

Recommendations of the European Pharmacopoeia. The use of ultrapure water is however strongly recommended for conventional and high-flux dialysis modalities.

(Evidence level: C)

Commentary on Guideline IV.1

According to the degree of desired water purity, the complexity and the cost of the water treatment system may differ significantly. Two different grades of water purity may be used for haemodialysis: (i) pure water and (ii) ultrapure water.

Pure water is the basic form of treated water that is suitable for conventional haemodialysis modalities [9]. Water treatment relies on different technical options that are detailed in the Appendix [10]. Briefly, purified water is easily obtained from a purification system consisting of pre-treatment (softener, activated carbon, downsizing microfilters), a reverse osmosis (RO) unit [11], and deionizer [12,13] implemented in series to prevent mainly aluminum intoxication [14–16] but also the accumulation of various water pollutants [17–20]. Microbiological contamination of the delivered water should comply with the recommendations of the European Pharmacopoeia (bacterial count <100 CFU/ml and endotoxin content <0.25 EU/ml). Maximum levels of the different water purity grades are presented in Table 1.

Maximum water contaminant levels and methods of analysis recommended by the European Pharmacopoeia and compared with those of the AAMI are presented in Table 2.

Ultrapure water may be used alternatively on a regular basis with all kinds of haemodialysis modalities. Ultrapure water is highly desirable with high-flux haemodialysis modalities and represents a basic prerequisite for dialysis modalities using on-line production of substitution fluid (on-line haemofiltration HF or haemodiafiltration HDF) [21]. Several technical options and arrangements may be used to reach this goal [22]. They are presented in the Appendix. The most common water treatment system option is based on pre-treatment and a double-stage RO module in series. Microbiological contamination in this case complies with more stringent standards (Table 1).

IV.1 Water treatment system

From the early days of dialysis it is known that water used for haemodialysis must be purified in order to prevent severe clinical side effects due to contamination of the water [3–8]. In brief, this means that all water contaminants (particles, dissolved substances such as ions, trace elements, organic substances, nitrogen compounds, and micro-organisms) should be removed prior to entering the dialysis machine.

Guideline IV.1

A. Contemporary haemodialysis requires the use of pure water complying at a minimum with the

Table 1. Maximum levels of the different water purity grades

Maximum levels	AAMI Water	European Pharmacopoeia		
		Regular water	Ultrapure water	Sterile water
Microbial contamination (CFU/ml)	200	<100	<0.1	<0.000001
Bacterial endotoxins (IU/ml)	<2	<0.25	<0.03	<0.03

Table 2. Comparison of maximum water contaminant levels and methods of analysis recommended by the European Pharmacopoeia and the AAMI

Contaminant	Methods of analysis	Maximum concentration (mg/l)	
		AAMI	European Pharmacopoeia
Aluminum	Atomic absorption spectrometry	0.0100	0.0100
Antimony	Atomic absorption spectrometry	0.0060	0.0060
Arsenic	Atomic absorption spectrometry	0.0050	0.0050
Barium	Atomic absorption spectrometry	0.1000	0.1000
Beryllium	Atomic absorption spectrometry	0.0004	0.0004
Cadmium	Atomic absorption spectrometry	0.0010	0.0010
Calcium	Atomic absorption spectrometry	2 (0.05 mmol/l)	2 (0.05 mmol/l)
Chloramines	Colorimetry	0.1000	0.1000
Chromium	Atomic absorption spectrometry	0.0140	0.0140
Copper	Atomic absorption spectrometry	0.1000	0.1000
Cyanide	Spectrophotometric	0.0200	0.0200
Fluoride	Molecular photoluminescence	0.2000	0.2000
Free chlorine	Colorimetry	0.5000	0.5000
Lead	Atomic absorption spectrometry	0.0050	0.0050
Magnesium	Atomic absorption spectrometry	4 (0.16 mmol/l)	2 (0.08 mmol/l)
Mercury	Atomic absorption spectrometry	0.0002	0.0010
Nitrate	Colorimetry	2.0000	2.0000
Potassium	Flame photometry	8 (0.2 mmol/l)	2 (0.08 mmol/l)
Selenium	Atomic absorption spectrometry	0.0900	0.0900
Silver	Atomic absorption spectrometry	0.0050	0.0050
Sodium	Flame photometry	70 (3.0 mmol/l)	50 (2.2 mmol/l)
Sulfate	Turbidimetric method	100	100
Thallium	Atomic absorption spectrometry	0.0020	0.0020
Zinc	Atomic absorption spectrometry	0.1000	0.1000

IV.2 Technical design of the water treatment system

To ensure a consistent production of adequately purified water, it is necessary to optimize the design of the water purification system [23].

Guideline IV.2

A. The water treatment system should consist of pre-treatment and RO modules feeding directly the dialysis machines. Storage tanks should be avoided. Pipe tubing material and plumbing should be designed to prevent bacterial contamination and to be easily disinfected.

(Evidence level: C)

Commentary on Guideline IV.2

Water engineering consists of the optimal assembly of the different components of the water treatment in terms of size, position, and purity to guarantee the appropriate quality of the water product. A combination of water pre-treatment (softener, activated carbon, down sizing microfiltration), RO module, and a direct delivery pipe system (no storage tank if possible) represents the minimal technical configuration intended to produce pure water and to prevent microbiological contamination [24].

An alternative option to produce higher quality grade water consists of a system to ‘polish’ the water by passing through a second RO module and/or electrochemical continuous deionizer in series. Such a technical configuration is used in the semiconductor industry and is able to produce ultrapure water with

very stringent purity criteria. In this case resistivity exceeds 1.5 MOhms and bacterial contamination is <0.1 CFU/ml.

To prevent bacterial contamination and biofilm formation, the water distribution circuit should be particularly well engineered [25,26]. Suitable materials for pipe network are made of stainless steel, polyethylene tubing, acrylonitrile-butadiene-styrene plastic, polyvinylidene fluoride, polypropylene, or polyvinyl chloride of sanitary grade. High shear stress should be maintained by means of small internal diameter pipes and high flow rates. The greatest effort should be made to obtain an adequate plumbing of the water distribution loop, with a linear pipe configuration favouring continuous high-speed water circulation and preventing water stagnation by avoiding dead space and lateral arms.

Table 3. Validation and monitoring frequency of water treatment system for chemical purity

Frequency	Validation for 3 months	Monitoring for 12 months
Tap water	X	X
Soft water	X	
RO/DI/UF water	X	X
Loop entrance	X	
Feeding machine water	X	X

Table 4. Validation and monitoring frequency for chemical parts

Frequency	Validation* for 3 months	Monitoring** for 12 months
Aluminum	Monthly	Monthly
Antimony	Monthly	Half-yearly
Arsenic	Monthly	Half-yearly
Barium	Monthly	Half-yearly
Beryllium	Monthly	Half-yearly
Cadmium	Monthly	Half-yearly
Calcium	Daily	Daily
Chlorine and Chloramines	Daily	Daily
Chromium	Monthly	Quarterly
Copper	Monthly	Monthly
Fluoride	Monthly	Half-yearly
Lead	Monthly	Monthly
Magnesium	Daily	Daily
Mercury	Monthly	Half-yearly
Nitrate	Monthly	Half-yearly
Potassium	Daily	Daily
Selenium	Monthly	Half-yearly
Silver	Monthly	Half-yearly
Sodium	Daily	Daily
Sulfate	Monthly	Half-yearly
Thallium	Monthly	Half-yearly
Zinc	Monthly	Half-yearly

*Re-validation (technical intervention, annual check-up).

