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Preparation and quality management of fluids for haemodialysis and related therapies —

Part 1: General requirements

Préparation et management de la qualité des liquides d'hémodialyse et de thérapies annexes —

Partie 1: Exigences générales

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 2, *Cardiovascular implants and extracorporeal systems*.

This first edition cancels and replaces ISO 23500:2014, which has been technically revised. The main changes compared to the previous edition are as follows:

— The document forms part of a revised and renumbered series dealing with the preparation and quality management of fluids for haemodialysis and related therapies. The series comprise ISO 23500-1 (previously ISO 23500), ISO 23500-2, (previously ISO 26722), ISO 23500-3, (previously ISO 13959), ISO 23500-4, (previously ISO 13958), and ISO 23500-5, (previously ISO 11663).

A list of all parts in the ISO 23500 series can be found on the ISO website.

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

Introduction

This document is the base standard for a number of other standards dealing with water treatment and the production of dialysis fluid (ISO 23500 series).

The objective of the ISO 23500 series is to provide users with guidance for handling water and concentrates and for the production and quality oversight of dialysis fluid used for haemodialysis. The need for such guidance is based on the critical role of dialysis fluid quality in providing safe and effective haemodialysis, and the recognition that day-to-day dialysis fluid quality is under the control of the healthcare professionals who deliver dialysis therapy.

Annex A provides further information on the rationale for the development and provisions of this document.

The equipment used in the various stages of dialysis fluid preparation is generally obtained from specialized vendors. Dialysis practitioners are generally responsible for maintaining that equipment following its installation. Therefore, this document provides guidance on quality oversight and maintenance of the equipment to ensure that dialysis fluid quality is acceptable at all times. At various places throughout this International Standard, the user is advised to follow the manufacturer's instructions regarding the operation and maintenance of equipment. In those instances in which the equipment is not obtained from a specialized vendor, it is the responsibility of the user to validate the performance of the equipment in the haemodialysis setting and to ensure that appropriate operating and maintenance manuals are available.

Annex B to this document provides further information on the system components that are used for water treatment, concentrate, and dialysis fluid preparation at a dialysis facility. These descriptions are intended to provide the user with a basis for understanding why certain equipment might be required and how it should be configured; they are not intended as detailed design standards. Requirements for water treatment equipment are provided in ISO 23500-2.

Increasingly, self-contained, integrated systems designed and validated to produce water and dialysis fluid are becoming available and used clinically. This document applies to systems assembled from individual components. Consequently, some of the requirements in ISO 23500-1 and ISO 23500-2 might not apply to integrated systems, however such systems are required to comply with the requirements of ISO 23500-3, ISO 23500-4, and ISO 23500-5. In order to ensure conformity when using such systems, adherence to the manufacturer's instructions regarding the operation, testing, and maintenance of such systems is required to ensure that the system is being operated under the validated conditions.

This document reflects the conscientious efforts of healthcare professionals, patients, and medical device manufacturers to develop recommendations for handling water and concentrates and for the production and surveillance of dialysis fluid for haemodialysis and protecting haemodialysis patients from adverse effects arising from known chemical and microbial contaminants that might be found in improperly prepared dialysis fluid. Annexes F and G provide further information in respect of special considerations for home and acute haemodialysis The standard together with its constituent parts is directed towards the healthcare professionals involved in the management or routine care of haemodialysis patients and responsible for the quality of dialysis fluid. However, the physician in charge of dialysis has the ultimate responsibility for ensuring that the dialysis fluid is correctly formulated and meets the requirements of all applicable quality standards.

The provisions contained in this document might not be applicable in all circumstances and they are not intended for regulatory application.

Preparation and quality management of fluids for haemodialysis and related therapies —

Part 1:
General requirements

1 Scope

1.1 General

This document is the base standard for a number of other standards dealing with water treatment equipment, water, dialysis water, concentrates, and dialysis fluid (ISO 23500 series) and provides dialysis practitioners with guidance on the preparation of dialysis fluid for haemodialysis and related therapies and substitution fluid for use in online therapies, such as haemodiafiltration and haemofiltration. As such, this document functions as a recommended practice.

This document does not address clinical issues that might be associated with inappropriate usage of the water, dialysis water, concentrates, or dialysis fluid. Healthcare professionals involved in the provision of treatment for kidney failure should make the final decision regarding the applications with which these fluids are used, for example, haemodialysis, haemodiafiltration, high-flux haemodialysis, and the reprocessing of dialysers, and need to be aware of the issues that the use of inappropriate fluid quality raises in each of the therapies.

The concepts incorporated in this document should not be considered inflexible or static. The recommendations presented here should be reviewed periodically in order to assimilate increased understanding of the role of dialysis fluid purity in patient outcomes and technological developments.

1.2 Inclusions

This document addresses the user's responsibility for dialysis fluid once the equipment used in its preparation has been delivered and installed.

For the purposes of this document, dialysis fluid includes:

a) dialysis water (see 3.17 for definition) used for the preparation of dialysis fluid and substitution fluid,

b) dialysis water used for the preparation of concentrates at the user's facility,

c) concentrates,

d) the final dialysis fluid and substitution fluid.

The scope of this document includes

a) the quality management of equipment used to treat and distribute water used for the preparation of dialysis fluid and substitution fluid, from the point at which municipal water enters the dialysis facility to the point at which the final dialysis fluid enters the dialyser or the point at which substitution fluid is infused,

b) equipment used to prepare concentrate from powder or other highly concentrated media at a dialysis facility, and

c) preparation of the final dialysis fluid or substitution fluid from dialysis water and concentrates.

NOTE Because water used to prepare dialysis fluid can also be used to reprocess dialysers not marked intended for single use, this aspect of water use is also covered by this document.

1.3 Exclusions

This document does not apply to sorbent-based dialysis fluid regeneration systems that regenerate and recirculate small volumes of dialysis fluid, systems for continuous renal replacement therapy that use pre-packaged solutions, and systems and solutions for peritoneal dialysis.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For undated references, the latest edition of the referenced document (including any amendments) applies. For dated references, only the edition cited applies.

ISO 23500-3, *Preparation and quality management of fluids for haemodialysis and related therapies — Part 3: Water for haemodialysis and related therapies*

ISO 23500-4, *Preparation and quality management of fluids for haemodialysis and related therapies — Part 4: Concentrates for haemodialysis and related therapies*

ISO 23500-5, *Preparation and quality management of fluids for haemodialysis and related therapies — Part 5: Quality of dialysis fluid for haemodialysis and related therapies*

3 Terms and definitions

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

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

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
acetate concentrate
concentrated solution of salts containing acetate, which, when diluted with dialysis water, yields bicarbonate-free dialysis fluid for use in dialysis

Note 1 to entry: Acetate concentrate can contain glucose.

Note 2 to entry: Sodium acetate is used to provide buffer in place of sodium bicarbonate.

Note 3 to entry: Acetate concentrate is used as a single concentrate.

3.2
acid concentrate
A-concentrate
acidified concentrated mixture of salts that, when diluted with dialysis water and bicarbonate concentrate, yields dialysis fluid for use in dialysis

Note 1 to entry: The term “acid” refers to the small amount of acid (for example, acetic acid or citric acid) that is included in the concentrate.

Note 2 to entry: Acid concentrate can contain glucose.

Note 3 to entry: Acid concentrate can be in the form of a liquid, a dry powder, other highly concentrated media, or some combination of these forms.

3.3
action level
concentration of a contaminant at which steps should be taken to interrupt the trend toward higher, unacceptable levels

3.4
additive
spike
small amount of a single chemical that, when added to the concentrate, will increase the concentration of a single existing chemical by a value labelled on the additive packaging

3.5
bicarbonate concentrate
B-concentrate
concentrated preparation of sodium bicarbonate that, when diluted with dialysis water and acid concentrate, makes dialysis fluid used for dialysis

Note 1 to entry: Sodium bicarbonate is also known as sodium hydrogen carbonate.

Note 2 to entry: Some bicarbonate concentrates also contain sodium chloride.

Note 3 to entry: Bicarbonate concentrate can be in the form of a liquid or a dry powder.

Note 4 to entry: Dry sodium bicarbonate, without added sodium chloride, is also used in concentrate generators to produce a concentrated solution of sodium bicarbonate used by the dialysis machine to make dialysis fluid.

3.6
biofilm
microbially-derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are imbedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription

Note 1 to entry: The matrix, a slimy material secreted by the cells, protects the bacteria from antibiotics and chemical disinfectants.

Note 2 to entry: A certain amount of biofilm formation is considered unavoidable in dialysis water systems. When the level of biofilm is such that the action levels for microorganisms and endotoxins in the dialysis water are routinely reached or exceeded, the operation of the system is compromised from a medical and technical point of view. This level of biofilm formation is often referred to as bio-fouling.

3.7
bulk delivery
delivery of large containers of concentrate to a dialysis facility

Note 1 to entry: Bulk delivery includes containers such as drums, which can be pumped into a storage tank maintained at the user's facility. Alternatively, the drums can be left at the facility and used to fill transfer containers to transfer the concentrate to the dialysis machines. Bulk delivery can also include large containers for direct connection to a central concentrate supply system.

Note 2 to entry: Bulk delivery also includes dry powder concentrates intended to be used with an appropriate concentrate mixer.

3.8
central concentrate system
system that prepares and/or stores concentrate at a central point for subsequent distribution to its points of use

3.9
central dialysis fluid delivery system
system that produces dialysis fluid from dialysis water and concentrate or powder at a central point and distributes the dialysis fluid from the central point to individual dialysis machines

3.10
combined chlorine

chlorine that is chemically combined

EXAMPLE Chloramine compounds.

Note 1 to entry: There is no direct test for measuring combined chlorine, but it can be established indirectly by measuring both total and free chlorine and calculating the difference.

3.11
free chlorine

chlorine present in water as dissolved molecular chlorine (Cl), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-)

Note 1 to entry: The three forms of free chlorine exist in equilibrium.

3.12
total chlorine

sum of free and combined chlorine

Note 1 to entry: Chlorine can exist in water as dissolved molecular chlorine, hypochlorous acid, and/or hypochlorite ion (free chlorine) or in chemically combined forms (combined chlorine). Where chloramine is used to disinfect water supplies, chloramine is usually the principal component of combined chlorine.

3.13
colony-forming unit
CFU

measure of bacterial or fungal cell numbers that theoretically arise from a single cell when grown on solid media

Note 1 to entry: Colonies can also form from groups of organisms when they occur in aggregates.

3.14
concentrate generator

system where the concentrate is delivered to the user as a powder in a container, suitable for attachment to the dialysis machine with which it is intended to be used, and then the powder is converted into a concentrated solution by the dialysis machine

Note 1 to entry: The solution produced by the concentrate generator is used by the dialysis machine to make the final dialysis fluid delivered to the dialyser.

3.15
dialysis fluid
dialysate
dialysis solution

aqueous fluid containing electrolytes and, usually, buffer and glucose, which is intended to exchange solutes with blood during haemodialysis and haemodiafiltration

Note 1 to entry: The term “dialysis fluid” is used throughout this document to mean the fluid made from dialysis water and concentrates that is delivered to the dialyser by the dialysis fluid delivery system. Such phrases as “dialysate” or “dialysis solution” are used in place of dialysis fluid in some countries; however, that usage is discouraged to avoid confusion.

Note 2 to entry: ISO 23500-5 defines three levels of dialysis fluid: standard dialysis fluid, ultrapure dialysis fluid, and online-prepared substitution fluid used for haemodiafiltration.

Note 3 to entry: The dialysis fluid entering the dialyser is referred to as “fresh dialysis fluid”, while the fluid leaving the dialyser is referred to as “spent dialysis fluid”.

Note 4 to entry: Dialysis fluid does not include prepackaged parenteral fluids used in some renal replacement therapies, such as haemodiafiltration and haemofiltration.

3.16
dialysis fluid delivery system

device that prepares dialysis fluid online from dialysis water and concentrates or that stores and distributes premixed dialysis fluid; circulates the dialysis fluid through the dialyser; monitors the dialysis fluid for temperature, conductivity (or equivalent), pressure, flow, and blood leaks; and, prevents dialysis during disinfection or cleaning modes

Note 1 to entry: The term includes reservoirs, conduits, proportioning devices for the dialysis fluid, and monitors and associated alarms and controls assembled as a system for the purposes listed above.

Note 2 to entry: The dialysis fluid delivery system can be an integral part of the dialysis machine or a centralized preparation system which feeds multiple individual dialysis consoles.

Note 3 to entry: Dialysis fluid delivery systems are also known as proportioning systems and dialysis fluid supply systems.

3.17
dialysis water

water that has been treated to meet the requirements of ISO 23500-3 and which is suitable for use in haemodialysis applications, including the preparation of dialysis fluid, reprocessing of dialysers, preparation of concentrates and preparation of substitution fluid for online convective therapies

3.18
disinfection

destruction of pathogenic and other kinds of microorganisms by thermal or chemical means

Note 1 to entry: Disinfection is a less lethal process than sterilization because it destroys most recognized pathogenic microorganisms but does not necessarily destroy all microbial forms.

Note 2 to entry: Appropriate disinfection strategies need to include: disinfection type, disinfectant concentration, exposure time and temperature

3.19
empty-bed contact time

EBCT

time taken by a feed water to pass through an empty volume equal to the volume of a particle bed

Note 1 to entry: EBCT (min) is calculated from the following formula:

$$\text{EBCT} = V/Q$$

where

V is the volume of the particle bed, in cubic metres (m^3);

Q is the flow rate of water through the bed, in cubic metres per minute (m^3/min).

Note 2 to entry: EBCT is used as an indirect measure of how much contact occurs between particles, such as activated carbon, and water as the water flows through a bed of particles.

3.20
endotoxin

major component of the outer cell wall of gram-negative bacteria

Note 1 to entry: Endotoxins are lipopolysaccharides, which consist of a polysaccharide chain covalently bound to lipid A. Endotoxins can acutely activate both humoral and cellular host defences, leading to a syndrome characterized by fever, shaking, chills, hypotension, multiple organ failure, and even death if allowed to enter the circulation in a sufficient dose. [See also *pyrogen* (3.35)].

3.21
endotoxin-retentive filter
ETRF
membrane filter used to remove endotoxins and microorganisms from dialysis water or dialysis fluid

Note 1 to entry: The performance of an endotoxin-retentive filter is usually expressed as the logarithmic reduction value (LRV), defined as $\log_{10}$(inlet concentration)/(outlet concentration).

Note 2 to entry: Endotoxin-retentive filters can be configured in a cross-flow or dead-end mode. Some endotoxin-retentive filters also remove endotoxins by adsorption.

3.22
endotoxin unit
EU
unit assayed by the *Limulus* amoebocyte lysate (LAL) test when testing for endotoxins

Note 1 to entry: Because activity of endotoxins depends on the bacteria from which they are derived, their activity is evaluated by reference to a standard endotoxin.

Note 2 to entry: In some countries, endotoxin concentrations are expressed in international units (IU). Since the harmonization of endotoxin assays, EU and IU are equivalent.

3.23
feed water
water supplied to a water treatment system or to an individual component of a water treatment system

Note 1 to entry: the water supplied to the water treatment system is potable water that meets drinking water requirements.

3.24
germicide
agent that kills microorganisms

3.25
haemodiafiltration
form of renal replacement therapy in which waste solutes are removed from blood by a combination of diffusion and convection through a high-flux membrane

Note 1 to entry: Diffusive solute removal is achieved using a dialysis fluid stream as in haemodialysis. Convective solute removal is achieved by adding ultrafiltration in excess of that needed to obtain the desired weight loss; fluid balance is maintained by infusing a replacement solution into the blood either before the dialyser (predilution haemodiafiltration), after the dialyser (postdilution haemodiafiltration), or a combination of the two (mixed dilution haemodiafiltration).

3.26
haemodialysis
form of renal replacement therapy in which waste solutes are removed primarily by diffusion from blood flowing on one side of a membrane into dialysis fluid flowing on the other side

Note 1 to entry: Fluid removal that is sufficient to obtain the desired weight loss is achieved by establishing a hydrostatic pressure gradient across the membrane. This fluid removal provides some additional waste solute removal, particularly for solutes with higher molecular weight.

3.27
haemofiltration
form of renal replacement therapy in which waste solutes are removed from blood by convection

Note 1 to entry: Convective transport is achieved by ultrafiltration through a high-flux membrane. Fluid balance is maintained by infusing a replacement solution into the blood either before the haemofilter (predilution haemofiltration), after the haemofilter (postdilution haemofiltration), or a combination of the two (mixed dilution haemofiltration).

Note 2 to entry: There is no dialysis fluid stream in haemofiltration.

3.28

heterotrophic

not self-sustaining, i.e. a type of nutrition in which organisms derive energy from the oxidation of organic compounds by either consumption or absorption of other organisms

3.29

LAL test

***Limulus* amoebocyte lysate test**

assay used to detect endotoxin

Note 1 to entry: The detection method uses the chemical response of an extract from blood cells of a horseshoe crab (*Limulus polyphemus*) to endotoxins.

Note 2 to entry: Amebocyte lysate from a second horseshoe crab, *Tachypleus tridentatus*, may also be used to detect endotoxin.

3.30

manufacturer

entity that designs, makes, fabricates, assembles, or processes a particular item or object.

Note 1 to entry: Manufacturer includes, but is not limited to, those who perform the functions of contract sterilization, installation, relabelling, remanufacturing, repacking, or specification development, and initial distributions of foreign entities performing these functions.

Note 2 to entry: Manufacturer does not cover the preparation of concentrates from pre-packaged dry chemicals at a dialysis facility or the handling of bulk concentrates at a dialysis facility as responsibility for the concentrate is transferred from the manufacturer to the user.

3.31

microbiological contamination

contamination with any form of microorganism (e.g. bacteria, yeast, fungi, and algae) or with the by-products of living or dead organisms, such as endotoxins, exotoxins, and cyanobacterial toxins (derived from blue-green algae)

3.32

non-pyrogenic

not eliciting a pyrogen reaction

Note 1 to entry: This definition is applicable for fluids produced by online techniques, e.g. substitution and priming fluids.

Note 2 to entry: For medical devices and injectable fluids, the threshold pyrogenic dose (the minimum dose that produces fever) is set at 5 EU/kg/h. The commonly used gel clot method has a sensitivity limit of 0,03 EU/ml, enabling the volume of fluid that may be administered without breaching the threshold pyrogenic dose to be established.

3.33

product water

water produced by a water treatment system or by an individual device thereof

3.34

proportioning system

apparatus that proportions dialysis water and haemodialysis concentrate to prepare dialysis fluid

3.35

pyrogen

fever-producing substance

Note 1 to entry: Pyrogens are most often lipopolysaccharides of gram-negative bacterial origin [see also *endotoxin* (3.20)].

3.36
sodium hypochlorite
chemical used for disinfection of haemodialysis systems

Note 1 to entry: Commercially available solutions of sodium hypochlorite are known in different countries by terms such as bleach and L'eau de Javel. These solutions are used for disinfection at concentrations recommended by equipment manufacturers.

3.37
source water
water entering a dialysis facility from an external supplier, such as a municipal water supply

Note 1 to entry: Source water, sometimes referred to as feed water, is potable water meeting the requirements for drinking water

3.38
sterile
free from viable microorganisms

Note 1 to entry: "Sterile" can be used to describe a packaged solution that was prepared using a terminal sterilization process validated according to the methods of the applicable pharmacopoeia. A terminal sterilization process is commonly defined as one that achieves a sterility assurance level (SAL) of 10^{-6}, i.e. assurance of less than one chance in a million that viable microorganisms are present in the sterilized article.

Note 2 to entry: Alternatively, "sterile" can be used to describe a solution prepared for immediate use by a continuous process, such as filtration, that has been validated according to the methods of the appropriate sections of the applicable pharmacopoeia to produce a solution free from microorganisms for the validated life of the filter.

3.39
storage tank
tank at the user's facility for storage of dialysis water or concentrate from bulk deliveries, or for concentrate prepared in bulk at the user's facility from powder and dialysis water

3.40
substitution fluid
fluid used in haemofiltration and haemodiafiltration treatments which is infused directly into the patient's blood as a replacement for the fluid that is removed from the blood by ultrafiltration

Note 1 to entry: Substitution fluid is also referred to as substitution solution or replacement solution.

Note 2 to entry: Substitution fluid can also be used for bolus administration, for priming of an extracorporeal blood circuit, and for returning blood to the patient at the end of a treatment.

3.41
total dissolved solids
TDS
sum of all ions in a solution, often approximated by means of electrical conductivity or resistivity measurements

Note 1 to entry: TDS measurements are commonly used to evaluate the performance of reverse osmosis units. TDS values are often expressed in terms of $CaCO_3$, NaCl, KCl, or 442 equivalents, in milligrams per litre (mg/L). [442 is a solution of sodium sulfate (40 %), sodium bicarbonate (40 %), and sodium chloride (20 %) that closely represents the conductivity to concentration relationship, on average, for naturally occurring fresh water.]

3.42
ultrapure dialysis fluid
highly purified dialysis fluid that can be used in place of conventional dialysis fluid

Note 1 to entry: A widely accepted specification of ultrapure dialysis fluid is <0,1 CFU/ml and <0,03 EU/ml.

3.43
user
responsible physician, their representative, or healthcare professional with a responsibility for the prescription, production, and delivery of dialysis fluid

Note 1 to entry: In this context the user refers to the decision maker who is accountable for the medical decision for the care of the patients.

3.44
validation
process of documenting that the dialysis water treatment and dialysis fluid production systems, when installed and operated according to the manufacturer's recommendations, consistently produce dialysis water or dialysis fluid meeting the stipulated quality levels

Note 1 to entry: In this context, validation also includes demonstrating that the system is "fit for purpose".

3.45
verification
process of demonstrating that the system complies with applicable regulations, specifications, or other conditions

3.46
water treatment system
collection of water treatment devices and associated piping, pumps, valves, gauges, etc., that together produce water for dialysis meeting the requirements of ISO 23500-3 for haemodialysis applications and deliver it to the point of use

4 Quality requirements

4.1 General

The quality requirements set forth in respect of dialysis water (4.2), concentrates (4.3), and dialysis fluid (4.4) are identical to those in ISO 23500-4, ISO 23500-3, and ISO 23500-5. The latest editions of these documents should be consulted to ascertain if there have been any changes to quality requirements before implementing the recommendations of this document.

4.2 Dialysis water

4.2.1 General

The requirements contained in this clause apply to dialysis water at its point of use. As such, these requirements apply to the water treatment system as a whole and not to each of the devices that make up the system. However, collectively, the individual devices shall produce water that, at a minimum, meets the requirements of this clause.

4.2.2 Chemical contaminants in dialysis water

Dialysis water shall not contain substances at levels greater than those listed in Tables 1 and 2. The manufacturer or supplier of a complete water treatment system should recommend a system that is capable of meeting these requirements based on a feed water analysis. The system design should reflect possible seasonal variations in feed-water quality. The manufacturer or supplier of a complete water treatment and distribution system should demonstrate that the complete water treatment, storage, and distribution system is capable of meeting the requirements of ISO 23500-3 at the time of installation.

NOTE The maximum allowable levels of contaminants listed in Tables 1 and 2 include the anticipated uncertainty associated with the analytical methodologies listed in Table 4 of ISO 23500-3:2019. Other analytical methods can be used, provided that such methods have been appropriately validated and are comparable to the cited methods.

Following installation of a water treatment, storage, and distribution system, the user is responsible for regular surveillance of the levels of chemical contaminants in the dialysis water and for complying with the requirements of this document.

Table 1 — Maximum allowable levels of toxic chemicals and dialysis fluid electrolytes in dialysis water[a,b]

Contaminant	**Maximum concentration** mg/l[c]
Contaminants with documented toxicity in haemodialysis	
Aluminium	0,01
Total chlorine[d]	0,1
Copper	0,1
Fluoride	0,2
Lead	0,005
Nitrate (as N)	2
Sulphate	100
Zinc	0,1
Electrolytes normally included in dialysis fluid	
Calcium	2 (0,05 mmol/l)
Magnesium	4 (0,15 mmol/l)
Potassium	8 (0,2 mmol/l)
Sodium	70 (3,0 mmol/l)

[a] A dialysis facility's Medical Director has the ultimate responsibility for ensuring the quality of dialysis water.

[b] The reader is cautioned to refer to the latest version of ISO 23500-3 to ensure that there have been no changes to this table.

[c] Unless otherwise noted.

