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Quality of dialysis fluid for haemodialysis and related therapies

Qualité des fluides de dialyse 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 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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 150, *Implants for surgery*, Subcommittee SC 2, *Cardiovascular implants and extracorporeal systems*.

This second edition cancels and replaces the first edition (ISO 11663:2009) which has been technically revised.

Introduction

Haemodialysis patients are directly exposed to large volumes of dialysis fluid, with the dialyser membrane being the only barrier against transfer of hazardous contaminants from the dialysis fluid to the patient. It has long been known that there could be hazardous contaminants in the water and concentrates used to prepare the dialysis fluid. To minimize this hazard, ISO 13958 and ISO 13959 set forth quality requirements for the water and concentrates used to prepare dialysis fluid. However, if the dialysis fluid is not prepared carefully, it could contain unacceptable levels of contaminants even though it is prepared from water and concentrates, meeting the requirements of ISO 13958 and ISO 13959. Further, the dialysis fluid might be used as the starting material for the online preparation of fluids intended for infusion into the patient, for example, in therapies such as online haemodiafiltration. For these reasons, this International Standard for dialysis fluid quality was developed to complement the existing International Standards for water and concentrates, ISO 13959 and ISO 13958, respectively. Guidelines to aid the user in routinely meeting the requirements of this International Standard and ISO 13959 can be found in ISO 23500.

Within these International Standards, measurement techniques current at the time of preparation have been cited. Other standard methods can be used, provided that such methods have been appropriately validated and compared to the cited methods.

This International Standard reflects the conscientious efforts of healthcare professionals, patients, and medical device manufacturers to develop recommendations for the quality of dialysis fluid. This International Standard is directed at the healthcare professionals involved in the management of dialysis facilities and the routine care of patients treated in dialysis facilities, since they are responsible for the final preparation of dialysis fluid. The recommendations contained in this International Standard are not intended for regulatory application.

The requirements of this International Standard aim to help protect haemodialysis patients from adverse effects arising from known chemical and microbiological contaminants that can be found in improperly prepared dialysis fluid. However, the physician in charge of dialysis has the ultimate responsibility for ensuring that the dialysis fluid is correctly formulated and meets the requirements of all applicable quality standards.

The verbal forms used in this International Standard conform to usage described in Annex H of the ISO/IEC Directives, Part 2. For the purposes of this International Standard, the auxiliary verb

— “shall” means that compliance with a requirement or a test is mandatory for compliance with this International Standard,

— “should” means that compliance with a requirement or a test is recommended but is not mandatory for compliance with this International Standard, and

— “may” is used to describe a permissible way to achieve compliance with a requirement or test.

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

Quality of dialysis fluid for haemodialysis and related therapies

1 Scope

This International Standard specifies minimum quality requirements for dialysis fluids used in haemodialysis and related therapies.

This International Standard includes dialysis fluids used for haemodialysis and haemodiafiltration, including substitution fluid for haemodiafiltration and haemofiltration.

This International Standard does not address the requirements for the water and concentrates used to prepare dialysis fluid or the equipment used in its preparation. Those areas are covered by other International Standards.

Sorbent-based dialysis fluid regeneration systems that regenerate and recirculate small volumes of dialysis fluid, systems for continuous renal replacement therapy that use prepackaged solutions, and systems and solutions for peritoneal dialysis are excluded from this International Standard.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13958, *Concentrates for haemodialysis and related therapies*

ISO 13959, *Quality of water for haemodialysis and related therapies*

3 Terms and definitions

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

3.1
acid concentrate
A-concentrate
acidified concentrated mixture of salts that, when diluted with dialysis water and bicarbonate concentrate, yields dialysis fluid for use in dialysis

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

Note 2 to entry: Acid concentrate may contain glucose.

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

3.2
action level
concentration of a contaminant at which steps should be taken to interrupt the trend toward higher, unacceptable levels

3.3
bicarbonate concentrate
B-concentrate
concentrated preparation of sodium bicarbonate that, when diluted with dialysis water and acid concentrate, makes dialysis fluid used for dialysis

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

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

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

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

3.4
central dialysis fluid delivery system
system that produces dialysis fluid from dialysis water and concentrate or powder at a central point and distributes the dialysis fluid from the central point to individual dialysis machines

3.5
chlorine, combined
chlorine that is chemically combined, such as in chloramine compounds

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

3.6
chlorine, free
chlorine present in water as dissolved molecular chlorine (Cl), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-)

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

3.7
chlorine, total
sum of free and combined chlorine

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

3.8
colony-forming unit
CFU
measure of bacterial or fungal cell numbers that theoretically arise from a single cell when grown on solid media

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

3.9
concentrate generator
system where the concentrate is delivered to the user as a powder in a container, suitable for attachment to the dialysis machine with which it is intended to be used, and then the powder is converted into a concentrated solution by the dialysis machine

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

3.10
device
individual water purification unit, such as a softener, carbon bed, reverse osmosis unit, or deionizer

Note 1 to entry: This term is synonymous with the term “component” as used by the US Food and Drug Administration (see Reference [48]).

