. The only method of control therefore is to prevent the growth of significant numbers of Gram-negative bacteria within the product or in any component or material which directly comes into contact with it.
- A3.19 Gram-positive bacteria, with the exceptions noted above, do not produce endotoxins. The Gram-positive bacteria include organisms such as the family *Micrococcaceae*, which contains the genera *Staphylococcus* and *Micrococcus*, and the spore formers of the genera *Bacillus* and *Clostridium*. It is among these organisms that those species most resistant to radiation and thermal sterilization are found.

### Regulatory requirements

- A3.20 Pharmacopoeial specifications for water include several different grades of which the two principal grades are Purified Water and Water for Injections (WFI).
- A3.21 In the European Pharmacopoeia (EP) WFI is required to be prepared from potable water or purified water “by distillation in an apparatus of which the parts in contact with the water are of neutral glass, quartz or suitable metal and which is fitted with an effective device to prevent the entrainment of droplets. The apparatus must produce water free from pyrogens and to ensure this correct maintenance is essential. The first portion of the distillate obtained when the apparatus begins to function is discarded.”
- A3.22 The United States Pharmacopoeia (USP), however, permits the use of reverse osmosis for the preparation of WFI. In all other respects the limits set, and the test to determine compliance, are essentially similar.
- A3.23 USP XXII suggests an aerobic viable count limit of 500 cfu/ml for potable water and 100 cfu/ml for purified water (although normal practice would be not to accept >50 cfu/ml for purified water).
- A3.24 WFI (both USP and EP) is required to be free from pyrogens and there is a specified limit for bacterial endotoxins of < 0.25 EU ml<sup>-1</sup>.



- A3.25 Where a product, such as a wound irrigation solution, is required under the terms of the product licence to be “non-pyrogenic” the endotoxin standard for WFI would apply even though the product is not actually for parenteral administration.

### Requirements for clean steam

- A3.26 The requirement for parenterally administered medicinal products to be free from pyrogens is immediately apparent. It is not always recognised, however, that a similar requirement exists for medical devices or that the steam sterilization process can be a source of pyrogen contamination.
- A3.27 In the sterilization of solid goods (as opposed to aqueous fluids) steam in the sterilizer chamber condenses on the surface of the goods. This condensation process is necessary to heat the goods to the required temperature and provide the moist conditions necessary for rapid sterilization. At the end of the sterilization stage the condensate is evaporated from the load by reducing the pressure in the sterilizer chamber (drying vacuum) to produce a cooler, dry load.
- A3.28 Bacterial endotoxin carried in the steam supply will be deposited with the condensate and tends to become concentrated on the surface of the goods when the condensate is evaporated off during the vacuum drying stage. In consequence, items intended for use in invasive procedures, or for use in the preparation or administration of parenteral products, should be sterilized in a sterilizer which is supplied with “pyrogen-free” steam.
- A3.29 For practical purposes steam for use in sterilizers may be regarded as pyrogen-free when a condensed, representative, sample meets the European Pharmacopoeial standard for Water for Injections, i.e. less than  $0.25 \text{ EU ml}^{-1}$ .
- A3.30 Two factors are of greatest importance in ensuring that the steam supply is pyrogen free:
- the quality of the feedwater to the steam raising plant, high levels of pyrogens or high bacterial counts in the feedwater will ensure that limited carry-over of water as droplets in the steam will make a significant contribution to the pyrogen level;
  - the performance of the steam raising plant, in particular that its design, construction and mode of operation ensure that there is the minimum carry-over of entrained droplets of water.



## Summary

A3.31 The following key points summarise the topics discussed above:

- a. most pyrogens are bacterial endotoxins;
- b. endotoxins are lipopolysaccharides formed by the cell wall of Gram-negative bacteria;
- c. endotoxins are very stable molecules and are not destroyed by normal sterilization processes;
- d. 90% of the bacteria growing in purified waters are Gram-negatives;
- e. pyrogen testing was traditionally done by administering the substance to rabbits and observing whether there is a temperature rise;
- f. endotoxin testing may be done *in-vitro* using the Limulus Amoebocyte Lysate (LAL) test;
- g. the endotoxin limit for WFI (EP) is  $< 0.25 \text{ EU ml}^{-1}$ ;
- h. endotoxins are also of significance for medical devices, surgical equipment and equipment used to prepare parenteral medicinal products;
- i. if the steam when condensed is within the endotoxin limit for WFI (EP) it may be regarded as “pyrogen-free”;
- j. control of pyrogens in the steam is achieved by appropriate control of the boiler and its feedwater.

## Appendix 4: Tests for clean steam

### Introduction

- A4.1 This appendix contains procedures for the testing of steam condensate samples for compliance with the clean-steam specification of Chapter 3. The tests for chemical purity and the test for bacterial endotoxins are derived from the tests for Water for Injections in the British Pharmacopoeia. A procedure for the field measurement of electrical conductivity is also given.