[d] When chlorine is added to water, some of the chlorine reacts with organic materials and metals in the water and is not available for disinfection (the chlorine demand of the water). The remaining chlorine is the total chlorine, and is the sum of free chlorine or non-bound chlorine and combined chlorine.

There is no direct method for the measurement of chloramine. It is generally established by measuring total and free chlorine concentrations and calculating the difference. When total chlorine tests are used as a single analysis the maximum level for both chlorine and chloramine shall not exceed 0,1 mg/l. Since there is no distinction between chlorine and chloramine, this safely assumes that all chlorine present is chloramine.

Table 2 — Maximum allowable levels of other trace elements in dialysis water[a]

Contaminant	Maximum concentration mg/l
Antimony	0,006
Arsenic	0,005
Barium	0,1
Beryllium	0,000 4
Cadmium	0,001
Chromium	0,014
Mercury	0,000 2
Selenium	0,09
Silver	0,005
Thallium	0,002

[a] The reader is cautioned to refer to the latest version of ISO 23500-3 to ensure that no changes have been made to the maximum concentrations shown.

No limits in respect of organic compounds are included in the above table. Currently, there is limited documentary evidence to suggest that exposure impacts on patients' wellbeing. A suggested starting point to establish whether such compounds are of concern is the National Drinking Water requirement for organic compounds.

Since organic compounds can be effectively removed by the use of activated carbon beds or filters, the dialysis facility should consider carefully their dimensioning to ensure that there is sufficient capacity to remove organic compounds should the need arise.

4.2.3 Organic Carbon, pesticides and other chemicals

The presence of organic compounds, such as pesticides, polycyclic aromatic hydrocarbons and other chemicals such as pharmaceutical products and endocrine disruptors in respect of hemodialysis patients are difficult to define. Consequences of exposure are probably of a long-term nature and it is technically difficult and costly to measure these substances on a routine basis. Furthermore, there is an absence of evidence of their widespread presence in water although it is recognized that inadvertent discharges are possible. In view of this, It is not at present possible to define limits for their presence in water used in the preparation of dialysis fluid.

Nanofiltration and reverse osmosis are capable of significant rejection of many such compounds. Granular Activated Carbon (GAC) is also highly effective at removing the majority of these chemicals. However, as Granular Activated Carbon is widely used in the removal chlorine/chloramine, their use in the removal or organic carbons, pesticides and other chemicals will be dependent upon the size of the carbon filters and/or beds and users shall be aware of appropriate dimensioning since the majority of carbon valences might be already occupied and not available for further removal activity.

4.2.4 Microbiological contaminants in dialysis water

The total viable microbial count and endotoxin concentration in dialysis water shall comply with the maximum allowable levels specified Table 3. Action levels for the total viable microbial count and endotoxin concentration shall also be set, based on knowledge of the microbial dynamics of the system. Typically, the action level is set at 50 % of the maximum allowable level for bacteria and endotoxins. If a total viable microbial count or endotoxin concentration at or above the action level is observed in the dialysis water, corrective measures should be taken promptly to reduce the level. The manufacturer or supplier of a complete water treatment and distribution system should demonstrate that the complete water treatment, storage, and distribution system is capable of meeting the requirements of ISO 23500-3 at the time of installation.

Following installation of a water treatment, storage and distribution system, the user is responsible for regular surveillance of the microbiology of the system and for complying with the requirements of this document, including those requirements related to action levels. Clause 8 of this document details strategies for microbiological control. Annex C provides further information relating to surveillance.

In association with the presence of bacteria and endotoxin in the water, yeast and filamentous fungi may also be present. No specific recommendations have been made in respect of the routine measurement of such contaminants, nor have action limits been set.

Table 3 — Maximum allowable levels for total viable microbial count (TVC) and endotoxins in dialysis water[a]

Contaminant	Maximum allowable level	Typical action level[b]
TVC	<100 CFU/ml	50 CFU/ml
Endotoxin	<0,25 EU/ml	0,125 EU/ml

[a] The reader is cautioned to refer to the latest version of ISO 23500-3 to ensure that there have been no changes to the values presented in this table.

[b] Typically set at 50 % of the maximum allowable level. Other values may be set.

4.3 Requirements for concentrate

4.3.1 Chemical and microbiological contaminants in concentrate

Concentrates used to prepare dialysis fluid shall comply with the quality requirements specified in ISO 23500-4.

Bicarbonate concentrate can grow bacteria and caution should be used to limit the bacterial levels in bicarbonate concentrate. Dialysis fluid can also be prepared from acetate concentrate used as a single concentrate that can be metabolized by the patient to bicarbonate.

4.3.2 Water used to prepare concentrate

Water used to prepare concentrates at a dialysis facility shall meet the requirements of ISO 23500-3. Any concentrate prepared at a dialysis facility shall permit the dialysis machine to prepare dialysis fluid meeting the requirements of ISO 23500-5.

4.4 Requirements for dialysis fluid

4.4.1 General

The requirements contained in this clause apply to a sample of the dialysis fluid collected as close as practicable to the inlet to the dialyser.

ISO 23500-5 defines three levels of dialysis fluid: standard dialysis fluid, ultrapure dialysis fluid, and online-prepared substitution fluid used for haemodiafiltration.

Standard dialysis fluid shall be regarded as the minimum acceptable quality. Ultrapure dialysis fluid is a step forward in improving biocompatibility, reducing inflammation and preventing dialysis related complications.

Tests for bacterial growth and endotoxins are not required if the dialysis machine fluid pathway is fitted with an appropriate capacity bacteria retentive and endotoxin-retentive filter validated by the manufacturer and operated and monitored according to the manufacturer's instructions, unless the manufacturer requires such tests in the instructions for use.

4.4.2 Microbiological requirements for standard dialysis fluid

The total viable microbial count and endotoxin concentration in standard dialysis fluid shall comply with the maximum allowable levels specified in ISO 23500-5 and reproduced in Table 4. Action levels for the total viable microbial count and endotoxin concentration shall also be set, based on knowledge of the microbial dynamics of the system as specified in ISO 23500-5. Typically, the action level is set at 50 % of the maximum allowable level for total viable microbial count and endotoxins. If total viable microbial counts or endotoxin concentrations at or above the action levels are observed in the dialysis fluid, corrective measures, such as disinfection and retesting, should promptly be taken to reduce the levels.

Table 4 — Maximum allowable levels for total viable microbial count (TVC) and endotoxins in standard and ultrapure dialysis fluid[a]

Contaminant	Standard dialysis fluid		Ultrapure dialysis fluid
	Maximum allowable level	Action level[b]	Maximum allowable level
TVC	<100 CFU/ml	50 CFU/ml	<0,1 CFU/ml
Endotoxin	<0,5 EU/ml	0,25 EU/ml	<0,03 EU/ml

[a] The reader is cautioned to refer to the latest version of ISO 23500-5 to ensure that there have been no changes to this table.

[b] Typically set at 50 % of the maximum allowable level. Other values may be set.

4.4.3 Microbiological requirements for ultrapure dialysis fluid

The total viable microbial count and endotoxin concentration in ultrapure dialysis fluid shall comply with the maximum allowable levels specified in ISO 23500-5 and reproduced in Table 4. If those limits are exceeded in ultrapure dialysis fluid, corrective measures should be taken to reduce the levels into an acceptable range. The user is responsible for surveillance of the dialysis fluid bacteriology of the total system following installation.

Tests for bacterial growth and endotoxins are not required if the dialysis machine fluid pathway is fitted with an appropriate capacity bacteria retentive and endotoxin-retentive filter validated by the manufacturer and operated and monitored according to the manufacturer's instructions, unless the manufacturer requires such tests in the instructions for use.

4.4.4 Microbiological requirements for online-prepared substitution fluid

The recommendations contained in this clause apply to substitution fluid as it enters the patient's blood.

This fluid shall be sterile and non-pyrogenic according to ISO 23500-5.

Substitution fluid for convective therapies, such as haemodiafiltration and haemofiltration, or for priming the extracorporeal circuit or bolus administration of fluid during a treatment, may be produced online by a process of ultrafiltration with endotoxin-retentive filters. The online process shall be validated by the manufacturer to produce substitution fluid that is sterile and nonpyrogenic.

The user shall follow the manufacturer's instructions for the installation, use, maintenance, and conformity of the validated system. The function of the validated system shall be verified according to the manufacturer's instructions at the time of installation and confirmed by the user with regular surveillance. Surveillance shall include confirmation that the dialysis water and concentrates used by the validated system to prepare the substitution fluid continue to meet the specifications of ISO 23500-4 and ISO 23500-3.

4.5 Record retention

Records of installation, surveillance, maintenance, and disinfection of the water treatment and dialysis fluid preparation systems, medical observation, and personnel education shall be kept according to national regulations. In the absence of national regulations, it is recommended that these records should be retained for the same period as clinical records.

5 Critical aspects of system design

5.1 General

The preparation of dialysis fluid, from inlet municipal water and acquisition of concentrates to discharge of spent dialysis fluid into the drain system, involves numerous components that, together, form a dialysis fluid handling system.

The technical features of the water treatment component of that system should be based on the criteria listed in ISO 23500-2, with special regard to the aspects related to the dialysis water quality, disinfection and maintenance. In addition to the general specifications the system design should also comply with local water and building regulations. The treatment of water for haemodialysis applications is an energy and resource intensive process and in designing the system, appropriate consideration should also be given to ensure optimal use of resources e.g. water and energy.

Concentrates used with the treated water to prepare dialysis fluid may be obtained from a supplier in a ready-to-use form or prepared at the dialysis facility from dialysis water and prepackaged salts. The technical features of the concentrate preparation and distribution component of the system should be based on the criteria listed in ISO 23500-4.

The spent or used dialysis fluid is discharged into the public sewage system without treatment. Disinfectant solutions may also be similarly discharged. The risks to public health and the environment from such discharges are negligible, however such discharges may impact on the effectiveness of domestic anaerobic digestion systems.

5.2 Technical aspects

In accordance with the requirements listed in ISO 23500-2, the system design should specifically address the following points:

a) The choice of the water treatment system should consider the following aspects concerning the feed-water supply:

   1) full chemical analysis of the feed water and silt density index (SDI);

   2) microbiological load, which may require the introduction of an additional chlorination step;

   3) flow rates, pressure, and temperature;

   4) treatment techniques used by the provider of the feed water (for example, addition of chloramine, fluoride, aluminium sulfate, or other chemicals.). Because treatment techniques can change, ongoing communication with the provider of the feed water is recommended;

   NOTE 1 Source water is assumed to be potable water meeting national drinking water requirements.

   If the water supply to the treatment system, is not directly from the municipal distribution network, but comes via an existing hospital water supply, there should be awareness of the potential risks that might arise from the introduction of chemicals into the hospital water supply by hospital engineering staff. To prevent the occurrence of adverse effects arising from such actions, the introduction or addition of chemicals into the hospital water supply should only be undertaken after prior consultation with renal services. It should be further noted, that if such chemicals are introduced, it might necessitate additional surveillance prior to water being used for dialysis applications.

b) product water capacity during sanitization:

   if heat sanitization is planned for the system, the distribution loop is sanitized along with the links from the distribution loop to the dialysis machines. The demand for water during such sanitization is higher than required by the dialysis machines during operation;

c) product water capacity during the winter months:

commonly, reverse osmosis systems capacity is rated at a specified incoming water temperature. There should be awareness that such temperatures may not be achieved during the winter months, leading to a reduced system efficiency. To meet the required water demand there may be a need to pre-heat the feed water or to install a system with increased capacity to compensate for the reduction in reverse osmosis efficiency during the winter months;

d) sanitization of the system:

disinfection is the only effective method of diminishing and inactivating microflora: First and foremost, the frequency of disinfection is important. Disinfection should be performed on a regular basis to limit bio-fouling within the fluid pathways of the system. Second, all surfaces in the circuit should be included in the disinfection procedure. This includes the reverse osmosis membranes (especially the clean side), the distribution piping, the inlet lines to the dialysis machines (located before the disinfection circuit of the dialysis machine), and the dialysis machines (which have their own disinfection circuit and programme). Third, the disinfection procedure, when applied with a given frequency and with inclusion of all critical areas, should be capable of minimizing the effects of bio-fouling.

Disinfection can be performed using heat or chemicals. UV lamps can be used to inactivate planktonic cells but are of no value against any biofilm that has formed in the system.

Hot water may be used to control bacterial proliferation in dialysis water storage and distribution systems. The exposure time should be according to the manufacturer's instructions. The water heater of a hot water disinfection system should be capable of delivering hot water at the temperature and for the exposure time specified by the manufacturer to any site in the dialysis water storage and distribution system. The manufacturer's instructions for using hot water disinfection systems should be followed.

If chemical sanitization is to be used, the period prior to the next dialysis treatment should be sufficient to enable the chemicals to be rinsed completely from the system.

If it is possible to sanitize the haemodialysis machines at the same time as the distribution loop, then this should be performed since this is the easiest and simplest.

e) provision for adequate process surveillance;

f) service and maintenance.

If the entire fluid handling system is not obtained from a single supplier, then the user becomes responsible for ensuring that the separate parts of the system are compatible. For example, it is important that the pre-treatment section of the water treatment system be designed to supply the main purification device (usually reverse osmosis) with feed water meeting the specifications for that device, i.e. pressure, temperature, flow, and quality (for example, elimination of chemicals which cannot be effectively removed by the main purification device or that could damage it).

NOTE There is a possibility that mandatory local water and building regulations for system design exist.

5.3 Microbiological aspects

The required microbiological quality of the dialysis water and dialysis fluid is achieved by paying adequate attention to the entire water treatment and dialysis fluid preparation cascade including central concentrate systems. For this reason, the system should be designed to reduce, as much as possible, potential sources of contamination and to allow effective surveillance and disinfection of the critical parts.

When applicable, equipment should be operated on a regular basis to reduce stagnation.

The distribution system should be designed to maintain dialysis water or dialysis fluid quality and, therefore, should fulfil the following criteria:

- the distribution loop should be of the minimum length, and avoid multiple branches and dead ends;
- materials shall be compatible with the different operational conditions (i.e. supply, disinfection, cleaning);
- there should be no release of chemicals and nutrients for microorganisms;
- temperature increases or exposure to sunlight should be minimized.

Suitable sampling ports should be available at the start and the end of the distribution loop.

5.4 Environmental impact

The environmental aspect of haemodialysis is considerable in terms of the resources used. e.g. water and electricity). In planning the water treatment, consideration should be given to minimizing the water that is rejected by the reverse osmosis system, for example by the use of a dual pass RO system in which the rejected water passes through a second RO system to improve recovery and/or the use of rejected water for applications that do not require drinking water, e.g. flushing toilets.

Treatment of patients may take place in a hospital, clinic or a home environment. When the patient is receiving treatment in their home, there should be awareness that the discharge of large volumes of fluid associated with dialysis treatment in the form of used dialysis fluid, or disinfection products associated with the water treatment maintenance may impact on the effectiveness of a domestic anaerobic digestion system. Furthermore, there may be local regulations in place limiting such discharges into public sewage systems.

6 Validation of system performance

6.1 General

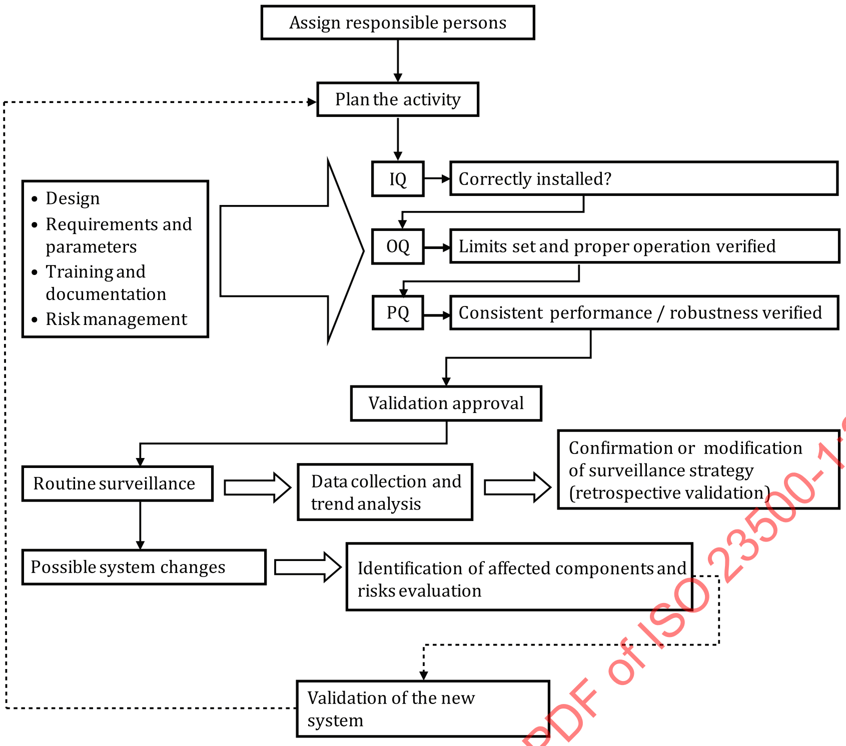
The validation process should provide documentary evidence that the system consistently produces dialysis water and dialysis fluids meeting the quality requirements of ISO 23500-3 or ISO 23500-5.

The validation process consists of the following:

- assignment of operational responsibility;
- assignment of clinical responsibility;
- assignment of legal responsibility;
- validation plan;
- installation and operational qualification;
- performance qualification;
- revalidation as it relates to routine surveillance.

Annex E provides further information on the validation process.

An example of the validation process is presented schematically in Figure 1.



Key

IQ	installation qualification
OQ	operational qualification
PQ	performance qualification

Figure 1 — Example of a validation process for a fluid preparation and distribution system

6.2 Validation plan

The validation plan should be a clear and concise document covering the following:

- description of relevant systems, equipment, or processes;
- current status of those systems, equipment, or processes;
- procedures for making changes to systems, equipment, or processes;
- planning and scheduling, including the addition of new systems, activities driven by change, and periodic review.

The validation plan should be approved by the person at the dialysis facility with overall responsibility for dialysis fluids.

6.3 Installation and operational qualification

The installation qualification defines and provides the documented proof that the system is installed in accordance with the approved plans and the manufacturer's technical requirements and specifications. Full system documentation should be available on completion of this procedure, including system flow diagrams and layout, log books, and operator's manuals. The installation should be carried out by qualified personnel in accordance with the manufacturer's documented recommendations.

The installation qualification should be followed by an operational qualification that verifies the proper operation of the system, including range of operation, set point and interlock testing, and functional testing, and that compares the system performance with the functional specifications of the system. On completion of this phase, the following information should be available:

- test records;
- set-up record;
- calibration schedule;
- sampling procedures;
- maintenance (e.g. disinfection, filter changes,) and surveillance (e.g. conductivity, microbiological analysis) plans;
- record of operator(s) training.

6.4 Performance qualification

The performance qualification should demonstrate the consistency and robustness of the system under local operational conditions. During this phase, information about system behaviour should be collected and action levels should be reviewed or defined. On completion of the performance qualification, the following information should be available:

- test records;
- chemical and microbial analyses;
- key performance indicators [for example, pre-treatment efficiency, reverse osmosis (RO) recovery/rejection rate];
- (initial) trend analysis.

For newly installed systems, the person with overall clinical responsibility for dialysis (possibly supported by technical experts) may authorize use of dialysis fluid for patient treatments once chemical and microbiological analyses are available that show full conformity with the quality requirements of Clause 4, the manufacturer's specifications, and any applicable regulatory requirements.

In some instances the schematic approach shown in Figure 1 cannot be followed, and concurrent validation may be appropriate e.g. following major refurbishment of an already existing system. The person with overall clinical responsibility for dialysis (supported by technical experts) may authorize use of dialysis fluid for patient treatments provided an appropriate risk assessment has been performed and recorded.

6.5 Routine surveillance and revalidation

This clause describes the surveillance activities to be conducted as part of the quality control and quality assurance process. Annex C provides further information on the surveillance guidelines for water treatment equipment, distribution systems and dialysis fluid. Guidance on parameters that should be subject to regular surveillance is provided in Table C.1.

Routine surveillance should begin following the performance qualification (see 6.4) to ensure ongoing conformity with the dialysis water and dialysis fluid quality requirements set forth in Clause 4. Trend analysis of surveillance data should be used to provide advanced information on system performance, thus enabling a preventive rather than a reactive approach to system maintenance.

Surveillance is achieved with online and offline measurements. Online surveillance of suitable parameters (such as conductivity) provides immediate identification of deviations from normal operating conditions and can identify potential problems in their early stages, and could trigger specific additional offline measurements. A purely time-based offline sampling regime has inherent limitations for the surveillance of a continuous production process since deviations can occur between samples.

Fluid sampling for microbiological testing should be performed before disinfection or no sooner than 24 h after disinfection (to avoid false-negative results). When disinfection is performed on consecutive days, or more frequently, samples should be taken before, and as close as practicable, to a disinfection procedure.

Samples for routinely scheduled microbiology surveillance should be obtained in as short a possible time before the scheduled disinfection.

A revalidation should be performed following:

- modification of the maintenance and surveillance plans;
- modification of the system;
- changes in the requirements for dialysis fluid quality.

In the absence of system modification, regular revalidation is still required. This revalidation consists of a retrospective assessment of the routine results of the previous year (no additional tests are required for the revalidation process). The purpose of this review is to prove the adequacy of the maintenance and surveillance plan under the local operating conditions. The revalidation should include a report submitted to, and approved by, the person with overall clinical responsibility for dialysis.

7 Quality management

7.1 General

Quality control and quality assurance procedures should be established to ensure on going conformance to policies and procedures regarding fluid quality. This clause defines some of the surveillance activities to be performed at the dialysis facility as part of the quality assurance process. The microbiological surveillance methods described in 8.3 and Table 4 are intended to provide examples of acceptable methods. Other test methods, provided that they have been appropriately validated and are comparable to the methods cited in 8.3 and Table 4 may also be used. The frequency of surveillance is generally recommended by the equipment manufacturer or by local organizations that oversee dialysis facilities. In the absence of a manufacturer's recommendations, guidance on tests to include in a quality assurance surveillance programme can be found in Annex C. This guidance can also be used to supplement the manufacturer's recommendations.

Water utility companies' primary obligation is to provide the domestic user with water that meets the requirements of national or local drinking water regulations. In order to meet such requirements, the water company may introduce new methods of treatment or sanitization without prior notification to end users. Whilst the impact of any such change is unlikely to affect the domestic user, such changes can have an impact on dialysis providers and dialysis patients. In view of this, the clinic should establish robust communications with the feed-water supplier so that the feed-water supplier is aware that the feed water is used for the production of dialysis fluid [i.e. for life-saving treatment of end-stage renal disease (ESRD) patients] and the clinic should request that formal procedures be established for the feed water supplier to provide the clinic with correct and timely information about any change in the supplied water quality (e.g. change of source or type of raw water treatment), changes in chemicals or procedures used for the disinfection/sanitization used by the feed water supplier or in the delivery (e.g. water supply interruptions). If such procedures cannot be established, additional surveillance of feed-water quality is highly desirable to maximize patient safety.

7.2 Surveillance of fluid quality

7.2.1 Surveillance of dialysis water quality

Dialysis water quality shall be monitored on a regular basis for the chemical and microbiological contaminants listed in 4.2.2 and 4.2.4. The surveillance schedule shall be based on the results of the system validation. For an established water treatment system operating under stable conditions, chemical contaminants in dialysis water should be monitored at least annually. The exception is for total chlorine, which should be monitored as described in 7.3.5.

Methods for the surveillance of chemical and microbiological contaminants in dialysis water are given in ISO 23500-3.

For water treatment and distribution systems that are integrated into a single system and that have been validated to produce water meeting the quality requirements of ISO 23500-3, the manufacturer's recommendations for surveillance may be followed for the manufacturer's recommended maximum period of use according to the instructions for use, provided that there is assurance that the system is being operated under the validated conditions.

7.2.2 Surveillance of concentrate quality

Users are not required to test concentrates to demonstrate conformity with the requirements of ISO 23500-4 when using commercially available packaged chemicals intended for use in preparing liquid concentrates at a dialysis facility or when using commercially available liquid concentrates, provided that the concentrates are manufactured in accordance with the requirements of ISO 23500-4. If the user prepares concentrate from raw chemicals, the resulting concentrate should meet the requirements of ISO 23500-4.