3.11
dialysis fluid
dialysate
dialysis solution
aqueous fluid containing electrolytes and, usually, buffer and glucose, which is intended to exchange solutes with blood during haemodialysis

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

Note 2 to entry: The dialysis fluid entering the dialyser is referred to as “fresh dialysis fluid”, while the fluid leaving the dialyser is referred to as “spent dialysis fluid”.

Note 3 to entry: Dialysis fluid does not include prepackaged parenteral fluids used in some renal replacement therapies, such as haemodiafiltration and haemofiltration.

3.12
dialysis fluid delivery system
device that prepares dialysis fluid online from dialysis water and concentrates or that stores and distributes premixed dialysis fluid; circulates the dialysis fluid through the dialyser; monitors the dialysis fluid for temperature, conductivity (or equivalent), pressure, flow, and blood leaks; and, prevents dialysis during disinfection or cleaning modes

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

Note 2 to entry: The dialysis fluid delivery system might be an integral part of the single-patient dialysis machine or a centralized preparation system which feeds multiple bedside monitoring systems.

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

3.13
dialysis water
water that has been treated to meet the requirements of ISO 13959 and which is suitable for use in haemodialysis applications, including the preparation of dialysis fluid, reprocessing of dialysers, preparation of concentrates, and preparation of substitution fluid for online convective therapies

3.14
disinfection
destruction of pathogenic and other kinds of microorganisms by thermal or chemical means

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

3.15
endotoxin
major component of the outer cell wall of gram-negative bacteria

Note 1 to entry: Endotoxins are lipopolysaccharides, which consist of a polysaccharide chain covalently bound to lipid A. Endotoxins can acutely activate both humoral and cellular host defences, leading to a syndrome characterized by fever, shaking, chills, hypotension, multiple organ failure, and even death if allowed to enter the circulation in a sufficient dose. [see also *pyrogen* (3.25)].

3.16
endotoxin units
EU
units assayed by the *Limulus* amoebocyte lysate (LAL) test when testing for endotoxins

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

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

3.17
haemodiafiltration
form of renal replacement therapy in which waste solutes are removed from blood by a combination of diffusion and convection through a high-flux membrane

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

3.18
haemodialysis
form of renal replacement therapy in which waste solutes are removed primarily by diffusion from blood flowing on one side of a membrane into dialysis fluid flowing on the other side

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

3.19
haemofiltration
form of renal replacement therapy in which waste solutes are removed from blood by convection

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

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

3.20
***Limulus* amoebocyte lysate test**
LAL test
assay used to detect endotoxin

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

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

3.21
manufacturer
entity that designs, manufactures, fabricates, assembles, or processes a finished device

Note 1 to entry: Manufacturers include, but are not limited to, those who perform the functions of contract sterilization, installation, relabelling, remanufacturing, repacking or specification development, and initial distributions of foreign entities performing these functions. The term does not cover preparation of concentrates from prepackaged dry chemicals at a dialysis facility or the handling of bulk concentrates at a dialysis facility after responsibility for the concentrate is transferred from the manufacturer to the user.

3.22
microbiological contamination
contamination with any form of microorganism (e.g. bacteria, yeast, fungi, and algae) or with the by-products of living or dead organisms such as endotoxins, exotoxins, and cyanobacterial toxins (derived from blue-green algae)

3.23
nonpyrogenic
not eliciting a pyrogen reaction

Note 1 to entry: Historically, the threshold pyrogenic dose of 5 EU/kg/h (the minimum dose that produces fever) has been used to set endotoxin limits of devices and injectable medications.

Note 2 to entry: The volume of fluid administered should not exceed the volume that would result in a total dose of endotoxin of ≥5 EU/kg/h.

Note 3 to entry: This definition is applicable for fluids produced by online techniques, e.g. substitution and priming fluids.

Note 4 to entry: The commonly used gel clot method has a sensitivity limit of 0,03 EU/ml.