### Laboratory tests for chemical purity

- A4.2 The tests in this section are extracted from the British Pharmacopoeia 1993. They are essentially identical to corresponding tests in the European Pharmacopoeia. The tests should be conducted only by suitably trained persons familiar with pharmacopoeial custom and practice.
- A4.3 The following tests are for Sterilized Water for Injections.

#### Acidity or alkalinity

- A4.4 To 20 ml add 0.05 ml of *phenol red solution*. If the solution is yellow, it becomes red on the addition of 0.1 ml of 0.01 M *sodium hydroxide VS*; if red, it becomes yellow on the addition of 0.15 ml of 0.01 M *hydrochloric acid VS*.

**NOTE:** The method given in the 1993 edition of the BP is incorrect and is amended in the BP Addendum 1995.

#### Ammonium (0.2 ppm)

- A4.5 To 20 ml add 1 ml of *alkaline potassium tetraiodomercurate solution* and allow to stand for 5 minutes. When viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 ml of *alkaline potassium tetraiodomercurate solution* to a mixture of 4 ml of ammonium standard solution (1 ppm  $\text{NH}_4$ ) and 16 ml of *ammonia-free water* (0.2 ppm).

#### Calcium and magnesium

- A4.6 To 100 ml add 2 ml of *ammonia buffer pH 10.0*, 50 mg of *mordant black 11 triturate* and 0.5 ml of 0.01 M *disodium edetate*. A pure blue colour is produced.

**Heavy metals (0.1 ppm)**

- A4.7 In a glass evaporating dish evaporate 150 ml to 15 ml on a water bath. 12 ml of the resulting solution complies with *limit test A for heavy metals*. Use *lead standard solution (1 ppm Pb)* to prepare the standard (0.1 ppm).
- A4.8 Limit test A for heavy metals: To 12 ml of the prescribed aqueous solution add 2 ml of *acetate buffer pH 3.5*, mix, add 1.2 ml of *thioacetamide reagent*, mix immediately and allow to stand for 2 minutes. Any brown colour produced is not more intense than that obtained by treating in the same manner a mixture of 10 ml of either *lead standard solution (1 ppm Pb)* or *lead standard solution (2 ppm Pb)*, as prescribed, and 2 ml of the solution being examined. The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of water and 2 ml of the solution being examined.

**Chloride (0.5 ppm)**

- A4.9 When the nominal volume of the final container is 100 ml or less, 15 ml complies with the *limit test for chlorides (0.5 ppm)*. Use a mixture of 1.5 ml of *chloride standard solution (5 ppm Cl)* and 13.5 ml of water to prepare the standard. Examine the solutions down the vertical axes of the tubes.
- A4.10 Limit test for chlorides: To a solution of the specified quantity of the substance being examined in 15 ml of *water* or to 15 ml of the prescribed solution add 1 ml of 2 M nitric acid, pour the mixture as a single addition into 1 ml of *silver nitrate solution R2* and allow to stand for 5 minutes protected from light. When viewed transversely against a black background any opalescence produced is not more intense than that obtained by treating a mixture of 10 ml of *chloride standard solution (5 ppm Cl)* and 5 ml of water in the same manner.

**Nitrate (0.2 ppm)****Sulphate**

- A4.12 To 10 ml add 0.1 ml of 2 M hydrochloric acid and 0.1 ml of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 hour.
- A4.11 To 5 ml in a test tube immersed in ice add 0.4 ml of a 10% w/v solution of *potassium chloride*, 0.1 ml of *diphenylamine solution* and, dropwise with shaking, 5 ml of *sulphuric acid*. Transfer the tube to a water-bath at 50°C and allow to stand for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 ml of *nitrate-free water* and 0.5 ml of *nitrate standard solution (2 ppm NO<sub>3</sub>) (0.2 ppm)*.

### Oxidisable substances

- A4.13 Boil 100 ml with 10 ml of 1 M *sulphuric acid*, add 0.2 ml of 0.02 M *potassium permanganate* and boil for 5 minutes. The solution remains faintly pink.

### Residue on evaporation (30 ppm)

- A4.14 Evaporate 100 ml to dryness on a water bath and dry the residue to constant weight at 100°C to 105°C. For containers with a nominal volume of 10 ml or less, the residue weighs not more than 4 mg (0.004%) and for containers with a nominal volume greater than 10 ml, the residue weighs not more than 3 mg (0.003%).

### Laboratory test for pyrogens

- A4.15 The following text is based on the bacterial endotoxin test from the British Pharmacopoeia 1993. Additional information pertinent to the analysis of steam condensate samples is given in notes.
- A4.16 The test for bacterial endotoxins (LAL test) uses a lysate of amoebocytes from the horseshoe crab, *Limulus polyphemus*. The addition of a solution containing endotoxins to a solution of the lysate produces turbidity, precipitation or gelation of the mixture. The rate of reaction depends on the concentration of endotoxin, the pH and the temperature. The reaction requires the presence of certain bivalent cations, a proclotting enzyme system and clottable protein; these are provided by the lysate.
- A4.17 The limit for a given material or preparation is expressed as the maximum allowable endotoxin concentration (MAEC) in endotoxin units per millilitre (EU ml<sup>-1</sup>) for a defined solution of that material or preparation.