7.2.3 Surveillance of dialysis fluid quality

Dialysis fluid quality shall be monitored on a regular basis for the microbiological contaminants listed in 4.4.2 Methods for the surveillance of microbiological contaminants in dialysis fluid are described in 8.3.

NOTE 1 Tests for bacterial growth and endotoxins are not required if the dialysis machine fluid pathway is fitted with an appropriate capacity bacteria retentive and endotoxin-retentive filter validated by the manufacturer and operated and monitored according to the manufacturer's instructions, unless the manufacturer requires such tests in the instructions for use.

NOTE 2 It is not possible to monitor conformity with the microbial quality requirements for substitution solutions (see A.8).

Because dialysis fluid is prepared from dialysis water and concentrates meeting the quality requirements of ISO 23500-3 and ISO 23500-4, respectively, and since the dialysis water distribution system and dialysis fluid delivery system are required to be constructed of materials that do not contribute chemical contaminants to the water, routine surveillance of chemical contaminants in the dialysis fluid is not required.

7.3 Surveillance of water treatment equipment

7.3.1 General

A brief description of the individual water treatment devices is provided in Annex B, and Annexes C and G provide further information in respect of surveillance guidance for water treatment equipment, distribution systems and dialysis fluid.

7.3.2 Surveillance of sediment filters

There is no easy test to determine the effectiveness of a sediment filter; however, pressure drop (ΔP) across the filter can be used to determine when the filter is retaining particulate matter to the point that the filter will no longer allow the required water flow without an excessive reduction in pressure at the outlet of the filter. A backwash cycle is used as a preventive measure to remove particulate matter from the sediment filter and avoid the development of an excessive pressure drop. The frequency of backwashing should follow the manufacturer's recommendations. Sediment filter surveillance should include verification that the timer used to initiate backwashing cycles is set to the correct time of day. A log sheet should be developed to record the pressure drop measurements and timer verifications.

7.3.3 Surveillance of cartridge filters

Cartridge filters should be monitored on a regular basis. There is no easy test to determine the effectiveness of a cartridge filter in removing particulate matter; however, pressure drop (ΔP) across the filter can be used to determine when the filter is retaining particulate matter to the point that the filter will no longer allow the required water flow without an excessive reduction in pressure at the outlet of the filter. A marked decrease in ΔP without a corresponding decrease in flow rate can indicate a loss of filter integrity. Cartridges are usually replaced when ΔP increases to or above a specified value or at a predetermined interval. A log sheet should be developed to record the pressure drop measurements.

7.3.4 Surveillance of softeners

Softener surveillance consists of the following: testing softened water for residual hardness; for automatically regenerating softeners, checking that the brine tank contains a sufficient supply of undissolved sodium chloride; and, for time-controlled softeners, checking that the timer indicates the correct time of day. The frequency of surveillance should be based on the hardness of the feed water and the capacity of the softener. When reverse osmosis is used, surveillance should be used to ensure that hardness limits established for the reverse osmosis membrane are not exceeded. A temporary rise in hardness will have a minimal effect on the performance of the reverse osmosis system, as calcium and magnesium are removed by reverse osmosis.

Testing for hardness should be performed using an ethylenediaminetetraacetic acid (EDTA) titration test, with “dip and read” test strips, or a similar method. Online hardness monitors are also available. If an online monitor is used, it should be used and maintained in accordance with the manufacturer's instructions. Regardless of the method chosen, users should ensure that test accuracy and sensitivity are sufficient to satisfy the hardness surveillance requirements of the reverse osmosis system manufacturer when the softener is used as pre-treatment for a reverse osmosis system.

When timer-controlled softeners are used, it is recommended that the hardness of the water exiting the softener be measured as near as practicable to the end of the duty cycle. The hardness test at the end of the duty cycle will indicate the overall effectiveness of the water softener under worst-case conditions and will ensure that the softener is sized properly and that the regeneration schedule is adequate. Timers should be checked at the beginning of each day. For volume-controlled duplex softeners, testing for hardness can be performed at any time of the day.

The softener brine tank should be monitored on a regular basis to ensure that enough salt is present in the brine tank to form a saturated salt solution of sufficient volume for a minimum of one regeneration cycle. Salt used for regeneration should meet the specifications of the softener manufacturer. In particular, salt designated as rock salt should not be used for softener regeneration since it is not refined and typically contains sediments and other impurities that can damage O-rings and pistons and clog orifices in the softener control head. The frequency of surveillance should be based on the length of the duty cycle.

Water softeners should be fitted with a mechanism to prevent water containing the high concentrations of sodium chloride used during regeneration from entering the product-water line during regeneration.

Water hardness test results and verification of timer settings and the assessment of sufficient quantity of salt pellets according to manufacturer’s instructions should be recorded in a water softener log.

7.3.5 Surveillance of carbon media

If chlorine is not used in the feed water, the performance of the carbon bed is monitored by measuring the pressure drop across the carbon bed and by time in use.

If chlorine is used in the feed water, the performance of carbon beds is monitored by measuring the total chlorine concentration in the water exiting the carbon bed, or exiting the first carbon bed when a series-connected pair of beds is used. The total chlorine level should not exceed 0,1 mg/l.

Testing for total chlorine can be accomplished using the N,N-diethyl-p-phenylenediamine (DPD)-based test kits, dip-and-read test strips based on Michler's thioketone (MTK or TMK), or other methods where comparable sensitivity and specificity can be demonstrated. Online monitors can also be used to measure total chlorine concentrations. If an online monitor is used, it should be used and maintained in accordance with the manufacturer's instructions. Whichever test system is used, it should have sufficient sensitivity and specificity to resolve the maximum levels described in 4.2.2 (see Table 1).

When offline tests are used, testing for total chlorine should be performed at the beginning of each treatment day prior to the patient's initiating treatment. Where chloramine is used to disinfect the potable water supply at a level of 1 mg/l or more, testing should be repeated prior to the beginning of each patient shift; if there are no set patient shifts, testing should be performed approximately every 4 h during operation. More frequent surveillance could be appropriate during temporary operation with a single carbon bed, which can occur following breakthrough of the first bed. In such instances, testing is performed on water exiting the second carbon bed in a series-connected pair. The decision to change the frequency of surveillance should be based on the past performance of the system and on whether changes in feed-water quality have occurred. The system should be flushed for a sufficient period of time to ensure that water sampled is representative of the water to be used for treatment. Sufficient flushing is necessary to ensure that sampled water has not been resident in the removal system between treatments, such that it would under-represent the chloramine level that would be delivered during normal operation. The minimum flush time should be 15 minutes unless otherwise directed by the manufacturer of the equipment. The analysis should be performed onsite since total chlorine levels will decrease if the sample is not assayed promptly.

Results of surveillance should be recorded on a log sheet.

7.3.6 Surveillance of chemical injection systems

Systems for chemical injection should be monitored according to the manufacturer's instructions. If a facility designs its own chemical injection system, procedures should be developed to ensure proper preparation of the chemical, adequate mixing of the injected chemical with the water flowing through the pre-treatment cascade, and reduction to a safe level of the concentration of any chemical residuals before the point of water use. The facility should also verify that the injected chemical does not degrade the performance of downstream devices, such as the reverse osmosis system. Verification can be accomplished by testing samples from the chemical reservoir and the water line after the point of injection for at least three batches of chemical.

When the chemical to be injected is prepared at a facility from powder or by dilution of a liquid concentrate, the chemical injection reservoir should be labelled with the name of the chemical and its concentration, the date the solution was prepared, and the name of the person who mixed the solution. Each batch of chemical should be tested for correct formulation before use. A batch of chemical should not be used or transferred to the injection system reservoir until all tests are completed. The test results, and verification that they meet all applicable criteria, should be recorded and signed by the trained personnel performing the tests.

Protective clothing and an appropriate environment, including ventilation adequate to meet applicable environmental exposure limits, and national standards or regulations should be provided when chemicals for injection are prepared in a dialysis facility.

7.3.7 Surveillance of reverse osmosis

Reverse osmosis systems should be monitored using continuously-reading monitors that measure product-water conductivity [sometimes displayed as total dissolved solids (TDS)]. The measurements can be used to calculate rejection of solutes by the reverse osmosis membrane and provide a measure of equipment performance. Percent rejection is calculated using the Formula (1):

$$\text{Rejection}\left(\%\right)=\frac{\sigma_{\text{F}}-\sigma_{\text{P}}}{\sigma_{\text{F}}}\times 100 \qquad (1)$$

where

σ_F is the feed water conductivity (S/m);

σ_P is the permeate conductivity (S/m).

Many reverse osmosis systems have a direct reading for percent rejection.

The permeate conductivity and rejection are both affected by a number of factors, such as the salinity and composition of the feed water, the water temperature, the level of dissolved gases, and the pressure in the system. Therefore, neither of them should be regarded as a true indicator of the water's suitability for dialysis. No limits are therefore set for these parameters in ISO 23500-3. Instead, they should be used to monitor changes in performance over time rather than an absolute measure of the quality. This can only be established by performing a chemical analysis of the product water in accordance with ISO 23500-3.

NOTE 1 For two-stage reverse osmosis systems, the percent rejection of the second stage will be lower than that of the first stage because of physicochemical phenomena.

Other parameters that should be measured include product and reject stream flow rates and various internal pressures to the extent permitted by the system's instrumentation. Although these parameters are not directly indicative of treated water quality, surveillance of them can help ensure that the system is operating within the manufacturer's specifications and, thus, will aid in maintaining the performance of the reverse osmosis membranes. Flow rates can be used to calculate the percent recovery of the reverse osmosis system using Formula (2):

$$\text{Recovery}\left(\%\right)=\frac{qv_P}{qv_P+qv_R}\times 100 \qquad (2)$$

where

qv_P is the permeate volumetric flow rate;

qv_R is the reject water volumetric flow rate.

NOTE 2 The percent recovery is also known as the “water conversion factor”. The terms are equivalent if none of the reject water stream is recycled to the feed-water stream (see B.2.7). If some of the reject water stream is recycled, Formula (2) provides a measure of overall water utilization by the reverse osmosis system, rather than the recovery of water during a single pass through the membrane module.

NOTE 3 The permeate water flow rate varies with operating pressure and temperature. To enable comparisons to be made under different operating conditions, a normalized permeate flow rate can be calculated. Methods for calculating the normalized permeate flow rate are available from reverse osmosis membrane manufacturers or can be found in ASTM D4516–00 (2010).

When reverse osmosis is the final process in the water treatment system for removing chemical contaminants, analysis for the contaminants listed in Tables 1 and 2 should be performed when the reverse osmosis system is installed to ensure that the limits specified are met. It is also recommended that chemical analyses be performed when the following situations occur:

- information is obtained from the water supplier that significant changes in the source water, such as seasonal variations, have occurred;
- significant deviations are observed in process parameters, such as pH, conductivity, chlorine concentration and hardness, that can affect the performance of components of the water treatment system;
- reverse osmosis rejection rates decrease by more than 10 %.

All results of measurements of reverse osmosis performance should be recorded daily in an operating log that permits trending and historical review.

7.3.8 Surveillance of deionization

Deionizers shall be monitored continuously using resistivity monitors that compensate for temperatures up to 25 °C and that are equipped with audible and visual alarms. Resistivity monitors shall have a minimum sensitivity of 1 MΩ·cm (1 µS/cm or 0,1 mS/m). If the resistivity of the water at the outlet of a deionizer is less than 1 MΩ·cm, the water shall not be used for dialysis. When deionization is employed as the primary method for removing inorganic contaminants (reverse osmosis is not employed), or when deionization is necessary to polish reverse-osmosis-treated water, chemical analyses to ensure that the requirements of 4.2.2 are met should be performed when the system is installed. Resistivity monitor readings should be recorded on a log sheet twice each treatment day.

7.3.9 Surveillance of endotoxin-retentive filters

The performance of endotoxin-retentive filters in dialysis water distribution, bicarbonate concentrate distribution, or dialysis fluid delivery systems can be monitored by testing the fluid that is directly exiting the filter for bacteria and endotoxins. Endotoxin-retentive filters should be fitted with a means of evaluating filter integrity and fouling. One suitable means is to monitor the pressure drop (ΔP) across the filter at a given product fluid flow rate using pressure gauges on the inlet (feed) and outlet (product) streams. Alternatively, product fluid flow rate can be measured at a given pressure drop. Such surveillance can indicate when membrane fouling has progressed to the point that membrane replacement or cleaning is needed. Surveillance is also necessary to ensure that the device is being operated in accordance with the manufacturer's instructions. Endotoxin-retentive filters operated in the cross-flow mode should also be monitored in terms of the flow rate of fluid being directed to drain at a given pressure drop. Results of pressure measurements and bacteria and endotoxin levels should be recorded on a log sheet.

7.4 Surveillance of dialysis water storage and distribution

7.4.1 Surveillance of water storage tanks

For a system that distributes dialysis water to single-patient proportioning systems, routine surveillance of water storage tank for total viable microbial count and endotoxin concentration is generally accomplished indirectly by surveillance of the dialysis water at the first outlet to the distribution loop. For routine surveillance of a storage tank that supplies dialysis water to a central dialysis fluid delivery system, or when direct surveillance of a dialysis water storage tank is performed as part of a troubleshooting process, the total viable microbial count and endotoxin concentration should be determined using samples drawn from a port at the outlet of the storage tank. When a change has been made to an existing storage tank, more frequent testing should be considered to verify that bacteria or endotoxin levels are consistently within the allowed limits. The need for additional testing should be based on the original validation plan (see 6.2) and a risk analysis of the likely impact of the change on system performance. All total viable microbial counts and endotoxin results should be recorded on a log sheet and should be subject to trend analysis.

7.4.2 Surveillance of the water distribution systems

Distribution piping systems used for dialysis water should be monitored for total viable microbial count and endotoxin concentration to demonstrate the adequacy of the disinfection programme described in 8.2. The total viable microbial count and endotoxins shall not exceed the levels specified in 4.2.4 and Table 3. Surveillance should be accomplished by taking samples from the last outlet of the distribution loop and the outlets supplying reuse equipment and bicarbonate concentrate mixing tanks. If the results of this testing are unsatisfactory, the disinfection programme should be re-evaluated and additional testing (for example, endotoxin-retentive filter inlet and outlet, reverse osmosis product water, and storage tank outlet) should be undertaken as part of a troubleshooting strategy to identify the source of contamination, after which appropriate corrective actions can be taken. Total viable microbial counts and endotoxin testing should be conducted on a regular schedule according to system validation data and local regulations and in accordance with 8.3. When a change has been made to an existing system, more frequent testing should be considered to verify that bacteria or endotoxin levels are consistently within the allowed limits. The need for additional testing should be based on the original validation plan (see 6.2) and a risk analysis of the likely impact of the change on system performance. All bacteria and endotoxin results should be recorded on a log sheet to permit identification of trends and the need for corrective action.

7.4.3 Surveillance of bacterial control devices

7.4.3.1 Surveillance of ultraviolet irradiators

Ultraviolet irradiators intended for use as a direct means of bacterial control should be monitored for radiant energy output. UV irradiators are available equipped with radiant energy intensity sensors. Either a visual alarm or an output meter is acceptable for determining if the UV lamp is emitting sufficient radiant energy. UV irradiators should be monitored at the frequency recommended by the manufacturer. Because the radiant energy decreases with time, annual lamp replacement is typically required. Periodic cleaning of the quartz sleeve may also be required, depending on the water quality. A log sheet should be used to indicate that surveillance has been performed.

7.4.3.2 Surveillance of ozone generators

Ozone generators should be monitored for ozone output at a level specified by the manufacturer. The output of the ozone generator should be measured by determining the ozone concentration in the water at the most distal point from the ozone generator. A test based on indigo trisulfonate chemistry, DPD, or the equivalent, or ozone-in-water test strips should be used to measure the ozone concentration. It is recommended that ozone concentration be measured each time disinfection is performed. An ozone-in-ambient-air test should be conducted on a periodic basis, as recommended by the manufacturer, to ensure conformity with National Standards and regulations for the permissible exposure limit. A log sheet should be used to indicate that surveillance has been performed.

7.4.3.3 Surveillance of hot water disinfection systems

Hot water disinfection systems should be monitored for temperature and time of exposure to hot water, as specified by the manufacturer. The temperature of the water should be recorded at a point farthest from the water heater; that is, where the lowest water temperature is likely to occur. It is recommended that the water temperature be measured each time a disinfection cycle is performed. A record that verifies successful completion of the heat disinfection should be maintained. Successful completion is defined as meeting the temperature and time requirements specified by the equipment manufacturer.

NOTE 7.4.1 to 7.4.3 relate to and help explain Table C.1.

7.5 Surveillance of concentrate preparation

7.5.1 Surveillance of mixing systems

Systems for preparing either bicarbonate or acid concentrate from powder or other highly concentrated media at a dialysis facility should be monitored according to the mixing system manufacturer's instructions to ensure appropriate dissolution. If a facility designs its own system it is considered a manufacturer, requiring that verification, validation and surveillance procedures shall be developed and implemented in an equivalent manner to a manufacturer.

Acid and bicarbonate concentrates may be tested by measuring their conductivity, density with a density meter, or specific gravity with a hydrometer according to the manufacturer's instructions. Although not required, some manufacturers of concentrate powder or other highly concentrated media may provide allowable ranges for either the conductivity, density, or the specific gravity of concentrates prepared from their powder. The use of pH, alone, as an indicator of proper dissolution is inappropriate for both acid and bicarbonate concentrates because large variations in concentration do not produce significant changes in pH. Concentrates should not be used or transferred to holding tanks or distribution systems until all tests are completed. The test results and verification that they meet all applicable criteria should be recorded and signed by the individuals performing the tests.

7.5.2 Surveillance of additives

When additives are prescribed for a specific patient, the container holding the prescribed concentrates should be labelled with the name of the patient, the final concentration of the added electrolyte, the date and time when the prescribed concentrate was mixed, and the name of the person who mixed the additive. This information should also be recorded in accordance with the requirements of 4.5. The label should be affixed to the container when the mixing process begins.

7.6 Surveillance of concentrate distribution

A daily check to ensure that the appropriate acid and bicarbonate concentrate is connected to the corresponding concentrate delivery line is recommended if the storage tank is not permanently connected to its distribution piping.

Once a bicarbonate distribution system has been activated, dialysis fluid should be monitored weekly until sufficient data have been obtained to demonstrate the adequacy of the disinfection programme for the bicarbonate distribution system. The frequency of surveillance may then be reduced, but surveillance should be performed at least monthly. If elevated total viable microbial counts or endotoxin levels are found in the dialysis fluid, the disinfection programme for all systems involved in dialysis fluid preparation, including the bicarbonate concentrate distribution system, should be evaluated and revised. The frequency of surveillance should then be increased until it can be demonstrated that the revised disinfection programme is adequate to provide concentrate that allows preparation of dialysis fluid meeting the quality requirements of ISO 23500-5.

There are no published reports of acid concentrate supporting bacterial growth; therefore, it is not necessary to perform routine testing for total viable microbial count and endotoxins on those systems. However, it should be possible to disinfect these systems.

7.7 Surveillance of dialysis fluid proportioning

Dialysis fluid proportioning should be monitored following the procedures specified by the equipment manufacturer. When the user has specific requirements for surveillance of dialysis fluid proportioning, such as when dialysis machine settings are changed to allow the use of concentrates with a different proportioning ratio, the user should develop procedures for the routine surveillance of dialysis fluid electrolyte values, such as by surveillance of sodium (Na^+), potassium (K^+), or dialysis fluid conductivity.

8 Strategies for microbiological control

8.1 General

The strategy for controlling the proliferation of microorganisms in haemodialysis systems primarily involves appropriate system design and operation, and regular disinfection of the entire fluid system. This includes the reverse osmosis membranes (especially the clean side), the distribution piping, the inlet lines to the dialysis machines (located before the disinfection circuit of the dialysis machine), and the dialysis machines (which have their own disinfection circuit and programme). A key concept in ensuring conformity with the requirements of 4.2.4 and 4.4 is that disinfection schedules should be designed to prevent microbial proliferation, rather than to eliminate microorganisms once they have proliferated to an unacceptable level. With such a strategy, the surveillance levels of microorganisms and endotoxin demonstrate the effectiveness of the disinfection programme, rather than indicating when disinfection should be performed.

Microorganisms in fluid colonize surfaces resulting in biofilm formation, even when levels of microbial contamination are low. Microorganisms living within biofilms produce an extracellular polysaccharide or slime matrix, which protects them against disinfection. Microorganisms also excrete both simple and complex metabolites. Their effect on patients, and whether or not they are removed by endotoxin-retentive filters, is largely unknown. All strategies for microbial control of the system should, therefore, be proactive in order to limit microbial growth and biofilm formation and prevent bio-fouling. It is of utmost importance that the disinfection procedure is applied from the start of the operation of the haemodialysis system as once formed, biofilm is difficult, if not impossible, to eradicate.

The combined efforts of disinfection and employing strategies for bacterial control make it possible to minimize microbiological growth and biofilm formation.

8.2 Disinfection

8.2.1 General

Disinfection is the only effective method of diminishing and inactivating microflora.

First, and most of all, the frequency of disinfection is important. Disinfection should be performed on a regular basis to limit biofilm formation and prevent bio-fouling within the fluid pathways of the system. Depending on the circumstances, different levels of disinfection may be required to comply with the fluid quality requirements of Clause 4.

Second, all surfaces in the circuit should be included in the disinfection procedure. This includes the reverse osmosis membranes (especially the clean side), the distribution piping, the inlet lines to the dialysis machines (located before the disinfection circuit of the dialysis machine), and the dialysis machines (which have their own disinfection circuit and programme).

Third, the disinfection procedure, when applied with a given frequency and with inclusion of all critical areas, should be capable of minimizing the effects of bio-fouling.

Disinfection can be performed using heat or chemicals. UV lamps can be used to inactivate planktonic cells but are of no value against any biofilm that has formed in the system.

8.2.2 Microbiological aspects of fluid system design

The circuit downstream of the reverse osmosis system, including the clean side of the reverse osmosis membranes, the distribution piping, tanks and filters in the distribution piping, and the inlet lines to the dialysis machines are to be maintained and disinfected so that it is possible to fulfil the microbiological requirements of ISO 23500-5 and ISO 23500-3.

Examples of good system design include

- use of a recirculation-type system,
- avoidance of dead ends and dead space areas,
- a high-quality finish to joints and connections,
- use of materials compatible with the planned methods of disinfection, and
- avoidance of storage tanks. If a storage tank is necessary it should be designed and constructed in such a manner that it can be cleaned and disinfected.

Once the water treatment system has been installed, water flow should be maintained to limit biofilm formation.

NOTE 1 Biofilm formation on a surface is a function of many factors including surface roughness and flow velocity. Experimental studies have demonstrated that turbulent flow in a piping system is able to minimize but not totally eliminate the formation of biofilm.

NOTE 2 In dialysis facilities, there might not be continuous flow through the system, since patients may not be treated overnight or the system is undergoing maintenance. Consequently it is not feasible to specify a minimum flow velocity that is effective in reducing biofilm formation and bacterial contamination. Furthermore, the use of such a flow velocity would not provide a substitute for regular disinfection of the distribution system.

The system design should also take into consideration preventive maintenance of the fluid distribution system, as well as education and training of staff in order to create awareness about disinfection and microbiological control.

The disinfection programme could compensate for a weakness in system design but will not totally prevent the formation of biofilm, which can become difficult to eradicate.

8.2.3 Disinfection frequency

8.2.3.1 General

A key concept in ensuring conformity with the requirements of 4.2.4, and 4.4.2 is that disinfection schedules should be designed to prevent bacterial proliferation, rather than being designed to eliminate bacteria once they have proliferated to an unacceptable level (i.e. above the action level). With this strategy, surveillance levels of bacteria and endotoxins serve to demonstrate that the disinfection programme is effective, not to indicate when disinfection should be performed.

Disinfection strategy is preventive and should be applied from the start of operation of the system. The disinfection frequency may be modified, based on results obtained during validation, surveillance and revalidation activities. Any such modification should be appropriately documented.

8.2.3.2 Dialysis water storage and distribution systems

Storage and distribution systems should be disinfected on a schedule that allows the water quality recommendations of 4.2.4 to be met routinely. For integrated treatment and distribution systems, the manufacturer's instructions for disinfection should be followed, provided routine surveillance shows that they are adequate to meet the requirements of 4.2.4. For systems assembled from individual components, the frequency of disinfection necessary to minimize bio film formation will vary with the design of the system and, in the case of existing systems, the extent to which any bio-fouling has already occurred. Annex C provides further information on surveillance of water treatment equipment and distribution systems. Annex D provides further information on strategies for microbiological control.