3.24
proportioning system
apparatus that proportions dialysis water and haemodialysis concentrate to prepare dialysis fluid

3.25
pyrogen
fever-producing substance

Note 1 to entry: Pyrogens are most often lipopolysaccharides of gram-negative bacterial origin [see also *endotoxin* (3.15)].

3.26
sterile
free from viable microorganisms

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

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

3.27
substitution fluid
fluid used in haemofiltration and haemodiafiltration treatments which is infused directly into the patient's blood as a replacement for the fluid that is removed from the blood by filtration

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

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

3.28
ultrapure dialysis fluid
highly purified dialysis fluid that can be used in place of conventional dialysis fluid

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

3.29
user
physician or physician's representative or healthcare professional with a responsibility for the prescription, production, and delivery of dialysis fluid

3.30
water treatment system
collection of water treatment devices and associated piping, pumps, valves, gauges, etc., that together produce dialysis water meeting the requirements of ISO 13959 for haemodialysis applications and deliver it to the point of use

4 Requirements

4.1 Microbiological contaminants in dialysis fluid

4.1.1 General

The requirements contained in this clause apply to a sample of the dialysis fluid collected at the inlet to the dialyser or the reinfusion point.

4.1.2 Microbiological requirements for standard dialysis fluid

Standard dialysis fluid shall contain a total viable microbial count of less than 100 CFU/ml (when tested in accordance with Clause 5) and an endotoxin concentration of less than 0,5 EU/ml (when tested in accordance with Clause 5).

Action levels for the total viable microbial count and endotoxin concentration in dialysis fluid should also be set based on knowledge of the microbial dynamics of the system. Typically, the action levels are set at 50 % of the maximum allowable levels for total viable microbial count and endotoxin; other levels may be set.

If microbial counts exceeding the action levels are observed in the dialysis fluid, corrective measures, such as disinfection and retesting, should be taken promptly to reduce the levels.

4.1.3 Microbiological requirements for ultrapure dialysis fluid

Ultrapure dialysis fluid shall contain a total viable microbial count of less than 0,1 CFU/ml (when tested in accordance with Clause 5) and an endotoxin concentration less than 0,03 EU/ml (when tested in accordance with Clause 5). If those limits are exceeded in ultrapure dialysis fluid, corrective measures should be taken to reduce the levels to an acceptable range. The user is responsible for monitoring the dialysis fluid bacteriology of the system following installation. It is incumbent on the user to establish a regular monitoring routine.

4.1.4 Microbiological requirements for online prepared substitution fluid

The requirements contained in this clause apply to online prepared fluid intended to be infused into the patient as it enters the patient's blood.

This fluid shall be sterile and nonpyrogenic.

Substitution fluid for convective therapies, such as haemodiafiltration and haemofiltration, may be produced online by a process of ultrafiltration with bacteria-retentive and endotoxin-retentive filters. This online process shall be validated to produce fluid that is sterile and nonpyrogenic.

Compliance of online produced fluid with the requirements of this International Standard cannot be demonstrated with traditional test procedures. For this reason, compliance with this International Standard shall be ensured by proper operation of a validated system, verified according to the manufacturer's instructions at the time of installation, and confirmed by the user with a regular

monitoring and maintenance schedule. The user shall follow the manufacturer's instructions for use of the validated system, and the user's monitoring and maintenance schedule shall be designed to confirm that the water and concentrates used to prepare the substitution fluid continue to meet the specifications of ISO 13958 and ISO 13959.

4.2 Chemical contaminants in dialysis fluid

Dialysis fluid shall be prepared from water meeting the requirements of ISO 13959 and acid and bicarbonate concentrates meeting the requirements of ISO 13958. The water and concentrates shall be combined using individual dialysis fluid delivery systems or a central dialysis fluid delivery system constructed from materials that do not contribute chemical contaminants to the final dialysis fluid. The maximum levels of chemical contaminants permitted in water used to prepare dialysis fluid and concentrates are given in Tables B.1 and B.2.

5 Tests for compliance with microbiological requirements

Samples shall be collected where the dialysis fluid enters the dialyser.

NOTE In some newer dialysis machines, dialysis fluid flow stops when the effluent line is disconnected from the dialyser. In these instances, the machines are equipped with dialysis fluid sampling ports that can be accessed using a syringe. Sample ports can be disinfected with alcohol and allowed to air-dry. A sterile syringe can then be used to aspirate dialysis fluid out of and into the port before filling the syringe. The filled syringe is discarded, and a fresh sample of dialysis fluid is collected using a new sterile syringe. For sample ports consisting of a simple septum penetrated with a needle, the use of a second syringe is not necessary. Alternatively, if the dialysis machine permits, samples can be collected immediately after the dialyser by disconnecting the effluent connector and aseptically collecting a "free/clean" catch sample after allowing dialysis fluid to run for 30 s to 60 s.

