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Preparation and quality management of fluids for haemodialysis and related therapies – Part 3: Water for haemodialysis and related therapies (ISO 23500-3:2019); English version EN ISO 23500-3:2019, English translation of DIN EN ISO 23500-3:2019-11

Herstellung und Qualitätsmanagement von Flüssigkeiten für die Hämodialyse und verwandte Therapien –
Teil 3: Wasser für die Hämodialyse und verwandte Therapien (ISO 23500-3:2019);
Englische Fassung EN ISO 23500-3:2019,
Englische Übersetzung von DIN EN ISO 23500-3:2019-11

Préparation et management de la qualité des liquides d'hémodialyse et de thérapies annexes –
Partie 3: Eau pour hémodialyse et thérapies apparentées (ISO 23500-3:2019);
Version anglaise EN ISO 23500-3:2019,
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In case of doubt, the German-language original shall be considered authoritative.

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National foreword

This document (EN ISO 23500-3:2019) has been prepared by Technical Committee ISO/TC 150 “Implants for surgery” in collaboration with Technical Committee CEN/TC 205 “Non-active medical devices” (Secretariat: DIN, Germany).

The responsible German body involved in its preparation was *DIN-Normenausschuss Medizin* (DIN Standards Committee Medicine), Working Committee NA 063-01-03 AA “Extracorporeal circuits, equipment and disposables”.

The DIN documents corresponding to the international documents referred to in this document are as follows:

ISO 23500-1	DIN EN ISO 23500-1
ISO 23500-4	DIN EN ISO 23500-4
ISO 23500-5	DIN EN ISO 23500-5

Amendments

This standard differs from DIN EN ISO 13959:2016-03 as follows:

a) this document forms part of a new standards series dealing with the preparation and quality management of fluids for haemodialysis and related therapies, water quality, concentrates for the preparation of dialysis fluid as well as quality of dialysis fluid;

b) the standard has been editorially revised.

Previous editions

DIN EN ISO 13959: 2016-03

National Annex NA
(informative)

Bibliography

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

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

DIN EN 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*

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EUROPEAN STANDARD

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English Version

Preparation and quality management of fluids for haemodialysis and related therapies - Part 3: Water for haemodialysis and related therapies (ISO 23500-3:2019)

Préparation et management de la qualité des liquides d'hémodialyse et de thérapies annexes - Partie 3: Eau pour hémodialyse et thérapies apparentées (ISO 23500-3:2019)

Herstellung und Qualitätsmanagement von Flüssigkeiten für die Hämodialyse und verwandte Therapien - Teil 3: Wasser für die Hämodialyse und verwandte Therapien (ISO 23500-3:2019)

This European Standard was approved by CEN on 14 January 2019.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

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European foreword

This document (EN ISO 23500-3:2019) has been prepared by Technical Committee ISO/TC 150 "Implants for surgery" in collaboration with Technical Committee CEN/TC 205 “Non-active medical devices” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2019, and conflicting national standards shall be withdrawn at the latest by September 2019.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 13959:2015.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 23500-3:2019 has been approved by CEN as EN ISO 23500-3:2019 without any modification.

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 13959:2014, 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

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 minimum requirements, chemical and microbiological, for the water to be used for preparation of dialysis fluids, 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 revised document.

Within this document, measurement techniques current 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. Operation of water treatment equipment and haemodialysis systems, including on-going 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 informative Annex A.

1 Scope

This document specifies minimum requirements for water to be used in haemodialysis and related therapies.

This document includes water to be used in the preparation of concentrates, dialysis fluids for haemodialysis, haemodiafiltration and haemofiltration, and for the reprocessing of haemodialysers.

This document excludes the operation of water treatment equipment and the final mixing of treated water with concentrates to produce dialysis fluid. Those operations are the sole responsibility of dialysis professionals. This document does not apply to dialysis fluid regenerating systems.

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 terminological databases for use in standardization at the following addresses:

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

— IEC Electropedia: available at http://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, or as required by national legislation or regulations. Table 1 does not include any recommendation in respect of 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 define 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 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 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 can be already occupied and not available for further removal activity.

NOTE 1 See A.3 for an explanation of values supplied.