**NOTE:** The maximum allowable concentration for clean steam condensate is 0.25 EU ml<sup>-1</sup>.

- A4.18 Before carrying out the test for endotoxins on the sample, it is necessary to verify:
- that the equipment used does not absorb endotoxins;
  - the sensitivity of the lysate; and
  - the absence of interfering factors.

**NOTE:** New borosilicate glass test tubes have a relatively high affinity for endotoxin in aqueous solution and may give rise to artificially low readings if used to make dilutions of endotoxin. Strict adherence to the reagent manufacturer's recommendations for choice and preparation of test equipment is necessary.

- A4.19 Carry out the test in a manner that avoids microbial contamination. If necessary, treat equipment to eliminate endotoxins.

### Reagents

- A4.20 **Limulus amoebocyte lysate.** A lysate of amoebocytes from the horseshoe crab, *Limulus polyphemus*. Reconstitute the lysate as stated on the label. For each batch, confirm the stated sensitivity as prescribed under Sensitivity of the lysate. The sensitivity of the lysate is defined as the lowest concentration of endotoxin that yields a firm gel in the test conditions and is expressed in EU ml<sup>-1</sup>.

**NOTE:** The LAL reagent is selected for the required sensitivity to endotoxin. 0.125 EU ml<sup>-1</sup> is used to test for compliance with a maximum allowable endotoxin concentration of 0.25 EU ml<sup>-1</sup>. The various commercially available LAL reagents differ in product compatibility, inhibition endpoints and buffer capacity. There may also be lot-to-lot variation within supplies from any one manufacturer.

- A4.21 **Water BET.** Water that gives a negative result in the conditions prescribed in the test for bacterial endotoxins on the preparation being examined. It may be prepared by distilling water three times in an apparatus fitted with an effective device to prevent the entrainment of droplets or by other means that give water of the requisite quality.
- A4.22 **0.1 M hydrochloric acid BET.** 0.1 M *hydrochloric acid* that has been prepared using *water BET*. After adjustment to pH 6.5 to 7.5 with 0.1 M *sodium hydroxide BET* it gives a negative result in the conditions of the test.

**NOTE:** The reconstituted lysate may be subdivided into suitable aliquots and frozen and stored at -20°C or below for up to 3 months. Frozen lysate should only be thawed once.

- A4.23 **0.1 M sodium hydroxide BET.** 0.1 M *sodium hydroxide* that has been prepared using *water BET*. After adjustment to pH 6.5 to 7.5 with 0.1 M *hydrochloric acid BET* it gives a negative result in the conditions of the test.

### Standard preparation

- A4.24 The Standard Preparation is the 1st International Standard for Endotoxin, established in 1986, consisting of freeze-dried endotoxin from *Escherichia coli* 0113:H10:K(-ve) with trehalose (supplied in ampoules containing 14 000 EU), or another suitable preparation the activity of which has been determined in relation to the International Standard using a gelation method. (For this purpose the European Pharmacopoeia Biological Reference Preparation is recommended.)

**NOTE:** Reconstitute the Standard Preparation according to the manufacturer's instructions. Mix by repeated vortexing and prepare working standards by serial dilution using *water BET* and pyrogen-free dilution tubes.

### Procedure

- A4.25 Unless otherwise prescribed, prepare the solutions and dilutions used in the test using *water BET*.

**NOTE:** Lyophilised endotoxin has a threshold limit value of  $5 \text{ EU kg}^{-1} \text{ h}^{-1}$ . Over-exposure may result in fever, nausea and shock. Avoid inhalation of the powder and injection of the reconstituted solution.

- A4.26 If necessary, adjust the solution being examined to pH of 6.5 to 7.5 using 0.1 M *hydrochloric acid BET*, 0.1 M *sodium hydroxide BET* or a suitable buffer.

**NOTE:** The "solution being examined" may be the sample, dilutions of the Standard Preparation, control solutions, as appropriate.

- A4.27 Add a volume of the lysate appropriate to the chosen receptacle (for example a slide or tube) to each of the requisite number of such receptacles maintained at 36°C to 38°C.

**NOTE:** Reaction tubes are recommended. The volume of lysate added should be 0.1 ml.

- A4.28 At intervals that will permit the examination of each receptacle and the recording of each result, add to each receptacle an equal volume of the solution being examined and immediately mix gently with the lysate.

- A4.29 Incubate the reaction mixture, without vibration and avoiding loss of water by evaporation, for a constant period that has been found suitable in the chosen experimental conditions (usually 20 to 60 minutes), examine the receptacle and record the result.

**NOTE:** Vibration during the incubation period can prevent stable gel formation. An unstirred water bath should be used since vibration from the motor in a stirred bath may interfere with the reaction. The tube should be incubated for 1 hour.