Microbial analysis of water and dialysis fluid samples should be conducted as soon as possible after collection to avoid unpredictable changes in the microbial population. If samples cannot be analysed within 4 h of collection, follow the laboratory's instructions for sample storage and shipping. Samples intended for colony counts should not be frozen.

Total viable microbial counts (standard plate counts) shall be obtained using conventional microbiological assay procedures (pour plate, spread plate, membrane filter techniques). The calibrated loop technique shall not be used.

Preferred methods and sample volumes:

Dialysis fluid, routine test:

— spread plate, 0,1 ml to 0,3 ml;

— pour plate, typically 1 ml.

Ultrapure dialysis fluid, routine test:

— membrane filtration, 10 ml to 1 000 ml.

Substitution fluid:

— sterility cannot be proven by sampling.

Culture media shall be tryptone glucose extract agar (TGEA) or Reasoner's 2A supplemented with 4 % sodium bicarbonate, or equivalent. Blood or chocolate agar shall not be used. Incubation temperatures of 17 °C to 23 °C, and an incubation time of 168 h (7 d) are recommended. Other test methods may also be used, provided such methods have been appropriately validated and compared to the cited methods.

Compliance with the microbial standards for ultrapure dialysis fluid and substitution fluid prepared online with a validated system can be met by following the requirements and instructions of the manufacturer of the dialysis fluid delivery system.

The presence of pyrogens shall be determined by the *Limulus* amoebocyte lysate test for endotoxins.

Annex A
(informative)

Rationale for the development and provisions of this International Standard

A.1 Microbiological contaminants in dialysis fluid

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

Pyrogenic reactions are caused by lipopolysaccharides or endotoxins that are associated with gram-negative bacteria. Furthermore, gram-negative water bacteria have been shown to have the capability of multiplying rapidly in a variety of hospital-associated fluids, including distilled, deionized, reverse osmosis, and softened water, all of which have been used in the past as supply water for haemodialysis systems. The dialysis fluid, which is a balanced salt solution made with this water, likewise provides a very good growth medium for these types of bacterium. Several studies have demonstrated that the incidence of pyrogenic reactions can be related directly to the number of bacteria in dialysis fluid.[10][14][15] 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).[18]

Several investigators[22][23] have shown that bacteria growing in dialysis fluid can produce products that cross dialysis membranes. It has also been shown[17][37] that gram-negative bacteria growing in dialysis fluid produced endotoxins, that in turn stimulated the production of anti-endotoxin antibodies in haemodialysis patients. These data suggest that bacterial endotoxins, although relatively large molecules, do indeed cross dialysis membranes, either intact or as fragments. The use of the very permeable membranes known as high-flux membranes has raised the possibility of a greater likelihood of passage of endotoxins into the blood path. Several studies support this contention. Vanholder et al.[52] observed an increase in plasma endotoxin concentrations during dialysis against dialysis fluid containing 10^3 CFU/ml to 10^4 CFU/ml *Pseudomonas* species. *In vitro* studies using both radiolabelled lipopolysaccharide and biological assays have demonstrated that biologically active substances derived from bacteria found in dialysis fluid can cross a variety of dialysis membranes.[26][13][29][50][9][43][53][44] Also, patients treated with high-flux membranes are reported to have higher levels of anti-endotoxin antibodies than normal subjects or patients treated with conventional low-flux membranes.[54] Finally, it was reported that the use of high-flux dialysers is a significant risk factor for pyrogenic reactions.[46] Although other investigators have not been able to demonstrate endotoxin transfer across dialysis membranes,[6][8] the preponderance of reports now supports the ability of endotoxin to transfer across at least some high-flux membranes under some operating conditions. Furthermore, in a Japanese Society for Dialysis Therapy (JSDT) survey, the 1-year mortality rate was significantly higher at facilities with a dialysis fluid endotoxin concentration of >0.100 EU/ml.[55] Consequently, it seems prudent to impose an upper limit on the endotoxin content of dialysis water and dialysis fluid. A level of 2 EU/ml was chosen by AAMI in 2001 as the upper limit for endotoxin, since these levels were easily achieved with contemporary water treatment systems using reverse osmosis, ultrafiltration, or both. At the same time, the European Community chose to use 0,25 EU/ml as the maximum allowable level of endotoxin in dialysis water. When ISO 13959 was revised in 2009, the 0,25 EU/ml limit for dialysis water was included. In developing this International Standard for dialysis fluid quality, the maximum allowable level of endotoxin was set at 0,5 EU/ml by the *Limulus* amebocyte lysate test.