8.2.3.3 Concentrate mixing systems

Concentrate mixing equipment should be either

a) completely emptied, cleaned, and/or disinfected according to the manufacturer's instructions, or

b) cleaned and/or disinfected using a procedure demonstrated by the facility to be effective in routinely producing concentrate that allows the recommendations of 4.3.1 to be met.

The mixing and disinfection data should be recorded for each mix and disinfection cycle using a dedicated log.

8.2.3.4 Concentrate distribution systems

Piped bicarbonate concentrate distribution systems should be disinfected either according to the manufacturer's instructions or using a procedure that has been demonstrated by the facility to be effective in routinely producing concentrate that allows the recommendations of 4.3.1 to be met. If the manufacturer does not supply disinfection procedures, the user should develop and validate a disinfection protocol. It is recommended that surveillance of concentrate distribution systems be performed on a routine basis.

When centrally produced bicarbonate concentrate is decanted into reusable concentrate containers, the containers and pick-up tubes should be disinfected at least weekly or at a frequency required by local regulations. Bicarbonate concentrate containers and concentrate pick up tubes should be rinsed with treated water allowed to air dry and stored inverted at the end of each treatment day. Because there are no published reports of acid concentrate supporting bacterial growth, disinfection of acid concentrate distribution systems is not normally required. However, it should be possible to disinfect these systems.

8.3 Microbiological surveillance methods

8.3.1 General

The fluid system shall be routinely monitored in order to verify that the microbiological quality indicators (bioburden represented by total plate count and endotoxin concentration) are being met.

The frequency of sampling should meet applicable local recommendations. If no such recommendations exist, the following are recommended.

a) Water system: The number of samples and positions of sampling should be based on the complexity and size of the water system. The frequency will depend on the analysis of the data collected during the validation and revalidation activities. Monthly surveillance is most frequently adopted but less or more frequent surveillance may be possible based on data collected during the validation and revalidation.

b) Dialysis fluid/haemodialysis machines without a validated bacteria- and endotoxin-retentive filter: Machines should be sampled on a regular basis to provide verification of the effectiveness of the disinfection process. The schedule of sampling will depend on the type of disinfection process being used. Each machine should be sampled at least once per year and different machines should be sampled on each occasion. Monthly surveillance is most frequently adopted.

c) It is not necessary to take samples of ultrapure dialysis fluid or substitution fluids if their production paths are fitted with bacteria- and endotoxin-retentive filters validated by the manufacturer and operated and monitored according to the manufacturer's instructions. It could be necessary to sample the dialysis fluid entering such bacteria- and endotoxin-retentive filters depending on the manufacturer's instructions for use of the filters; for example, when the instructions for use specify the quality of the fluid entering the filter. (See also Annexes D and E.)

The results of testing should be subjected to trend analysis. When results exceed the action levels, or in the case of a patient's pyrogenic reaction or suspected bacteraemia/fungemia, an investigation and follow-up should be initiated. This investigation could include additional sampling and extra disinfection procedures carried out as per the manufacturer's recommendations.

8.3.2 Sample collection

8.3.2.1 Dialysis water sample sites

Samples are to be taken at outlets of the distribution system, according to the sampling instructions provided by the manufacturer.

In the absence of manufacturer’s instructions, the following may be used to ensure that the sample taken is not contaminated by any microbial growth at the point of sampling.

- Any hose attached to the sampling point should be removed. The port should be cleaned with a sterile gauze or cotton swab wetted with alcohol. Bleach or other disinfectant solutions should not be used. The sampling port is opened and fluid allowed to run to waste for at least 60s unless the port manufacturer instructions for use state otherwise, prior to the aseptic collection of the sample. The sample volume collected should be 5 ml to 1 000 ml depending upon the test to be run and/or as specified by the laboratory performing the test. The containers used for the samples to be cultured should be sterile and endotoxin free.
- Other sampling methods may be used provided they have been validated.

8.3.2.2 Dialysis fluid samples

Samples should be collected from the dialysis fluid line according to the sampling instructions provided by the manufacturer of the dialysis fluid delivery system. If not specified, the exterior of the sample port (not the lumen) may be disinfected by wiping with alcohol, 70 % ethanol or isopropanol (isopropyl alcohol). Bleach or other disinfectant solutions should not be used. The sample should not be collected until all the alcohol has evaporated so as to leave no disinfectant residual in the sample.

The sample volume collected should be 5 ml to 1 000 ml depending upon the test to be performed and/or as specified by the laboratory performing the test.

Containers used for samples to be cultured shall be sterile and endotoxin free.

NOTE Where the dialysis fluid delivery system is equipped with a dialysis fluid sampling port that can be accessed using a syringe, the sample port can be disinfected with alcohol and allowed to air dry. A sterile syringe should be used to aspirate at least 10 ml of dialysis fluid out of the sampling port. The filled syringe is discarded and a fresh sample of dialysis fluid collected using a new sterile syringe. For sample ports consisting of a simple septum penetrated with a needle, the use of a second syringe is not necessary. Alternatively, if the haemodialysis machine permits, samples can be collected immediately after the dialyser by disconnecting the effluent connector and aseptically collecting a “free/clean” catch sample after allowing dialysis fluid to run for at least 60 s.

8.3.3 Heterotrophic plate count

8.3.3.1 Storage of samples

Microbial analysis of any fluid sample should be conducted as soon as possible after collection to avoid unpredictable changes in the microbial population. Samples intended for colony counts should not be frozen but kept at a temperature of <10 °C until ready for shipping or collection by the laboratory. If samples cannot be analysed within 4 h of collection, they should be stored at <10 °C. Sample storage for more than 24 h should be avoided.

Storage of samples for endotoxin analysis may be different from what is given above, provided the complete procedure follows the manufacturer's instructions for use of the LAL assay.

8.3.3.2 Analytical methods

8.3.3.2.1 Membrane filtration

The recommended methods (e.g. membrane filtration, spread plate, pour plate,), media, and incubation ranges allow each dialysis center to develop a surveillance program appropriate to their facility.

The culture medium and incubation times selected should be based on the type of fluid to be analysed e.g. standard dialysis fluid, water used in the preparation of standard dialysis fluid, ultrapure dialysis fluid, water used for the preparation of ultrapure dialysis fluid or fluid used for online therapies such as haemodiafiltration. The method selected, should be based on the analysis of the advantages, disadvantages and sensitivity, of each of the suggested methods. It should also ensure that the patient is provided with the necessary safeguards within any constraints imposed by local laboratory working practices and reimbursement.

Membrane filtration is filtration of the sample through a membrane filter with pore diameter of 0,45 µm or less. Membrane filtration is a method used when the sample is to be concentrated to detect low levels of contamination (usually <1 CFU/ml), and can also be used for quantification of yeast and filamentous fungi should this be required. The volume to be filtered should be determined by the suspected level of contamination and should be between 10 ml and 1 000 ml.

NOTE Alternate membrane filtration techniques may be used provided that they have been appropriately validated and are comparable to the analytical methods outlined in this section.

8.3.3.2.2 Spread-plate technique

A pipette is used to apply 0,1 ml to 0,3 ml of a sample to a Petri dish (typically 90 mm in diameter) containing agar medium and spread over the surface of the agar. The detection limit of this technique is 5 CFU/ml when 0,2 ml of sample is used as the inoculum.

NOTE New methods may become available. Such methods may be used provided that they have been appropriately validated and are comparable to the analytical methods outlined in this section.

8.3.3.2.3 Pour-plate technique

A sample (typically 1 ml) is placed in a Petri dish and 15 ml to 20 ml of molten medium is added. The sample and medium are mixed carefully by gentle rotation and allowed to set. The time between addition of the sample and addition of the molten medium should not exceed 15 min. The plate is inverted and incubated. If 1 ml of sample is used, the detection limit of this technique is 1 CFU/ml.

Molten media should be <45 °C at the time the plate is poured.

NOTE New methods may become available. Such methods may be used provided that they have been appropriately validated and are comparable to the analytical methods outlined in this section.

8.3.3.2.4 Dip samplers

Currently available dip samplers are not suitable for use in dialysis applications.

8.3.3.3 Cultivation methods and conditions

The recommended methods and cultivation conditions can be found in ISO 23500-3, ISO 23500-4 and ISO 23500-5. The methodologies detailed use Tryptone Glucose Extract Agar (TGEA) or Reasoner's Agar No. 2 (R2A) incubated at 17 °C to 23 °C for a period of 7 days, or Tryptic Soy Agar incubated at 35 °C for 48 hours for the culturing of standard dialysis fluid and dialysis water. The rationale for the use of TSA and its inclusion is explained in ISO 23500-3:2019, Annex A (A.3 Microbiology of water).

Culture results obtained using the methods outlined are only a relative indicator of the bioburden and do not provide a measure of the absolute bacterial burden. Different media types and incubation periods can result in varying colony concentrations and types of microorganisms recovered[10][11][12].

The use of Reasoner's 2A agar (R2A) has been reported in previous studies to result in higher colony counts than tryptic soy agar (TSA) for water samples and dialysis fluids[11][12][13]. In a more recent publication, the authors indicated that there were no significant differences when bacterial burden in standard dialysis water and standard dialysis fluid were compared using ≥50CFU/ml as the action limit when assayed using R2A and TSA at the conditions stated above[11].

Tryptone glucose extract agar (TGEA), also has reported higher colony counts than TSA.[10] Maltais et al.[11] in their comparison of this medium with TSA showed that the proportion of standard dialysis water samples yielding colony counts ≥50 CFU/ml was significantly different from that found using TSA ($p = 0{,}001$), with each medium using the respective incubation conditions stated above. The proportions of dialysis fluid samples in which microbial burden was ≥50 CFU/ml were not significantly different on the two media and incubation conditions.

The culture medium and the assay method conditions selected should be based on the type of fluid to be analysed; dialysis water, standard dialysis fluid, ultrapure dialysis fluid, or online substitution fluid used for online therapies such as haemodiafiltration and the purpose of the analysis. It should also ensure that patient safety is safeguarded and allow for consideration of local laboratory working practices, and that local regulations and reimbursement requirements can be met.

8.3.4 Bacterial endotoxin test

Bacterial endotoxins are assayed using the *Limulus* amoebocyte lysate (LAL) test. Current pharmacopoeias [United States (USP), European, and Japanese pharmacopoeias] acknowledge six different testing techniques.

Endotoxin testing requires training and should be performed by fully trained personnel.

Tests shall follow official applicable requirements and manufacturer's instructions.

NOTE It is important to use the correct types of sample containers, labelled or validated for storage of endotoxin samples. Appropriate containers are usually specified by the testing laboratory or the manufacturer of the LAL testing kit.

8.3.5 Determination of yeast and mould

Currently there is no requirement for the routine surveillance for yeast and mould. If quantification of such species is required, the culture media used should be Sabouraud or Malt Extract Agar, with a 7 d incubation period at 20 °C to 22 °C. Alternate techniques validated against Sabouraud or Malt Extract Agar, with a 7 d incubation period at 20 °C to 22 °C, can also be used provided it has been demonstrated that such methods have been appropriately validated and are comparable to the cited methods.

9 Location of and access to the water treatment system

The water treatment and storage system should be located in a secure locked area, accessible only by personnel authorized by the dialysis facility. An up to date list with contact details should be held centrally. Spare keys to the facility should be kept in a secure location.

The location for the water treatment system should be chosen with a view to minimizing the length and complexity of the distribution system. Access to the treatment system should be restricted to individuals responsible for surveillance and maintenance of the system.

The layout of the water treatment system should provide easy access to all components of the system, including all meters, gauges, and sampling ports used for surveillance system performance. An area for processing samples and performing onsite tests is also recommended. Critical alarms, such as those associated with deionizer exhaustion or low water levels in a storage tank, should be configured to notify staff in the patient treatment area, as well as in the water treatment room.

Water systems should include schematic diagrams that identify components, valves, sample ports and flow direction. Additionally, piping should be labelled to indicate the contents of the pipe and direction of flow. The use of text labels, such as “RO Water”, and colour-coded “arrow tape” provides a convenient means of identifying the pipe content and flow direction. Sampling points should be marked as such and identified in schematic diagrams.

If water system manufacturers have not done so, users should identify major water system components and describe their function. How performance is verified, and what actions to take in the event that performance is not within an acceptable range, should be readily available to the user.

10 Personnel

Policies and procedures that are understandable and accessible are mandatory, along with a training programme that includes quality testing, the risks and hazards of improperly prepared concentrate, and bacterial issues. Operators should be trained in the use of equipment by the manufacturer or should be trained using materials provided by the manufacturer. Additional training may be provided using materials from other sources. The training should be specific to the functions performed (i.e. mixing, disinfection, maintenance, and repairs). Periodic audits of the operator’s conformity with procedures should be performed. The user should establish an ongoing training programme designed to maintain the operator's knowledge and skills.

Annex A
(informative)

Rationale for the development and provisions of this document

A.1 Scope

It has long been known that chemical and microbiological contamination of dialysis fluid places haemodialysis patients at risk of acute and chronic adverse events. From the beginning, it was recognized that there was a problem with including fluid quality requirements in a document directed at manufacturers of water treatment equipment or haemodialysis machines. Although a manufacturer might be responsible for providing equipment that, when assembled as a system, provides water, concentrate, or dialysis fluid that meets certain quality requirements, that manufacturer has no control over the equipment once it is installed. For example, the treated water quality could change if a water treatment system is not well maintained or if there is some change in the municipal water feeding the system. Therefore, fluid quality standards were established independent of the standards for the equipment used to prepare those fluids. Since the day-to-day responsibility for maintaining fluid quality lies with the healthcare professionals at each dialysis facility under the direction of the facility's Medical Director, this document was prepared to provide guidance to those healthcare professionals on how to manage fluid preparation systems so as to comply with the requirements of the fluid quality standards.

A.2 Chemical contaminants

This clause provides the rationale for the requirements in 4.2.2.

ISO 23500-3 sets forth maximum levels of chemical contaminants for dialysis water in three groups: chemicals with documented toxicity in haemodialysis patients, such as aluminium[15] to [30], fluoride[31] to [41], nitrates[42][43][44], chlorine its by-products and chloramine (47-63), lead (64-67), electrolytes normally included in dialysis fluid, and trace elements[62][63][64][65]. The rationale for including particular chemicals in ISO 23500-3, and for setting their maximum levels, is set forth in Annex A of ISO 23500-3:2019.

Hazards to patients receiving haemodialysis treatment associated with the presence of organic compounds such as pesticides, polycrylic aromatic hydrocarbons and other chemical products present in the water are difficult to define. Consequences are probably of a long-term nature and routine measurement of such substances is difficult and costly. In view of this, no recommendation in respect of such compounds is made in this version of the standard.

The ability of water treatment systems to remove such compounds depends on the chemical structure and the concentration of the contaminant. Microfiltration and ultrafiltration are less effective than nanofiltration and reverse osmosis. Granular activated carbon (GAC) is on the other hand highly effective in removing such chemical contaminants. Compounds with a high degree of hydrophilicity may breach activated carbon faster than hydrophobic compounds. Backwashing cycles also play an important role in this process. Since granular activated carbons provide the primary method for the removal of chlorine and chloramine from the feed water, proper dimensioning of the carbon beds or filters is essential to ensure that the majority of the carbon valences are not already occupied limiting the removal of organic contaminants should there be a requirement to remove such compounds.

A.3 Microbiological contaminants

This clause provides the rationale for the requirements in 4.2.4.

ISO 23500-3 sets forth maximum levels of bacteria and endotoxins in dialysis water, along with action levels for these contaminants. The rationale for the maximum levels of bacteria and endotoxins is set forth in Annex A of ISO 23500-3:2019.

A.4 Requirements for concentrate

The rationale in this clause addresses the requirements in 4.3. Requirements for commercially available concentrates are given in ISO 23500-4. It was decided not to recommend limits on bacteria and endotoxins for concentrate prepared at a facility, even for bicarbonate concentrate. This decision was based on the difficulty of performing cultures and endotoxin assays in samples with high concentrations of salts. High concentrations of bicarbonate require special culturing techniques and are inhibitory in the LAL assay. It was determined that it was unreasonable to require an individual dialysis facility to meet the special conditions required for proper testing of bicarbonate concentrate and that patients would be adequately safeguarded by the quality recommendations for the water used to prepare the concentrate and for the final dialysis fluid. For users who wish to establish bacterial levels and endotoxin concentrations as part of a troubleshooting investigation, guidelines on performing cultures and endotoxin assays in bicarbonate concentrate are included in 8.3.3.3 and A.10.

A.5 Microbiological contaminants in dialysis fluid

The rationale in this clause addresses the requirements in 4.4. ISO 23500-5 sets forth maximum levels of bacteria and endotoxins in three categories of dialysis fluid: standard dialysis fluid, ultrapure dialysis fluid, and online-prepared substitution fluid. Where appropriate, action levels for bacteria and endotoxins are also given. The rationale for the maximum levels of bacteria and endotoxins is set forth in Annex A of ISO 23500-5:2019.

ISO 23500-3 sets a maximum allowable level for endotoxins in dialysis water of 0,25 EU/ml, while ISO 23500-5 sets a maximum allowable level for endotoxins in dialysis fluid of 0,5 EU/ml. The level for dialysis fluid is set higher than that for dialysis water in recognition that powder concentrates can contribute endotoxins, but not volume, to the final dialysis fluid. No such accommodation is made for bacteria since most bacteria will not survive in a viable form in powder concentrate.

A.6 Surveillance of carbon media

The rationale in this clause addresses the requirements in 7.3.5. Intensive surveillance of carbon beds is recommended because of the long history of adverse events related to chloramine contamination of dialysis fluid[45] to [61]. Chloramine concentrations in municipal water can change from day to day and the capacity of carbon beds to remove chloramine can vary with the pH and temperature of the water, the nature of the chloramine compounds present, and the presence of other substances in the water. The dependence of chloramine removal on multiple factors makes the performance of carbon beds unpredictable. Therefore, patient safety can only be ensured by intensive surveillance the performance of the carbon bed. Configuring carbon beds in series and sampling from a port located between the two beds provides one margin of protection against chloramine breakthrough. When chloramine is first detected in the effluent from the first bed, the second bed will still have some capacity for chloramine removal. This reserve capacity allows the user to conveniently replace the exhausted bed without risk to patients. The exhausted bed is discarded, the second bed is moved into the first position, and a new bed is placed in the second position. A new bed of virgin carbon should be used for replacement. Continuous surveillance with an online monitor provides the best protection of the patient. The online monitor should be capable of activating a means of diverting the water from the reverse osmosis system, for example by shutting down the reverse osmosis system, should the total chlorine level exceed 0,1 mg/l.

In situations where chloramine is not used to disinfect the water, and the ammonia level in the water is low, one carbon bed or a carbon cartridge filter with a shorter EBCT could be sufficient. Carbon cannot be regenerated in a dialysis facility, and the use of regenerated carbon is prohibited. Backwashing of carbon beds does not regenerate the carbon, although it can allow more efficient use of the bed's capacity by removing channels that can form in the bed during routine operation. The recommendation that the water purification system should operate for at least 15 min, before samples of water from a carbon bed are drawn, is to guard against inadvertently sampling water that has been in the bed for an extended period.

A.7 Strategies for microbiological control

The rationale in this clause addresses the requirements in Clause 8. Microbial growth within a fluid can occur in two forms; planktonic, i.e freely existing in the bulk solution, and sessile, i.e. attached to a surface.

Biofilm formation occurs in a sequence of distinct events, including initial cell-to-surface or cell-to-cell attachment, microcolony formation, biofilm maturation, and biofilm dispersal.

The ability of bacteria to produce extracellular matrix components that enable them to stick to surfaces and to each other is a prerequisite for biofilm formation. As the biofilm matures the resident bacteria become embedded and protected in this self-produced extracellular matrix which is composed of polysaccharide, protein and DNA.

In view of this no testing is able to show a complete picture of bacterial growth and the tests described and referred to in this document do not measure many microbial contaminants, including: cell wall fragments from microorganisms; nucleic acids DNA and RNA; and metabolites of various kinds.

For this reason, a proactive disinfection programme is recommended to combat bacterial growth in the dialysis water and dialysis fluid systems[15] to [80].

A.8 Heterotrophic plate count

The rationale in this clause addresses the requirements in 8.3.3.

Sensitive culturing methods should be used to measure the low total viable microbial counts permitted for water used for haemodialysis applications. The membrane filter technique is particularly suited for this application because it permits large fluid volumes to be assayed. This technique increases the sensitivity of the method and the likelihood of detection of the bacteria present in low numbers. Because the membrane filter technique might not be readily available in clinical laboratories, the spread plate assay or the pour plate assay can be used as an alternative for water and standard dialysis fluid[81]. If the spread plate assay is used, a calibrated loop should not be used to apply sample to the plate. The sensitivity of an assay performed with a calibrated loop is low. A standard calibrated loop transfers 0,001 ml of sample to the culture medium so that the minimum sensitivity of the assay is 1 000 CFU/ml. This sensitivity is unacceptable when the maximum allowable limit for microorganisms is 100 CFU/ml. Therefore, when the spread plate method is used, a pipette should be used to place 0,1 ml to 0,3 ml of water or dialysis fluid on the culture medium. The pour-plate method may be used as an alternative method to the spread-plate method. If a sample volume of 1 ml is used, the detection limit of the pour-plate method is 1 CFU/ml.

Culture results obtained using any of the methods outlined in this document are only a relative indicator of the bioburden in dialysis water or dialysis fluid and do not provide a measure of the absolute bacterial burden. The original clinical observations on which the microbiological requirements were based used standard methods agar (SMA), a medium containing relatively few nutrients. Later, the use of trypticase soy agar (TSA), a general-purpose medium for isolating and cultivating fastidious organisms was added. Several studies have shown that the use of nutrient-poor media, such as tryptone glucose extract agar (TGEA) or Reasoner's agar No. 2 (R2A), results in an increased recovery of bacteria from water[12][13][14][82]. Extending the culturing time up to 7 d and using incubation temperatures of 23 °C to 28 °C have also shown an increase the recovery of bacteria compared to incubation for 48 h at 35 °C to 37 °C (14, 84).

The use of Reasoner's 2A agar (R2A) and Tryptone glucose extract agar (TGEA) has been reported in some publications to result in higher colony counts than tryptic soy agar (TSA) for culturing of water samples and dialysis fluids[10][12][13][14]. In a more recent (2016) publication, the authors indicated that there were no significant differences for comparisons of bacterial burden in the proportion of standard water and standard dialysis fluid yielding colony counts ≥50 CFU/ml when assayed using R2A and TSA at the conditions stated above[11].

Maltais et al.[11] in their comparison of this medium (TGEA) with TSA when quantifying the microbial burden in water and dialysis fluid used for standard dialysis showed that the proportion of water samples yielding colony counts ≥50 CFU/ml incubated at 17 °C to 23° C for a period of 7 days was significantly different from the proportion established by TSA at an incubation temperature of 35 °C -37 °C and an incubation time of 48 hours ($p = 0{,}001$). The proportions of dialysis fluid samples in which microbial burden was ≥50 CFU/ml were not significantly different.

The TSA method balances the increased recovery against the shorter time for actionable results.

Tryptone Glucose Extract Agar (TGEA) or Reasoner's Agar No. 2 (R2A) incubated at 17 °C to 23 °C for a period of 7 days, or Tryptic Soy Agar incubated at 35 °C for 48 hours are all validated and acceptable methods. The user should determine which of these methodologies is appropriate for the circumstance, taking into account the advantages of each.

According to the United States Pharmacopeia, "the decision to use longer incubation times, should be made after balancing the need for timely information and the type of corrective actions required when alert or action level is exceeded with the ability to recover the microorganisms of interest. The advantages gained by incubating for longer times namely recovery of injured microorganisms, slow growers, or more fastidious microorganisms, should be balanced against the need to have a timely investigation and take corrective action, as well as the ability of these microorganisms to detrimentally affect products or processes" (e.g. patient safety).

The culture medium and incubation times selected should also be based on the type of fluid to be analysed e.g. standard dialysis fluid, water used in the preparation of standard dialysis fluid, ultrapure dialysis fluid, water used for the preparation of ultrapure dialysis fluid or fluid used for online therapies such as haemodiafiltration. The method selected, should be based on the analysis of the advantages, disadvantages and sensitivity of each of the suggested methods. It should also ensure that the patient is provided with the necessary safeguards within any constraints imposed by local laboratory working practices and reimbursement.