- A4.30 A positive result is indicated by the formation of a firm gel that does not disintegrate when the receptacle is gently inverted. A result is negative if such a gel is not formed.

**NOTE:** Turbidity can be confused with initial stages of gelation. It is essential that all apparent gel formation is verified by demonstrating a stable gel on inversion through 180°.



## Sensitivity of the lysate

- A4.31 Prepare not fewer than four replicate series each of not fewer than three dilutions of the Standard Preparation such that at least the final dilution in each series gives a negative result. Examine the dilutions, and a negative control solution consisting of *water BET*, as described under *Procedure*. Calculate the average of the logarithm of the lowest concentration of endotoxin in each series of dilutions for which a positive result is found. The antilogarithm of this average gives the estimated lysate sensitivity. The estimated lysate sensitivity is confirmed if it does not differ by more than a factor of 2 from the stated sensitivity. The estimated sensitivity is then used in all tests performed using this lysate.

## Interfering factors

- A4.32 Operate as prescribed under *Sensitivity of the lysate* but to prepare the dilutions of the Standard Preparation use the sample at the maximum valid dilution calculated from the expression:

$$\frac{\text{Maximum allowable endotoxin concentration}}{\text{Sensitivity of the lysate}}$$

both values being expressed in EU ml<sup>-1</sup>.

- A4.33 If the sensitivity of the lysate determined in the presence of the sample does not differ by more than a factor of 2 from that determined in the absence of the sample, the latter does not contain factors that interfere in the experimental conditions and it may be examined without further treatment.
- A4.34 If the sensitivity of the lysate determined in the presence of the sample differs by more than a factor of 2 from that determined in the absence of the sample, the sample acts as an inhibitor or an activator. The interfering factors must be eliminated by suitable treatment such as dilution, filtration, neutralisation, dialysis or addition of substances that displace absorbed endotoxins. The use of a more sensitive lysate permits the use of a greater dilution of the sample and this contributes to the elimination of interference.
- A4.35 Ultrafiltration may be used when the interfering factor passes through a filter with a nominal separation limit corresponding to a molecular weight of 10 000 to 20 000. Asymmetrical membrane filters of cellulose triacetate or polysulphone have been found to be suitable. The material retained on the filter, which contains the endotoxins, is rinsed with *water BET* or a suitable buffer and endotoxins are recovered in *water BET* or a suitable buffer. The test volume and the final volume used to recover the endotoxins are determined for each preparation being examined.
- A4.36 Establish that the chosen method effectively eliminates interference without removing endotoxins by repeating the test for interfering factors using the sample to which the Standard Preparation has been added and which is then submitted to the chosen treatment.



### Test for bacterial endotoxin in the sample

- A4.37 Carry out the method described under *Procedure* in duplicate using the maximum valid dilution of the sample which has been treated if necessary to eliminate interfering factors. Examine at the same time a negative control consisting of *water BET* and two positive controls each of which contains the Standard Preparation at a concentration corresponding to twice the stated sensitivity of the lysate and one which contains the sample (treated if necessary to eliminate interfering factors after the addition of the Standard Preparation) at the concentration being used in the test. The test is not valid unless the negative and both positive controls give the appropriate results.

### Interpretation of results

- A4.38 The sample complies with the test if a negative result is found for both test mixtures. The sample does not comply with the test if a positive result is found for both test mixtures. If a positive result is found for one test mixture and a negative result for the other, repeat the test; the sample complies with the test if a negative result is found for both test mixtures.

**NOTE:** For a given batch of lysate of calibrated sensitivity  $\lambda$  EU ml<sup>-1</sup>, a positive result will indicate the presence of endotoxin within the range 0.5  $\lambda$  to 2  $\lambda$  EU ml<sup>-1</sup>.

### Field test for electrical conductivity

- A4.39 The only test of steam condensate or feedwater that can be reliably carried out on site is a test for electrical conductivity. Guidance on the interpretation of conductivity measurements is given in Chapter 7.
- A4.40 A portable conductivity meter is required, accurate to 1% over a range which includes 1.0 to 30  $\mu\text{S cm}^{-1}$  with a resolution of 0.1  $\mu\text{S cm}^{-1}$ . It should be temperature-compensated over the range 0°C to 40°C, so that it gives readings standardised to 25°C. The instrument should be designed to measure the conductivity of very pure water.
- A4.41 Commercially available meters usually have temperature compensation set at 2% per °C either as standard or as a default value. The compensation effect is often user-adjustable over the range 0-5% per °C, but unless there are unusual local circumstances (such as a particularly ubiquitous contaminant) the temperature compensation value should be set at 2% per °C.
- A4.42 Several standard conductivity reference solutions are also required, preferably with conductivities which bracket the expected value. A range of such standards, including pure water standards (also known as absolute water) is available commercially, standardised at 25°C and traceable to national standard reference materials. The standards should be allowed to equilibrate to room temperature in the area in which the tests will be conducted.