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 for the reprocessing of 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 mg[b]
Contaminants with documented toxicity in haemodialysis	
Aluminium	0,01
Total chlorine[1]	0,1
Copper	0,1
Fluoride	0,2
Lead	0,005
Nitrate (as N)	2
Sulfate	100
Zinc	0,1

[a] A physician in charge of dialysis has ultimate responsibility for ensuring the quality of water used for dialysis.

[b] Unless otherwise indicated.

[1] 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 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 1 *(continued)*

Contaminant	Maximum concentration mg[b]
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 ultimate responsibility for ensuring the quality of water used for dialysis.

[b] Unless otherwise indicated.

[1] 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 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

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

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 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 at present not possible to define 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, or lower if required by national legislation or regulations. 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, or lower if required by national legislation or regulations. 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.

NOTE See 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 in accordance with 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 bacterial 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 (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 a period of 7 days and Tryptic Soy Agar (TSA) at an incubation temperature of 35 °C to 37 °C and an incubation time of 48 h[8]. The background for the inclusion of TSA for standard water and standard dialysis fluid used for standard dialysis is explained in detail in A.4.

Different media types and incubation periods can result in varying colony concentrations and types of microorganisms recovered[8][9][10]. The use of Reasoner's 2A agar (R2A) has been shown in previous studies to result in higher colony counts than tryptic soy agar (TSA) for water and dialysis fluids samples[10][11][12]. In a more recent publication, in 2016, the authors indicated that there were no significant differences for comparisons of bacterial burden of standard dialysis water and standard dialysis fluid yielding colony counts ≥50 CFU/ml when assayed using R2A and TSA at the conditions stated in the preceding paragraph of this subclause [8].

Historic studies with tryptone glucose extract agar (TGEA) incubated at 17 °C to 23 °C for a period of 7 days also yielded higher colony counts than TSA.[13] Maltais et al.[8] 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 at an incubation temperature of 35 °C to 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 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 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 methods detailed above. 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].

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 indication of their presence is required, membrane filtration is the preferred method for the provision of 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.

The presence of endotoxins shall be determined by a *Limulus* amoebocyte lysate (LAL) assay or other validated method.

Table 3 — Culture techniques

Culture medium	Incubation temperature	Incubation time
Tryptone Glucose Extract Agar (TGEA)	17 °C to 23 °C	7 d
Reasoner's Agar no. 2 (R2A)	17 °C to 23 °C	7 d
Sabouraud or Malt Extract Agar[a]	17 °C to 23 °C	7 d
Tryptic Soy Agar (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 been only validated for measurement of standard dialysis water.		

5.3 Chemical contaminants test methods

Conformity with the requirements listed in Table 1 can be shown by using chemical analysis methods referenced by the ISO[1][2][3], the American Public Health Association[4] or the US Environmental Protection Agency[5][6] methods referenced in applicable pharmacopoeia, or by any other equivalent validated analytical method.

Conformity to the requirements listed in Table 2 can be shown in one of the three ways below.

— Where such testing is available, the individual contaminants in Table 2 can be determined using chemical analysis methods referenced by ISO[1][2][3], the American Public Health Association[4] or the US Environmental Protection Agency[5][6], or other equivalent analytical methods.

— 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 defined by the WHO or local regulations[7], 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 or local regulations 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 lists for information suitable test methods for each contaminant, along with an appropriate reference.