**More frequently according to the local risk or the country regulation.

IV.3 Water treatment monitoring and maintenance

Water treatment quality control covers two aspects: one is the chemical purity and the other is the microbiological purity.

Guideline IV.3.1

A. The chemical and bacteriological purity of the dialysis water must be monitored routinely and regularly and the results should be documented. There should be documented procedures, which come into effect once these limits are exceeded. These procedures will include temporary closure of the dialysis unit when the safe limits for contaminants are exceeded.

(Evidence level: C)

Commentary on Guideline IV.3.1

Basic requirements for an adequate control of the chemical purity of the produced water are summarized in this section.

It must be acknowledged that monitoring of the water treatment system relies on two levels: first, the validation of a new water treatment system after its installation; secondly, the surveillance of the water treatment system in routine functioning. Frequent monitoring (weekly) is mandatory during the validation phase (3 months) to confirm that the objective is achieved. Quarterly monitoring is desirable during the surveillance and/or maintenance phase. Detailed monitoring (frequency, analytical methods) is provided in Tables 3 and 4. Validation of the system should be undertaken each time the water treatment system has been opened or works have been performed on the water treatment and distribution system.

The effectiveness of the softener should be monitored daily (before starting dialysis sessions) by

measuring the hardness of the effluent water immediately downstream of the softener using disposable titration kits (resolution < 1 mg/l) or permanently by a sensitive automated probe (Testomat®) equipped with an appropriate alarm. The softener regeneration cycle must be adapted to the volume of the resin and salt. Regeneration should be checked daily.

Removal of chloramine by activated charcoal is important to prevent RO membrane damage and patient reaction (haemolysis) [27]. A control must be performed daily with appropriate dosage kits or alternatively chloramine should be monitored continuously by measuring free chlorine (Chlorometer) in the circuit.

The effectiveness of the RO system and/or the deionizer must be monitored daily by measuring the resistivity of the effluent water using an adequate resistivity meter [28,29].

Monitoring of the quality of the final water product is required on a monthly to a half-yearly basis according to the phase of use. Major water contaminants must be checked by appropriate analytical methods as defined in Table 4. The maximum allowed concentration should be respected to prevent intoxication on a long-term basis [30]. Data storage of water chemical monitoring is an essential part of the quality assurance process.

Guideline IV.3.2

A. Monitoring the microbiology of the water feeding dialysis machine should be performed weekly during the validation phase and at least monthly during the surveillance period.

(Evidence level: C)

Commentary on Guideline IV.3.2

Microbiological water contamination is a common feature of all water treatment systems that could be a source of water-borne bacteraemia and/or pyrogenic reactions [31–33]. It is facilitated by the use of resins, activated charcoal, filters that represent an excellent medium for bacterial growth, and by a poor design of the system, which facilitates water stagnation. It is therefore mandatory to establish hygienic and handling rules to sanitize and disinfect regularly the water treatment system in order to prevent the formation of a biofilm [34,35]. The maintenance of water treatment systems requires pre-emptive measures including frequent disinfection cycles (either chemical or heat or mixed) of the complete chain, filter and resin changes according to the size and the contamination level, and destruction of microbial biofilm in the circuit.

Water microbiological monitoring is an integral part of the quality assurance process. A surveillance protocol documenting the degree of water contamination along the water chain must be adopted. Water sampling devices should be placed at key points of the chain to facilitate the microbiological surveillance. Samples of water should be cultured regularly following the methods proposed in the Appendix. Frequency and methods used for this bacteriological surveillance must be adapted to the degree of contamination of the water treatment chain and to the frequency of disinfection of the system (Table 5).

The methods used for the microbiological monitoring are detailed in the Appendix. Contrary to the European Pharmacopoeia recommendations, we strongly recommend using the most sensitive method. It consists in culturing the covering layer of a 0.45- μm membrane after filtration of a large volume (100 ml at a minimum) of the water to be tested [36]. Following sample filtration, the membrane is placed on a culture medium favouring the growth of water-borne bacteria. The use of poor nutrient medium (R2A) maintained

Table 5. Validation and monitoring frequency for microbiological contamination

Frequency Sampling sites	Validation for 1 month	Monitoring for 12 months
Tap water	Weekly	Monthly
Soft water	Weekly	
RO/DI/UF water	Weekly	
Loop entrance	Weekly	
Feeding machine water	Weekly	Monthly
Dialysate outlet	Weekly	Monthly

*Re-validation (technical intervention, annual check-up).

at 20–22°C observed over a long period (7 days) of time will offer a more sensitive method to detect and quantify the degree of water contamination [37,38]. Microbiological monitoring should concentrate on critical areas of the chain: beyond the softener and the activated charcoal and at the entrance of the dialysis machines. The frequency of this bacteriological surveillance should be performed on a monthly basis during the surveillance phase and weekly during the validation phase. The presence of bacterial endotoxin must be evaluated using a sensitive Limulus Amoebocyte Lysate (LAL) test with a sensitive assay and a detection limit of 0.03 EU/ml [39–42]. Recently, a new assay has been proposed to detect the presence of peptidoglycans, which are strong cytokine-inducing substances, not recognized by the LAL test [43]. Endotoxin content of the water feeding the dialysis machine should be evaluated on a quarterly basis or more frequently when renal replacement modalities producing on-line substitution fluid are used. Documenting and storing data related to water microbiological purity is an essential component of the quality assurance process.

Guideline IV.3.3

A. Regular and effective disinfection procedures are an integral part of the hygienic maintenance of the water treatment system. Periodicity, type of disinfection (chemical, heat, mixed), periodic changes of components (filters, resins, filters) should be performed in accordance with manufacturer recommendations and adapted to microbiology monitoring results. Complete disinfection of the water treatment chain should be performed at least monthly.

(Evidence level: B)

Commentary on Guideline IV.3.3

Although it is virtually impossible to establish common rules, it is acceptable to claim that frequent

disinfection of the water treatment system is required to prevent contamination. When starting a new water treatment system it is recommended to disinfect on a weekly basis to sanitize the resins and the pipe system. Afterward, the periodicity of disinfection cycles should be adapted to the configuration of the system and on the results of the microbiological monitoring [44]. The optimal periodicity of disinfection measures should be established according to the degree and the kinetics of recontamination after a disinfection procedure [45]. Starting early and effective disinfection procedures is the only means to prevent microbial biofilm formation in the pipe system.

Periodicity, type of disinfection, concentration, and time of exposure to the agent are dependent upon the nature of the material composing the dialysis fluid circuit and must be done in accordance with manufacturer recommendations to prevent damaging effects. A complete disinfection of the water treatment system including RO and/or desionizer and distribution loop at least once per month appears highly desirable.