The level is set higher than that for dialysis water in recognition that both the water and concentrates used in the preparation of dialysis fluid can contribute endotoxin.

In addition to the acute risk of pyrogenic reactions, there is increasing indirect evidence that chronic exposure to low amounts of endotoxin might play a role in some of the long-term complications of

haemodialysis therapy. Patients treated with ultrafiltered dialysis fluid have demonstrated a decrease in serum β_2-microglobulin concentrations,[35][33] a decrease in markers of an inflammatory response and oxidant stress,[42][45][40][36][19][4][20][16] and an increased responsiveness to erythropoietin.[45][31][19][36] In longer term studies, use of ultrafiltered dialysis fluid has been associated with a decreased incidence of β_2-microglobulin-associated amyloidosis,[5][25][39] better preservation of residual renal function,[32][41] and improved nutritional status.[40][4][33] These observations have led to the recommendation that dialysis fluid of a higher microbiological quality, so-called "ultrapure" dialysis fluid, should be used for routine haemodialysis (see Reference [12]). Ultrapure dialysis fluid is defined as one having a bacterial content of less than 0,1 CFU/ml and an endotoxin content of less than 0,03 EU/ml using sensitive assays.[27] This definition is now widely accepted, particularly in Europe, as the standard for dialysis fluid used to prepare substitution solution for online convective therapies. In developing this International Standard, the desirability of using ultrapure dialysis fluid was recognized, but it was accepted that obtaining this level of purity on a routine basis might not yet be feasible in all dialysis settings.

Because 7 d can elapse between sampling dialysis fluid for the determination of microbiological contamination and receiving results, and because bacterial proliferation can be rapid, action levels for microbial counts were introduced into this International Standard. These action levels allow the user to initiate corrective action before levels exceed the maximum levels established by this International Standard.

In haemodialysis, the net movement of water is from the blood to the dialysis fluid, although within the dialyser there can be movement of dialysis fluid to the blood due to the phenomenon of back-filtration, particularly in dialysers with highly permeable membranes.[28] In contrast, haemofiltration and haemodiafiltration feature infusions of large volumes of electrolyte solution (20 l to more than 100 l) into the blood. Increasingly, this electrolyte solution is being prepared online from water and concentrate. The large volumes of fluid infused in haemofiltration and haemodiafiltration, and general concerns about the transfer of endotoxin and endotoxin fragments across high-flux membranes, necessitate the use of ultrapure dialysis fluid in the online preparation of substitution fluid for haemofiltration and haemodiafiltration.

A.2 Chemical contaminants in dialysis fluid

When this International Standard was being developed, the need to include maximum levels for chemical contaminants in dialysis fluid was discussed. It was proposed that the maximum allowable levels of chemical contaminants in dialysis fluid should be the same as those in the water used to prepare the dialysis fluid since there were no data supporting the need for lower levels. Dialysis fluid is prepared from water and concentrates, meeting the requirements of ISO 13959 and ISO 13958, including the same requirements for maximum levels of chemical contaminants that were proposed for inclusion in this International Standard. Because the water and concentrates are combined using individual dialysis machines or central dialysis fluid delivery systems that are required to be constructed of materials that do not contribute chemical contaminants to the dialysis fluid, it was concluded that including maximum allowable levels of chemical contaminants in the dialysis fluid would be redundant and impose an unnecessary burden on dialysis facilities.

A.3 Tests for compliance with microbiological requirements

The original clinical observations showing a relationship between bacterial levels in dialysis fluid and pyrogenic reactions were based on cultures performed with standard methods agar (SMA), a medium containing relatively few nutrients.[14] Later, the use of tryptic soy agar (TSA), a general-purpose medium for isolating and cultivating fastidious organisms was recommended because it was thought more appropriate for culturing bicarbonate-containing dialysis fluid. However, several studies have shown that the use of tryptone glucose extract agar (TGEA) or R2A results in an increased recovery of bacteria from water and dialysis fluid.[27][51][34][38] Extending the culturing time up to 168 h and using a lower incubation temperature have also been shown to increase the recovery of bacteria compared to incubation for 48 h at 35 °C as recommended in ANSI/AAMI RD52.[2][27][34][38] The membrane filtration technique is applied when higher sensitivity is required or desired. The use of larger volumes (up to 1 000 ml) will provide greater sensitivity, but the improved sensitivity needs to be balanced against

the increased risk of contamination in collecting and handling the sample. Even with the most sensitive techniques, compliance with the stringent requirements for online prepared substitution fluid cannot be demonstrated by culturing; it has to be ensured by use of a validated process.

