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

Part 3: Water for haemodialysis and related therapies

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

Partie 3: Eau pour hémodialyse et thérapies apparentées

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document 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).

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This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 2, *Cardiovascular implants and extracorporeal systems*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 205, *Non-active medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 23500-3:2019), which has been technically revised.

The main changes are as follows:

— the use of WHO Drinking Water Guideline as the drinking water quality reference has replaced the previously used EPA Water quality requirements;

— thallium has been removed from the list of contaminants of other trace elements in dialysis water as no published study reports that this contaminant is of particular concern in the setting of haemodialysis;

— alternatives to classic microbial analytical methods (endotoxin testing using recombinant Factor C [rFC]) have been incorporated.

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

Assurance of adequate water quality is one of the most important aspects of ensuring a safe and effective delivery of haemodialysis, haemodiafiltration or haemofiltration.

This document contains the minimum chemical and microbiological requirements for the water to be used for preparation of dialysis fluids and concentrates, and for the reprocessing of haemodialysers and the necessary steps to ensure conformity with those requirements.

Haemodialysis and related therapies such as haemodiafiltration can expose the patient to more than 500 l of water per week across the semi-permeable membrane of the haemodialyser or haemodiafilter. Healthy individuals seldom have a weekly oral intake above 12 l. This over 40-fold increase in exposure requires control and regular surveillance of water quality to avoid excesses of known or suspected harmful substances. Since knowledge of potential injury from trace elements and contaminants of microbiological origin over long periods is still growing and techniques for treating drinking water are continuously developed, this document will evolve and be refined accordingly. The physiological effects attributable to the presence of organic contaminants in dialysis water are important areas for research, however, the effect of such contaminants on patients receiving regular dialysis treatment is largely unknown, consequently no threshold values for organic contaminants permitted in water used for the preparation of dialysis fluids, concentrates and reprocessing of haemodialysers has been specified in this document.

Within this document, current measurement techniques at the time of publication have been cited. Other standard methods can be used, provided that such methods have been appropriately validated and are comparable to the cited methods.

The final dialysis fluid is produced from concentrates or salts manufactured, packaged and labelled according to ISO 23500-4 mixed with water meeting the requirements of this document. The operation of water treatment equipment and haemodialysis systems, including ongoing surveillance of the quality of water used to prepare dialysis fluids, and handling of concentrates and salts are the responsibility of the haemodialysis facility and are addressed in ISO 23500-1. Haemodialysis professionals make choices about the various applications (haemodialysis, haemodiafiltration, haemofiltration) and should understand the risks of each and the requirements for safety for fluids used for each.

This document is directed towards manufacturers and providers of water treatment systems and also to haemodialysis facilities.

The rationale for the development of this document is given in Annex A.

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

Part 3:
Water for haemodialysis and related therapies

1 Scope

This document specifies the minimum chemical and microbiological quality requirements, for water used for preparation of dialysis fluids, concentrates, and for the reprocessing of haemodialysers, together with the necessary steps to ensure conformity with the requirements. The document also provides guidance for the ongoing monitoring of the purity of such water in terms of chemical and microbiological quality.

This document is applicable to

— water used in the preparation of dialysis fluids for haemodialysis, haemodiafiltration and haemofiltration and the reprocessing of haemodialysers, and

— water used in the preparation of concentrates.

This document does not apply to dialysis fluid regenerating systems.

The operation of water treatment equipment and the final mixing of treated water with concentrates to produce dialysis fluid are the sole responsibility of dialysis professionals.

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 dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 23500-1, *Preparation and quality management of fluids for haemodialysis and related therapies — Part 1: General requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23500-1 apply.

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

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

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

4 Requirements

4.1 Dialysis water quality requirements

The quality of the dialysis water, as specified in 4.2 and 4.3, shall be verified upon installation of a water treatment system. Regular surveillance of the dialysis water quality shall be carried out thereafter.

NOTE Throughout this document, it is assumed that the water undergoing treatment is potable water and therefore meets the appropriate regulatory requirements for such water. If the water supply is derived from an alternate source such as a privately-owned borehole or well, contaminant levels cannot be as rigorously controlled.

4.2 Chemical contaminant requirements

4.2.1 General

Dialysis water shall not contain chemicals at concentrations in excess of those listed in Tables 1 and 2. Table 1 does not include any recommendation for organic carbon, pesticides and other chemicals such as pharmaceutical products and endocrine disruptors that can be present in feed water. It is technically difficult and costly to measure such substances on a routine basis. The effect of their presence on haemodialysis patients is difficult to specify and consequences of exposure are probably of a long-term nature. 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 specify 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 majority of these chemicals. However, as granular activated carbon is widely used in the removal chlorine/chloramine, their use in the removal of 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 can be already occupied and not available for further removal activity.

NOTE 1 See Clause A.3 for an explanation of the values in Tables 1 and 2.

NOTE 2 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.

Where the dialysis water is used to reprocess haemodialysers (cleaning, testing and mixing of disinfectants), the user is cautioned that the dialysis water shall meet the requirements of this document. The dialysis water should be measured at the input to the dialyser reprocessing equipment.