Periodic changes of water treatment components such as resins (softeners, deionizer), activated carbon, and filters should be performed according to microbiological results and to their life expectancy [46]. This is an efficient way to prevent downstream seeding from highly contaminated resins.

of use, a checklist defining the parameters that must be evaluated before dialysis start. For this purpose, the user should have validated the correct development of this entire preparation phase:

- The haemodialysis machine has been correctly disinfected before use.
- Chemical analysis of the dialysis fluid composition performed in a certified laboratory is required on a monthly basis to ensure the correct function of the proportioning dialysis machine.
- The used disinfectant has been removed completely from the dialysis fluid. This is a major step that should not be bypassed in any circumstances to prevent serious adverse events [47]. According to the type of chemical disinfectant used, a highly sensitive strip or chromogenic liquid reagent test will be performed [48]. Negativity of the test will be documented on the dialysis machine and dialysis prescription sheet form.

Guideline IV.4.2

A. In order to reduce the risk of pyrogenic reactions and water-borne bacteraemia, the dialysis fluid must routinely meet as a minimum the European Pharmacopoeia microbiological standards.
(*Evidence level: B*)

Commentary on Guideline IV.4.2

The regular use of ultrapure water to supply the dialysis machine is not sufficient to guarantee the microbiologic purity of the dialysis fluid. Bicarbonate dialysis fluid offers an excellent seeding medium for bacterial growth that could be the cause of bacteraemia and/or pyrogenic reactions in dialysis [49,50]. The dialysis-proportioning machine facilitates the contamination of dialysis fluid, by the complexity of its hydraulic circuit. Several factors including design of the circuit of the machine and inadequate disinfection procedures favour bacterial growth and biofilm formation in the hydraulic circuit [51,52]. The microbial dialysis fluid contamination and the presence of microbial-derived products create new potential hazards (pyrogenic reactions, silent cell, and protein activation) for the haemodialysis patient that must be prevented by the regular use of ultrapure dialysis fluid (UPD) [53,54].

Guideline IV.4.3

A. UPD in which endotoxin and bacteriae are undetectable is absolutely required when it is used as substitution fluid for on-line haemofiltration or haemodiafiltration. In order to minimize inflammation, dialysis units should also work towards supplying UPD routinely for all dialysis modalities. The regular and routine production

IV.4 Haemodialysis-proportioning machine

The haemodialysis machine is the proportioning system that mixes the electrolyte concentrate solution (or powder) with the purified water to produce the dialysis fluid with characteristics (electrolytic composition, pH, temperature, dialysis fluid flow) complying with the medical prescription set on the dialysis monitor. The final composition of the dialysis fluid relies on the dialysis-proportioning machine itself that is warranted by the manufacturer and its conditions of use. This specific point of view will not be discussed here. However, from the safety point of view, it is important to remind that any user is responsible for the safe use of a well functioning dialysis machine to treat a patient. This basic safety precaution is valid in any circumstances and applies to every dialysis session.

Guideline IV.4.1

A. The safe accomplishment of any dialysis session requires that the dialysis fluid composition is correct and that any disinfectant is completely removed before the start.

(*Evidence level: C*)

Commentary on Guideline IV.4.1

To comply with this basic rule of safety one must define, according to the type of machine and the mode

of UPD relies on the implementation of ultrafilter(s) on the dialysis fluid pathway.
(*Evidence level: B*)

Commentary on Guideline IV.4.3

Renal replacement therapies favouring convective solute mass transfer (haemofiltration and haemodiafiltration) require the on-line production of substitution fluid [55–57]. Such renal replacement modalities require more stringent maintenance rules. Disinfection either by heat or chemical agent must be performed after each dialysis session. Cleansing and descaling of the haemodialysis-proportioning machines should be performed on a daily basis. On-line methods such as haemodiafiltration or haemofiltration require the use of certified haemodialysis-proportioning machines ensuring ‘cold sterilization’ of the dialysis fluid by means of ultrafilters [58–60]. Until now, dialysis fluid ultrafiltration is the only method that has been proven to be efficient in routine clinical use [61]. Ultrafiltration is effective in two ways: one is to retain microorganisms in the incoming fluid solution by sieving exclusion and the other is to remove endotoxins from the incoming solution by adsorption [62,63]. Two ultrafilters placed in series appear to be an optimal number to warrant an absolute safety for the dialysis fluid ultrapurity. However, it must be underlined that the implementation of such a sterilizing module has shortfalls. First, ultrafilters installed in the dialysis fluid circuit are captive and exposed to disinfection cycles. Prolonged and/or repeated exposure to a chemical disinfecting agent may alter the retentive capacities of the ultrafilter [64]. Therefore, it is mandatory to change the filters periodically to preserve the safety margin of the cold sterilizing process. Secondly, including ultrafilters into the dialysis fluid circuit will prolong the rinsing time of the chemical disinfecting agent. Rinsing times must be readjusted according to manufacturer recommendations based on precise kinetics of the disappearance of the disinfecting agent. Thirdly, handling and disinfection procedure of dialysis machines equipped with on-line and/or dialysis fluid ultrafilters must strictly comply with manufacturer’s instructions.

Guideline IV.4.4

A. Regular disinfection and hygienic maintenance of the haemodialysis-proportioning machine are mandatory to prevent microbial proliferation and biofilm formation in the hydraulic circuit. Disinfection of the dialysis machine after each session is recommended to prevent microbial contamination and viral transmission.
(*Evidence level: C*)

Commentary on Guideline IV.4.4

Hygienic maintenance of all haemodialysis delivery machines is required to ensure the regular production of clean dialysis fluid [65]. This includes regular cleaning of the hydraulic tubing with a detergent agent to remove organic deposits, descaling with acid solution to dissolve phosphate and calcium precipitates, and disinfection with an appropriate chemical and/or heat-sterilizing agent. Replacement of hydraulic tubing is advised in case of a high contamination and/or presence of a biofilm in the dialysis circuit. In all cases, cleaning, descaling, and disinfection procedures must comply with recommendations of the manufacturers in order to prevent material damage and to assure efficiency [64]. Finally, a precise microbial inventory of the machine as well as a comprehensive hydraulic scheme are required to define disinfection cycles. Regular microbiological control of dialysis fluid is necessary to optimize the disinfection cycles and to check their efficacy.

IV.5 Electrolytic concentrates

Electrolytic concentrates are required for the preparation of the dialysis fluid. Originally liquid electrolyte concentrates provided in two independent plastic containers (A for acid; B for bicarbonate) were extemporaneously reconstituted into dialysis fluid by the dialysis machine. Nowadays, bicarbonate is mainly distributed as a soluble powder, which is diluted to a saturated solution by the dialysis machine. Concentrates may be the source of bacterial dialysis fluid contamination, particularly the bicarbonate solution, which is an excellent medium for bacterial growth [66]. Salts used in the preparation of concentrate may as well be the source of metal intoxication [67].

Guideline IV.5

A. Bicarbonate concentrate must be handled with care to prevent bacterial contamination after container opening. The use of already open containers should be discouraged.