- A4.43 Carry out the BP test for acidity or alkalinity (see paragraph A4.4). If the sample is fresh condensate, there is no need to boil the sample as described in the test. If the sample complies with the test, then it may be tested for conductivity.
- A4.44 Wash the meter probe with Purified Water BP or with the sample water. Measure the conductivity of the standards. Use the results to calibrate the meter in accordance with the manufacturer's instructions.
- A4.45 Measure the temperature of the sample. For effective temperature compensation, this test is best carried out with both sample and standards near a temperature of 25°C. If the sample is hotter, allow it to cool until the temperature is approximately 25°C.
- A4.46 Wash the meter probe either with Purified Water BP or with the sample water. Measure the conductivity of the sample.
- A4.47 The test should be considered satisfactory if the measured conductivity:
- does not exceed the value specified for clean steam in Table 2;
  - is consistent within experimental errors with the value measured during validation.
- A4.48 If the conductivity has risen substantially from the value determined during validation, the cause should be identified and corrected.

## Glossary

The following terms have been used in this SHTM. Chapter or paragraph references to where more information may be found are given in brackets. Cross-references are shown in bold.

### **bacterial endotoxins**

A group of compounds derived predominantly from **Gram-negative** bacteria, which give rise to high temperatures and fever-like reactions when injected into mammals. Also known as **pyrogens** (Appendix 3).

### **blow-down**

The process of removing sludge from a boiler by using the internal pressure to expel it from a valve in the bottom of the vessel (4.15, A2.8).

### **carry-over**

The delivery of substantial quantities of liquid water in steam due to **priming** or **foaming** (4.5).

### **clean steam**

Steam whose **condensate** meets the purity requirements of **Water for Injections BP** with additional limits for phosphate and silicate (3.24).

### **clean-steam generator**

A boiler designed to produce **clean steam** (4.26).

### **condensate**

Water formed by the condensation of steam.

### **Conductivity**

A measure of the ability of a material to pass an electric current. Reciprocal of resistivity (7.25).

### **cyclonic separator**

A device forming part of a **clean-steam generator** that removes entrained water droplets from steam by causing the steam to rotate at high speed (4.29).

### **degassing**

A pre-heating treatment of boiler **feedwater** to reduce the amount of **non-condensable** gases in the steam supply (4.48).

**deionisation (DI)**

A water purification process in which ions and other electrically charged particles are removed from solution either by the influence of an electric field or by ion exchange columns (4.46).

**dryness value**

A dimensionless quantity, approximating to the dryness fraction, derived to determine whether steam is of the correct dryness for sterilization purposes. A dryness value of 1.0 represents saturated steam (3.10).

**EN 285 steam**

Steam whose **condensate** meets the recommended purity requirements for steam contained in the European Standard EN 285 (3.20).

**endogenous infection**

Infection due to re-activation of organisms in a dormant focus.

**endotoxin unit (EU)**

A measure of the potency of **bacterial endotoxins** in relation to those derived from *E. coli* (A3.13).

**feedwater**

Water that is to be used for the generation of steam.

**foaming**

The production of a head of foam within a boiler, often due to a raised level of **total dissolved solids**, which is drawn off with the steam so leading to a wet and contaminated steam supply (4.6).

**Gram-negative**

A class of bacteria that do not take Gram's stain and which are also sources of **bacterial endotoxins (pyrogens)** (A3.14).

**hot well**

A tank in which **feedwater** is maintained at a high temperature to drive off dissolved gases before it is admitted to a boiler (4.48).

**make-up water**

Freshly treated water often mixed with returned steam **condensate** to make feedwater for a boiler.

**medical device**

Any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of diagnosis, prevention, monitoring, treatment or alleviation of disease; diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap; investigation, replacement or modification of the anatomy or of a physiological process; control of conception; and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be

assisted in its function by such means (source: EU Council Directive 93/42/EEC) (3.6).

**medicinal product**

Any substance or combination of substances presented for treating or preventing disease in human beings or animals. Any substance or combination of substances which may be administered to human beings or animals with a view to making a medical diagnosis or to restoring, correcting, or modifying physiological functions in human beings or in animals is likewise considered a medicinal product (source: EU Council Directive 65/65/EEC) (3.3).

**non-condensable gas**

Gases which cannot be liquefied by compression under the range of conditions of temperature and pressure used during the **operating cycle** of a sterilizer (3.10).

**packaged boiler**

A small local boiler used to supply steam for a **clean-steam generator** (4.33).

**parenteral**

Of a **medicinal product**, administered by means other than through the digestive tract, and especially by injection.

**pitot**

A metal tube of narrow bore inserted along the axis of a steam pipe and designed to extract a sample of steam for testing or collection (6.9).

**potable steam**

Process steam intended for culinary applications and meeting the purity requirements of drinking water (3.18).

**priming**

Of a boiler, the delivery of steam containing water in suspension due to violent boiling or frothing (4.5).