When surveillance for microbial contamination in ultrapure dialysis fluid, the maximum allowable level of bacteria requires that culturing be performed using the membrane filtration method with a minimum of 10 ml of ultrapure dialysis fluid being passed through the filter. The use of larger volumes (up to 1 000 ml) will provide greater sensitivity, but the improved sensitivity needs to be balanced against the increased risk of contamination in collecting and handling the sample. Even with these more sensitive techniques, conformity with the stringent requirement that online-prepared substitution fluid be sterile cannot be demonstrated by culturing; it has to be ensured by use of a validated process. Surveillance of the production of online-prepared substitution fluid will depend on the production system and should be performed according to the manufacturer's instructions.

Furthermore, the culturing conditions recommended in this document could fail to identify the presence of some organisms. Specifically, the recommended methods might not detect the presence of various non-tuberculous mycobacteria that have been associated with several outbreaks of infection in dialysis units[83][84]. Also, the recommended methods will not detect fungi and yeast, which have been shown to contaminate water used for haemodialysis applications[85].

The microbiological purity of packaged liquid concentrates and dry powder cartridges is the responsibility of the manufacturer. Surveillance of bicarbonate concentrate produced at a dialysis facility from powder and water, though not required routinely, may be undertaken as part of a troubleshooting investigation. The sodium content of TSA is sufficient for use in culturing bicarbonate concentrate without supplementation. Salt tolerance studies showed that optimal growth of organisms found in bicarbonate concentrate occurred when the sodium chloride concentration was approximately 3 % to 6 %[86]. Therefore, if a low-salt medium, such as Reasoner's 2A or TGEA, is used to monitor microbial contamination in bicarbonate concentrate, it should be supplemented by the addition of 4 % sodium bicarbonate.

A.9 Cultivation conditions

The rationale in this clause addresses the requirements in 8.3.3.3.

Due to cultivation of the samples, there is a delay between sampling and obtaining the results. If using techniques that require seven days for the results to be available, significant contamination may be detected earlier than seven days if intermediate counts (e.g. every two days) are used.

A.10 Bacterial endotoxin test

The rationale in this clause addresses the requirements in 8.3.4. Bicarbonate concentrate inhibits the LAL assay. Inhibition is caused by the high concentration of solutes in the concentrate and the pH. In the gel-clot assay, this inhibition results in a failure of the positive control to clot. In kinetic assays, inhibition results in a failure to recover the positive control to within −50 % to +200 % of the nominal value.

Dilution of the bicarbonate concentrate with water is the usual method for overcoming inhibition. A minimum dilution of 1:16 is necessary; however, higher dilutions are recommended for more sensitive assays. For example, a 1:20 dilution is recommended when using an assay with a sensitivity of 0,03 EU/ml. Dilution reduces the detection limit of the assay. In kinetic methods, the sensitivity is the lowest concentration used to construct the standard curve. A kinetic assay is recommended because kinetic assays generally are more sensitive than gel-clot assays.

Standard gel-clot tests are incubated at 37 °C for the time recommended by the manufacturer. At the end of the recommended time, the tubes are inverted to detect the presence of a clot. A positive test will have a clot, which will remain in the end of the tube as long as it is not shaken or bumped. A negative test will not have a clot and will tend to flow out of the tube, when inverted. A clot that is semisolid and flows slowly is classified as a negative clot. For example, when bicarbonate concentrate is tested using a gel-clot assay with a sensitivity of 0,03 EU/ml and the concentrate is diluted 1:20 (1,0 ml concentrate plus 19 ml LAL reagent water or equivalent), the positive control sample should clot, indicating that there is no inhibition of the assay. If the diluted bicarbonate concentrate sample clots, it indicates that the sample contains at least 0,6 EU/ml of endotoxins. A new version of the gel-clot assay has a matched positive control, which simplifies the testing and increases the reliability of the results.

Annex B
(informative)

Equipment

B.1 General

This annex provides a brief description of the different components that may be included in systems to treat and distribute dialysis water and dialysis fluid and to prepare and distribute concentrates for haemodialysis applications. Two types of systems are used: systems in which dialysis water is distributed to a dialysis machine at an individual patient treatment station, which prepares and controls the dialysis fluid for use at that station; and central dialysis fluid delivery systems, which prepare dialysis fluid centrally and supply it to multiple-patient treatment stations. Since feed-water quality and product-water requirements can vary from facility to facility, not all of the components described in the following clauses will be necessary in every water treatment and distribution system. Also, not every dialysis facility will prepare concentrate at the dialysis facility.

Requirements for water treatment equipment used for haemodialysis and related therapies can be found in ISO 23500-2 and the requirements for equipment used to prepare concentrates at a dialysis facility can be found in ISO 23500-4.

Routine dialysis requires a well-functioning water treatment and distribution system, since dialysis cannot be performed without an adequate supply of water. In addition, certain components of the water treatment and distribution system are critical to its operation. An example of such a critical component is the circulating pump in an indirect feed system. A dialysis facility should develop contingency plans to cover the failure of its water treatment and distribution system or a critical component of that system. Such contingency plans should describe how to deal with events that completely prevent dialysis from being performed, such as failure of the facility's municipal water supply or electrical service following a natural disaster or water main break. Other plans should address how to deal with sudden changes in municipal water quality, as well as with failure of a critical component of the water treatment and distribution system. Similar contingency plans should be developed to deal with the failure of concentrate preparation systems.

B.2 Water treatment systems

B.2.1 General

Water treatment systems consist of three basic sections: a pre-treatment section that conditions the water supplied to the primary purification device, which may be followed by other devices that increase final water quality. The pre-treatment section commonly includes a sediment filter, cartridge filters capable of retaining particles of various sizes, a softener, and carbon beds. The primary purification process most commonly used is reverse osmosis, which may be followed by deionization and ultrafiltration for polishing the product water from the reverse osmosis system. Whether a particular device is included in an individual water treatment system will be dictated by local conditions.

This clause provides a brief description of the principal equipment used to purify water used in haemodialysis applications. Devices used to treat water for haemodialysis should comply with the requirements of ISO 23500-2, including certain design and performance specifications for individual water treatment devices.

General information is provided in B.2.2 to B.2.9. The design and instrumentation of individual water treatment devices may vary from these general descriptions. For example, softeners may be configured as a single resin bed that is regenerated outside the normal operating hours of the dialysis unit, or they may have a dual-bed configuration that allows one bed to be regenerated while the other is used to provide water for normal dialysis operations.

Depending on the feed-water quality and product-water requirements, not every component in this clause might be required in a given facility. Likewise, additional components can be required in certain circumstances. For example, carbon might not provide adequate chloramine removal if the water contains substances, such as polyphosphates, that mask the reactive sites on the carbon particles. In those circumstances, other processes, such as infusion of sodium bisulfite, can be required to achieve product water that meets the requirements of 4.2.2.

Users are encouraged to obtain detailed descriptions of all water treatment components, together with operating manuals and maintenance procedures, from the manufacturer or the supplier of the water treatment and distribution system.

B.2.2 Sediment filters

Permanent, backwashable sediment filters, also known as “bed filters”, are frequently located at or near the beginning of haemodialysis water treatment systems and are intended to remove relatively coarse particulate materials from incoming water. Although a single filtration medium may be used, bed filters known as multimedia filters are more commonly selected. These units contain multiple layers, each layer retaining progressively smaller particles. In this way, the bed is used to its fullest extent; the largest particles are removed in the first layer contacted by the water and the smallest in the final layer.

As the bed accumulates particulate material, open passages begin to clog and resistance to the water flowing through the filter increases. Ultimately, the increased resistance to flow will lead to a reduction in water supply to downstream components. To prevent this situation from occurring, bed filters are cleaned by periodic backwashing, which is accomplished either manually or by using a timer-activated control valve. Sediment filters should have an opaque housing or other means to inhibit proliferation of algae. Bed filters should be fitted with gauges to measure the hydrostatic pressure at the filters' inlet and outlet. These values can be used to determine the dynamic pressure drop across the filter (delta pressure or ΔP), which serves as an index of resistance to flow and provides a basis, together with the manufacturer's recommendations, for setting the frequency of backwashing.

B.2.3 Cartridge filters

Cartridge filters consist of a cylindrical cartridge of the filter medium with a central drainage core. The cartridge is contained within a filter housing with seals to separate the feed and product-water streams. In the pre-treatment cascade, transparent filter housings can be useful because they allow any carbon or resin leakage to be seen without the need to break the integrity of the system. The housing can be cleaned to remove any algae growth when the filter cartridge is changed. For this reason, use of opaque housings for cartridge filters is recommended, but not required. If transparent housings are used, they should not be exposed to natural light, in order to minimize proliferation of algae. Cartridge filters should be fitted with gauges to measure the hydrostatic pressure at the filters' inlet and outlet. Although cartridge filters may be installed at the inlet to a water treatment system, their usual application is as a final filtration step prior to reverse osmosis. Resistance to flow through the filter increases as the cartridge accumulates particulate material, as indicated by an increase in ΔP. When the maximum ΔP recommended by the filter manufacturer is reached, the cartridge should be replaced according to the manufacturer's instructions.

B.2.4 Softeners

Water that contains calcium or magnesium can form relatively hard deposits and is called “hard water”. Water that has had these elements replaced by sodium ion exchange is called “soft water”, hence the term “softener” is used. Softeners also remove other polyvalent cations, most notably iron and manganese, although they are somewhat limited in this regard. However, if significant concentrations of iron and manganese are present, special procedures should be implemented in order to reduce those concentrations to levels that do not interfere with the softening process or cause membrane fouling. The primary use of softeners in haemodialysis water systems is to prevent hard water deposits from fouling reverse osmosis membranes.

A softener is a cylinder or vessel that contains insoluble spheres or beads, called “resin”, to which sodium ions are attached. During operation, exchangeable sodium ions in the resin are progressively replaced by calcium and magnesium ions. When all the sodium ions have been used, the resin bed has reached a condition referred to as “exhaustion”. Prior to exhaustion, softeners should be restored; that is, new exchangeable sodium ions are placed on the resin by a process known as “regeneration”, which involves exposure of the resin bed to a highly concentrated sodium chloride solution. The concentrated sodium chloride solution is prepared in a separate brine tank, from which the solution is drawn during the regeneration process. A control valve on the softener regulates the regeneration and service cycles.

Automatically regenerated water softeners should be fitted with a mechanism to prevent water containing the high concentration of sodium chloride used during regeneration from entering the product-water line during regeneration. For softeners with a time-controlled regeneration cycle, the face of the timer should be visible to the user. Operating controls should be positioned so as to minimize inadvertent resetting.

B.2.5 Carbon media

Carbon systems, often referred to as carbon filters, are the principal means of removing both free chlorine and chloramine. Removal of chloramine to a maximum level of 0,1 mg/l and removal of free chlorine to a maximum level of 0,5 mg/l are necessary to protect haemodialysis patients from red blood cell haemolysis. In addition, free chlorine can also degrade some reverse osmosis membranes, depending on the membrane material. Determining the level of chloramine typically involves measuring both total chlorine and free chlorine and assigning the difference in concentrations to chloramine. To permit a single test to be used, a maximum allowable level for total chlorine was set at the maximum allowable level for chloramine (0,1 mg/l).

In addition to removing free chlorine and chloramine, carbon also adsorbs a wide variety of other substances, including both naturally occurring and synthetic organic compounds. The capacity of carbon to remove free chlorine and chloramine can be reduced when other substances “mask” reactive sites on the carbon media. In addition, the rate of free chlorine and chloramine removal is reduced as pH increases or as temperature decreases. The net effect of those variables is that the finite capacity of carbon beds to remove free chlorine and chloramine cannot be predicted with any certainty. Therefore, their performance needs to be monitored frequently.

Carbon systems should be adapted specifically to the maximum anticipated water flow rate of the system. When carbon is used for the removal of chloramine at a level of 1 mg/l or more, two carbon beds should be installed in a series configuration. A means should be provided to sample the product water from the first bed. A sampling port should also be installed following the second bed for use in the event of total chlorine breaking through the first bed. In situations where chloramine is not used to disinfect the water, and the ammonium level in the water is low, one carbon bed or carbon cartridge filters could be sufficient. Exhausted carbon media should be discarded and replaced with new media, according to a replacement schedule determined by regular surveillance. For example, when testing between the beds shows that the first bed is exhausted, the second bed should be moved into the first position, the second bed replaced with a new bed, and the exhausted bed discarded. When samples from the first sampling port are positive for total chlorine, operation may be continued for a short time (up to 72 h) until a replacement bed is installed, provided that samples from the second sampling port remain negative. It was recognized that it might not be practical to rotate the bed positions in installations that use large, backwashable carbon beds. However, there was concern that the capacity of the second bed could decrease unpredictably and no longer provide adequate backup if there was breakthrough of the first bed. For this reason, replacing both beds if bed rotation was not possible was recommended.

Granular activated carbon with an iodine number greater than 900 is considered optimal for chlorine/chloramine removal. When granular activated carbon is used as the medium for the removal of chloramine from water containing >1 mg/l chloramine, each bed should have an empty-bed contact time (EBCT) of at least 5 min at the maximum product-water flow rate (a total EBCT of at least 10 min). Some source waters, such as those with a high organic content, could require alternative types of carbon that are more resistant to organic fouling. These types of carbon may have iodine numbers less than 900. When other forms of carbon or granular activated carbon with an iodine number of less than 900 are used, the manufacturer should provide performance data to demonstrate that each bed has the capacity to reduce the total chlorine concentration in the feed water to less than 0,1 mg/l when operating at the maximum anticipated flow rate for the maximum time interval between scheduled testing of the product water for total chlorine. Regenerated carbon should not be used. Some granular activated carbon contain aluminium, which can elute from the carbon and add to the burden of aluminium to be removed by reverse osmosis or ion exchange. The use of acid-washed carbon minimizes this source of aluminium in the water.

Where practical, portable dialysis systems supplied with water known to contain chloramine should include two carbon beds in series, which together provide a 10 min EBCT. Where that is not practical, alternative technologies can be used provided there is a redundant means of chloramine removal and that a total chlorine concentration of less than 0,1 mg/l is verified in a sample collected after the primary device before each treatment. Possible alternatives include: a granular activated carbon bed followed by a dense carbon block; and two carbon block filters in series.

Carbon beds used for free chlorine and chloramine removal are sometimes arranged as series-connected pairs of beds so that they need not be overly large. The beds within each pair are of equal size and water that flows through them are parallel. For removal of chloramine from water containing >1 mg/l, each pair of beds should have a minimum empty bed contact time of 5 min at the maximum flow rate through the bed. When series-connected pairs of beds are used, the piping should be designed to minimize differences in the resistance to flow from inlet and outlet between each parallel series of beds to ensure that an equal volume of water flows through all beds.

Although treatment of water by carbon is the method usually used to meet the requirement of 4.2.2 for total chlorine, it was recognized that, in certain situations, carbon might not adequately remove chloramine. Inadequate removal of chloramine might occur when chloramines in the form of naturally occurring *N*-chloramines are present or when practices such as the use of high pH or the inclusion of orthophosphate or polyphosphates are used. If the pH of the incoming water is out of the range specified by the manufacturer, the carbon might not function properly and deplete rapidly. In other situations, such as acute dialysis with portable water treatment systems, it could be impractical to use the volume of carbon required to ensure adequate chloramine removal. In such circumstances, other strategies for chloramine removal may be needed to supplement carbon. Acid injection can be used to decrease pH (see B.2.6), anion exchangers (also known as organic scavengers) can be installed before carbon beds to remove organic matter and other substances that might foul carbon beds, and dealkalizers can be used to reduce alkalinity. It is known that adding sodium bisulfite prior to the reverse osmosis system has been successful in eliminating chloramine in haemodialysis applications. Ascorbic acid has also been added to the acid concentrate used to eliminate chloramine from the final dialysis fluid. It should be noted that a minimum contact time is required for ascorbic acid to neutralize chloramine in water. If ascorbic acid is being used to neutralize chloramine, and if unexplained red blood cell destruction or anaemia occurs, the effectiveness of the ascorbic acid neutralization of chloramine should be investigated. Other means of removing chloramine, such as redox alloy media and ultraviolet irradiation, are used in the pharmaceutical and electronics industries. These processes are currently being evaluated for haemodialysis applications. The final choice of a system for chloramine removal in haemodialysis settings will depend on local conditions and might need to include more than one of the processes outlined above.

B.2.6 Chemical injection systems

Chemical injection systems may be used in the pre-treatment section of a water purification system to supplement the physical purification processes described in the previous clauses. Applications of chemical injection include the addition of sodium bisulfite to remove chloramine and the addition of acid to adjust pH.

Chemical injection systems consist of a reservoir that contains the chemical to be injected, a metering pump, and a mixing chamber located in the main water line. Chemical injection systems also include some means of regulating the metering pump to control the addition of a chemical. This system should be designed to tightly control the addition of the chemical. The control system should ensure that a chemical is added only when water is flowing through the pre-treatment cascade and that it is added in fixed proportion to the water flow or based on some continuously monitored parameter, such as pH, using an automated control system. If an automated control system is used to inject the chemical, the controlling parameter should be independently monitored. There should also be a means of verifying that the concentrations of any residuals arising from the chemical added to the water are reduced to a safe level before the water reaches its point of use.

When acid is added to adjust pH, a mineral acid should be used; organic acids can act as a nutrient and allow bacteria to proliferate.

Reservations were expressed about the addition of chemicals to the water. However, it was recognized that the addition of chemicals could be necessary, in some circumstances, if a facility is to meet the maximum contaminant levels set forth in 4.2.2. For example, if the municipal water contains high levels of *N*-chloramines or chloramine in the presence of orthophosphate or polyphosphate, injection of sodium bisulfite can be one of the few options available for chloramine removal.

If chemical injection is used in the pre-treatment cascade, users should ensure that the addition of the chemical does not interfere with the operation of subsequent purification processes, including the primary purification process. For example, the performance of thin-film composite reverse osmosis membranes might be affected by the pH of the feed water. At pH levels below 7, the rejection of fluoride can be substantially reduced, compared to its rejection at a pH of 8.

B.2.7 Reverse osmosis

Reverse osmosis (RO) systems have become widely used in haemodialysis water treatment systems, largely because these devices remove dissolved inorganic and organic solutes, as well as bacteria and bacterial endotoxins.

The following requirements apply to reverse osmosis systems.

a) When used to prepare water for haemodialysis applications, either alone or as the last chemical purification stage in a water treatment system, reverse osmosis systems should be shown to be capable, at installation, of meeting the requirements of ISO 23500-3 when tested with the typical feed water of the user.

b) Reverse osmosis devices should be equipped with online monitors that allow the determination of rejection rates and product-water conductivity. The product-water conductivity monitor should activate audible and visual alarms when the product-water conductivity exceeds the preset alarm limit. The alarms should be capable of notifying staff in the patient care area when reverse osmosis is the last chemical purification process in the water treatment system. Monitors that measure resistivity may be used in place of conductivity monitors.

The RO membrane separation process components are a semipermeable membrane, typically in a spiral-wound configuration, a pump, and various flow and pressure controls to direct the flow of water through the system. In operation, feed water is pressurized by the RO pump and is then directed along the surface of the semipermeable membrane. A portion of the water is forced through the membrane; a process that removes inorganic and organic solutes, bacteria and bacterial endotoxins. The remainder of the water continues along the membrane surface and is directed to the drain. Water passing through the membrane is referred to as “product water” or “permeate”. The water that flows along the membrane surface and to the drain is known as “reject water” or “concentrate”. This flow configuration, known as “cross-flow filtration”, prevents a progressive build-up of materials on the membrane surface that would eventually lead to fouling and membrane failure. In some reverse osmosis systems, a portion of the reject water stream is recycled to the feed-water stream. This recycling allows higher velocities across the membrane surface, which can help reduce membrane fouling, as well as allowing higher overall use of water. RO systems may operate in a single-stage or two-stage (double-pass) configuration depending on feed-water quality and/or local requirements and preferences. In a two-stage RO, the product water from the first stage acts as the feed water for the second stage.

NOTE The rejection rate of the second stage in a two-stage reverse osmosis system can be significantly lower than the rejection rate of the first stage. One reason for the difference in rejection rates is due to dissolved CO_2.

RO systems may also be fitted with flow meters, usually in the product water and the rejected water streams, to monitor the output of the RO system and gauges to monitor the pressure at various points in the system. Although not indicative of treated water quality, surveillance flow rates and pressures can help ensure that the system is operating within the manufacturer's specifications and thus will help ensure RO reliability.

In addition, it is recommended that, when a reverse osmosis system is the last chemical purification process in the water treatment system, means should be incorporated to prevent patient exposure to unsafe product water in the event of a product-water conductivity alarm. Such means could include diversion of the product water to the drain, in addition to activating the audible and/or visual alarms that should be situated to ensure a prompt response by personnel in the patient care area.

Depending on membrane configuration and materials of construction, RO systems are sensitive to various feed-water conditions that can lead to diminished performance or premature failure. To avoid such problems, users should carefully follow the manufacturer's instructions for feed-water treatment and surveillance to ensure that the RO is operated within its design parameters.

B.2.8 Deionization

Deionization (DI) is an ion exchange process that removes both anions (negatively charged ions) and cations (positively charged ions) from water. During the exchange process, hydroxyl ions replace other feed-water anions, and hydrogen ions replace other feed-water cations; the hydroxyl and hydrogen ions then combine to form pure water. DI systems may contain anion and cation resin in separate vessels, known as “dual-bed systems”, or may have both resin types mixed together in a single vessel, known as “mixed-bed” or “unibed systems”.

Deionizers are an effective means of removing ionic contaminants from water. However, they do not remove nonionic contaminants and they can contribute bacterial contaminants to the water rather than remove them. The inability of deionizers to remove nonionic contaminants could limit aluminium removal by deionization, since aluminium is an amphoteric substance that changes from being cationic to being anionic as the pH varies from acidic to basic[87]. At neutral pH, aluminium is present mostly as colloidal aluminium, which does not carry a charge and is not removed by deionization[17]. Furthermore, deionizers have a finite capacity for contaminant removal. Once the deionizer is depleted of hydrogen and hydroxyl ions, the next least avidly bound ions will be displaced by more avidly bound ions. For example, once the hydroxyl ions are depleted, anionic contaminants in the water will displace fluoride ions from the anion exchange resin[35]. This phenomenon has led to high levels of fluoride in the product water, with subsequent patient injury and death[33][36]. Deionization, even in combination with an endotoxin-retentive filter, does not remove certain low-molecular-weight toxic bacterial products, such as microcystins. For the above reasons, use of deionization as the primary means of treating water supplying multiple dialysis machines is strongly discouraged. Deionization may be used to polish product water from a reverse osmosis system or may be used as a standby if the reverse osmosis system fails. Many believe that a two-stage reverse osmosis system operated in a redundant configuration is preferable to a combination of reverse osmosis and deionization.

The most common configuration for DI is to have two mixed beds in series, with resistivity monitors being placed downstream of each bed. Upon exhaustion of the first bed, reliance for water of sufficiently high resistivity shifts to the second bed, and dialysis operations may be continued for a short time until a replacement bed is installed, provided that the product water from the second tank has a specific resistivity of 1 MΩ·cm or greater.

DI has a finite capacity that, when exceeded, will cause dangerously high levels of contaminants in the product water. Therefore, DI systems, when used to prepare water for haemodialysis applications, should be monitored continuously to produce water of 1 MΩ·cm or greater specific resistivity (or conductivity of 1 µS/cm or less) at 25 °C. An audible and visual alarm should be activated when the product-water resistivity falls below this level and the product-water stream should be prevented from reaching any point of use, for example by being diverted to drain. The alarm should be capable of notifying staff in the patient care area. Under no circumstances should DI be used when the product water of the final bed has a resistivity below 1 MΩ·cm.

Feed water for deionization systems should be pretreated with activated carbon, or a comparable alternative, to prevent nitrosamine formation.

If a deionization system is the last process in a water treatment system, it should be followed by an endotoxin-retentive filter or other bacteria- and endotoxin-reducing device. The tendency for deionizers to contribute bacterial contaminants to the water is greater when deionizers are kept as a backup for a reverse osmosis system, particularly if there is no flow through the deionizers. Some facilities counter this tendency by connecting the deionizers in parallel to the main water line and by maintaining a low flow through them. An alternative approach is to contract with a local supplier to provide backup deionizers on demand.