(Evidence level: C)

Commentary on Guideline IV.5

Electrolytic concentrate is a potential source for microbial dialysis fluid seeding. A centralized distribution system of bicarbonate concentrate is not recommended. The use of sterile liquid concentrates should be strongly recommended to reduce the risk of bacterial dialysis fluid contamination. However, due to the fact that concentrates are considered to be medical devices, sterility and apyrogenicity may not be guaranteed by the manufacturer. Liquid bicarbonate concentrate is usually produced in a sterile

condition, which is virtually maintained until the opening of the plastic container. Bacterial contamination occurs at the time of opening the container (air contamination) and is facilitated by the introduction of the sampling rod (rod contamination). Therefore, it is important to keep the concentrate sampling rod clean and disinfect it regularly to reduce the contaminating bacterial load. Bicarbonate containers may be the source of bacterial proliferation during the entire length of the dialysis session. Closing the container cap as soon as the sampling rod has been introduced in the container is mandatory to reduce contamination by air-borne bacteria and their proliferation. Bicarbonate solutions must be stored at a low temperature in a clean and dark warehouse to prevent bacterial and mycelial growth.

Bicarbonate powder reduces the risk of bacterial contamination before dilution. Since the saturated, warm bicarbonate solution still creates ideal conditions for bacterial growth during the dialysis session, it still remains to be proven that bicarbonate powder has a beneficial effect on the dialysis fluid contamination.

Dextrose addition to dialysis fluid is frequently advocated to improve dialysis tolerance particularly in diabetic or in elderly patients [68,69]. No study has evaluated the microbiological risk associated with dextrose addition to acid concentrate.

IV.6 Dialysis fluid purity: implications in the haemocompatibility network system

Over the last decade, microbiological contamination of the dialysis fluid has become a major concern [70–72]. In the early 1980s dialysis fluid purity was mainly targeted to prevent pyrogenic reactions associated with the use of bicarbonate buffer and back-transport phenomena (back-filtration, back-diffusion) of dialysis fluid to the blood [73]. From this perspective, the role of the dialyzer membrane is essential to prevent the blood passage of cytokine-inducing substances from dialysis fluid [74–77] and/or to prevent immunization against dialysis fluid endotoxin [78]. From the late 1990s the dialysis fluid purity was considered as one of the main factors in the complex haemocompatibility network [79–82].

UPD is a concept introduced in the mid 1990s that qualifies a sterile and non-pyrogenic dialysate produced extemporaneously by ‘cold sterilization’ by means of ultrafilters [83]. Over the last few years it has been recognized that dialysate purity should tend to ultrapure product for contemporary dialysis to prevent the potential hazards associated with contaminated dialysate [84–88].

Guideline IV.6.1

A. Regular use of UPD appears desirable in long-term haemodialysis patients to prevent and/or to delay the occurrence of dialysis related complications.
(*Evidence level: B*)

Commentary on Guideline IV.6.1

Several scientific reports have referred to the beneficial effects of the use of UPD in reducing the incidence of dialysis-related pathology [89,90].

Several studies (*in vitro*, *ex vivo*, *in vivo*) have shown that contaminated dialysate may be the source of cytokine production facilitated by the presence of blood and proteins [91–95]. On the contrary, the use of UPD prevented blood cell activation and reduced the production and/or release of pro-inflammatory cytokines [96–99]. Clinical studies have demonstrated *in vivo* the beneficial role of such a low level of scanty contamination [100,101]. Such beneficial effects of UPD remain, however, disputed in some studies showing no significant effect on the cytokine production despite the use of high-flux membrane and contaminated dialysate [102].

Clinical studies have shown that in the long-term, the use of UPDs was associated with several beneficial effects such as: suppression of haemodialysis-related pyrogenic reactions [103–105], prevention of silent chronic inflammation [106–109], significant reduction of the incidence of β 2m-amyloidosis [110–112], sparing effect on erythropoietin needs [113]. Unfortunately, most of these studies were performed in uncontrolled or non-randomized conditions reducing the power of their significance.

At this stage, it is important to remember that dialysate production is a complex chain of events that includes the water treatment system, the pipe distribution system, the dialysis-proportioning machine, and the electrolytic concentrates [114]. Accordingly, the microbial purity of the final dialysate should integrate efficacy of the different components. One must recognize that UPD would never be obtained safely only by implementing a series of ultrafilters on a dialysis machine [115]. It is a complete concept that should take into consideration all components described above. UPD is however, affordable with the utilization of modern water treatment systems, optimally designed haemodialysis machines and a global quality assurance concept [116].

Guideline IV.6.2

A. Success in achieving dialysate purity on a long-term basis relies on a quality assurance process that involves all dialysis staff members, requires strict protocols, a permanent documentation of results and quick corrective actions when results deviate from their norm.
(*Evidence level: C*)

Commentary on Guideline IV.6.2

Regular production of dialysate purity complying with standards defined above is a permanent challenge for any dialysis facility that is rarely achieved [117–120].

The probability of success in this procedure relies on a continuous quality assurance process involving all persons working in the haemodialysis field (technician, nurse, pharmacist, microbiologist, physician) and using very strict protocols of follow-up [121–123].

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Appendix: Water purification methods

Water treatment can be defined as any procedure or method used to alter the chemical composition of a water supply. Water supplies are classified as either surface water or remote groundwater. The origin of water (surface or remote) affects considerably the content of its contaminants. Water purity used in haemodialysis should satisfy specific needs and standards to prevent toxicity either in the acute or the chronic condition.

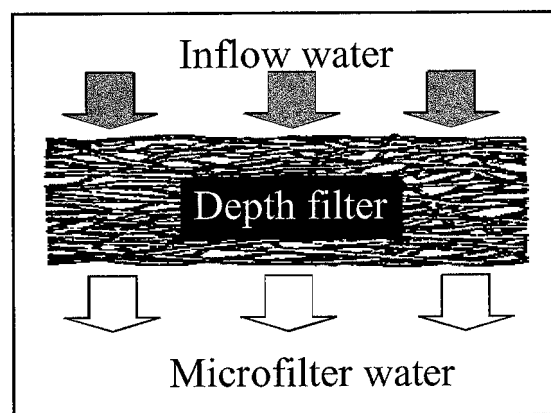


Fig. 1. Screen pre-filtration.

Municipal water treatment

Specific water treatment options are used by municipalities to meet local, regional, or national standards. They are schematically summarized below.

Screen pre-filtration

Pre-filtration consists in a rough screen, usually 300–150 μ , placed at the intake point of a surface water supply, to remove large particulate matter to protect downstream equipment from fouling (see Figure 1).

Clarification

Clarification is a multi-step process intent to reduce turbidity and suspended matter. The different steps include the addition of chemical coagulants or pH-adjustment chemicals that react to form a flocculate that is removed in gravity tanks or as the water percolates through a gravity filter. The clarification process effectively removes particles $>25 \mu$.