Table 4 — Analytical test methods for chemical contaminants

Contaminant	Analytical technique	Reference, method number
Aluminium	Inductively coupled plasma mass spectrometry or Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Antimony	Inductively coupled plasma mass spectrometry or Atomic absorption (platform)	ISO 17294–2:2016 US EPA, #200.9
Arsenic	Inductively coupled plasma mass spectrometry or Atomic absorption (gaseous hydride)	ISO 17294–2:2016 American Public Health Assn, #3114
Barium	Inductively coupled plasma mass spectrometry or Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Beryllium	Inductively coupled plasma mass spectrometry or Atomic absorption (platform)	ISO 17294–2:2016 US EPA, #200.9
Cadmium	Inductively coupled plasma mass spectrometry or Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Calcium	Inductively coupled plasma mass spectrometry or EDTA (Ethylene diamine tetraacetic acid) titrimetric method or atomic absorption (direct aspiration) or ion specific electrode	ISO 17294–2:2016 American Public Health Assn, #3500-Ca D American Public Health Assn, #3111B
Total chlorine	DPD(N-Diethyl-p-Phenylenediamine) ferrous titrimetric method or DPD (N-Diethyl-p-Phenylenediamine)colourimetric method Thio-Michler's Ketone (TMK/MTK) colourimetric method	American Public Health Assn, #4500-Cl F American Public Health Assn, #4500-Cl G
Chromium	Inductively coupled plasma mass spectrometry or Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Copper	Inductively coupled plasma mass spectrometry or Atomic absorption (direct aspiration) or neocuproine method	ISO 17294–2:2016 American Public Health Assn, #3111 American Public Health Assn, #3500-Cu D
Fluoride	Ion chromatography or Ion selective electrode method or sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonate (SPADNS) method	ISO 10304–1:2007 ISO 10359–1:1992 American Public Health Assn, #4500-F^- C American Public Health Assn, #4500-F^- D
Lead	Inductively coupled plasma mass spectrometry Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Magnesium	Inductively coupled plasma mass spectrometry or Atomic absorption (direct aspiration) Ion chromatography	ISO 17294–2:2016 American Public Health Assn, #3111 EPA 300.7:1986
Mercury	Flameless cold vapour technique (atomic absorption)	American Public Health Assn, #3112

Table 4 *(continued)*

Contaminant	Analytical technique	Reference, method number
Nitrate	Ion chromatography or Spectrophotometric method using sulfosalicylic acid or Cadmium reduction method	ISO 10304–1:2007 ISO 7890–3:1988 American Public Health Assn, #4500-NO3 E
Potassium	Inductively coupled plasma mass spectrometry or Atomic absorption (direct aspiration) or flame photometric method or ion specific electrode	ISO 17294–2:2016 American Public Health Assn, #3111 American Public Health Assn, #3500-K D American Public Health Assn, #3500-K E
Selenium	Inductively coupled plasma mass spectrometry or Atomic absorption (gaseous hydride) or atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3114 American Public Health Assn, #3113
Silver	Inductively coupled plasma mass spectrometry or Atomic absorption (electrothermal)	ISO 17294–2:2016 American Public Health Assn, #3113
Sodium	Inductively coupled plasma mass spectrometry or Atomic absorption (direct aspiration) or flame photometric method or ion specific electrode	ISO 17294–2:2016 American Public Health Assn, #3111 American Public Health Assn, #3500-Na D
Sulfate	Ion chromatography or Turbidimetric method	ISO 10304–1:2007 American Public Health Assn, #4500-SO_4^{2-} E
Thallium	Inductively coupled plasma mass spectrometry or Atomic absorption (platform)	ISO 17294–2:2016 US EPA, 200.9
Total heavy metals	Colourimetric	European Pharmacopoeia, 2.4.8 US Pharmacopoeia, < 231 >
Zinc	Inductively coupled plasma mass spectrometry or Atomic absorption (direct aspiration) or dithizone method	ISO 17294–2:2016 American Public Health Assn, #3111 American Public Health Assn, #3500-Zn D

Annex A
(informative)

Rationale for the development and provisions of this document

A.1 General

Water treated in accordance with the requirements of this standard 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 in accordance with ISO 23500-4:2019, the requirements detailed in ISO 23500-5:2019 apply.

A.2 Feed water

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 define 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 of this document.

If the feed water to the water treatment infrastructure is via an indirect feed, e.g. 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; *Guidance for the preparation and quality management of fluids for haemodialysis and related therapies — Part 2: Water treatment equipment for haemodialysis applications and related therapies.* Removal of those compounds renders the water susceptible to bacterial proliferation and biofouling unless appropriate preventative measures are taken as outlined in ISO 23500-1 *Guidance for the preparation and quality management of fluids for haemodialysis and related therapies — Part 1: General requirements*.

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[14]. Cyanobacterial blooms usually occur according to a combination of environmental factors e.g. 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[15].

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; 1) chemicals known to cause toxicity in dialysis patients; 2) physiological substances that can adversely affect the patient if present in the dialysis fluid in excessive amounts and 3) 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[16]. Isolated cases of acute exposure of dialysis patients to elevated levels of fluoride has been described in the scientific literature. Illness in a group of eight dialysis patients, with the death of one patient, was reported as a result of accidental over fluoridation of a municipal water supply[16]. 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. Three of the patients died from ventricular fibrillation. Fluoride concentrations in the water used to prepare the dialysis fluid were as high as 22,5 mg/l[17].