**process steam**

Steam whose quality is not optimised for sterilization (3.16).

**pyrogen**

A **bacterial endotoxin** that causes a rise in body temperature and which is not destroyed by steam **sterilization** (Appendix 3).

**residue on evaporation**

The mass of solid remaining when a given volume or mass of aqueous solution is evaporated. Unit: mg litre<sup>-1</sup> or ppm. See also **total dissolved solids** (7.16).

**reverse osmosis (RO)**

A water purification process in which impurities are filtered out by forcing the water through a semi-permeable membrane (4.46).

**Sterilized Water for Injections BP (Sterilized WFI)**

A grade of **Water for Injections BP** designed for dilution of sterile medicinal products intended for subsequent intravenous administration (3.31).

**Sterile Water for Irrigation**

Sterile Water for Irrigation is a sterile, nonpyrogenic preparation of Water for Injections BP, containing no antimicrobial agent or other substances.

**total dissolved solids**

The mass of solid material dissolved in a given volume or mass of aqueous solution. Unit: mg litre<sup>-1</sup> or ppm. See also **residue on evaporation**.

**Water for Injections BP**

A pharmaceutical preparation designed for administration by injection consisting of distilled water that meets the purity specifications of the British Pharmacopoeia (3.30).

**Water for Injections in Bulk BP**

A grade of **Water for Injections BP** designed for use in the manufacture of medicinal products that are to be terminally sterilized and intended for administration by injection (3.31).

## Abbreviations

<b>BET</b>	Bacterial Endotoxins
<b>BP</b>	British Pharmacopoeia
<b>BS</b>	British Standard
<b>°C</b>	Degree Celsius
<b>Ca</b>	Calcium
<b>CaCO<sub>3</sub></b>	Calcium carbonate
<b>cfu/ml</b>	Calony forming units/millimetre
<b>CIBSE</b>	Chartered Institute of Building Services Engineers
<b>Cl</b>	Chlorine
<b>DI</b>	De-ionised (referring to water)
<b>EEC</b>	European Economic Community
<b>EEF</b>	Estates Environmental Forum
<b>EN</b>	European Norm
<b>EO</b>	Ethylene oxide
<b>EU</b>	European Union
	Endotoxin unit
<b>EU ml<sup>-1</sup></b>	Endotoxin units per millilitre
<b>EP</b>	European Pharmacopoeia
<b>HEEU</b>	Health Engineering and Environment Forum
<b>H<sub>2</sub>O</b>	Water/water vapour
<b>HMSO</b>	Her Majesty's Stationary Office
<b>HSE</b>	Health and Safety Executive
<b>HTM</b>	Heath Technical Memorandum
<b>kg h<sup>-1</sup></b>	kilogram per hour
<b>kW</b>	kilowatt
<b>LAL</b>	Limulus amoebocyte lysate
<b>LPS</b>	Lipopolysaccharides
<b>LTS</b>	Low temperature steam
<b>LTSF</b>	Low temperature steam and formaldehyde
<b>M</b>	Molar
<b>mg l<sup>-1</sup></b>	milligram per litre
<b>ml</b>	millilitres
<b>mm</b>	millimetre
<b>mmol l<sup>-1</sup></b>	millimol per litre
<b>ms<sup>-1</sup></b>	metres per second
<b>mSm<sup>-1</sup></b>	milli Siemen per metre
<b>ng</b>	nanograms
<b>NH<sub>4</sub></b>	Ammonium
<b>NHS</b>	National Health Service
<b>OD</b>	Outside diameter
<b>P</b>	Phosphorus
<b>Pb</b>	Lead
<b>pH</b>	The inverse of the logarithm.....etc.



<b>Ph Euro</b>	Pharmacopoeia European
<b>P<sub>2</sub>O<sub>2</sub></b>	Phosphate
<b>PO<sub>4</sub></b>	Phosphate
<b>ppm</b>	Parts per million
<b>RO</b>	Reverse osmosis
<b>SI</b>	System International
	Statutory instrument
<b>SHG</b>	Scottish Health Guidance (note)
<b>SHTM</b>	Scottish Health Technical Memorandum
<b>TDS</b>	Total dissolved solids
<b>USP</b>	United States Pharmacopoeia
<b>WFI</b>	Water for injections BP
<b>:m</b>	micron (a unit of length equal to one millionth of a metre)
<b>:Scm<sup>-1</sup></b>	micro Siemens per centimetre
<b>&lt;</b>	greater than
<b>&lt;</b>	less than

## References

### NOTE:

Where there is a requirement to address a listed reference, care should be taken to ensure that all amendments following the date of issue are included.