NOTE The requirements given above for deionization might not apply to electrodeionization (EDI) technology, which can be used as an alternative to deionization following reverse osmosis in haemodialysis applications.

B.2.9 Endotoxin-retentive filters

Endotoxin-retentive filters are membrane-based separation devices that may be used to remove both bacteria and endotoxins. Endotoxin-retentive filters should be placed in dialysis water systems at locations downstream of deionization. They may also be used at the end of the purification cascade and in dialysis water or central dialysis fluid distribution systems. Endotoxin-retentive filters can also be used in the dialysis fluid line of individual dialysis machines as a final barrier against bacteria and endotoxins. These filters are considered part of the dialysis machine and might not be subject to all the recommendations that follow.

NOTE Endotoxin-retentive filters do not remove low-molecular-weight microbial metabolites.

Endotoxin-retentive membranes used for haemodialysis applications are typically in either a spiral-wound configuration or a hollow-fibre configuration. Spiral-wound ultrafilters are usually operated in a cross-flow mode, with a fraction of the feed water being forced through the membrane and the remainder being directed along the membrane surface to drain. As with reverse osmosis, cross-flow filtration is intended to minimize membrane fouling. Hollow-fibre endotoxin-retentive filters are typically housed in vessels similar to those used for cartridge sediment filters and may be operated in a cross-flow or a dead-end (no cross-flow) mode. When used in a water purification system for haemodialysis applications, an endotoxin-retentive filter should be shown to reduce the concentrations of bacteria and endotoxins entering the filter by factors at least as great as those specified in the manufacturer's labelling.

Endotoxin-retentive filters should be fitted with a means of evaluating filter integrity and fouling during use. One suitable means is to monitor the pressure drop (ΔP) across the filter at a given product-water flow rate using pressure gauges on the inlet (feed) and outlet (product) water lines. Alternatively, product-water flow rate can be measured at a given pressure drop. Such surveillance will indicate when membrane fouling has progressed to the point at which membrane replacement or cleaning is needed. Surveillance also ensures that the device is being operated in accordance with the manufacturer's instructions. Endotoxin-retentive filters should be included in routine disinfection procedures to prevent uncontrolled proliferation of bacteria in the filter. If bacterial proliferation is not controlled, bacteria can “grow through” the membrane and contaminate the product-water compartment of the filter. Endotoxin-retentive filters operated in the cross-flow mode should also be fitted with a flowmeter to monitor the flow rate of water being directed to the drain.

B.3 Dialysis Water storage and distribution

B.3.1 General

The function of the water storage and distribution system is to distribute dialysis water from the treatment cascade to its points of use, including individual haemodialysis machines, proportioning systems used to prepare dialysis fluid centrally, dialyser reprocessing equipment, and concentrate preparation systems. A water storage and distribution system typically contains a large volume of water exposed to a large surface area of piping and storage tank walls. Because chlorine and chloramine are removed in the purification process, the water does not contain a bacteriostatic agent. This combination of circumstances predisposes wetted surfaces to bacterial proliferation and biofilm formation. Therefore, any dialysis water storage and distribution system should be designed specifically to facilitate bacterial control, including measures to prevent bacterial colonization and to allow for easy and frequent disinfection.

B.3.2 Water storage

When used, storage tanks should have a conical or bowl-shaped base and should drain from the lowest point of the base. Storage tanks should have a tight-fitting lid and be vented through a hydrophobic 0,22 µm to 0,45 µm air filter. The filter should be changed on a regular schedule according to the manufacturer's instructions or if it becomes wet. A means should be provided to effectively disinfect any storage tank installed in a water distribution system. Internal spray mechanisms can facilitate effective disinfection and rinsing of a storage tank.

B.3.3 Water distribution

Two types of water distribution systems are used: direct feed systems and indirect feed systems. In a direct feed system, dialysis water flows directly from the last stage of the purification cascade to the points of use. In an indirect feed system, dialysis water flows from the end of the purification cascade to a storage tank. From there, it is distributed to the points of use. The water storage and distribution system chosen for a particular situation should provide the simplest possible flow path and contain the smallest volume of water consistent with the operating needs of the dialysis unit. The simplest system is generally a direct feed system. However, direct feed systems can be impractical. For example, the pressure at the end of the purification cascade could be insufficient to provide adequate flow and pressure at the points of use without a booster pump. If a direct feed system is used, it is also necessary to size the water treatment cascade to provide sufficient water to meet the peak demand. For these reasons, an indirect feed system with a storage tank may be used. Since storage tanks provide a large surface area for potential biofilm formation, their volume should be kept to a minimum in order to maximize water turnover in the tank. Whichever type of system is used, water distribution systems should be configured as a continuous loop and designed to minimize bacterial proliferation and biofilm formation (see Clause 8). A centrifugal pump made of inert materials is necessary to distribute the dialysis water and aid in effective disinfection. A multistage centrifugal pump is preferred for this purpose.

Direct feed distribution systems typically return unused dialysis water to the feed side of the reverse osmosis unit. If the pressure at the end of the distribution loop decreases to a value below the water pressure at the inlet to the reverse osmosis pressurizing pump, retrograde flow of untreated water into the distribution loop can occur. To minimize this risk, it is recommended that dual check valves or a break tank at the inlet to the reverse osmosis system with an air gap on the lines from the pre-treatment cascade and the distribution loop be used to prevent retrograde flow and that the pressure at the end of the distribution loop be monitored.

Distribution systems for dialysis water should be constructed of materials that do not contribute chemicals, such as aluminium, copper, lead, and zinc, or bacterial contaminants to dialysis water. The choice of materials used for a water distribution system will also depend on the proposed method of disinfection. Table B.1 provides guidance on the compatibility of different materials and disinfection agents. Whatever material is used, care should be taken to select a product with properties that provide the least favourable environment for bacterial proliferation, such as smooth internal surfaces.

Table B.1 — Guidance on piping materials used in dialysis water distribution systems and their compatibility with common disinfectants

Material	Sodium hypochlorite (bleach)	Peracetic acid	Formaldehyde	Hot water	Ozone[a]
PVC	X	X	X		X
CPVC	X	X	X		X
PVDF	X	X	X	X	X
PEX	X	X	X	X	X
SS		X	X	X	X
PP	X	X	X	X	
PE	X	X	X		X
ABS		X			
PTFE	X	X	X	X	X
Glass	X	X	X	X	X

NOTE 1 X denotes probable compatibility.

NOTE 2 PVC = polyvinylchloride, CPVC = chlorinated polyvinylchloride, PVDF = polyvinylidene fluoride, PEX = cross-linked polyethylene, SS = stainless steel, PP = polypropylene, PE = polyethylene, ABS = acrylonitrile butadiene styrene, PTFE = polytetrafluoroethylene.

[a] Ozone refers to ozone dissolved in water, not ozone gas.

Table B.1 is not intended as an exhaustive compilation of all possible compatible combinations of piping material and disinfectant. Considerations of compatibility should include any joint materials and pipe fittings, as well as the actual piping material. The concentration of germicide and the duration, frequency, and conditions (flow, pressure, temperature) of exposure should also be taken into account.

Users should verify compatibility between a given germicide and the materials of a piping system with the supplier of that piping system and/or the disinfectant supplier before using the germicide.

B.3.4 Bacterial control devices

B.3.4.1 General

Traditionally, chemical disinfection has been used to prevent bacterial proliferation in dialysis water storage and distribution systems. One consequence of the increased attention being paid to bacterial control in the dialysis water storage and distribution system is an interest in alternatives to traditional chemical disinfection, including ultraviolet irradiators, ozone generators, and hot water disinfection systems. Both ozone and hot water can allow more frequent disinfection of the dialysis water storage and distribution system because prolonged rinsing is not needed to remove residual disinfectant from the system before dialysis is recommenced. The use of ozone or hot water is possible only if the systems are constructed from appropriately resistant materials. This limitation applies not only to the piping and any storage tank that may be in the system but also to all pumps, valves, and other fittings, including any O-rings and seals they may contain. Ultraviolet irradiation can be used to kill planktonic cells, but it has no impact on bacteria located in biofilm. In order to achieve an effective and preventive disinfection with the respective system, the user should refer to the recommendations given by the manufacturer of the device or system.

B.3.4.2 Ultraviolet irradiators

When used to control bacterial proliferation in dialysis water storage and distribution systems, UV irradiation devices should be fitted with a low-pressure mercury lamp which emits light at a wavelength of 254 nm and provides a dose of radiant energy of 30 mW·s/cm^2. If the irradiator includes a calibrated ultraviolet intensity meter, the minimum dose of radiant energy should be at least 16 mW·s/cm^2. The device should be sized for the maximum anticipated flow rate according to the manufacturer's instructions. It is recommended that UV irradiators be followed by an endotoxin-retentive filter to remove pyrogens.

The recommendations provided in this clause concern UV irradiator used specifically for bacterial control. UV irradiators may also be used for other applications in a water purification and distribution system.

Ultraviolet irradiation can also be used to control bacteria in the pre-treatment section of a water treatment system, such as the following carbon beds to reduce the bacterial burden presented to a reverse osmosis unit.

UV irradiators should be equipped with a calibrated ultraviolet intensity meter, as described above, or with an online monitor of radiant energy output that activates a visible alarm, which indicates that the lamp should be replaced. Alternatively, the lamp should be replaced on a predetermined schedule according to the manufacturer's instructions to maintain the recommended radiant energy output.

When ultraviolet irradiators are dipped in the storage tank to control bacteria, they should be designed to keep the required energy at the farthest position in the tank, considering the flow situation during operation.

B.3.4.3 Ozone disinfection systems

When used to control bacterial proliferation in dialysis water storage and distribution systems, an ozone disinfection system should be capable of delivering ozone at the concentration and for the exposure time specified by the manufacturer. When ozone disinfection systems are used, it is recommended that an ambient air ozone monitor be installed in the area of the ozone generator.

Ozone generators convert oxygen to ozone using a corona discharge or ultraviolet irradiation. The ozone is then injected into the water stream. An ozone concentration of 0,2 mg/l to 0,5 mg/l, combined with a contact time of 10 min, measured at the end of the distribution loop, is capable of killing bacteria, bacterial spores, viruses, moulds, and yeast in water. Destruction of established biofilm could require longer exposure times and/or higher concentrations of ozone. Ozone can also degrade endotoxins.

Ozone can degrade many plastic materials, including PVC and elastomeric O-rings and seals. Therefore, ozone can be used for bacterial control only in systems constructed from ozone-resistant materials (see B.3.3).

B.3.4.4 Hot water disinfection systems

Hot water may be used to control bacterial proliferation in dialysis water storage and distribution systems. The exposure time should be according to the manufacturer's instructions. The water heater of a hot water disinfection system should be capable of delivering hot water at the temperature and for the exposure time specified by the manufacturer at any site in the dialysis water storage and distribution system. The manufacturer's instructions for using hot water disinfection systems should be followed. If no manufacturer's instructions are available, the effectiveness of the system can be demonstrated by validating the system maintains a specified temperature throughout the system for a specified time and by performing ongoing surveillance with bacterial cultures and endotoxin testing.

NOTE 1 The ability of hot water to disinfect a distribution system is a function of water temperature and time of exposure. For example, the minimum exposure time for hot water disinfection at 80 °C is 10 min.

NOTE 2 The concept A_0 can be used for quantification of heat disinfection between 65 °C to 100 °C (see ISO 15883-1:2006 Washer-disinfectors -- Part 1: General requirements, terms and definitions and tests). Temperature and time can be combined to yield a number representing a dose capable of achieving the required reduction in viable organisms.

$$A_0 = \sum 10^{(T-80)/z} \cdot \Delta t$$

where

T is the temperature in °C;

z is equal to 10 °C;

Δt is the selected time period in seconds.

An A_0 value of 1 is defined as an exposure to 80 °C for 1 s. Thus, A_0 = 600 represents 10 min at 80 °C, 1 min at 90 °C, or 100 min at 70 °C.

Hot water disinfection systems can be used only in systems constructed from heat-resistant materials, such as PVDF (polyvinylidene fluoride), PEX (cross-linked polyethylene), SS (stainless steel), PP (polypropylene), and PTFE (polytetrafluoroethylene) (see B.3.3).

B.4 Concentrate preparation

B.4.1 General

Dialysis fluid is customarily prepared from two concentrates: the bicarbonate concentrate, which contains sodium bicarbonate (and sometimes additional sodium chloride), and the acid concentrate, which contains all remaining ions, acetic acid, or citric acid and sometimes glucose. Some systems have also been developed that prepare acid concentrate from individual components, such as from a sodium chloride cartridge and a concentrated solution of the remaining minor electrolytes.

Acid concentrate can be supplied by the manufacturer in bulk or in single-use containers. In some cases, the manufacturer will pump the acid concentrate from bulk delivery containers into a holding tank at the dialysis facility. Systems have recently been introduced that allow a user at a dialysis facility to prepare acid concentrate from packaged powder and dialysis water using a mixer. If the acid concentrate is pumped into a bulk storage tank at the dialysis facility, the user is responsible for maintaining the concentrate in its original state and to ensure that the correct formula is used according to the patient's prescription. Acid concentrate prepared at the dialysis facility from powder and dialysis water is also the responsibility of the user.

Bicarbonate concentrate can be supplied by the manufacturer in one of the following three ways:

a) in powder cartridges that are used to prepare concentrate online at the time of dialysis;

b) as packaged powder that is mixed with dialysis water at the dialysis facility;

c) in single-treatment containers of liquid concentrate.

Dialysis fluid can also be prepared from a single concentrate that contains acetate, which is metabolized by the patient to yield bicarbonate. Acetate-based dialysis fluid is rarely used in routine clinical practice. In general, acetate-based concentrate is handled in a similar manner to that of acid concentrate, except that acetate-based concentrate systems use only one concentrate which is mixed with dialysis water. The labels of acetate-based concentrate are colour-coded white.

B.4.2 Materials compatibility

All components used in concentrate preparation systems (including mixing and storage tanks, pumps, valves, and piping) should be fabricated from materials (e.g. plastics or appropriate stainless steel) that do not interact chemically or physically with the concentrate to affect its purity, or with the germicides or germicidal procedure used to disinfect the equipment. The use of materials that are known to cause toxicity in haemodialysis, such as copper, brass, zinc, galvanized material, lead, and aluminium, are specifically prohibited.

B.4.3 Labelling

B.4.3.1 General

Labelling strategies should permit positive identification by anyone using the contents of concentrate mixing tanks, bulk storage/dispensing tanks, and small containers intended for use with a single haemodialysis machine. Requirements for such positive identification will vary among facilities, depending on the differences between concentrate formulations used and on whether single or multiple dialysis fluid proportioning ratios are used. The use of multiple dialysis fluid proportioning ratios in a single facility is strongly discouraged.

In addition to the container labelling described below, there should be permanent records of all batches of concentrate produced at a dialysis facility. These records should include the concentrate formula produced, the volume of the batch, the lot numbers of powdered concentrate packages, the manufacturer of the powdered concentrate, the date and time of mixing, any test results, the person performing the mixing, the person verifying mixing and test results, and the expiration date, if applicable.

Although it is the responsibility of facilities to develop and use labelling to positively identify the contents of mixing tanks, bulk storage/dispensing tanks, and concentrate containers, the guidelines in the following subclauses are suggested.

B.4.3.2 Mixing tanks

Prior to batch preparation, a label should be affixed to the mixing tank that includes the date of preparation and the chemical composition or formulation of the concentrate being prepared. This labelling should remain on the mixing tank until the tank has been emptied. Using a photocopy of the concentrate manufacturer's package label provides a convenient and comprehensive means of identifying the chemical composition or formulation of the concentrate; however, the lot number and expiration date should be marked out because they apply only to the dry powder.

B.4.3.3 Bulk storage/dispensing tanks

These tanks should be permanently labelled to identify the chemical composition or formulation of their contents. As with mixing tanks, bulk storage/dispensing tank labelling can be conveniently accomplished by affixing a copy of the concentrate manufacturer's package label.

B.4.3.4 Concentrate containers

Concentrate containers may be non-disposable vessels provided by haemodialysis machine manufacturers and having a capacity sufficient for one or two haemodialysis sessions. The extent of labelling for these containers depends on the variety of concentrate formulations used and on whether the facility uses dialysis machines with different proportioning ratios; the latter practice is strongly discouraged.

At a minimum, concentrate containers should be labelled with sufficient information to differentiate the contents from other concentrate formulations used at the facility. If a chemical spike is added to an individual container to increase the concentration of an electrolyte, the label should show the added electrolyte, the date and time added, and the name of the person making the addition (see B.4.5). The additional information may be simple or extensive, but in all cases it should permit users to positively identify the container's contents.

B.4.4 Concentrate mixing systems

B.4.4.1 General

Concentrate mixing systems require a source of dialysis water, a suitable drain, and a ground-fault-protected electrical outlet. Protective measures should be used to ensure a safe work environment. For example, ventilation and personal protective equipment should be used to handle any residual dust that is introduced into the atmosphere as powdered concentrates are added to the system and to handle any additional heat produced by the device. Structural issues, such as the facility's weight-bearing capacity, should be addressed if systems are to be installed above ground level. Operators should at all times use appropriate personal protective equipment, such as face shields, masks, gloves, gowns, and shoe protectors, as recommended by the manufacturer.

If a concentrate mixing system is used, the preparer should follow the manufacturer's instructions for mixing the powder with the correct amount of dialysis water. The number of bags or the weight of powder added should be determined and recorded.

The manufacturer's recommendations should be followed regarding any preventive maintenance and disinfection procedures. Records should be maintained indicating the date, time, person performing the procedure, and results (if applicable).

B.4.4.2 Acid concentrate mixing systems

Acid concentrate mixing tanks should be designed to allow the inside of the tank to be completely emptied and rinsed according to the manufacturer's instructions when concentrate formulas are changed. Use of a tank with a sloping bottom that drains from the lowest point is one means of facilitating this process. Because concentrate solutions are highly corrosive, mixing systems should be designed and maintained to prevent corrosion. Acid concentrate mixing tanks should be emptied completely and rinsed with dialysis water before mixing another batch of concentrate. If another batch of concentrate is not to be mixed promptly, the mixing tank should be rinsed again with dialysis water before the next batch is mixed.

B.4.4.3 Bicarbonate concentrate mixing systems

Bicarbonate concentrate mixing tanks should be designed to drain completely; for example, they should have a sloping bottom and a drain at the lowest point. High- and low-level alarms can prevent overfilling and air damage to the pump. Because concentrate solutions are highly corrosive, mixing systems should be designed and maintained to prevent corrosion. Mixing tanks should have a tight-fitting lid and should be designed to allow all internal surfaces to be disinfected and rinsed. A translucent tank allows users to see the liquid level; the use of sight tubes is not recommended because of the potential for microbial growth, such as bacteria, algae, and fungi.

Once mixed, bicarbonate concentrate should be used within the time specified by the manufacturer of the concentrate. The concentrate should be shown to routinely produce dialysis fluid meeting the recommendations of 4.4.2. Overagitating or overmixing of bicarbonate concentrate should be avoided, as this can cause CO_2 loss and can increase pH. (Systems designed for mixing dry acid concentrates may use methods that are too vigorous for dissolving dry bicarbonate.)

The mixing tank should be either

- completely emptied and disinfected according to the manufacturer's instructions, or
- disinfected using a procedure demonstrated by the facility to be effective in routinely producing dialysis fluid that allows the recommendations of 4.4.2 to be met.

B.4.5 Additives

Manufacturers provide acid concentrates with a wide range of electrolyte compositions for different proportioning ratios. Most typical dialysis fluid prescriptions can be obtained by using one or more of these commercially available concentrates. If particular formulations are not available, manufacturers provide additives that can be used to adjust the level of potassium or calcium in the dialysis fluid. These additives are commonly referred to as “spikes”.

NOTE The use of additives is not approved in some countries.

Concentrate additives should be mixed with liquid acid concentrates according to the manufacturer's instructions, taking care to ensure that the additive is formulated for use in concentrates of the appropriate dilution ratio. When liquid additives are used, the volume contributed by the additive should be considered when calculating the effect of dilution on the concentration of the other components in the resulting concentrate. When powder additives are used, care should be taken to ensure that the additive is completely dissolved and mixed before the concentrate is used.

B.5 Concentrate storage and distribution

B.5.1 Materials compatibility

All components used in concentrate distribution systems (including concentrate containers, storage tanks, and piping) that contact the fluid should be fabricated from nonreactive materials (e.g. plastics or appropriate stainless steel) that do not interact chemically or physically with the concentrate to affect its purity. The use of materials that are known to cause toxicity in haemodialysis, such as copper, brass, zinc, galvanized material, lead, and aluminium, are specifically prohibited.

B.5.2 Bulk storage tanks (acid concentrate)

Procedures should be in place to control the transfer of the acid concentrate from the delivery container to the storage tank to prevent the inadvertent mixing of different concentrate formulations. If possible, the tank and associated plumbing should form an integral system to prevent contamination of the acid concentrate. The storage tanks and inlet and outlet connections, if remote from the tank, should be secure and labelled clearly.

B.5.3 Distribution systems

Concentrate may be distributed from a central preparation point using reusable concentrate containers that contain sufficient concentrate for one to two treatments, or it may be distributed through a piping system that provides concentrate connections at each treatment station. A combination of these two systems may also be used, with some concentrates distributed by concentrate container and others through a piping system. Two common configurations used for distributing concentrate through a piping system are gravity feed and pressurized. Gravity feed systems require an elevated tank; pressurized systems deliver the concentrate using a pump and motor and do not require an elevated tank. The maximum allowable concentrate delivery pressure is specified by the manufacturer of the dialysis fluid delivery machine and should not be exceeded.

Elevated tanks are usually smaller than those used for preparing concentrates. Elevated tanks for bicarbonate concentrate distribution should be equipped with conical or bowl-shaped bottoms, tight-fitting lids, a spray mechanism, and high- and low-level alarms. Any air vents should have 0,45 µm hydrophobic vent filters.

B.5.3.1 Acid concentrate distribution systems

Acid concentrate delivery piping should be labelled and colour-coded red at the point of use (at the container filling station or the dialysis machine connection). More than one type of acid concentrate may be delivered, and each line should clearly indicate the type of acid concentrate it contains. Even though there are no published reports of acid concentrate supporting bacterial growth, every effort should be made to keep the system closed to prevent contamination and evaporation. If the acid system remains intact, no rinsing or disinfection is necessary.

B.5.3.2 Bicarbonate concentrate distribution systems

Bicarbonate concentrate delivery piping should be colour-coded blue at the point of use (at the concentrate container filling station or dialysis machine connection). All joints should be sealed to prevent leakage of concentrate.

Because bicarbonate concentrates provide excellent media for bacterial proliferation, bicarbonate concentrate delivery systems should be disinfected on a regular basis to ensure that the dialysis fluid routinely achieves the level of bacteriological purity recommended in 4.4.2. The manufacturer's instructions can provide an initial disinfection schedule. However, this schedule might need to be adjusted on the basis of the user's bacteriological surveillance. For piped distribution systems, the entire system, including patient station ports, should be purged of bicarbonate concentrate before disinfection. Each patient station port should be opened and flushed with disinfectant and then rinsed; otherwise, it would be a "dead leg" in the system. Also, prompt use of bicarbonate concentrates prepared in dialysis facilities from powder and dialysis water is strongly recommended.

When reusable concentrate containers are used to distribute bicarbonate concentrate, they should be rinsed free of residual concentrate before disinfection.

All chemical disinfectants (e.g. sodium hypochlorite and peracetic acid products) that are compatible with dialysis machines can be used to disinfect bicarbonate concentrate distribution systems. However, some disinfectants attack biofilm better than others. Appropriate dwell times and concentrations should be used as recommended by the manufacturer of the concentrate system. If this information is not available, sodium hypochlorite solutions, such as bleach, may be used at a dilution of 1:100 and proprietary disinfectants at the concentration recommended by the manufacturer for disinfecting piping systems. In the event that precipitation or salt build-up impedes flow through a piping system, cleaning with a 1:34 solution of 5 % acetic acid (e.g. distilled white vinegar) is recommended. Some manufacturers supply bicarbonate concentrate systems with UV irradiation or ozone systems for bacterial control.