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

Contaminant	Maximum concentration[b] mg/l
Contaminants with documented toxicity in haemodialysis	
Aluminium	0,01
Total chlorine[c]	0,1
Copper	0,1
Fluoride	0,2
Lead	0,005
Nitrate (as N)	2
Sulfate	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 physician in charge of dialysis has the ultimate responsibility for ensuring the quality of water used for dialysis.

[b] The reader is cautioned to refer to the latest edition of this document to ensure that there have been no changes to this table.

[b] Unless otherwise indicated.

[c] 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 (i.e. the chlorine demand of the water). The remaining chlorine is the total chlorine and is the sum of free or non-bound chlorine and combined chlorine.

Total chlorine is usually measured on site by appropriately trained personnel in water prior to entering the treatment system. Additional measurements in the treated water are not necessary provided that the pre-treatment concentration level is below the permitted limit.

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.

NOTE The maximum allowable levels of contaminants listed include the anticipated uncertainty associated with the analytical methodologies used to establish the values shown.

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

Contaminant	Maximum concentration[a] 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

[a] The reader is cautioned to refer to the latest edition of this document to ensure that no changes have been made to the maximum concentrations shown.

NOTE The maximum allowable levels of contaminants listed in include the anticipated uncertainty associated with the analytical methodologies to establish the values shown.

4.2.2 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 haemodialysis patients are difficult to specify. 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 at present not possible to specify limits for their presence in water used in the preparation of dialysis fluid.

4.3 Dialysis water microbiological requirements

Total viable microbial counts in dialysis water shall be less than 100 CFU/ml. An action level shall be set based on knowledge of the microbial dynamics of the system. Typically, the action level will be 50 % of the maximum allowable level.

Endotoxin content in dialysis water shall be less than 0,25 EU/ml. An action level shall be set, typically at 50 % of the maximum allowable level.

Fungi (yeasts and filamentous fungi) can coexist with bacteria and endotoxin in the dialysis water. Further studies on the presence of fungi in haemodialysis water systems, their role in biofilm formation and their clinical significance are required and in view of this, no recommendation in respect of permitted maximum limits is made.

Some integrated, validated systems, and other new systems by alternative design can provide ultrapure dialysis water with <0,1 CFU/ml and <0,03 EU/ml. By mixing with sterile and non-pyrogenic concentrates and by utilising sterile and non-pyrogenic dialysis fluid pathway, ultrapure dialysis fluid can be produced in such integrated and validated systems.

NOTE See Clause A.4 for a history of these requirements.

5 Tests for microbiological and chemical requirements

5.1 Dialysis water microbiology

Samples shall be collected where a dialysis machine connects to the water distribution loop, and from a sample point in the distal segment of the loop or where such water enters a mixing tank.

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 until ready to transport to the laboratory for analysis. Sample storage for more than 24 h should be avoided and sample shipping should be done according to the laboratory's instructions.

Total viable counts (standard plate counts) shall be obtained using conventional microbiological assay procedures (pour plate, spread plate, membrane filter techniques). Membrane filtration is the preferred method for this test. Other methods may be used, provided that such methods have been appropriately validated and are comparable to the cited methods. The use of the calibrated loop technique is not acceptable.

5.2 Microbial contaminant test methods

Methodology to establish microbial contaminant levels is given in Table 3. Such methods provide only a relative indication of the bioburden rather than an absolute measure.

Recommended methods and cultivation conditions can also be found in ISO 23500-4 and ISO 23500-5 as well as this document (see Table 3). The methodology detailed uses tryptone glucose extract agar (TGEA) and Reasoner's agar no. 2 (R2A) incubated at 17 °C to 23 °C for 7 d and tryptic soy agar (TSA) at an incubation temperature of 35 °C to 37 °C and an incubation time of 48 h.[17] The background for the inclusion of TSA for dialysis water and dialysis fluid used for standard dialysis is explained in Clause A.4.

Different media types and incubation periods can result in varying colony concentrations and types of microorganisms recovered.[18]-[21] Furthermore, the use of R2A has been shown to result in higher colony counts than TSA for dialysis water and dialysis fluids samples. In a more recent publication, the authors indicated that there were no significant differences when comparing the microbial burden of dialysis water and dialysis fluid used for the standard dialysis fluid yielding colony counts ≥50 CFU/ml when assayed using R2A incubated at 17 °C to 23 °C for 7 d and TSA incubated at 35 °C to 37 °C for 48 h.[17]

Historic studies with TGEA incubated at 17 °C to 23 °C for a period of 7 d also yielded higher colony counts than TSA. Maltais et al.[17] in their comparison of TGEA with TSA showed that the proportion of dialysis water samples yielding colony counts ≥50 CFU/ml was significantly different from that found using TSA at an incubation temperature of 35 °C to 37 °C and an incubation time of 48 h (p = 0,001). 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 incubation times selected should be based on the type of fluid to be analysed for example, 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 methods detailed above in this subclause. According to the United States Pharmacopeia (USP), 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 the 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). Other methods may be used, provided that such methods have been appropriately validated and are comparable to the cited methods. Blood agar and chocolate agar shall not be used.

Currently, there are no requirements for routine surveillance for the presence of fungi (i.e. yeasts and filamentous fungi) which can coexist with other microbial species, however if an indication of their presence is required, membrane filtration is the preferred method to provide a sample suitable for analysis. Culture media used should be Sabouraud or malt extract agar (MEA) media. Other methods may be used, provided that such methods have been appropriately validated and are comparable to the cited methods. An incubation temperature of 17 °C to 23 °C and an incubation time of 168 h (7 d) are recommended. Other incubation times and temperatures can be used, provided it has been demonstrated that such methods have been appropriately validated and are comparable to the cited methods.