Aluminium is toxic to hemodialysis 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 hemodialysis patients, exposure to aluminium can result in severe neurologic symptoms[18][19].

The maximum aluminium level set for dialysis water has been specified to prevent accumulation of this toxic metal in the patient[20][21]. Despite this, occasional sporadic outbreaks of aluminium intoxication have been reported (e.g. an outbreak in 1993 was traced to aggressive alum flocculation of water under conditions of extreme drought while, in 2001, acute aluminium encephalopathy in a dialysis centre was attributed to aluminium leaching from a cement mortar water distribution pipe[22][23].

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 byproducts[24].

Exposure of hemodialysis 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 hemodialysis patient from haemolytic reactions (haemolysis, haemolytic anaemia, and methaemoglobinaemia) and, EPO resistance[24][25][26][27][28][29]. 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, the working group chose to simplify this situation by setting a maximum allowable level for total chlorine at the same value used previously for chloramine (0,1 mg/l), thus permitting a single test to be used. It should be noted that total chlorine is defined 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 should 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 could 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[30]. 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 may switch to chlorine dioxide as a disinfectant.

Sulfate can be found in almost all natural water. 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[31].

Nitrates are a marker for bacterial contamination and fertilizer runoff and have caused methaemoglobinaemia[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 may be necessary.

Both copper and zinc toxicity have been demonstrated when these substances are present in dialysis fluid at levels below those permitted by the US Environmental Protection Agency (EPA) standard for drinking water[33][34]. Both levels for dialysis water are set below that permitted in drinking water.

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. Dialysis fluid lead levels of 52 µg/l to 65 µg/l have been associated with abdominal pain and muscle weakness[35]. There

is no evidence of lead toxicity when lead levels in water or dialysis fluid are below 5 µg/l. 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[36]. 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[37].

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 %.

A.3.4 Trace metals and other compounds

Little, if any, 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 Safe Drinking Water Act when the document was originally developed and was based on the 1992 version of ANSI/AAMI RD5, Hemodialysis systems. 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) (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 defined 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 nonenforceable public health goals.

At this time the compounds included are barium, selenium, chromium, silver, cadmium, mercury, and arsenic. Selenium and chromium levels in dialysis water were set at the “no-transfer” level· The “no-transfer” level was chosen even though it is 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 could occur in the blood, and because there is reduced renal excretion of these substances. These reduced limits were selected using the following assumptions: 1) feed water entering dialysis systems typically meets the drinking water requirements (i.e. complies with regulatory requirements in respect of contaminant levels); 2) the water treatment system incorporates reverse osmosis, which typically, would remove 90 % to 99 % of dissolved inorganic solids; and 3) reverse osmosis-treated water is a suitable standard for safety of water used in dialysis. These assumptions are based on the recommendations of a report “Investigation of the Risks and Hazards Associated with Hemodialysis Systems” authored by Keshaviah et al.[38] 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.

It should be noted that in respect of arsenic, the above reference contains a typographical error and the value given in Table 2 is incorrect. The correct value is 0,005 mg/l as given in Table 2 of this document. It should further be noted that the current drinking water maximum contaminant level for this compound is set at 0,01 mg/l (effective from 01/23/2016).

Several changes occurred in the *Safe Drinking Water Act* after these levels were included; specifically, antimony, beryllium, free cyanide, and thallium were added to the list of contaminants covered by the Act and the maximum allowable level for cadmium decreased. For consistency, antimony, beryllium, and thallium were added to the list of contaminants.

The maximum allowable levels of antimony and thallium were set at values above one-tenth of the US EPA maximum allowable level because of limitations in the sensitivity of commonly available analytical methods for these two contaminants. After considerable discussion, it was decided not to add free cyanide to the list of contaminants. There was concern that special requirements for sample collection and shipment, together with the need to pre-treat the sample before analysis to eliminate interfering substances, would impose a burden on dialysis facilities that could not be justified in the absence of specific toxicity data. It was also decided not to decrease the maximum allowable level of cadmium in the list of contaminants. This decision was based on the absence of toxicity data in dialysis patients treated with water that meets this document and the minimum detection levels of currently used analytical methods.