UV irradiation devices that are used to control bacterial proliferation in the pipes of bicarbonate concentrate distribution systems should be fitted with a low-pressure mercury lamp that emits light at a wavelength of 254 nm and provides a dose of radiant energy of 30 mW·s/cm^2. The device should be sized for the maximum anticipated flow rate according to the manufacturer's instructions and be equipped with an online monitor of radiant energy output that activates a visual alarm indicating that the lamp should be replaced. Alternatively, the lamp should be replaced on a predetermined schedule according to the manufacturer's instructions to maintain the recommended radiant energy output. It is recommended that UV irradiators be followed by an endotoxin-retentive filter. Disinfection of the bicarbonate concentrate distribution system should continue to be performed routinely.

When used to disinfect the pipes of a bicarbonate concentrate distribution system, an ozone generator should be capable of delivering ozone at the concentration and for the exposure time specified by the manufacturer. When ozone disinfection systems are used, ambient air should be monitored for ozone according to national standards and regulations.

When heat is used to disinfect the bicarbonate distribution system, the time and temperatures should be validated by the manufacturer.

Overagitation or mixing of bicarbonate concentrate can result in loss of CO_2 from the solution. Loss of CO_2 results in an increase in pH and favours the formation of carbonate that can lead to precipitation of calcium carbonate in the fluid pathways of the dialysis machine following dialysis fluid proportioning.

B.5.3.3 Concentrate outlets

For piped concentrate distribution systems, each treatment station is equipped with a concentrate outlet for bicarbonate, one or more outlets for acid concentrate, and a dialysis water outlet for connection to the inlet line of the dialysis machine (optional). To prevent mix-ups with delivery of two or more types of acid concentrate, each concentrate should have its own outlet. Concentrate outlets should be compatible with the dialysis machine and have a means of minimizing the risk that the wrong concentrate will be connected to an outlet. The dispensing outlets should be labelled with the appropriate symbol (see Table B.2) indicating the proportioning ratio for the dialysis machine, if required, and should be colour-coded blue for bicarbonate and red for acid.

B.6 Dialysis fluid proportioning

Historically, dialysis fluid was buffered with acetate. For acetate buffered dialysis fluid, dialysis water is mixed with an acetate containing concentrate to produce the dialysis fluid. In such a system, the pH can vary depending on the supply water. Although a single concentrate is used to prepare acetate dialysis fluid, consideration should be given to checking both conductivity and pH because mix-ups involving acid concentrate and other chemicals can result in an acceptable conductivity with an incorrect pH.

One of the functions of the dialysis fluid is to correct metabolic acidosis present in patients undergoing dialysis treatment. With acetate buffered dialysis fluid, the acetate is converted by the body to bicarbonate, acetate intolerance can be present and is characterized by vasodilation and smooth muscle relaxation leading to hypotension. Current haemodialysis treatments utilize proportionating or mixing technology which uses two separate concentrates mixed with dialysis water; an acid concentrate, and a bicarbonate concentrate. It is important that the acid and bicarbonate concentrates are matched with respect to the proportioning ratio and with the model and setup configuration of the dialysis machine.

Several types of three-stream concentrates are available, with different ratios of acid concentrate to bicarbonate concentrate to dialysis water (see Table B.2). The different proportioning types are not compatible with one another. Generally, bicarbonate is available in one or two forms for each proportioning type (in liquid, cartridge, or dry powder, and in various sizes). Each proportioning type has numerous acid concentrate formulations (“codes”) with different amounts of potassium, calcium, and magnesium ions, plus glucose. To help differentiate between concentrates of different proportioning types, ISO 23500-4 recommends that the manufacturer include a geometric symbol on the labels, along with acid/base colour coding.

Table B.2 — Symbols and colour coding for different concentrate proportioning ratios

Concentrate type	**Acid proportioning ratio**[a] (red colour coding)	**Geometric symbol**	**Bicarbonate concentrate** (blue colour coding)	**Comments**
35X	1+34[a]	Square	Dry, liquid, or cartridge	
36,83X	1+35,83[a]	Circle	Dry or liquid	Bicarbonate concentrate contains some NaCl.
45X	1+44[a]	Triangle	Dry, liquid, or cartridge	
36,1X	1+35,1[a]	Diamond	Cartridge	Powder cartridges may be used for other proportioning ratios, except for 36,83X, in which the bicarbonate concentrate also contains NaCl.

NOTE Acetate-containing concentrate is colour-coded white.

[a] Acid+bicarbonate + water.

Different manufacturers of dialysis machines use different methods of controlling the proportions of the concentrates. Such control may be: “fixed proportioning” or “servo-control”. With both methods, the operator can select a desired sodium and bicarbonate level, or conductivities corresponding to defined sodium and bicarbonate levels, and the machine will make the necessary adjustments to achieve the selected levels. Both types use a redundant system of controls and surveillance. With fixed proportioning systems, the pumps are set to established volumes, and the final conductivity is verified. With servo-control machines, the individual concentrates are added until the conductivity achieves the expected value. A final redundant conductivity monitor monitors the conductivity. Some machines also monitor the pH of the dialysis fluid as an additional safeguard against gross errors in dialysis fluid formulation. A different type of machine with a batch tank and dedicated concentrates is also available.

Depending on the type of acidified concentrate in use, the acid component may be in the form of sodium acetate, sodium di-acetate or citric acid. Acetate is metabolized to bicarbonate in a 1:1 ratio, whilst citric acid generates bicarbonate in a 3:1 molar ratio.

In selecting the dialysis fluid bicarbonate, the physician should consider all sources of buffer delivered to the patient during the dialysis treatment, including the bicarbonate in the bicarbonate concentrate, the acetate, citrate or lactate in the acid concentrate which, when metabolized to form bicarbonate. In selecting the bicarbonate prescription, the physician should consider the patient’s nutritional status, assessed by history, physical examination, anthropometrics, serum albumin and protein nitrogen appearance, since individuals whose metabolism results in a small acid load are at higher risk of developing metabolic alkalosis following treatment. Decisions regarding the bicarbonate prescription should also take into account changes in serum potassium, magnesium and calcium concentrations during dialysis, and the presence and severity of heart disease.

Some models of dialysis machines use a fixed proportionating ratio, whilst others may be set up or calibrated for use with concentrates of more than one proportioning ratio. (Note that changing from one proportioning ratio to another requires recalibration for some models of dialysis machines.) Thus, for such machines, the type of concentrate should be labelled on the machine or clearly indicated by the machine display. It is strongly recommended that facilities configure every machine to use only one type of concentrate.

Injuries related to incorrect dialysis fluid composition are rare, but they can and do happen when all procedures are not followed. Frequently, when the error occurs, several patients have been exposed before the facility recognizes the mistake. For example, because one of the concentrates is acidic and the other is basic, connecting the wrong concentrates to the machine could result in dialysis fluid that could harm the patient. Thus, it is necessary for the operator to follow the manufacturer's instructions regarding dialysis fluid conductivity, including measuring the approximate pH with an independent method before starting the treatment of the next patient, if recommended by the dialysis machine manufacturer. More recently, systems have been developed that use three concentrates (bicarbonate, sodium chloride, and an acid concentrate containing the remaining electrolytes) to allow more sophisticated variation of the dialysis fluid composition during dialysis.

B.7 Central dialysis fluid storage and delivery systems

B.7.1 General

Dialysis fluid may be prepared centrally and distributed to individual dialysis consoles at the treatment stations using a central dialysis fluid delivery system (CDDS). Central dialysis fluid delivery systems incorporate many of the features found in dialysis water storage and distribution systems (see B.3) and concentrate preparation systems (see B.4) and most of the recommendations in those clauses are applicable to central dialysis fluid delivery systems; however, there are additional factors to be considered.

B.7.2 Design and maintenance

Central dialysis fluid delivery systems are usually designed as single-pass systems, although a distribution loop can also be used.

If a distribution loop is used, it is necessary to pay attention to preventing calcium carbonate precipitation and an increase in pH resulting from loss of CO_2 and an increase in temperature as the dialysis fluid is circulated.

Central dialysis fluid delivery systems should be disinfected daily to limit biofilm formation using a chemical disinfectant or hot water. Such disinfection should include the tubing connection to the individual dialysis console.

Microbiological surveillance methods for central dialysis fluid delivery systems should be similar to that described in 8.3. Surveillance should include the individual dialysis consoles located at each treatment station, as well as the dialysis fluid distribution system. Sampling should include samples collected from the inlet to dialysis fluid proportioning system and the inlet to individual dialysis consoles. The frequency of surveillance should meet applicable local recommendations; if no such recommendations exist the following are suggested.

a) Water system: The number of samples and positions of sampling should be based on the complexity and size of the water system. The frequency will depend on the analysis of the data collected during the validation and revalidation activities. Monthly surveillance is most frequently adopted but less frequent surveillance may be possible based on data collected during the validation and revalidation.

b) Dialysis fluid/haemodialysis machines without a validated bacteria- and endotoxin-retentive filter: Machines should be sampled on a regular basis to provide affirmation of the effectiveness of the disinfection process. The schedule of sampling will depend on the type of disinfection process being used. Each machine should be sampled at least once per year and different machines should be sampled on each occasion. Monthly surveillance is most frequently adopted.

c) It is not necessary to take samples of ultrapure dialysis fluid or substitution fluids if their production paths are fitted with bacteria- and endotoxin-retentive filters validated by the manufacturer and operated and monitored according to the manufacturer's instructions. It could be necessary to sample the dialysis fluid entering such bacteria- and endotoxin-retentive filters depending on the manufacturer's instructions for use of the filters; for example, when the instructions for use specify the quality of the fluid entering the filter. (See also Annexes D and E of this document)

The results of testing should be subjected to trend analysis. When results exceed the action levels, or in the case of a patient's pyrogenic reaction or suspected bacteremia/fungemia, an investigation and follow-up should be initiated. This investigation could include additional sampling and extra disinfection procedures carried out as per the manufacturer's recommendations.

B.7.3 Dialysis fluid storage

Central dialysis fluid delivery systems usually include a dialysis fluid storage tank. The tank should be designed to drain completely; for example, it should have a sloping bottom and a drain at the lowest point, and be ventilated through a hydrophobic 0,45 µm air filter.

B.7.4 Materials compatibility

All components used in dialysis fluid storage and delivery systems (including storage tanks, pumps, valves and piping) should be fabricated from materials (e.g. plastics or appropriate stainless steel) that do not interact chemically or physically with the dialysis fluid to affect its purity, or with the germicides or germicidal procedure used to disinfect the system. The use of materials that are known to cause toxicity in haemodialysis, such as copper, brass, zinc, galvanized material, lead, and aluminium, is specifically prohibited.

Annex C
(informative)

Surveillance guidelines for water treatment equipment, distribution systems, and dialysis fluid

C.1 Surveillance systems

Table C.1 provides guidelines on surveillance systems used for preparing and distributing dialysis fluid. The recommendations given in Table C.1 can be used as a starting point for developing a quality management programme for dialysis fluid when the manufacturer or supplier of the system does not provide adequate instructions. Not every item listed in Table C.1 will be required in all dialysis facilities and the frequency of surveillance may differ depending on the nature of the water supplied to the dialysis facility; for example, whether or not the water supply is disinfected using chloramine. The actual quality management programme for a given facility will depend on the components used in that facility's water treatment system, the purposes for which the fluids are to be used, the results of validation procedures, and any applicable local regulations.

NOTE Refer to footnotes [a] and [b] for an explanation of the use of Xs in the "Typical range of values" column.

Table C.1 — Suggested framework for surveillance water treatment equipment, distribution systems, and dialysis fluid

Item to monitor	What to monitor	Typical range of values	Typical interval	Comments
Sediment filter	Pressure drop across the filter (see 7.3.2)	Pressure drop less than XXXX	Daily	NA
Sediment filter backwashing cycle	Backwash cycle timer setting (see 7.3.2)	Backwash clock set to XX:XX	Daily	NA
Cartridge filter	Pressure drop across the filter (see 7.3.3)	Pressure drop less than XXXX	Daily	NA
Water softener	Residual hardness of product water (see 7.3.4)	Hardness as specified by the manufacturer of the reverse osmosis equipment.	Daily	NA
Water softener brine tank	Level of undissolved salt in tank (see 7.3.4)	Salt level at XXX	Daily	NA
Water softener regeneration cycle	Regeneration cycle timer setting (see 7.3.4)	Regeneration cycle timer set to XX:XX	Daily	NA

Table C.1 *(continued)*

Item to monitor	What to monitor	Typical range of values	Typical interval	Comments
Carbon beds	Product-water total chlorine between the beds (see 7.3.5)	≤0,1 mg/l of total chlorine	Daily	Prior to each patient shift if chloramine is present in the feed water at 1 mg/l or more (see 7.3.5 for exceptions to these typical intervals). (Note that use of an online monitor can provide continuous surveillance and avoid the need for offline surveillance.)
Chemical injection system	Level of chemical in the reservoir, injector function, value of the controlling parameter (e.g. pH) (see 7.3.6)	Chemical level in reservoir ≥ XXX; controlling parameter in range XX to XX	Daily (continuous surveillance is preferable)	NA
Reverse osmosis	Product water conductivity, total dissolved solids (TDS), or resistivity and calculated rejection (see 7.3.7)	Rejection ≥ XX % Conductivity < XX µS/cm	Daily (continuous surveillance is preferable)	NA
Reverse osmosis	Product and reject flow rates, and calculated recovery (see 7.3.7)	Product water flow rate ≥ X,X l/min XX % < recovery < XX %	Daily (continuous surveillance is preferable)	NA
Deionizers	Product water resistivity or conductivity (see 7.3.8)	Resistivity ≥ 1 MΩ·cm Conductivity ≤ 1 µS/cm	Continuous surveillance	NA
Endotoxin-retentive filters	Pressure drop across the filter at a fixed flow rate or product-water flow rate at a fixed pressure drop (see 7.3.9)	Pressure drop less than XXXX or flow rate greater than XXX	Daily	NA
Water system chemical contaminants	Chemical contaminants as listed in Tables 1 and 2 of ISO 23500-3	Maximums as listed in Tables 1 and 2 The parameters to be monitored should be defined by the validation process on the basis of the expected contaminants.	Yearly	These recommendations apply to dialysis water. However, chemical analysis of source water (or analysis results from the water supplier) is necessary to evaluate the overall performance of the water treatment system.

Table C.1 *(continued)*

Item to monitor	What to monitor	Typical range of values	Typical interval	Comments
Dialysis water storage tanks	Bacterial growth and endotoxins (see Clause 8)	Total viable microbial count < action level (typically 50 CFU/ml); (see 4.2.4) Endotoxin < action level (typically 0,125 EU/ml); (see 4.2.4)	Monthly, or as defined by the results of the validation process for storage tanks supplying a central dialysis fluid delivery system	Specific testing at this location is performed to troubleshoot contamination of the distribution system for tanks connected to a water distribution piping system until a pattern of consistent conformity with limits can be demonstrated.
Water distribution piping system	Bacterial growth and endotoxins (see 7.4)	Total viable microbial count < action level (typically 50 CFU/ml); (see 4.2.4) Endotoxin < action level (typically 0,125 EU/ml); (see 4.2.4)	Monthly, or as defined by the validation process results	NA
UV irradiators	Energy output and/or the lamp life span (see 7.4.3.1)	Light output > XXX Lamp life span < XXXX	Monthly	NA
Ozone generators	Concentration in the water and contact time (see 7.4.3.2)	Ozone concentration > XXX Contact time > XXX Residual ozone after disinfection < X,XX mg/l	During each disinfection	NA
Hot water disinfection systems	Temperature and time of exposure of the system to hot water (see 7.4.3.3)	Temperature not less than XX °C; minimum exposure time at temperature ≥ XX min	During each disinfection	This information might be available from the data logs of automated systems.
Chemical disinfection systems	Concentration of germicide in water and contact time	Germicide concentration > X,X mg/l; residual germicide concentration < X,XX mg/l after rinsing	During each disinfection	NA
Dialysis fluid	Conductivity, pH, electrolyte concentrations	XX,X mS/cm < conductivity < XX,X mS/cm pH in the range 6,9 to 8,0 for dialysis fluid containing bicarbonate, or as otherwise specified by the manufacturer	In accordance with local regulations or as specified by the manufacturer of the dialysis fluid delivery system (continuous surveillance for proportioning systems)	pH surveillance is necessary only if recommended by the manufacturer of the dialysis fluid delivery system.

Table C.1 *(continued)*

Item to monitor	What to monitor	Typical range of values	Typical interval	Comments
Standard dialysis fluid	Bacterial growth and endotoxin concentration in standard dialysis fluid (see 4.4.2)	Total viable microbial count < action level (typically 50 CFU/ml); (see 4.4.2) Endotoxin < action level (typically 0,25 EU/ml); (see 4.4.2)	Monthly, rotated among machines so that each machine is tested at least once per year and different machines are sampled on each occasion	The sample should be collected at worst-case time (for example, Monday morning) if possible.
Ultrapure dialysis fluid	Bacterial growth and endotoxins in the ultrapure dialysis fluid as it enters the dialyser (see 4.4.3)	Total viable microbial count < 0,1 CFU/ml; endotoxin < 0,03 EU/ml (see 4.4.3) (see 8.3.1 and Annex E)	see footnote b	NA
Substitution fluid	Bacterial growth and endotoxins in the ultrapure dialysis fluid as it enters the dialyser (see 8.3.1 and Annex E)	Sterile and non-pyrogenic (see 8.3.1 and Annex E)	see footnote b	NA

[a] It is not possible to specify universally acceptable operating ranges for each device listed in the table since some of the specifications will be system-specific. In those cases, the facility should define an acceptable operating range based on the manufacturer's instructions or measurements of system performance.

[b] The actual interval for surveillance, testing, cleaning, and/or disinfection should be based on the results of the validation process and on going trend analysis (see Clause 6, 7.2.3, and 8.2.3).

C.2 Cleaning/disinfection strategies

Cleaning/disinfection strategies for dialysis water treatment systems, dialysis water storage and distribution systems, concentrate distribution systems, and dialysis fluid distribution systems are given in Table C.2.

Prior to referring to the “Typical interval” column. Consult the manufacturer's instructions for more detail

Table C.2 — Summary of cleaning/disinfection strategies for dialysis water treatment systems, dialysis water storage and distribution systems, concentrate distribution systems, and dialysis fluid distribution systems

Item for cleaning/ disinfection	Element(s) to be cleaned/disinfected	Cleaning/ disinfection	Typical interval	Comments
Reverse osmosis	The membrane module should be disinfected, paying particular attention to the product side (See 8.2, 8.2.1)	Disinfection	Monthly, or according to manufacturer's instructions (See D.1)	The product side of the membrane is considered to be a part of the dialysis water distribution system. It should be disinfected at an interval sufficient to routinely produce dialysis water meeting the quality requirements of Clause 4. (See D.1, 3rd paragraph.) If needed, the feed side of the membrane should be cleaned periodically to remove foulants that can degrade membrane performance.
Water storage tanks	Tanks and pipes (See 8.2.3.2)	Disinfection	Monthly, or according to manufacturer's instructions	More frequent disinfection might be necessary if indicated by microbiological testing results.
Water distribution piping system	Piping system (See 8.2.3.2, D.1)	Disinfection	Monthly, or according to manufacturer's instructions	More frequent disinfection might be necessary if indicated by microbiological testing results.
UV irradiators	Quartz sleeve (See 7.4.3.1)	Periodic cleaning (See 7.4.3.1)		
Concentrate mixing systems	Tanks and piping (See 8.2.2.3)	Cleaning and/or disinfection		Disinfection is usually not needed for acid concentrate mixing systems.
Concentrate distribution systems (bicarbonate)	Tanks and piping (See 8.2.3.3, B.5.3.2)	Disinfection	Weekly (See D.1, last paragraph)	If using sodium hypochlorite for disinfection, a concentration of 0,5 % to 1 % is recommended. If cleaning with acetic acid, a concentration of approximately 0,15 % acetic acid is recommended. (See B.5.3.2, 4th paragraph.) Disinfection is usually not needed for acid concentrate distribution systems.
Dialysis machine	System (See 8.2)	Disinfection	According to manufacturer's instructions	By its own disinfection circuit and programme. (See 8.2, 3rd paragraph.)
Central dialysis fluid delivery system, CDDS	Dialysis fluid delivery system Individual dialysis console (See B.7.2)	Disinfection	Daily (See B.7.2)	Use chemical disinfectant or hot water. (See B.7.2, 3rd paragraph.)
NOTE The actual interval for cleaning and/or disinfection should be based on the results of the validation process and ongoing trend analysis (see Clause 6 and 8.2.3).				

Annex D
(informative)

Strategies for microbiological control

D.1 General

The strategy for controlling the proliferation of microorganisms in haemodialysis systems primarily involves proper system design and operation, and regular disinfection of water treatment system and haemodialysis machines. A key concept in ensuring conformity with the requirements of 4.2.4 and 4.4 is that disinfection schedules should be designed to prevent bacterial proliferation, rather than being designed to eliminate bacteria once they have proliferated to an unacceptable level. With this strategy, surveillance levels of bacteria and endotoxins serves to demonstrate that the disinfection programme is effective, not to indicate when disinfection should be performed. Gram-negative water bacteria, their associated lipopolysaccharides (bacterial endotoxins), and nontuberculous mycobacteria (NTM) most frequently come from the community water supply, and levels of those bacteria can be amplified depending on the water treatment system, dialysis fluid distribution system, type of dialysis machine, and method of disinfection.

All components of dialysis water treatment and distribution systems, and dialysis fluid preparation and distribution systems, can serve as reservoirs of microbial contamination. Dialysis water distribution systems frequently use pipes that are of larger diameter and longer than are needed to handle the required flow. Oversized piping increases both the total fluid volume and the wetted surface area of the system. Gram-negative bacteria in fluids remaining in pipes overnight multiply rapidly and colonize the wet surfaces, thus producing bacterial populations and endotoxin quantities in proportion to the volume and surface area. Such colonization results in the formation of protective biofilm that is difficult to remove once formed and that provides a barrier between the bacteria and germicide during disinfection.

Biofilm is a community of microorganisms consisting of cells that are irreversibly attached to a surface or interface or to each other[88]. Biofilms can occur at solid-liquid, solid-air, and liquid-air interfaces. Most microorganisms can form biofilms and more than 99 % of all microorganisms live in such aggregates. A feature of all biofilms is that the organisms are embedded in a matrix of microbial origin, consisting of extracellular polymeric substances (EPS). The EPS comprises mainly polysaccharides and proteins, which form hydrogel matrices[89]. The structure of biofilm, and the physiological attributes of biofilm organisms, confer an inherent resistance to antimicrobial agents, whether those agents are antibiotics, disinfectants or germicides.

Mechanisms responsible for resistance can include

- delayed penetration of the antimicrobial agent through the biofilm matrix,
- altered growth rate of biofilm organisms, and
- other physiological changes related to the mode of growth of the biofilm.

A certain amount of biofilm formation is considered unavoidable in dialysis water systems. When the level of biofilm is such that the action levels for microorganisms and endotoxins in the dialysis water cannot be routinely achieved, the operation of the system is compromised from a medical and technical point of view. This level of biofilm formation is often referred to as bio-fouling. The key to avoiding bio-fouling is to minimize biofilm development. The extent of biofilm growth is dependent on the availability of nutrients. Classic biocidal approaches usually do not limit nutrient availability. In fact, some biocides increase nutrient availability by oxidizing recalcitrant organics and making them more bioavailable[90].

Routine disinfection should be performed to control bacterial contamination of distribution systems. The frequency of disinfection will vary with the design of the system and the extent to which biofilm has already formed in existing systems. Sodium hypochlorite and ozone are generally the most effective agents against biofilm, and their use might be more efficacious if the pipes are treated first with a descaling agent. However, in some cases, complete or partial replacement of a distribution system might be the only way to re-establish control over mature biofilm.

It is commonly believed that maintaining flow through piping systems at all times minimizes biofilm formation. However microbial growth and biofilm formation in hydraulic systems cannot be controlled by the fluid velocity[91]. Data from the semiconductor industry show that a Reynolds number of 3 000 in a piping system is insufficient to prevent bacterial contamination in the water as biofilm was found on the internal surfaces of pipes[92]. [A Reynolds number of approximately 3 000 is obtained with a flow velocity of 0,15 m/s in a 2-cm-diameter pipe (0,5 ft/s in a 3/4" diameter pipe).]