Microbial endotoxins are assayed using the *Limulus* amoebocyte lysate (LAL) test. Current pharmacopoeias (USP, European and Japanese pharmacopoeias) acknowledge different testing techniques.

The most frequently used test for microbial endotoxins, the LAL test is based on the humoral coagulation cascade of the horseshoe crab *Limulus polyphemus*. The first enzyme in this coagulation cascade reacts with endotoxin and is called Factor C. This factor is now produced recombinantly (i.e. using biotechnology) and offered as the rFC test by several manufacturers for the determination of microbial endotoxins. Compared to the LAL test, the rFC test has proven to be at least as sensitive and reliable, but less susceptible to certain interfering factors and batch fluctuations. Due to biotechnological production, no live animals are required as blood donors.

This new method has been incorporated into the European Pharmacopoeia (Ph.Eur) (<2.6.32> Endotoxins using recombinant factor C)[44], the Japanese Pharmacopeia (<G4-4-180> Bacterial Endotoxins Test and Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay)[45] and the USP and the National Formulary (USP–NF) (<1085.1> Use of Recombinant Reagents in the Bacterial Endotoxins Test - Photometric and Fluorometric Methods Using Recombinantly Derived Reagents)[46].

Table 3 — Culture techniques

Culture medium	Incubation temperature	Incubation time
TGEA	17 °C to 23 °C	7 d
R2A	17 °C to 23 °C	7 d
Sabouraud or malt extract agar[a]	17 °C to 23 °C	7 d
TSA[b]	35 °C to 37 °C	48 h

[a] Intended for the quantification of yeasts and filamentous fungi. Currently, there are no requirements in this document for their routine surveillance; they have been included for completeness.

[b] The use of TSA has only been validated for dialysis water and standard dialysis fluid.[11]

5.3 Chemical contaminants test methods

Conformity with the requirements listed in Table 1 and 2 can be shown by using the chemical analysis methods detailed in Table 4 or by any other equivalent validated analytical method. Where testing for the individual trace elements listed in Table 2 is not available, and the source water can be demonstrated to meet the standards for potable water as specified by the WHO[16], an analysis for total heavy metals can be used with a maximum allowable level of 0,1 mg/l. If neither of these options is available, conformity with the requirements of Table 2 can be met by using water that can be demonstrated to meet the potable water requirements of the WHO and a reverse osmosis system with a rejection of ≥90 % based on conductivity, resistivity or TDS. Samples shall be collected at the end of the water purification cascade or at the most distal point in each water distribution loop.

Table 4 — Analytical test methods for chemical contaminants

Contaminant	Analytical technique	Reference, method
Aluminium	Inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3113[14]
Antimony	ICP-MS or atomic absorption (platform)	ISO 17294-2[4], EPA Method 200.7[9]
Arsenic	ICP-MS or atomic absorption (gaseous hydride)	ISO 17294-2[4], APHA Method 3114[14]
Barium	ICP-MS or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3113[14]
Beryllium	ICP-MS or atomic absorption (platform)	ISO 17294-2[4], EPA Method 200.7[9]
Cadmium	ICP-MS or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3113[14]
Calcium	ICP-MS or ethylene diamine tetraacetic acid (EDTA) titrimetric method or atomic absorption (direct aspiration) or ion specific electrode	ISO 17294-2[4], APHA Method 3500-Ca D[14], APHA Method 3111B[14]
Total chlorine	N-diethyl-p-phenylenediamine (DPD) ferrous titrimetric method or DPD colorimetric method or Thio-Michler's Ketone (TMK/MTK) colorimetric method	APHA Method 4500-Cl F[14], APHA Method 4500-Cl G[14]
Chromium	ICP-MS or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3113[14]
Copper	ICP-MS or atomic absorption (direct aspiration) or neocuproine method	ISO 17294-2[4], APHA Method 3111[14], APHA Method 3500-Cu D[14]
Fluoride	Ion chromatography or ion selective electrode method or sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonate (SPADNS) method	ISO 10304-1[2], ISO 10359-1[3], APHA Method 4500-F^- C[14], APHA Method 4500-F^- D[14]
Lead	ICP-MS or atomic absorption (electrothermal)	ISO 17294–2[4], APHA Method 3113[14]
Magnesium	ICP-MS or atomic absorption (direct aspiration) or ion chromatography	ISO 17294–2[4], APHA Method 3111[14], EPA 300.7[8]
Mercury	Flameless cold vapour technique (atomic absorption)	APHA Method 3112[14]
Nitrate	Ion chromatography or spectrophotometric method using sulfosalicylic acid or cadmium reduction method	ISO 10304–1[2], ISO 7890-3[1], APHA Method 4500-NO_3 E[14]
Potassium	Inductively coupled plasma mass spectrometry or atomic absorption (direct aspiration) or flame photometric method or ion specific electrode	ISO 17294-2[3], APHA Method 3111[14], APHA Method 3500-K D[14], APHA Method 3500-K E[14]
Selenium	ICP-MS or atomic absorption (gaseous hydride) or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3114[14], APHA Method 3113[14]
Silver	Inductively coupled plasma mass spectromety or atomic absorption (electrothermal)	ISO 17294-2[4], APHA Method 3113[14]
Sodium	ICP-MS or atomic absorption (direct aspiration) or flame photometric method or ion specific electrode	ISO 17294-2[4], APHA Method 3111[14], APHA method 3500-Na D[14]