Disinfection

Disinfection is one of the most important steps of the municipal water treatment. Chlorine is added into the water supply system after the water has been clarified to kill bacteria. In order to maintain the 'biocidal effect', an excess of chlorine is added into the water to maintain a residual concentration. Therefore, chlorine levels must be constantly monitored to prevent harmful levels of chloramines or chlorinated hydrocarbons in the supply.

pH adjustment

Municipal waters may be pH-adjusted to a pH of approximately 7.5–8.0 to prevent corrosion of water pipes (release of lead) into the water supply. In the case of excessive alkalinity, the pH may be reduced by the addition of CO_2 .

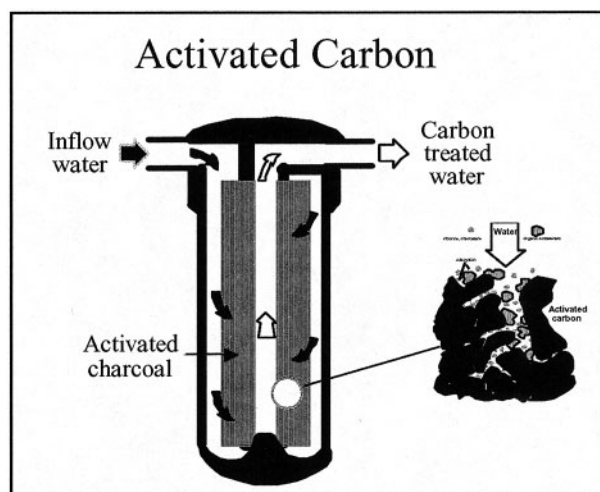


Fig. 2. AC filter.

Haemodialysis facility treatment

Many technical options for water treatment may be used to meet specific requirements of haemodialysis. They are schematically summarized below.

Chemical addition

Dispersants. Dispersants may be added to disrupt the scale formation, preventing growth of precipitate or crystals.

Chelating agents. Chelating agents may be used to prevent the negative effects of hardness in preventing the deposition of Ca, Mg, Fe, and Mn.

Oxidizing agents. Oxidizing agents may be used with a dual action: as a biocide, or to neutralize reducing agents. Potassium permanganate, a strong oxidizing agent, may be used to oxidize organic compounds and ferrous iron facilitating precipitation and filtration.

pH adjustment. Certain chemicals, membranes, ion exchange resins, reverse osmosis (RO) membranes, and other materials are sensitive to specific pH conditions. The adjustment of the pH is recommended in this case.

Reducing agents. Reducing agents, such as sodium metabisulfite, are added to neutralize oxidizing agents such as chlorine or ozone. These agents are useful with membrane and ion exchange systems, as they prevent their degradation.

Activated carbon filters

Activated carbon (AC) is similar to an ion exchange resin. AC absorbs low molecular weight organics and reduces chlorine or other halogens from water, but is not active on salts. AC filters must be changed periodically to avoid bacterial growth. To prevent

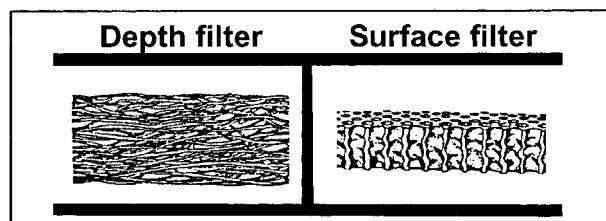


Fig. 3. Cartridge filter.

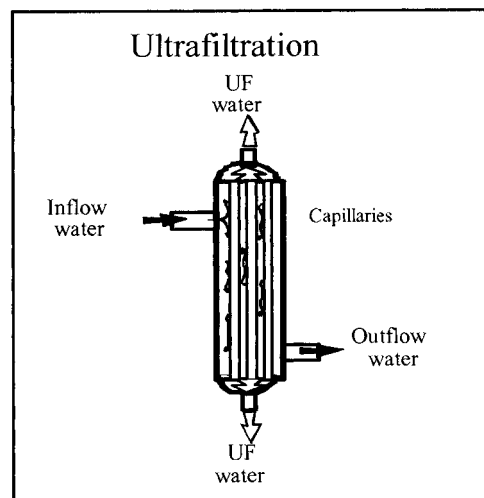


Fig. 4. UF cartridge filter.

fouling from accumulated solids frequent backwashing of the filter is required (see Figure 2).

Cartridge filters

A typical filter consists of a tank to house the filter media. Cartridge filters are of two main types: depth filters or surface filters (see Figure 3).

Depth cartridge filters. In a depth cartridge filter, the water flows through the thick wall of the filter, where the particles are trapped, throughout the inner part of the media. The filter is made of cotton, cellulose, synthetic yarns, or synthetic bundle of polypropylene fibres. The efficacy of the filter is related to the 'density' of the media and its capacity to trap particles. Depth cartridge filters are usually disposable and retain particles in the range of 1–100 μ .

Surface filtration–pleated cartridge filters. Pleated cartridge filters act as absolute particle filters using a flat sheet media, to trap particles. The media are pleated to increase usable surface area. Pleated membrane filters are used for sub-micron particle or bacteria filters in the 0.1–1.0 μ range. Newer cartridges also perform in the ultrafiltration (UF) range: 0.005–0.15 μ . Positively charged membranes may be used as well to expand retention capacity removing negatively charged pyrogens.

UF cartridge filters. UF cartridges may be used to remove pyrogens and other macromolecular compounds from ultrapure water. Usually they are built in a spiral-wound configuration allowing a cross-flow mode of filtration. They are also produced as fibres assembled in a bundle (see Figure 4).

Ion exchange systems

An ion exchange module consists of a tank containing small beads of synthetic resin. The beads are treated to selectively adsorb either cations or anions and exchange certain ions based on their relative affinity. The ion exchange process continues until all exchange sites are saturated; at this stage the resin is exhausted and must be regenerated by suitable chemicals.

Water softening. The ion exchange water softener is one of the most common options of water treatment. Water softeners remove scale-forming calcium and magnesium ions from hard water. A standard water softener has four major components: a resin tank, resin, a salt tank, and an electronic controller. Water softeners release sodium ions due to the ion exchange process, which may be difficult to remove downstream (see Figure 5).

Demineralization/deionization. Ion exchange deionizers (DI) use synthetic resins similar to those used in water softeners. DI use a two-stage process to remove virtually all ionic material remaining in pre-treated water. Two types of synthetic resins are used; one to remove positively charged ions (cations) and another to remove negatively charged ions (anions). The two basic configurations of deionizers are two-bed and mixed-bed (single tank of resins). Resins have limited capacities and must be regenerated upon exhaustion (see Figure 6).

Continuous desionizer. Continuous desionizers are based on the principle of an electrodialysis and employ specially prepared membranes, which are semi-permeable to ions related to their charge, and they employ electrical current to reduce the ionic content of water. Two flat sheet membranes, one that preferentially is permeable to cations and the other to anions, are stacked alternately with flow channels between them. Cathode and anode electrodes are placed on each side of the alternating stack of membranes to draw most ions through the membranes (see Figure 7).

Deionization produces extremely high quality water in terms of dissolved ions or minerals (charged), but does not remove organic substances.