There was discussion about moving Table 2 to this Annex during previous revisions. This discussion was prompted by the on-going 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, 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 are provided. The preferred option is 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 of this document should not be taken as a definitive list of harmful substances but as a partial listing of those that might 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. The concern was raised that this practice could cause significant problems in water purification.

Water used in the preparation of water for dialysis can also contain organic contaminants. However, the long-term effects of organic contaminants on hemodialysis patients are unknown. To date, there has been only one report of contamination of hemodialysis water supply with an organic compound (trichloroethylene)[39].

In view of the limited availability of data in respect of patient exposure to organic compounds, the working group, has 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[40].

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 bacterial proliferation and the formation of biofilm. Once formed, the biofilm is difficult to remove and results in the release of bacteria and bacterial fragments (endotoxins, muramylpeptides, and polysaccharides)[41].

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[42] to [51]. Such transfer induces cytokines and contributes to pyrogenic reactions and microinflammation seen in haemodialysis patients[52][53].

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 studies which demonstrated that the incidence of pyrogenic reactions were linked to bacterial load in the dialysis fluid[54][55][56]. Later, 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 to 7 d can elapse between sampling water for the determination of microbiological contamination and receiving results, and because bacterial 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 bacterial 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 could 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 for the second edition of this document in 2008, 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 during the most recent revisions.

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[57][58].

Cyanotoxin species have been involved in dialysis patient exposure which occurred in Brazil in 1996 and 2001. During the first, 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[59] to [64]. In the course of the current revision the working group discussed the issue of cyanotoxins. 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 the working group felt that there should be awareness of the 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 cyanobacterial 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 bacterial 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 the purpose of the analysis. The method selected should also consider the advantages, disadvantages and sensitivity of each of the suggested methods. 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][67].

Recommended methods and cultivation conditions can be found in ISO 23500-4:2019 and ISO 23500-5:2019 as well as this document (Table 3). In the current revision as well as Tryptone Glucose Extract Agar (TGEA) and Reasoner’s Agar No. 2 (R2A) incubated at 17 °C to 23 °C for a period of 7 days, Tryptic Soy Agar (TSA) at an incubation temperature of 35 °C to 37 °C at an incubation time of 48 hours has been included for analysis of water and dialysis fluid used in standard haemodialysis based upon a recent (2016) publication supporting comparable methods to those of the previously recommended methods and cultivation conditions.

The rationale for inclusion of TSA in the current revision merits an explanation. The working group were requested by the US to consider including TSA as a method. The working group debated this request, and consented to its inclusion, provided an up to date validation of the TSA method compared to the ISO recommended R2A and TGEA methods was produced. To comply with this, Maltais et al. in 2016 published a study in which dialysis water and fluid samples collected from 41 US dialysis programs between 2011 and 2014 were cultured at two US laboratories[8]. Each sample was cultured using either TGEA-7d or R2A-7d and TSA-48h. The findings of this publication were discussed at the most recent group meeting. Briefly, the authors found that there were no significant differences for comparisons of bacterial burden yielding colony counts ≥ 50 CFU/ml for both standard dialysis water and dialysis fluid when assayed using R2A or TSA at the conditions stated above. A comparison of TSA with TGEA however showed that the proportion of standard dialysis water samples yielding colony counts ≥ 50 CFU/ml quantified using TGEA incubated at 17 °C to 23 °C for a period of 7 days showed a statistically significant difference from the proportion established by 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 did not show a statistically significant difference. Based on these observations, the working group agreed to include TSA as a recommended method for indicating microbial burden in water and dialysis fluid used for conventional or standard dialysis.

In addition to bacteria and endotoxins, yeasts and filamentous fungi can also be present and their presence implies a potential risk to the patient[65][66]. Further studies are required to investigate the organism’s ability to persist, their role in biofilm formation and their clinical significance. In view of this, no limits in respect of yeasts and filamentous fungi have been set in this revision. Should the

presence of yeasts and filamentous fungi in fluids be of clinical concern, Malt Extract Agar (MEA) can be used to identify the species rather than Sabouraud Agar which is less effective. For mould, Corn Meal Agar or Czapek-Dox Agar are suitable growth media.

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