Table 4 *(continued)*

Contaminant	Analytical technique	Reference, method
Sulfate	Ion chromatography or turbidimetric method	ISO 10304-1[3], APHA Method 4500-SO_4^{2-} E[14]
Total heavy metals	Colorimetric method	European Pharmacopoeia, 5.20[13], European Pharmacopoeia 2.4.20[13], USP–NF <232>[10], USP–NF <233>[11]
Zinc	ICP-MS or atomic absorption (direct aspiration) or dithizone method	ISO 17294-2[4], APHA Method 3111[14], APHA Method 3500-Zn D[14]

Annex A
(informative)

Rationale for the development and provisions of this document

A.1 General

Water treated according to the requirements of this document is predominantly used for the preparation of dialysis fluid but can also be used for other applications such as the reprocessing of haemodialysers intended for multiple use. When dialysis water is mixed with concentrated electrolyte solutions manufactured according to ISO 23500-4, the requirements detailed in ISO 23500-5 apply.

A.2 Feed water

The water used in the preparation of dialysis fluid usually originates as potable water from a municipal water supply, although in some instances the water can be from a local borehole or well. Potable water complies with the WHO Guidelines for drinking water, or its local equivalent. These requirements specify the permitted water contaminants and their levels. As dialysis patients are exposed to larger volumes of water than the general population, the water needs to undergo additional treatment to reduce any risk from water contaminants and to meet the appropriate requirements detailed in 4.2 and 4.3.

If the feed water to the water treatment infrastructure is via an indirect feed, for example, a hospital water system, disinfectants and antimicrobial agents can be added to supress the development of legionella within the water system. Commonly used agents include hydrogen peroxide and silver stabilized hydrogen peroxide. Unintended exposure to both have resulted in adverse events in dialysis patients as remaining residues cannot be removed by reverse osmosis and rely on the use of activated carbon.

If drinking water has chlorine and/or chloramine added to minimize bacterial content, both of these compounds are toxic to dialysis patients and are removed by the water treatment system as outlined in ISO 23500-2. Removal of those compounds renders the water susceptible to microbial proliferation and biofouling unless appropriate preventative measures are taken as outlined in ISO 23500-1.

While the majority of bacteria in the feed water are faecal in origin and the measures that the water utility takes are intended to minimize their proliferation, the feed water can also contain other microbial compounds such as cyanotoxins that occur in the presence of cyanobacteria or blue green algae. Cyanotoxins are considered natural contaminants that occur worldwide. Specific classes of cyanotoxins have shown regional prevalence. The Americas encompassing North Central and South America often show high concentrations of microcystin, anatoxin-a and cylindrospermopsin in freshwater, whereas those in Australia often show high concentrations of microcystin, cylindrospermopsin and saxitoxins. Other less frequently reported cyanotoxins include lyngbyatoxin A, debromoaplysiatoxin and beta-N-methylamino-L-alanine.[17] Cyanobacterial blooms usually occur according to a combination of environmental factors, for example, nutrient concentration, water temperature, light intensity, salinity, water movement, stagnation and residence time, as well as several other variables. Cyanotoxins are primarily produced intracellularly during the exponential growth phase. Release of toxins into water can occur during cell death or senescence but can also be due to evolutionary-derived or environmentally-mediated circumstances such as allelopathy or relatively sudden nutrient limitation[22].

In many countries, cyanotoxins have been viewed primarily as a recreational water issue. However, there is a growing awareness of the public health risk they pose in drinking water and thus the need to monitor and remove cyanotoxins in the drinking water treatment process. The WHO has established a suggested drinking water guideline value of 1 µg/l and a recreational exposure guideline value of 10 µg/l for microcystin-LR. Health Canada has also published a drinking water standard of 1,5 µg/l for microcystin-LR, while in the United States, the EPA has developed health advisory recommendations for concentrations of cyanotoxins in

drinking water, namely that for adults, the recommended levels for drinking water are at or below 1,6 µg/l for microcystins and 3,0 µg/l for cylindrospermopsin.

Currently, water utilities do not regularly look for cyanobacterial toxins in the water supply unless cyanobacteria are present in the source water. Once cyanobacteria are detected in the water supply, treatment can remove them using a variety of different methods, such as clarification or membrane filtration, adsorption on activated carbon or reverse osmosis and chemical oxidation by ozonation or chlorination.

A.3 Chemical contaminants in dialysis water

A.3.1 General

Chemical contaminants present in potable water can pose a risk to the patient receiving dialysis treatment. Contaminants identified as needing restrictions on their allowable level compared with potable water have been divided into three groups for the purposes of this document:

a) chemicals known to cause toxicity in dialysis patients,

b) physiological substances that can adversely affect the patient if present in the dialysis fluid in excessive amounts, and

c) trace elements.