Distillation

Distillation is the collection of condensed steam produced by boiling water. Most contaminants do not vaporize and, therefore, do not pass into the distillate. Distillation is a process that permits

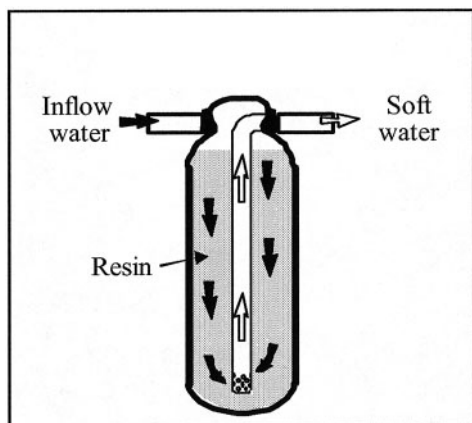


Fig. 5. Water softener.

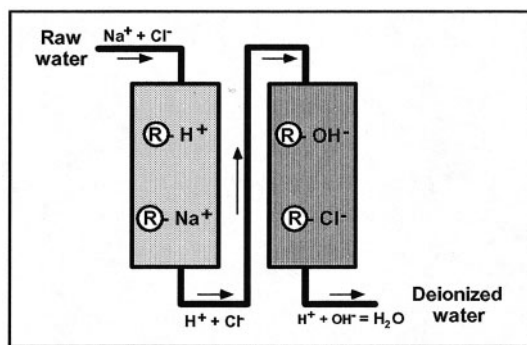


Fig. 6. Ion exchange deionizer.

removal of both organic and inorganic contaminants, including biological impurities and pyrogens. Distillation involves a phase change, which removes all impurities producing water of extremely high purity, but due to the high consumption of energy this is not a technical option used in dialysis (see Figure 8).

Filtration

Cross-flow filtration systems. Cross-flow or tangential flow filtration is based on the pressurized flow of incoming water (influent) flowing over a membrane, with a portion of the feed water permeating the membrane and the remaining sweeping tangentially along the membrane to exit the system without being filtered. The filtered stream is called the 'permeate', while the second stream is called the 'concentrate' or 'reject'. Because the feed water and the concentrate flow parallel to the membrane instead of perpendicular to it, the process is called 'tangential flow'. Depending on the size of the pores of the membrane, cross-flow filtering systems are effective in the range of reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and more recently microfiltration (see Figure 9).

Cross-flow membrane filtration allows continuous removal of contaminants and prevents plugging of the membrane pores.

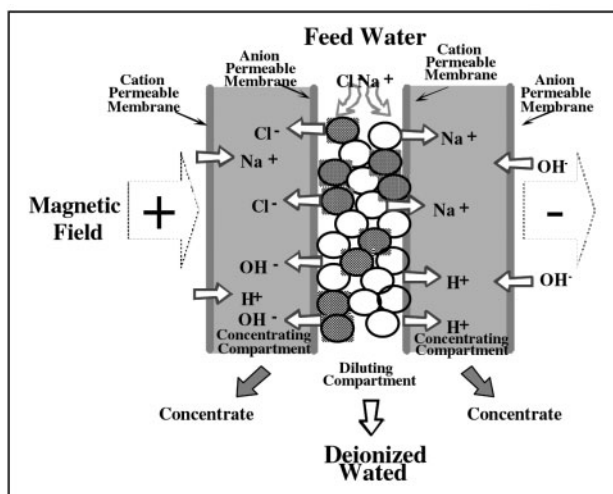


Fig. 7. Continuous deionizer.

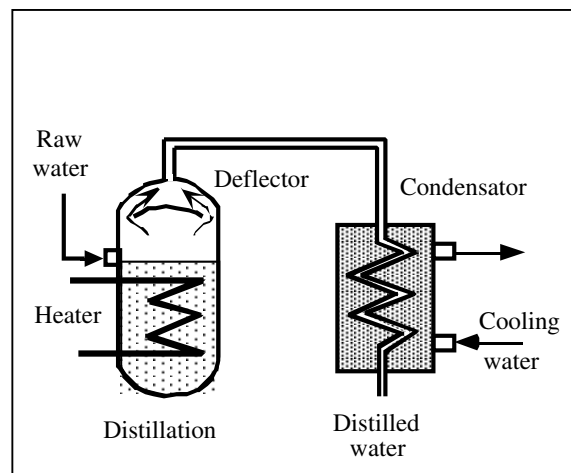


Fig. 8. Distillation.

Reverse osmosis. RO was the first membrane separation process commercially available. RO removes virtually all organic compounds and 90–99% of all ions. RO can meet most water standards with a single-pass system and the highest standards (ultrapurity) with a double-pass system. RO rejects viruses, bacteria, and pyrogens. High-pressure regimens (10–70 bar) are required to maintain the driving force of the RO purification process. RO requires less energy than heat-driven purification (distillation) and has the same efficiency as the ion exchange resins (see Figure 10).

Nanofiltration. NF equipment removes organic compounds in the 300–1000 molecular weight, rejects selected salts, and treats more water at lower pressure regimens than RO systems. NF softens water without the use of salt-regenerated systems.

Ultrafiltration. UF is a similar process to RO and NF, based on a cross-flow process that does not

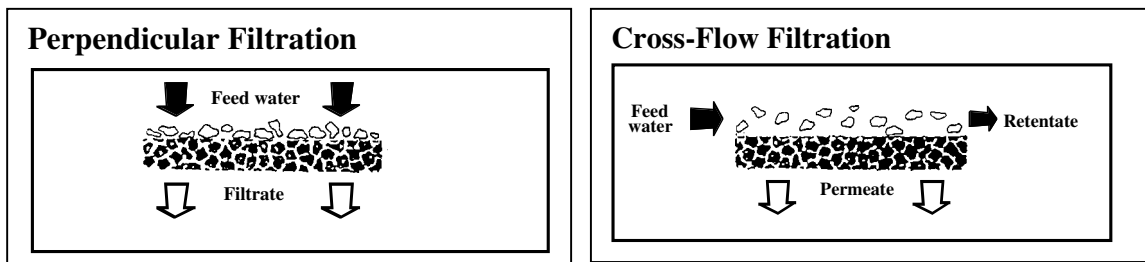


Fig. 9. Perpendicular vs cross-flow filtration.

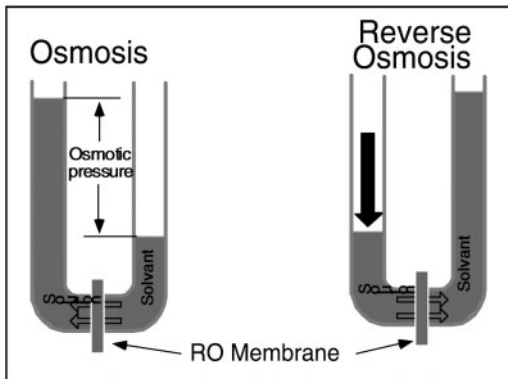


Fig. 10. Reverse osmosis.

reject ions. UF rejects contaminants in the range of 1000 Da to 0.1 μ particles. Because of the larger pore size in the membrane, UF requires a low operating pressure regimen. UF removes essentially organics, bacteria, and pyrogens.