The culturing conditions recommended in this International Standard could fail to identify the presence of some organisms. Specifically, the recommended method might not detect the presence of various non-tuberculous mycobacteria that have been associated with several outbreaks of infection in dialysis units,[7][30] or fungi, and yeast, which have been shown to contaminate dialysis fluid.[24]

Annex B
(informative)

Reference tables from ISO 13959

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

Contaminant	Maximum concetraion (mg/l)[b]
Contaminants with documented toxicity in haemodialysis	
Aluminum	0,01
Total chlorine	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)

NOTE This table is reproduced from ISO 13959.

[a] A dialysis facility's medical director has the ultimate responsibility for ensuring the quality of water used for dialysis.

[b] Unless otherwise noted.

Table B.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	0,0004
Cadmium	0,001
Chromium	0,014
Mercury	0,0002
Selenium	0,09
Silver	0,005
Thallium	0,002

NOTE This table is reproduced from ISO 13959.

Chemical analyses of the substances listed in Tables B.1 and B.2 can be obtained by using methods referenced by ISO, the American Public Health Association,[3] or the US Environmental Protection

Agency,[47] or other equivalent analytical methods. Where testing for the individual trace elements listed in Table B.2 is not available, an analysis for total heavy metals can be used with a maximum allowable level of at 0,1 mg/l.

NOTE The maximum allowable levels of contaminants listed in Tables B.1 and B.2 include the anticipated uncertainty associated with the analytical methodologies listed in Table B.3.

Table B.3 — Analytical test methods for chemical contaminants

Contaminant	Analytical technique	Reference, method number
Aluminium	Inductively coupled plasma mass spectrometry or Atomic Absorption (electrothermal)	ISO 17294-2:2003 American Public Health Assn, #3113
Antimony	Inductively coupled plasma mass spectrometry or Atomic Absorption (platform)	ISO 17294-2:2003 US EPA, #200.9
Arsenic	Inductively coupled plasma mass spectrometry or Atomic Absorption (gaseous hydride)	ISO 17294-2:2003 American Public Health Assn, #3114
Barium	Inductively coupled plasma mass spectrometry or Atomic Absorption (electrothermal)	ISO 17294-2:2003 American Public Health Assn, #3113
Beryllium	Inductively coupled plasma mass spectrometry or Atomic Absorption (platform)	ISO 17294-2:2003 US EPA, #200.9
Cadmium	Inductively coupled plasma mass spectrometry or Atomic Absorption (electrothermal)	ISO 17294-2:2003 American Public Health Assn, #3113
Calcium	Inductively coupled plasma mass spectrometry or EDTA Titrimetric Method, or Atomic Absorption (direct aspiration), or Ion Specific Electrode	ISO 17294-2:2003 American Public Health Assn, #3500-Ca D American Public Health Assn, #3111B
Total chlorine	DPD Ferrous Titrimetric Method, or DPD Colourimetric Method 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 17294-2:2003 American Public Health Assn, #3113
Copper	Inductively coupled plasma mass spectrometry or Atomic Absorption (direct aspiration), or Neocuproine Method	ISO 17294-2:2003 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-napthalenedisulfonate (SPADNS) Method	ISO 17294-2:2003 ISO 10359-1:1992 American Public Health Assn, #4500-F^- C American Public Health Assn, #4500-F^- D
Lead	Inductively coupled plasma mass spectrometry or Atomic Absorption (electrothermal)	ISO 17294-2:2003 American Public Health Assn, #3113
Magnesium	Inductively coupled plasma mass spectrometry or Atomic Absorption (direct aspiration) Ion chromatography	ISO 17294-2:2003 American Public Health Assn, #3111 EPA 300.7:1986
Mercury	Flameless Cold Vapor Technique (Atomic Absorption)	American Public Health Assn, #3112
Nitrate	Ion chromatography or Spectrophotometric method using sulfosalicylic acid or Cadmium Reduction Method	ISO 10304-1:2007 ISO 78901-3:1988 American Public Health Assn, #4500-NO_3 E
NOTE This table is reproduced from ISO 13959.		