A.3.2 Chemicals known to cause toxicity in dialysis patients

Chemicals known to cause toxicity to dialysis patients include those which are added to drinking water for public health benefits. Fluoride can be present naturally in potable water or be added in low concentrations to minimize dental caries. The maximum limit for this compound in drinking water is set at 1,5 mg/l. The toxicity of fluoride in dialysis patients at the levels present in fluoridated water, is questionable. In the absence of a consensus on fluoride's role in uraemic bone disease, it was initially thought prudent to restrict the fluoride level of dialysis fluid. Isolated cases of acute exposure of dialysis patients to elevated levels of fluoride has been described in the scientific literature. Fluoride levels of up to 50 mg/l were found in water used for dialysis that was treated only with a water softener. In another case, where deionizers were allowed to exhaust, 12 of 15 patients became acutely ill from fluoride intoxication and three of the patients died from ventricular fibrillation. In another publication, the death of one patient was reported as a result of accidental over fluoridation of a municipal water supply[23].

Aluminium is toxic to haemodialysis patients. Salts of aluminium, such as alum, are added to drinking water in order to facilitate chemical precipitation and flocculation of colloidal particles (turbidity). In haemodialysis patients, exposure to aluminium can result in severe neurologic symptoms.[24]

The maximum aluminium level set for dialysis water has been specified to prevent accumulation of this toxic metal in the patient. Despite this, occasional sporadic outbreaks of aluminium intoxication have been reported.[25],[26]

Aluminium in potable water can increase suddenly from changes in the method of water treatment. As with fluoride, water treatment would provide a measure of safety even if the aluminium levels increase dramatically between chemical tests of the dialysis water.

Chlorine and/or chloramines (reaction products of chlorine and ammonium) are added to drinking water as disinfectants. Chloramines are used in place of chlorine to minimize the toxicity of chlorine by-products.

Exposure of haemodialysis patients to free chlorine to a maximum level of 0,5 mg/l and combined chlorine/chloramines to a maximum level of 0,1 mg/l is necessary to protect the haemodialysis patient from haemolytic reactions (haemolysis, haemolytic anaemia and methemoglobinemia) and EPO resistance.[27]-[30] Chlorine can be present in water as both free chlorine and chlorine in chemically combined forms such as chloramine. Determining the level of chloramine typically involves measuring both total chlorine and free chlorine and assigning the difference in concentrations to chloramine. During the second revision of this document in 2008 (i.e. ISO 13959:2009), to simplify this, a maximum allowable level for total chlorine

at the same value used previously for chloramine (0,1 mg/l) was chosen thus permitting a single test to be used. It should be noted that total chlorine is specified as the sum of free chlorine and combined chlorine.

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.

At the time of revision of the previous versions of this document, some municipal water suppliers were considering the use of chlorine dioxide as a disinfectant for potable water supplies. Its use in the treatment of water for building services has grown significantly in recent years, driven by increased awareness of biological related health issues, the need to conserve energy and the simplicity of use of chlorine dioxide systems. When used, chlorine dioxide is termed a 'dispersive' treatment, this means that the chlorine dioxide is dosed into the water system and travels around the entire water system, providing a 'residual' level of treatment. This means that the applied chlorine dioxide can continue to kill bacteria in all areas of the system that it reaches and not just at the point of use.

When chlorine dioxide is used as a disinfectant, residual chlorine dioxide and a range of breakdown products namely chlorite, chlorate, and organic disinfection by products (DBPs) results. Little information can be found about the potential for chlorine dioxide and its daughter products to be toxic to haemodialysis patients. A limited study of 17 patients unknowingly treated with dialysis water prepared by carbon and reverse osmosis from water disinfected with chlorine dioxide showed no evidence of adverse effects.[31] In this study, the dialysis water used to prepare dialysis fluid contained 0,02 mg/l to 0,08 mg/l of chlorite ions and no detectable chlorate ions. However, the patient population was small, and potentially important haematological parameters were not measured. Further, there was only sparse data included on the removal of chlorine dioxide, chlorite ions and chlorate ions by carbon and reverse osmosis, and it was not clear that sufficiently sensitive methods were available for their analysis in a dialysis facility. In view of this, there is no basis for setting maximum allowable levels of chlorine dioxide, chlorite ions or chlorate ions in water to be used for dialysis applications, or for making recommendations on methods for their removal at this time. However, in specifying water purification systems for use in the production of dialysis water, users and providers should be aware of the possibility that municipal water suppliers can switch to chlorine dioxide as a disinfectant.

Sulfate can be found in almost all natural waters. The origin of most sulfate compounds is the oxidation of sulphite ores, the presence of shales or industrial wastes. Sulfate is one of the major dissolved components of rain. At levels above 200 mg/l, it has been related to nausea, vomiting and metabolic acidosis. The symptoms disappear when the level remains below 100 mg/l.

Nitrates are a marker for bacterial contamination and fertilizer runoff and have caused methemoglobinemia.[32] They should, therefore, be permitted only at very low levels. In areas where ground water nitrate content is high, reverse osmosis alone cannot always be guaranteed to reduce the levels to meet requirements. Additional nitrate removal using a nitrate selective anion, an ion-exchange resin to specifically remove nitrate installed upstream of the reverse osmosis system can be necessary.

Both copper and zinc toxicity have been demonstrated when these substances are present in dialysis fluid at levels below those permitted by drinking water standards[16]. Levels for dialysis water are set below that permitted in drinking water for both copper and zinc.