Microfiltration. Microfiltration (MF) membranes are considered as absolute filters rated in the 0.1–2 μ range. MF membranes are available in polymer or metal membrane discs or pleated cartridge filters. Positively charged MF systems remove more efficiently negatively charged pyrogens. MF is also available in a cross-flow configuration that reduces the frequency of filter media replacement.

Water treatment and distribution system for dialysis

The permanent production of purified water for dialysis requires a well-designed water purification and distribution system.

A combination of pre-treatment (softener, AC, down sizing microfiltration modules) completed by a RO with a piping system delivering directly the treated water to the dialysis machine is the basic water treatment system required to safely treat the haemodialysis patient.

Two different technical assembly options are presented here (see Figures 11 and 12). They correspond to

two different grades of water purification: water for conventional dialysis, ultrapure water for high-flux haemodialysis, and on-line haemodiafiltration or haemofiltration methods.

Note that in order to prevent bacterial contamination and biofilm formation, the design of the water distribution circuit should be as much as possible of straight pipes preventing water stagnation and ensuring a permanent high-flux water recirculation (loop system) including when the dialysis facility is closed. The nature of the material used in pipes is important to prevent biofilm formation and corrosive action of chemical disinfectants. The suitable materials for the pipe system are stainless steel, acrylonitrile-butadiene-styrene plastic, polyethylene tubing, polypropylene, polyvinylidene fluoride, and polyvinyl chloride. In all cases they should be labelled for sanitary use and preferably CE marked. The greatest effort should be made to obtain the best plumbing, with a linear pipe configuration favouring continuous high speed water circulation (small diameter) and preventing water stagnation (no dead-space) and recontamination.

Microbiological monitoring

Definition

Microbiological monitoring of the dialysis fluids requires a quantitative assessment of the contamination level. Bacteriometry is the term that expresses the number of viable microorganisms present in the water and/or the dialysate. Analysis of dialysis fluids should be performed according to the methods recommended by the European Pharmacopoeia or the AAMI [1] or ISO. Bacteriometry of water and dialysate should comply at a minimum with the European standards [2]. However, due to the fact that more sensitive culturing methods give a higher recovery rate for water-borne bacteria, it is recommended to use these highly sensitive methods [3,4].

Method

Recommended technique: membrane filtration culturing technique

- Sample water and dialysate as follows: for the water, draw a 100-ml sample under aseptic conditions using flame sterilization of the sampling ports.

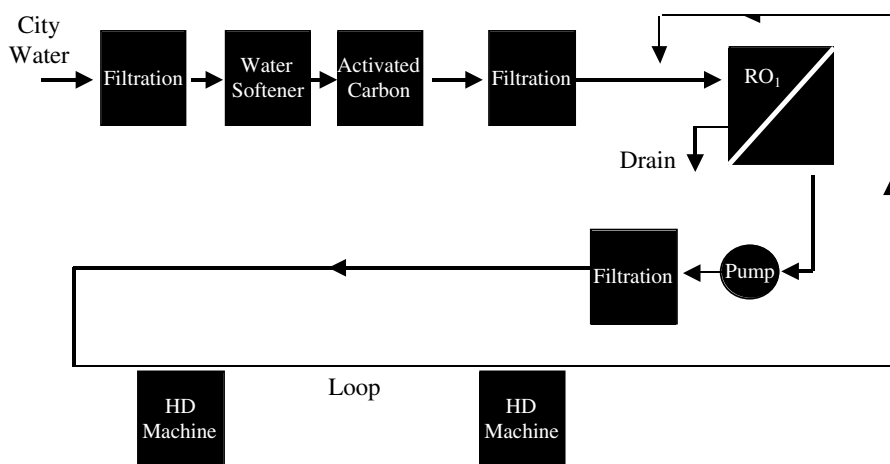


Fig. 11. Conventional water treatment system.

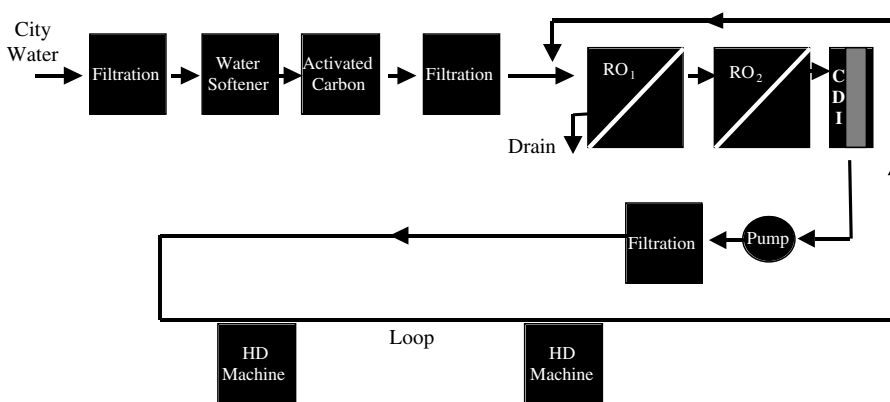


Fig. 12. Ultrapure water treatment system.

Before collecting the sample, discard 1000 ml of water; for the dialysate, draw a 100-ml sample on the effluent tubing either after disconnecting the dialysate line from the dialyzer or even better by using sterile specific sampling ports installed on the dialysate line.

- Collect the samples in sterile closed glass containers prepared by the laboratory of analysis.
- Send the containers immediately to the laboratory to be processed. If the sample cannot be processed, it is necessary to store the container in a refrigerator (3–6°C).
- Process the samples in a microbiology laboratory under strict aseptic conditions.
- Filter the water and dialysate samples through sterile disc microfilter (0.22–0.45 μm) hold in plastic container.
- Remove the membrane of the microfilter and lay it on a poor nutrient medium contained in a pour-plate. Use preferentially tryptone glucose extract agar (TGEA) or Reasoners 2A (R2A) media.

- Incubate the samples for at least 7 days at a controlled room temperature of 20–22°C.
- Count the number of colonies per plate at 7 days. The number of colonies should be <100/ml sample for bacteria and <10/ml for yeasts and fungi.
- Identify the type of microorganism with appropriate techniques when microorganism growth is observed.
- Check results and define a corrective action if required.
- Store results in a database for further documentation.

Alternative technique: pour-plate spreading technique

- Sample water and dialysate as follows: for the water, draw a 5 ml-sample (syringe) under aseptic conditions using flame sterilization of the sampling port. Before collecting the sample, discard 1000 ml of water; for the dialysate, draw a 5 ml sample (syringe) on the effluent tubing either after dis-

connecting the dialysate line from the dialyzer or best by using sterile specific sampling ports installed on the dialysate line.

- Send immediately the samples to the laboratory to be processed. In case of delay, store the samples in a refrigerator (3–6°C).
- Spread 0.5–1 ml of the samples on a pour-plate medium under strict aseptic conditions. Use a poor nutrient medium such as tryptone glucose extract agar (TGEA) or Reasoners 2A (R2A).
- Incubate the samples for at least 7 days at a controlled room temperature of 20–22°C.
- Count the number of colonies per plate at 7 days. The number of colonies should be <100/ml sample for bacteria and <10/ml for yeasts and fungi.
- Identify the type of microorganism with appropriate techniques when microbial colonies are detected on the plate.
- Check results and define a corrective action if required.
- Store results in a database for further documentation.