Even if it were possible to specify a minimum flow velocity that was effective in reducing biofilm formation and bacterial contamination, use of such a minimum flow velocity would not provide a substitute for regular disinfection of the distribution system. Other measures can also help protect pipes from contamination. A mechanism should be incorporated in a distribution system to ensure that disinfectant does not drain from pipes during the disinfection period. Dead-end pipes and unused branches and taps that can trap fluid should be eliminated because they act as reservoirs of bacteria and are capable of continuously inoculating the entire volume of the system. Joints between sections of piping and between piping and fittings should be formed in a manner that minimizes the formation of crevices and other voids that might serve as sites for bacterial colonization. Pipes should not be cut with a hacksaw. Any burrs should be removed before the joint is formed. These measures also minimize the possibility that pockets of residual disinfectant could remain in the piping system after disinfection.

A storage tank in the dialysis water or dialysis fluid distribution system greatly increases the volume of fluid and surface area available and can serve as a niche for water bacteria. Storage tanks are therefore not recommended for use in dialysis water or dialysis fluid distribution systems unless they are frequently drained and adequately disinfected. It could be necessary for the user to scrub the sides of the tank to remove bacterial biofilm if the tank design and maintenance are not adequate to prevent bacterial proliferation. A bacteria- and endotoxin-retentive filter, distal to the storage tank, or some other form of bacterial control device, is recommended.

For most haemodialysis machines, routine disinfection with hot water or with a chemical germicide connected to a disinfection port on the machine does not disinfect the line between the outlet from the dialysis water distribution system and the back of the dialysis machine. Users should establish a procedure for regular disinfection of this line. One approach is to rinse the haemodialysis machines with water containing germicide or hot water when the dialysis water distribution loop is disinfected. If this procedure is used with a chemical germicide, each haemodialysis machine should be rinsed and tested for the absence of residual germicide following disinfection.

Storage times for bicarbonate concentrate should be minimized (normally less than 24 h), as well as the mixing of fresh bicarbonate concentrate with unused portions of concentrate from a previous batch. The manufacturer's instructions should be followed if they are available. Facilities that reuse concentrate containers for bicarbonate concentrate should disinfect the containers at least weekly. Bicarbonate concentrate can support prolific growth of microorganisms. Containers and pick up tubes can be disinfected with household sodium hypochlorite solutions (300 mg/l to 600 mg/l free chlorine), with a contact time of about 30 min or according to another nationally approved standard or regulation, or according to the manufacturer's instructions.

The containers and pick-up tubes should be disinfected at least weekly or at a frequency required by local regulatory requirements. Following disinfection, the bicarbonate concentrate containers and concentrate pick up tubes should be rinsed with treated water, allowed to air dry and stored inverted at the end of each treatment day.

D.2 Microbial surveillance methods

D.2.1 General

The microbial quality of dialysis water is surveilled regularly to validate the effectiveness of the disinfection programme. The frequency of surveillance should be determined during the process of system validation. In the absence of a formal determination of frequency, surveillance is usually performed monthly. Surveillance can be accomplished by direct plate counts, in conjunction with the measurement of endotoxins.

Samples of dialysis water are collected from several places to give an indication of the microbial quality of the water throughout the dialysis water distribution system. For routine surveillance, samples should be collected from the last outlet of the dialysis water distribution loop, where dialysis water enters equipment used to reprocess dialysers, and where dialysis water enters equipment used to prepare bicarbonate concentrate or from the bicarbonate concentrate mixing tank. Additional testing, such as at the end of the water treatment cascade and at the outlet of the storage tank, if one is used, can be necessary during qualification of a newly installed system or when troubleshooting the cause of contamination within the dialysis water distribution loop. For central dialysis fluid distribution systems, samples should be collected from the last outlet of the dialysis fluid distribution loop.

For dialysis machines that are not fitted with validated endotoxin-retentive filters, dialysis fluid samples should be collected from enough machines so that each machine is tested at least once per year. For dialysis machines fitted with validated endotoxin-retentive filters, samples should be collected according to the filter manufacturer's instructions. If testing of any haemodialysis machine reveals a level of contamination above the action level, an investigation should be conducted. The investigation should be based on the presumption that other haemodialysis machines might also be contaminated. It should include a review of conformity with disinfection and sampling procedures and an assessment of microbiological data for the previous three months to look for trends. The offending machine should be re-tested and an additional sample of machines tested to determine if the contamination was limited to a single machine or more widespread. The person in charge should also be notified.

Cultures should be repeated when bacterial counts exceed the allowable levels. If culture growth exceeds permissible standards, samples from the dialysis water distribution system or dialysis fluid distribution system and haemodialysis machines should be cultured weekly until acceptable results are obtained. Additional samples should be collected when there is a clinical indication of a pyrogenic reaction or septicaemia, and following a specific request by the clinician or the infection control practitioner.

Samples are always collected before sanitization/disinfection or no sooner than 24 h after disinfection. For systems disinfected daily, samples should be collected before, and as close as practicable to, the next disinfection. Samples from haemodialysis machines should always be collected before disinfection. Culture dialysis water and dialysis fluid weekly for new systems until a pattern has been established. For established systems, culture monthly unless a greater frequency is dictated by historical data at a given institution. If bio-fouling is suspected, for example due to erratic microbiological test results, it could be necessary to check for the presence of biofilm (see D.2.3).

D.2.2 Sample collection

Samples are collected directly from sampling ports situated in different parts of the dialysis water or dialysis fluid distribution system. In general, the sampling ports should be opened and the dialysis water or dialysis fluid should be allowed to run for at least 60 s unless the sampling port manufacturer instructions for use state otherwise, before a sample is collected in a sterile, endotoxin-free container. Containers validated for collection of endotoxin samples should be used to collect samples. The sample volume collected should be 5 ml to 1 000 ml depending upon the test to be run and/or as specified by the laboratory performing the test. Sampling ports should be disinfected using a cotton swab or sterile gauze wetted with alcohol, or as recommended by the port manufacturer. The sample should be collected only when no disinfectant residual is present.

Dialysis fluid samples should be collected from a sampling port in the dialysis fluid inlet line to the dialyser, or from the dialysis fluid outlet port of the dialyser, or from a sampling port in the dialysis fluid outlet line of the dialyser. In some newer haemodialysis machines, dialysis fluid flow stops when the dialysis fluid lines are disconnected from the port. In these instances, the machines are equipped with dialysis fluid sampling ports that can be accessed using a syringe. Sampling ports may be disinfected with alcohol and allowed to air dry. A sterile syringe should be used to aspirate at least 10 ml of dialysis fluid out of the sampling port and be discarded. A new appropriately sized sterile syringe should be attached and used to draw the sample. The sample volume collected should be 5 ml to 1 000 ml depending upon the test to be run and/or as specified by the laboratory performing the test.

Containers used for samples to be cultured should be sterile and endotoxin free.

D.2.3 Heterotrophic plate count

Samples should be analysed as soon as possible after collection to avoid unpredictable changes in the microbial population. If samples cannot be analysed within 4 h of collection, they should be stored at <10 °C without freezing and during transit to the laboratory. Sample storage for more than 24 h should be avoided.

The reference method for culturing is the membrane filtration technique. With this method, a known volume of sample or diluted sample is filtered through a 0,45 µm membrane filter and the membrane filter is aseptically transferred to the surface of an agar plate. The spread-plate technique may also be used. With this method, an inoculum of at least 0,1 ml of sample is spread equally over the surface of the agar plate. Use of a calibrated loop to apply the sample to the agar plate is not permitted. The pour-plate technique may also be used. A sample volume of 0,1 ml to 0,3 ml is usually used with this method. Dip samplers should not be used. The culture medium used should be selected based on the type of fluid to be analysed e.g. standard dialysis fluid, water used in the preparation of standard dialysis fluid, ultrapure dialysis fluid, water used for the preparation of ultrapure dialysis fluid or fluid used for online therapies such as haemodiafiltration. Blood and chocolate agars should not be used.

Validated media selections, incubation times and temperatures are specified in ISO 23500-3, ISO 23500-4 and ISO 23500-5. During incubation, the plates can be sealed or kept in a plastic bag to avoid desiccation of the agar if that is a concern, e.g. for methods requiring 7-day incubation. Colonies should be counted using a magnifying device. If a more accurate count from plates containing fewer than 30 colonies or more than 300 colonies is desired, larger or smaller volumes may be cultured. Smaller volumes can be obtained by making 1:10 serial dilutions in sterile phosphate buffer. If larger volumes are required, the membrane filtration method should generally be used.

It is virtually impossible to keep a dialysis water or dialysis fluid distribution system sterile; microorganisms will always be present on surfaces waiting for nutrients which, in the case of lithotrophic organisms, can even be inorganic[93]. Heterotrophic plate counts do not provide a good measure of the presence of biofilm. Fluid samples give no information about the site, extent, or composition of a biofilm. Although biofilms contaminate the fluid in a distribution system, they do so only very irregularly. Erratic colony counts might indicate the presence of bio-fouling since clusters of cells might be sloughed from the biofilm with release of bacteria into flowing fluid. Currently, few practical methods are available for the routine detection of biofilm. Conventional methods rely on sampling-defined surface areas or on exposure of test surfaces (coupons) with subsequent analysis in the laboratory. A classic example is the so-called “Robbins device”, which consists of plugs inserted flush with pipe walls, thereby experiencing the same shear stress as the wall itself[94]. After given periods of time, they are removed and analysed in the laboratory for all biofilm-relevant parameters. The disadvantage of such systems is the time-lag between analysis and result. Jacobs et al. (1996)[95] described a simple spectrophotometric surveillance method, using a nucleotide-specific fluorescent stain (4′,6-diamidino-2-phenylindole), and automated measurement. Other methods which report biofilm growth online, non-destructively and in real time have been invented. They are all based on physical methods. If careful attention is paid to routine disinfection, routine surveillance for biofilm is not necessary. However, when the level of biofilm leads to a bio-fouling situation, it might be necessary to determine the level of biofilm in the system using the methods currently available.

D.2.4 Endotoxin test

Endotoxin testing is performed using the Limulus amoebocyte lysate (LAL) assay. A variety of different assay methods are available and a number of new methods are in development. Existing available methods include, gel-clot, which is semi-quantitative, kinetic which are chromogenic, turbidimetric, or end point.

The gel-clot LAL assay is not as sensitive as the kinetic assay and provides only a positive or negative result; that is, it shows if endotoxins are present, or not, at a particular concentration. Single-tube gel-clot tubes are available from several commercial sources, and kits with the typical following sensitivities available: 0,015 EU, 0,03 EU, 0,06 EU, 0,125 EU, 0,25 EU, and 0,5 EU. At a minimum, two tubes should be run each time the assay is performed. The first tube contains LAL reagent and the sample to be tested. The second tube contains LAL reagent, a known amount of endotoxins, and the sample to be tested. The second tube acts as a positive control to confirm the absence of any interference that might lead to a false-negative result. Positive control tubes are available from the suppliers of commercial LAL assays.

The kinetic LAL assay uses control standard endotoxins to generate a standard curve to which unknowns are compared and concentrations are determined using linear regression. The kinetic assays employed in laboratories generally use a computer-driven spectrophotometer that automatically calculates the amount of endotoxins on the basis of colour development, turbidimetric readings, or onset times for gel formation.

Apart from the LAL test a number of assays with varying specificities and sensitivities are available to quantify and define biologically active substances of microbial origin (e.g. silkworm larvae, mononuclear cell cytokine assay, and 1,3-β D-glucans).

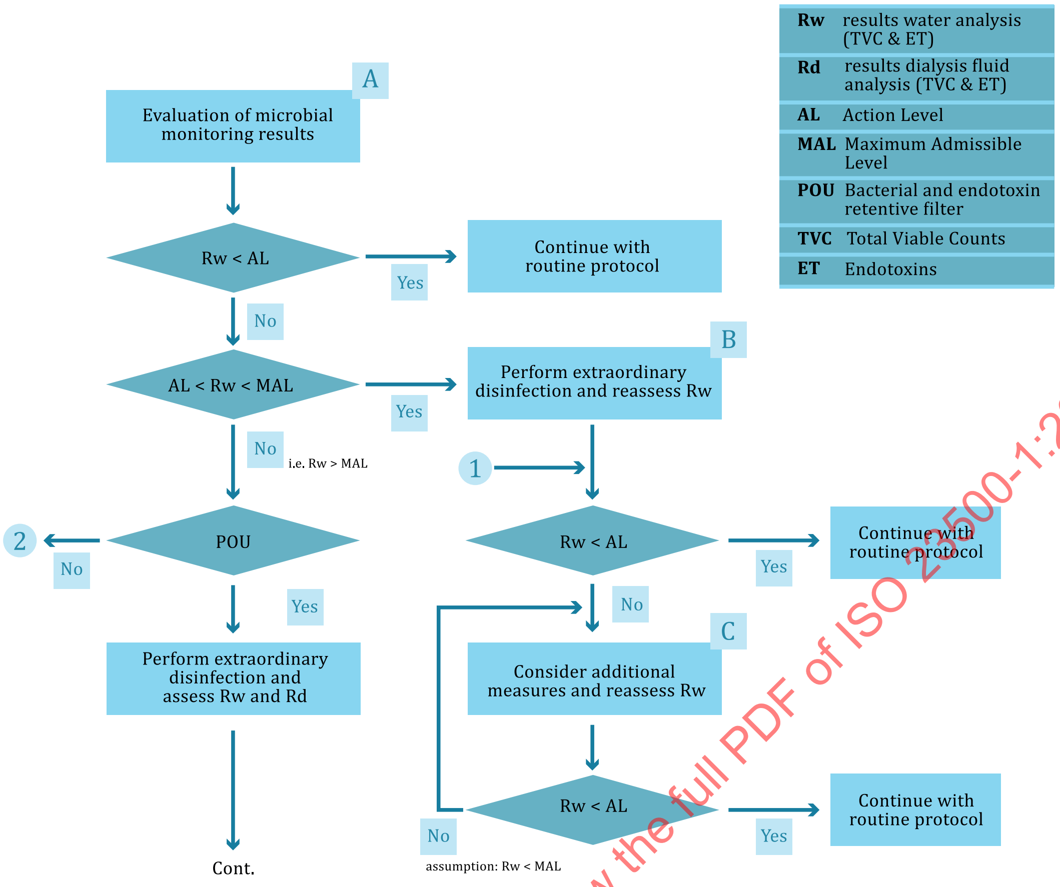
D.3 Interpreting the results of microbial surveillance

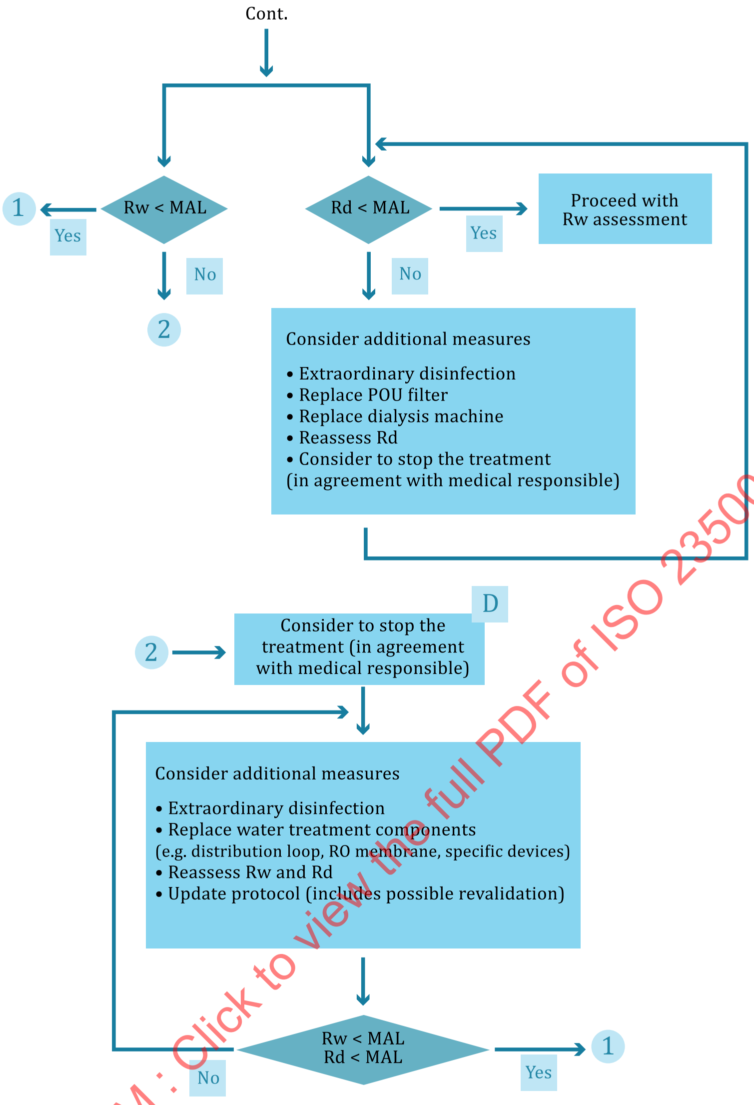
Microbial surveillance or culture results are dependent upon three basic parameters: culture medium, culture temperature, and culture duration. Recommended methods and cultivation conditions can be found in ISO 23500-3, ISO 23500-4 and ISO 23500-5. Accurate microbiological surveillance is important in establishing the microbial content of the water and dialysis fluid. Culture results obtained using the methods outlined in this document are only a relative indicator of the bioburden in dialysis water or dialysis fluid and do not provide a measure of the absolute bacterial burden.

Tryptone Glucose Extract Agar (TGEA) or Reasoner's Agar No. 2 (R2A) incubated at 17° C -23° C for a period of 7 days, or Tryptic Soy Agar incubated at 35 °C for 48 hours are all validated and acceptable methods. The user should determine which of these methodologies is appropriate for the circumstance, taking into account the advantages of each. According to the United States Pharmacopeia, "the decision to use longer incubation times, should be made after balancing the need for timely information and the type of corrective actions required when alert or action level is exceeded with the ability to recover the microorganisms of interest. The advantages gained by incubating for longer times namely recovery of injured microorganisms, slow growers, or more fastidious microorganisms, should be balanced against the need to have a timely investigation and take corrective action, as well as the ability of these microorganisms to detrimentally affect products or processes" [e.g. patient safety][9].

It should be further noted that measurements, reflect the presence of planktonic organisms in a fluid storage and distribution system. However, more than 99 % of all microorganisms in such a system live in biofilms on the surfaces of the system[96]. Thus, care should be taken in interpreting culture results since a low bacterial count might be obtained even though a system was contaminated with an established biofilm if the sample was taken at a point in time after disinfection, but before a biofilm shedding event re-established a population of planktonic organisms.

The primary approach in respect of interpretation of microbiological test results is the use of trend analysis. This enables the point at which corrective action should be taken to be identified. A suggested algorithm in respect of actions to be taken, in the event of endotoxin levels >0,25 EU/ml and a bacterial content of >100 CFU/ml is outlined in Figure D.1.


Rw results water analysis (TVC & ET)
Rd results dialysis fluid analysis (TVC & ET)
AL Action Level
MAL Maximum Admissible Level
POU Bacterial and endotoxin retentive filter
TVC Total Viable Counts
ET Endotoxins
A
Evaluation of microbial monitoring results
Rw < AL
Yes
Continue with routine protocol
No
AL < Rw < MAL
Yes
B
Perform extraordinary disinfection and reassess Rw
No
i.e. Rw > MAL
1
2
No
POU
Yes
Perform extraordinary disinfection and assess Rw and Rd
Cont.
Rw < AL
Yes
Continue with routine protocol
No
C
Consider additional measures and reassess Rw
Rw < AL
No
assumption: Rw < MAL
Yes
Continue with routine protocol



Key

A Microbial parameters assessed according to system validation results

B Extraordinary disinfection (might involve the need to change disinfection methods).
Samples shall be collected no sooner than 24 hours after disinfection

C Additional measures might involve the need to change disinfection methods, changing of components (e.g. RO membrane). Samples shall be collected no sooner than 24 hours after disinfection

D Interruption of the treatment must always be agreed with the medical user responsible on the basis of the risk analysis. Furthermore, the assessment of the dialysis fluid must be taken into account in this analysis

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Figure D.1 — Evaluation of microbial surveillance results and relevant (corrective) actions

Annex E
(informative)

Validation

E.1 General and background

This annex provides background for Clauses 6 and 8 as well as for Annex C.

The dialysis fluid and the substitution fluid used for online convective therapies are the result of an online process and are used immediately after their production. For this reason, the use of "batch control" techniques based on testing at fixed intervals is not the most effective way to ensure the required quality level is continuously reached. Periodic appraisal of the chemical and microbial fluid quality might not identify a potential problem; for example, if that problem arose just after a test sample was collected.

Furthermore, conformity with the requirement that substitution fluid used for online convective therapies be sterile cannot be demonstrated by culturing but is ensured by the application of a validated and adequately monitored process.

The surveillance plan of the overall system (i.e. including the steps from the dialysis water production to the generation of the dialysis fluid and substitution fluid) is based on the knowledge acquired with the validation plan for the specific dialysis water or dialysis fluid production system and the surveillance strategies applicable to the dialysis fluid production devices as validated by the manufacturer.

Moreover, when using a validated and monitored process for removal of bacteria and endotoxins at the dialysis machine, sampling of the substitution fluid is not needed. The presence in the system of parts and equipment (e.g. dialysis machine or endotoxin-retentive filters) that have already been validated by the manufacturer is sufficient to ensure fluid quality, provided those parts and equipment are operated in accordance with the manufacturer's instructions. Sampling of the dialysis water or dialysis fluid should be performed if required by the manufacturer of the validated process.

For these reasons, and under the above-mentioned conditions, an effective surveillance strategy should be based on the direct sampling of the dialysis water and/or dialysis fluid and on surveillance of the process parameters as recommended by the manufacturer and defined by knowledge of the water treatment or dialysis fluid preparation system acquired during the validation phase.

E.2 Validation programme

E.2.1 General

The performance of the dialysis water or dialysis fluid production system should be verified to demonstrate that the system is "fit for purpose". The validation procedure should provide documentary evidence that the process will consistently produce dialysis water, dialysis fluid, or substitution fluid meeting the quality requirements of ISO 23500-3 and/or ISO 23500-5.

E.2.2 Validation steps

As described in Clause 6, validation consists of the following:

- validation plan;
- installation and operational qualification;

- performance qualification;
- revalidation through the data collected during routine surveillance.

E.2.3 Validation plan

The validation plan is the road map for successful validation and provides a complete picture of the dialysis facility's validation activities.

The validation plan defines and lists all necessary activities and documentation for

- installation and operational qualification,
- performance qualification, and
- revalidation.

The level of detail in the plan should reflect the risk, complexity and novelty of the system. The validation plan should define all responsibilities during validation and subsequent system operation.

E.2.4 Performance qualification

As described in Clause 6, the aim of performance qualification is to establish that the system, as a whole, functions consistently to produce water and dialysis fluid of the required quality when operated in accordance with the defined procedures and with an incoming water supply of defined characteristics.

The prerequisites for performance qualification are as follows:

- a demonstration that the system has been installed in accordance with the design plans and following the manufacturer's procedures for installation (i.e. installation qualification);
- a demonstration that the system performs all the required actions and can be operated in accordance with relevant technical manuals (i.e. operational qualification).

Performance qualification includes periodic appraisal of a set of physical, chemical, and microbiological parameters to demonstrate that a consistent performance pattern can be achieved for the specific system design and performance requirements.

The sampling and testing pattern can be relaxed during the surveillance phase (normal operation) provided it can be demonstrated that the system consistently yields high quality results over an extended period and that continuously monitored parameters provide full surveillance of the system performance.

Under these assumptions, the following pattern for performance qualification can be adopted.

The first phase requires a full chemical and microbiological analysis of the dialysis water or microbiological analysis of the dialysis fluid, followed by regular microbiological analyses to demonstrate consistent quality in the interval between disinfections. During this period, all the information about the system behaviour should be collected and fine-tuning of the action levels performed.

In this phase, the testing frequency of the microbiological parameters is kept at a higher level to create a "trend analysis" and to identify any deviations to ensure the patients' safety.

The achievement of at least three consecutive results, consistently below the action level, allows the start of the second phase where the final frequency of testing of microbiological parameters and the final frequency of disinfection are implemented. Sampling should be performed prior to disinfection or, alternatively, not sooner than 24 h after the disinfection process, to avoid false-negative results and to demonstrate adequate system behaviour. Samples taken after disinfection are used to demonstrate the effectiveness of the disinfection procedure; they should be taken at least once after the first disinfection and following any significant deviations in the microbiological quality of the dialysis water or dialysis fluid.