Public health measures over the past four decades have reduced the level of lead in drinking water. Nevertheless, in older properties that have not been renovated, interior piping as well as the piping connecting the property to the municipal or main supply can still be made of lead and result in elevated blood lead levels[33]. The use of chloramines can increase exposure to lead in drinking water due to alterations in water chemistry. These changes lead to increased corrosion within the distribution network such as lead piping, and this in turn can be reflected in abnormal blood levels in haemodialysis patients.[39] Such corrosion of the municipal distribution system was responsible for the elevated lead levels found in Flint, Michigan, when the town changed its water supply in 2014[34].

There is no evidence of lead toxicity when lead levels in water or dialysis fluid are below 5 µg/l.

A.3.3 Physiological substances

Physiological substances that can adversely affect the patient if present in the dialysis fluid in excessive amounts include calcium, magnesium, potassium and sodium. Of these, calcium has been reduced from the 10 mg/l originally selected to 2 mg/l on the basis of the critical role of calcium in bone disorders associated with renal disease. A level of 10 mg/l would have allowed a potential 20 % error in dialysis fluid calcium, whereas a level of 2 mg/l reduces that error risk to less than 5 %.

During the latest revision of this document (i.e. ISO 23500-3:2024), a discussion took place to review adjustment of the levels of physiological substances (i.e. compounds normally present in the dialysis fluid) to match those published in the European Pharmacopoeia, namely a reduction from 4 mg/l to 2 mg/l for magnesium, a reduction from 8 mg/l to 2 mg/l for potassium and a reduction from 70 mg/l to 50 mg/l for sodium. Due to lack of clinical data supporting the rationale for such changes, the decision was made to retain the existing limits.

A.3.4 Trace metals and other compounds

Little data exist to indicate that haemodialysis patients are at particular risk from any members of this group of contaminants. These contaminants were included in the earlier version of this document solely by virtue of their inclusion in the US *Safe Drinking Water Act* when the document was originally developed and were based on the 1992 edition of ANSI/AAMI RD5. The limits for the compounds are based upon known toxicity of individual contaminants and upon technology available to remove contaminants. Similar drinking water legislation exists in many developed countries (e.g. the European Drinking Water Directive). Such limits are generally expressed in terms of enforceable maximum contaminant levels (MCL) expressed in mg/l – the highest level of a contaminant that is allowed in drinking water. MCL values are set as close to maximum contaminant level goal (MCLG) as possible. MCLG is specified as the level of a contaminant in drinking water below which there is no known or expected risk to health and which allow for a margin of safety but are non-enforceable public health goals.

At the time of the latest revision of this document (i.e. ISO 23500-3:2024), the compounds included were antimony, arsenic, barium, beryllium, cadmium, chromium, mercury, selenium, silver and thallium. Selenium and chromium levels in dialysis water were initially set at the "no-transfer" level. The historic "no-transfer" level was chosen even though it was above the US EPA limit for selenium and 28 % of the US EPA limit for chromium, because a restriction is not needed below the level at which there is no passage from the dialysis fluid to the blood. The document specified the maximum allowable limits for the other contaminants in this group to be one tenth of the US EPA maximum allowable limits as the volume of water used for dialysis far exceeds that used for drinking, because protein binding of these solutes and occur in the blood, and because there is reduced renal excretion of these substances. These reduced limits were selected using the following assumptions:

a) feed water entering dialysis systems typically meets the drinking water requirements;

   NOTE Regulatory requirements can apply with respect to contaminant levels.

b) the water treatment system incorporates reverse osmosis, which typically, would remove 90 % to 99 % of dissolved inorganic solids; and

c) reverse osmosis-treated water is a suitable standard for safety of water used in dialysis.

These assumptions are based on the recommendations of Reference [33]. Although these assumptions can be questioned, it was reasoned that setting standards in this way would cause little or no economic impact, even if feed water exceeds the maximum allowable levels.

In the latest revision of this document (i.e. ISO 23500-3:2024), it was decided to use WHO Drinking Water Guideline[16] replacing the previously used EPA water quality requirements. The list of contaminants of potential concern were also reviewed as with the exception of selenium and chromium, all contaminants listed in this group were set historically at values above one-tenth of the MCLG for drinking water.

The toxicity mechanisms of antimony are similar to those known in arsenic and include vomiting, diarrhoea, confusion, memory loss, cardiotoxicity and pancreatitis. Even though the current WHO drinking water

guideline value is set at 0,02 mg/l, a decision was taken not to change the previous limit (0,006 mg/l) since no studies reported data to indicate that this contaminant is of particular concern in a haemodialysis setting.

Regarding arsenic, the current WHO drinking water guideline value is set at 0,01 mg/l with haemodialysis exposes patients to large volumes of water (>120 l/week) in the form of dialysis fluid. Although levels of certain ions (such as potassium and calcium) are carefully regulated, many others are measured infrequently and trace elements such as arsenic present in water but not in blood can accumulate and cause toxicity. It has been hypothesized that the increased morbidity and mortality seen in haemodialysis patients can in part be due to the imbalance of trace elements that has not been recognized. A recent systematic review has shown that, compared with healthy controls, haemodialysis patients have significantly lower blood levels of zinc, manganese and selenium, while blood levels of lead can accumulate if the level in the water is high. Other trace elements, such as mercury and arsenic, are biologically plausible causes of excess mortality in dialysis patients. However, in a recent study by Tonelli[36], higher concentrations of lead, arsenic or mercury were not associated with higher risk of clinical outcomes. In view of this, a decision was taken not to change the previous limit (0,005 mg/l).