Endotoxin monitoring

Definition

Detection and quantification of bacterial endotoxin in the dialysis fluids should be performed using the Limulus Amoebocyte Lysate assay (LAL) and comply with the European Pharmacopoeia recommendations [5–10]. Three techniques may be currently used:

- Gel-clot method (Mallinckrodt[®], Inc.): based on gel formation when threshold limit of endotoxin is achieved (semi-quantitative method).
- Turbidimetric technique (Endosafe[®], Charles River Laboratories, Inc.): based on turbidity change occurring after cleavage of an endogenous substrate (kinetic method).
- Chromogenic kinetic technique (Endosafe[®], Charles River Laboratories, Inc.): based on colour change occurring after cleavage of a synthetic peptide complex (kinetic method).

Method

Any kind of dialysis fluid (water, dialysate, concentrate, infusate) should be processed in the same fashion.

- Sample with an appropriate technique and the ad hoc container to prevent non-related water contamination.
- Sample 5 ml of fluid with an aseptic technique described in the microbiology section. Discard 100 ml of liquid before sampling.
- Collect the sample in special closed plastic containers prepared by the laboratory.
- Store the sample in a refrigerator and send it as soon as possible to the laboratory to be processed.

- Choose a LAL method having at least a sensitivity of 0.03 IU/ml.
- Check results and define a corrective action if required.
- Store results in a database for further documentation.

Chemical contaminant monitoring

Monitoring chemical contaminants in dialysis fluids is mandatory to minimize the risk of dialysate-related toxicity either in acute or chronic conditions. The purity of dialysis fluids (water, dialysate, concentrate, infusate) should comply with standards established by the European Pharmacopoeia according to their reference methods [11,12].

Method

Water and dialysate sampling should be performed according to specific methods defined by the laboratories that perform the analysis. Dosing methods are not discussed in this appendix. Only sampling and storage methods applicable to dialysis fluids are summarized in this section.

- Select representative sampling site ports along the water treatment system as close as possible to the dialysate line of the dialysis machine.
- Define frequency of water and dialysate samplings according to the investigation phase, the water treatment system used and the risks related to the water feeding system.
- Sample water on selected sampling ports as discussed earlier in special containers (and or syringe) prepared by the laboratory performing the analysis. Such a precaution is essential to prevent non-water-related contamination (inadequate condition of sampling or storage or inadequate container material). Aluminum is an interesting contaminant to illustrate this problem. The use of plastic containers rinsed previously with HCl is mandatory to prevent the artifact induced by aluminum released from glass container.
- Send as soon as possible the water samples to a certified laboratory in order to perform the battery analysis of contaminants and trace elements.
- Check the results and adopt the corrective action if required.
- Store results in a database for further documentation.

Appendix: Quality assurance process

Chemical purity monitoring of water and dialysis fluid

On a daily basis, chemical purity of water may be ascertained at a high probability level by checking

the optimal functioning of the different components of the water treatment system and the absence of particular toxic contaminants. One of the most frequent acute injuries reported is related to the presence of chloramine in water [13,14]. This risk should be ruled out by checking the absence of chloramine daily.

On a quarterly basis, the absence of chronic toxic components must be confirmed by analysis of the water product feeding the dialysis machine with appropriate methods. The analytical methods are reported in Table 2.

Microbiological monitoring of water and dialysis fluid

Microbiological monitoring includes cultures of water and dialysis fluid samples to determine the number of colony-forming units per millilitre (CFU/ml).

Periodicity of sampling must be determined according to the design of the water treatment chain and the configuration of the dialysis-proportioning machine. However, it is strongly recommended that a microbiological check up should be performed monthly both on water and on dialysis fluid to provide a representative picture of the dialysis fluid purity [15]. The most sensitive methods to detect water-borne bacteria and endotoxin should be used to detect and quantify the degree of dialysis fluid contamination [16–20]. Because of its complexity, the monocyte test used to reveal cytokine-inducing substances, not recognized by the LAL assay, may not be recommended routinely [21,22].

Microbiological methods recommended for monitoring the dialysis fluids are detailed in the appendix.

Patient monitoring

All clinical or sub-clinical events should be documented and reported to ensure a complete tracking of adverse events in dialysis patients. It must be remembered that adverse events in dialysis, particularly those related to water, will occur frequently as a mini-epidemic. This was recently illustrated once more with the chloramine-induced haemolysis reports.

Aluminum as well as other metals should be periodically monitored to ensure optimal functioning of the water treatment system [23,24].

Body temperature check-up pre- and post-dialysis and during the interdialytic period in cases of problems is an important, sometimes neglected parameter that will draw attention to a specific infectious disorder of the haemodialysis patient.

Inflammatory state of haemodialysis patients, recently reported as a major cause of disease (MIA syndrome, atherosclerosis), may be partially induced by the dialysis fluid contamination reflecting a chronic cell and protein stimulation in dialysis [25–27].

C-reactive protein (CRP) is a very sensitive marker that seems particularly well adapted to detect and monitor this chronic inflammation [28,29]. CRP may be used as a surrogate of pro-inflammatory cytokine release related to dialysis fluid microbial and endotoxin contamination [30]. In the absence of dialysis fluid contamination, any increase of the CRP should lead to investigate potential causes of infection and/or inflammation.

Documentation

All results collected in the dialysis unit should be documented and stored for further analysis.

Optimal functioning of the different links of the dialysis fluid chain from the water treatment system to the dialysis fluid must be checked daily by means of simple indicators (hardness, resistivity, pressure drop, dialysis fluid conductivity) and quarterly or more by means of more complex dosing methods for toxic markers (e.g. aluminum). A schematic planning for dosing such toxic markers is provided in Table 4.

Microbiologic inventories (water and dialysis fluid) must be documented and stored. Regular reports of microbiologic contamination of the chain are important to determine timely the corrective actions to be taken (desinfection, filter changes, tubing change). A microbiologic database is, in this perspective, essential to authenticate and track over time the dialysis fluid purity and ensure maximum safety to the patients.

Patient-specific parameters (adverse events, body temperature, pyrogenic reactions, biochemical, inflammation proteins, etc.) must be collected to analyse the clinical impact of any new procedure introduced in dialysis.

Methods of reference

The monitoring methods to evaluate the microbial contamination, the presence of endotoxin or toxic contaminants are detailed in the Appendix.

A timetable schedule (Tables 3 and 4) to perform this monitoring is proposed as a minimum to guarantee the quality of dialysis fluids used.

Microbiological monitoring includes cultures of water and dialysis fluid samples to determine the number of colony-forming units per millilitre (CFU/ml). Bacteriometry should rely on very sensitive methods using poor nutrient media and prolonged time of incubation. Endotoxin content must be determined using the LAL assay with a threshold detection limit of 0.03 endotoxin units per millilitre (EU/ml).

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