Publication ID	Title	Publisher	Date	Notes
<b>Acts and Regulations</b>				
SI 3146	Building (Scotland) Act	HMSO	1959	
	Clean Air Act	HMSO	1968	
	Electricity Act	HMSO	1989	
	Health and Safety at Work etc Act	HMSO	1974	
	Registered Establishments (Scotland) Act	HMSO	1987	
	Water (Scotland) Act	HMSO	1980	
SI 2179 & 187	Active Implantable Medical Devices Regulations	HMSO	1998	
SI 1460	Building Standards (Scotland) Regulations	HMSO	1990	
	Building Standards (Scotland) Regulations: Technical Standards Guidance	HMSO	1998	
SI 437	Chemicals (Hazard Information and Packaging for Supply) Regulations (CHIP2)	HMSO	1997	
SI 3140	Control of Substances Hazardous to Health Regulations (COSHH)	HMSO	1999	
SI 1057	Construction (Design and Management) Regulations	HMSO	1994	
SI 635	Electricity Supply Regulations	HMSO	1988	
SI 2372	Electricity at Work Regulations	HMSO	1989	
SI 3080	Electromagnetic Compatibility Regulations	HMSO	1992	
SI 2451	Electromagnetic Compatibility (Amendment) Regulations	HMSO	1994	
SI 2792	Gas Safety (installation and use) Regulations	HMSO	1994	
SI 917	Health and Safety (Display Screen Equipment) Regulation (as amended)	HMSO	1992	
SI 682	Health & Safety (First Aid regulations)	HMSO	1981	
	Health & Safety Information for Employees Regulations	HMSO	1989	

<b>Publication ID</b>	<b>Title</b>	<b>Publisher</b>	<b>Date</b>	<b>Notes</b>
SI 1380	Health and Safety (Training for Employment) Regulations	HMSO	1990	
SI 341	Health and Safety (Signs and Signals) Regulations	HMSO	1996	
SI 2307	Lifting Operations and Lifting Equipment Regulations 1998 (LOLER)	HMSO	1998	
SI 2051	Management of Health and Safety at Work Regulations	HMSO	1992	
SI 2793	Manual Handling Operations Regulations	HMSO	1992	
SI 3017	Medical Devices Regulations	HMSO	1998	
SI 1790	Noise at Work Regulations	HMSO	1989	
SI 2966	Personal Protective Equipment at Work (PPE) Regulations	HMSO	1992	
SI 3139	Personal Protective Equipment (EC Directive) Regulations	HMSO	1992	
SI 2169	Pressure Systems and Transportable Gas Containers Regulations	HMSO	1989	
SI 2306	Provision and Use of Work Equipment Regulations (PUWER)	HMSO	1998	
SI 3163	Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (RIDDOR)	HMSO	1985	
SI 119	Water Supply (Water Quality) (Scotland) Regulations	HMSO	1990	
SI 3004	Workplace (Health, Safety and Welfare) Regulation	HMSO	1992	
<b>European Union Directives</b>				
65/65/EEC	Approximation of provisions laid down by law, regulation or administrative action relating to proprietary medicinal products.	Official Journal of the European Communities (OJEC), no 22, 9/2/65, p 369		
90/385/EEC	Approximation of the laws of the Member States relating to active implantable medical devices.	Official Journal of the European Communities (OJEC), L189 20/7/90, p 17		
91/356/EEC	Laying down the principles and guidelines of good manufacturing practice for medicinal products for human use.	Official Journal of the European Communities (OJEC). L193 17/7/91, p 30		

Publication ID	Title	Publisher	Date	Notes
93/42/EEC	Medical Devices Directorate	Official Journal of the European Communities (OJEC), L169 12/7/93, p 1		
<b>British Standards</b>				
EN 868	<b>Packaging materials for sterilization of wrapped goods.</b> <b>Part 1:</b> General requirements and requirements for the validation of packaging for terminally sterilized devices. <b>Part 2:</b> Sterilization wrap. Requirements and tests. <b>Part 3:</b> Paper for use in the manufacture of paper bags and in the manufacture of pouches and reels. Requirements and tests. <b>Part 4:</b> Paper bags. Requirements and tests. <b>Part 5:</b> Heat sealable pouches and reel materials of paper and plastic film construction. Requirements and tests. <b>Part 6:</b> Paper for the manufacture of packs for medical use for sterilization by ethylene oxide or irradiation. Requirements and tests. <b>Part 7:</b> Adhesive coated paper for the manufacture of packs for medical use for sterilization by ethylene oxide or irradiation. Requirements and tests. <b>Part 8:</b> Reusable sterilization containers. Requirements and tests. <b>Part 9:</b> Non-woven uncoated materials of high density polyethylene fibres (non-woven HDPE) for use in the manufacture of pouches, reels, etc. Requirements and tests. <b>Part 10:</b> Non-woven adhesive coated materials of high density polyethylene fibres (non-woven HDPE) for use in the manufacture of pouches, reels, etc. Requirements and tests. <b>Part 11:</b> Heat-sealable pouches and reel materials on non-woven high density polyethylene fibres (non-woven HDPE) and plastic film construction. Requirements and tests.	BSI Standards		Draft