The maximum concentration of barium in dialysis water was not changed. The set value of 0,1 mg/l is below 10 % of the current drinking water guideline value (set at 1,3 mg/l).

Beryllium is a carcinogenic agent affecting mainly the lungs and bones. It also affects the skin and mucous membranes competing with magnesium forming enzyme complexes interfering in ATP connections. There is no established drinking water guideline value for beryllium given in the WHO drinking water guideline and no studies have reported that this contaminant is of particular concern in the setting of haemodialysis, a decision was made not to take out this contaminant from the list and to retain the limit at 0,000 4 mg/l primarily because it is a compound that is released into the environment from industrial pollution and is an incineration by-product.

Regarding cadmium, the current drinking water guideline value is set at 0,01 mg/l. Since no documented arsenic intoxication via dialysis fluid contamination has been reported and there can be practical difficulties in the measurement of low levels the limit permitted in dialysis water was unchanged and remains at 0,005 mg/l.

The maximum concentration of mercury in dialysis water was not changed. The set level of 0,000 2 mg/l is below 10 % of the current WHO drinking water guideline value set at 0,006 mg/l.

Silver can be found in ground water, surface water and drinking water at low concentrations. Significantly higher concentrations may be present in drinking water if treated with silver for disinfection purposes. Contamination of dialysis water with silver is associated with enhanced trace metal levels. Even though there is no established drinking water guideline value for silver by WHO, a decision was made not to remove this contaminant from the list and to retain the limit at 0,005 mg/l.

After considerable discussion, it was decided to remove thallium from the list of contaminants, since no studies have reported that this contaminant is of particular concern in the haemodialysis setting.

Historically, there were discussions about moving Table 2 to this annex during previous revisions of this document. This discussion was prompted by the ongoing addition of contaminants to the US *Safe Drinking Water Act* as was the case with antimony, beryllium and thallium. In general, no data exist to indicate that these new contaminants are of particular concern in the setting of haemodialysis. On the other hand, adding new contaminants to Table 2 has the potential to increase the operating constraints to dialysis facilities for testing water samples for conformity. There was enough discomfort about removing the third category of contaminants from Table 2 that it was decided to leave the list of contaminants unchanged but to reorganize the table into three clear sections and not to add new contaminants to the table unless there was accompanying evidence of toxicity in the setting of haemodialysis. During the revision of this document in 2008 (i.e. ISO 13959:2009), a decision was made to separate the third group of contaminants into a separate table. One reason for this change was to allow alternative approaches to regular surveillance of these contaminants to facilitate use of this document in areas lacking the appropriate analytical tools for measuring trace elements at the levels listed in Table 2.

Three options were historically considered. The preferred option was to measure concentrations of the individual trace elements. If this option is not available, two other approaches can be used. The first, and preferred alternative, is to measure total heavy metals. The second, and least preferred alternative, is to

use reverse osmosis with a demonstrated rejection of at least 90 %. Both these alternative approaches are based on the use of feed water meeting applicable potable water standards and it is the responsibility of the dialysis facility to ensure that its water supply routinely meets potable water standards. Finally, it should be evident from the discussion in this annex that the maximum allowable levels for the contaminants listed in Tables 1 and 2 are not precisely determined values but represent reasonable estimates based on sparse clinical data. As a result, any uncertainty in the analytical methods listed in Table 4 is likely to be small compared to the uncertainty involved in establishing the maximum allowable level and, for that reason, analytical uncertainty is considered to be included in the values listed in Tables 1 and 2.

Tables 1 and 2 should not be taken as a definitive list of harmful substances but as a partial listing of those that can reasonably be expected to be present and have clinical implications. Iron is not included because it does not enter the patient's blood in sufficient quantities to cause toxicity. Iron can, however, cause fouling of water purification devices or dialysis fluid supply systems. While no specific limit has been set, water treatment equipment suppliers are encouraged to consider the iron content of the feed water when recommending suitable equipment. A concern was raised regarding the injection of formulated phosphates (known as polyphosphates) primarily to bind iron and manganese to avoid the staining of fixtures and clothing since the presence of polyphosphates can cause significant problems in water purification. However, to date, no reports have appeared in the literature regarding this issue.

Water used in the preparation of water for dialysis can also contain organic contaminants. However, the long-term effects of organic contaminants on haemodialysis patients are unknown. To date, there has been only a single report of contamination of haemodialysis water supply with an organic compound (trichloroethylene)[37].

In view of the limited availability of data on patient exposure to organic compounds, it was decided not to establish specific maximum permitted levels for organic contaminants nor for radioactive compounds. In general, the starting point to assess whether organic compounds are a cause for concern is the national drinking water requirement for such compounds.

If there is concern about specific organic compounds in the feed water, then taking the approach used in establishing limits for other compounds known to cause toxicity, namely an assessment of the compounds removal by granulated activated carbon (GAC) and reverse osmosis should be undertaken to quantify the reduction achieved. If the existing system does not reduce the levels sufficiently, for example in the case of hydrophilic compounds where breakthrough in the activated carbon can occur, consideration should be given to the use of alternate approaches, e.g. microfiltration to achieve the required reduction.

A.4 Microbiology of dialysis water

NOTE The information in this clause is intended to give the reader a historical perspective of how the microbial limits were developed for this document.

The water treatment applied to feed water to produce dialysis water to meet the chemical contaminant levels specified in Tables 1 and 2 removes chlorine and/or chloramine added to drinking water as a public heath safeguard. Consequently, the product water and the distribution network downstream of the water treatment infrastructure in a dialysis unit are susceptible to microbial proliferation and the formation of biofilm. Once formed, the biofilm is difficult to remove and results in the release of bacteria and microbial fragments (endotoxins, muramylpeptides and polysaccharides).

Historically, little emphasis was based on the microbiological quality of water used to prepare dialysis fluid since it was perceived that the dialysis membrane prevents transmembrane passage of intact bacteria. Subsequently, a number of publications demonstrated that bacterial fragments including short bacterial DNA fragments are able to traverse high and low flux haemodialysis membranes[38]-[41]. Such transfer induces cytokines and contributes to pyrogenic reactions and microinflammation seen in haemodialysis patients.

In earlier versions of this document, the maximum level of bacteria in dialysis water was set at 200 CFU/ml. This value was based on historic studies which demonstrated that the incidence of pyrogenic reactions were linked to bacterial load in the dialysis fluid. The European community chose to use a lower level of less than 100 CFU/ml as their bacterial limit for dialysis water and that value has been adopted in this document. Because 2 d to 7 d can elapse between sampling water for the determination of microbiological contamination and receiving results, and because microbial proliferation can be rapid, action levels for

microbial counts and endotoxin were also introduced into this document. These action levels allow the user to initiate corrective action before levels exceed the maximum levels established by this document.

Even at low levels of microbial contamination, pyrogenic reactions have been reported when the source of endotoxin was exogenous to the dialysis system (i.e. present in the community water supply). Consequently, it was considered prudent to impose an upper limit on the endotoxin content of dialysis water. A level of 2 EU/ml was chosen by AAMI in 2001 as the upper limit for endotoxin, since conformity with such a level can be easily achieved with contemporary water treatment systems using reverse osmosis, ultrafiltration, or both. At the same time, the European community chose to use an upper limit of 0,25 EU/ml for endotoxin. During the revision of this document in 2008 (i.e. ISO 13959:2009), the 0,25 EU/ml limit was included as the upper limit for endotoxin in dialysis water.

No changes have been made in respect of bacteria and endotoxin levels in dialysis fluid and the levels given in the previous edition of this document remain.

Cyanotoxins are considered natural contaminants that occur worldwide. Studies report only low (below WHO or local guidelines) or undetectable levels of cyanotoxins in treated drinking water even when cyanotoxins are present in the source water[42]. Cyanotoxin species have been involved in dialysis patient exposure which occurred in Brazil in 1996 and 2001[43]. During the first incident in 1996, patients were exposed to high (20 µg/l) levels of microcystin and suffered liver failure, visual abnormalities and death. The second incident in 2001 involved a lower level of exposure (0,32 µg/l) and resulted in milder clinical sequalae.

In the course of the latest revision of this document (i.e. ISO 23500-3:2024), the issue of cyanotoxins was discussed. The establishment of limits for compounds that can adversely affect dialysis patients has been set historically at 10 % of the levels allowed in drinking water in the absence of concentration-toxicity data. Using the WHO provisional drinking water guideline (microcystin-LR concentration ≤1 µg/l), the maximum concentration for microcystins in dialysis water would be 0,1 µg/l. This level is well below the levels monitored during outbreaks in Brazil, and it was difficult to be confident that an upper limit of 0,1 µg/l of microcystins can be accurately detected using current surveillance methodology. It was therefore decided not to introduce limits or regular surveillance for microcystins in dialysis water. Nevertheless, there should be awareness of the potential presence of such toxins in the feed water, and risk limitation should be in place in the event of the presence of microcystins in the public water supply. To facilitate such awareness, dialysis facilities should establish regular communication with their water provider, to ensure that they receive timely warning of the presence of cyanomicrobial blooms in any water used to supply the public water system.

Accurate and timely microbiological surveillance is important in indicating the microbial content of dialysis water. The culture results obtained using the methods outlined in this document are only a relative indicator of the bioburden and as with any microbiological method, they do not provide an absolute measure of the absolute microbial burden.

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 on the purpose of the analysis. The method selected should also consider the advantages, disadvantages and sensitivity of each of the suggested methods. 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 take early corrective action, as well as the ability of these microorganisms to detrimentally affect products or processes (e.g. patient safety).

Recommended methods and cultivation conditions can be found in ISO 23500-4 and ISO 23500-5 as well as in this document (see Table 3). In the latest revision of this document (i.e. ISO 23500-3:2024), TGEA and R2A incubated at 17 °C to 23 °C for a period of 7 d, TSA at an incubation temperature of 35 °C to 37 °C at an incubation time of 48 h has been included for analysis of dialysis water and dialysis fluid used in standard haemodialysis based upon a publication[17] supporting comparable methods to those of the previously recommended methods and cultivation conditions.