Publication ID	Title	Publisher	Date	Notes
BS 3970	<b>Sterilizing and disinfecting equipment for medicinal products</b> <b>Part 1:</b> Specification for general requirements	BSI Standards	1990 (1996)	
BS EN 550	<b>Sterilization of medical devices. Validation and routine control of ethylene oxide sterilization</b>	BSI Standards	1994	
BS EN 554	<b>Sterilization of medical devices. Validation and routine control of sterilization by moist heat.</b>	BSI Standards	1994	
EN 285	<b>Sterilization: steam sterilizers: large sterilizers.</b>	BSI Standards	1997	
EN 1422	<b>Sterilizers for medical purposes: ethylene oxide sterilizers. Specification</b>	BSI Standards	1998	
BS 6068	<b>Water quality</b> <b>Part 0:</b> Introduction  <b>Part 2:</b> Physical, chemical and biomedical methods <b>Section 2.28:</b> Method for the determination of phosphorus: ammonium molybdate spectrophotometric method.  <b>Part 6:</b> Sampling <b>Section 6.3:</b> Guidance on the preservation and handling of samples. <b>Section 6.7:</b> Guidance on sampling of water and steam in boiler plants.	BSI Standards	1995 1986 (1991)          1986 (1990) 1994	
<b>Scottish Health Technical Guidance</b>				
SHTN 2	Domestic hot and cold water systems for Scottish Healthcare Premises	EEF	1999	CD-ROM
SHTM 2007	Electrical services supply and distribution	EEF	1999	CD-ROM
SHTM 2011	Emergency electrical services	EEF	1999	CD-ROM
SHTN 4	General Purposes Estates and Facilities Model Safety Permit-to-Work System	EEF	1997	
SHPN 1	Health Service building in Scotland	HMSO	1991	
SHPN 2	Hospital briefing and operational policy	HMSO	1993	
	NHS in Scotland – Scotconcode	EEF	1999	Version 3
SHTN 1	Post commissioning documentation for health buildings in Scotland	HMSO	1993	
SHGN	Pressure Systems and Transportable Gas Containers Regulations 1989	EEF	1999	CD-ROM
SHGN	'Safe' hot water and surface temperatures	EEF	1999	CD-ROM
SHTM 2010	Sterilization	EEF	1999	CD-ROM

<b>Publication ID</b>	<b>Title</b>	<b>Publisher</b>	<b>Date</b>	<b>Notes</b>
SHTM 2030	Washer-disinfectors	EEF	1999	CD-ROM
<b>NHS in Scotland Firecode</b>				
HTM 82	Alarm and detection systems	EEF	1998	CD-ROM
Fire Practice Note 6	Arson prevention and control in NHS healthcare premises	EEF	1998	CD-ROM
Fire Practice Note 5	Commercial enterprises on hospital premises	EEF	1998	CD-ROM
Fire Practice Note 3	Escape bed lifts	EEF	1998	CD-ROM
HTM 85	Fire precautions in existing hospitals	EEF	1998	CD-ROM
HTM 81	Fire precautions in new hospitals	EEF	1998	CD-ROM
Fire Practice Note 7	Fire precautions in patient hotels	EEF	1998	CD-ROM
HTM 86	Fire risk assessment in hospitals	EEF	1998	CD-ROM
HTM 83	Fire safety in healthcare premises: general fire precautions	EEF	1998	CD-ROM
HTM 84	Fire safety in NHS residential care properties	EEF	1998	CD-ROM
Fire Practice Note 4	Hospital main kitchens	EEF	1998	CD-ROM
Fire Practice Note 10	Laboratories on hospital premises	EEF	1998	CD-ROM
HTM 87	Textiles and furniture	EEF	1998	CD-ROM
<b>UK Health Technical Guidance</b>				
EH 40	HSE Occupational Exposure limits	HSE	Annual	
	Model Water Byelaws: Dept. of the Environment	HMSO	1986	
<b>Miscellaneous References</b>				
	British Pharmacopoeia. Medicines Commission	HMSO	1993	
	British Pharmacopoeia Addendum. Medicines Commission	HMSO	1995	
	CIBSE Guides and Commissioning Codes, A, R and W	CIBSE	1986 1991 1994	
	European Pharmacopoeia. 3rd edition		1996	
	General principles of sampling waters and associated materials (methods for the exhibition of waters and associated materials)	Strasbourg Council of Europe Publishing	1996	



<b>Publication ID</b>	<b>Title</b>	<b>Publisher</b>	<b>Date</b>	<b>Notes</b>
MES C14	Model Engineering Specifications Sterilization	NHS Estates	1996	
	Report on analytical work to verify test methods for Clean Steam Analysis. (MTMCS/0021/9603/01)	Healthcare Science Ltd.	March 1996	
	Rules governing medicinal products in the European Community. Volume IV: Good manufacturing practice for medicinal products. (CO-71-91-760-EN-C)	Commission of the European Communities (HMSO)	1992	
	United States Pharmacopoeia	United States Pharmacopoeial Convention	1994	Updated by supplements