Chapter 3
Water Treatment and Safety Requirements

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Abstract On-line haemodiafiltration differs from other forms of haemodialysis in that up to 20 L/h of dialysis fluid can be infused directly into the bloodstream during each treatment. That infused fluid must be free of chemical contaminants, sterile and pyrogen-free. Compliance with that requirement cannot be demonstrated by testing at the time of infusion. Instead, the infused fluid must be prepared using equipment that has been validated to produce sterile and pyrogen-free fluid when operated in accordance with the machine manufacturer's instructions. It is the responsibility of the user of the machine to ensure those instructions are followed, including providing the dialysis machine with water and concentrates that meet the specifications set forth by the machine manufacturer. Properly designed systems for water treatment and distribution and concentrate preparation are central to achieving that goal. In addition, those systems must be subject to rigorous quality control that includes maintenance practices designed to prevent contamination of the dialysis and infusion fluids, coupled to monitoring that verifies the adequacy of the system design and maintenance program.

Keywords Dialysis fluid quality • Infusion fluid quality • Water treatment • Microbiological contaminants • Endotoxin • Equipment maintenance

Introduction

Like other forms of haemodialysis, HDF exposes patients to 30–60 l of dialysis fluid for each hour of treatment. Patients may be harmed by any contaminants in that dialysis fluid since water-soluble contaminants can diffuse across the dialyser

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membrane from the dialysis fluid to the blood as easily as uraemic toxins diffuse from the blood to the dialysis fluid. In this respect, the risk is the same for low-flux dialysis, high-flux dialysis and HDF and the concentration of any potentially toxic solute in the dialysis fluid must be reduced to safe levels regardless of the therapy.

Unlike other forms of haemodialysis, in on-line HDF, up to 20 L/h of dialysis fluid can be infused directly into the blood, bypassing the dialyser membrane. This fluid for infusion is required to be sterile and pyrogen-free. Since that requirement necessitates levels of microbiological contaminants far below detection limits, dialysis machines for HDF incorporate a process validated to produce sterile, pyrogen-free infusion fluid from standard dialysis fluid [1].

This chapter reviews the contaminants commonly encountered in preparing fluids for HDF, the hazards associated with those contaminants, and the steps that can be taken to ensure that they do not exceed safe levels in the dialysis and infusion fluids.

Contaminants Encountered in the Preparation of Fluids for HDF

Low-Molecular Weight Contaminants

A number of low-molecular-weight substances commonly found in drinking water supplies are toxic to haemodialysis patients. These substances, some of which are added to the drinking water supply for public health reasons, have been long known to cause a variety of toxicities, including anaemia (chloramine, aluminium), arrhythmias (fluoride), bone disease (aluminium), and a fatal encephalopathy (aluminium) [2]. The maximum allowable concentrations for these low-molecular-weight contaminants in water used to prepare dialysis fluid, and in the final dialysis fluid, are approximately tenfold lower than allowed in drinking water [3, 4]. This is because of the large volumes of fluid involved, the non-selective nature of toxin transfer across the dialyser membrane, and the lack of any excretory kidney function to eliminate any toxins that do enter the bloodstream (Table 3.1).

Microbial Contaminants

Microbial contaminants of water used for dialysis include viable microorganisms (e.g. bacteria, viruses, fungi, algae, prions) and organic fragments released by those microorganisms during their lifecycle and death. Viable microorganisms may be capable of causing infection, while microbial products can cause an inflammatory response in the patient. Safe limits have been set for the level of microbiological contaminants in dialysis fluid [5] (Table 3.2).

Table 3.1 Comparison of standards for drinking water and dialysis water

Contaminant	WHO recommendations for drinking water (mg/L) [3]	ISO 11663:2014 standards dialysis water (mg/L) [4]
Aluminium	0.1	0.01
Arsenic	0.01	0.005
Barium	0.7	0.1
Cadmium	0.003	0.001
Calcium	200	2.0
Total chlorine	5	0.1
Chromium	0.05	0.014
Copper	2	0.1
Fluoride	1.5	0.2
Lead	0.01	0.005
Magnesium	50	4.0
Mercury	0.006	0.0002
Nitrate (N)	50	2.0
Potassium	–	8.0
Selenium	0.04	0.09
Silver[a]	0.05	0.005
Sodium[a]	200	70.0
Sulphate	–	100.0
Zinc	5	0.1

[a]Canadian standard (WHO determined evidence inadequate to set safe limit)

Table 3.2 Maximum allowable levels of microbiological contaminants in dialysis fluids

	Endotoxin EU/mL	Bacteria CFU/mL
Standard dialysis fluid	<0.5	<100
Ultrapure dialysis fluid	<0.03	<0.1
Sterile, pyrogen-free infusion fluid[a]	<0.03	<0.000001

Based on data from Ref. [5]

[a]Must be ensured by proper operation of a validated system, verified by the manufacturer

Viable bacteria are monitored by culturing fluid samples in a low-nutrient medium in the dark, at room temperature, for several days. Viable bacterial contamination is quantified by counting the colonies growing on the medium and reporting as colony-forming units per ml (CFU/mL).

The microbial fragments contaminating dialysis water vary widely in size and composition. The components likely to cause inflammation are those which are small enough to pass through the dialyser membrane into the bloodstream, yet large enough to provoke an inflammatory response. Endotoxin, a lipopolysaccharide originating from the bacterial cell wall, is a representative inflammatory microbial contaminant. Since endotoxin molecules vary in size, endotoxin contamination is typically measured as its inflammatory potential in Endotoxin Units/mL (EU/mL) using the *Limulus amebocyte* lysate (LAL) assay. LAL assay kits,

capable of detecting as little as 0.001 EU/mL are commercially available. Short fragments of bacterial DNA are another potential contaminant originating from bacterial colonization of the treated water distribution system. These fragments have been shown to induce inflammation. Compared to endotoxin, they are smaller molecules and can more easily pass through a dialyser or filter membrane. There is currently no test that can be used to routinely monitor these DNA levels [6].

In conventional haemodialysis, the dialysis fluid is separated from the blood by the dialyser membrane. Because microbial contaminants are typically large solutes which diffuse slowly, they do not pass through low-flux dialyser membranes to a significant extent. For that reason, it is considered acceptable for the dialysis fluid to contain up to 100 viable bacteria/mL and up to 0.25 EU/mL of endotoxin. In high-flux dialysis, the membrane is typically about 50 μm thick and is highly porous. Viable bacteria are still too large to pass through those membranes. However, naturally occurring endotoxins are of variable size and some are small enough to potentially pass through the pores. Nevertheless, a high flux dialyser is an effective barrier to endotoxin because endotoxin is absorbed onto the membrane surfaces before it can reach the blood [7].

In high-flux dialysers, there is inevitably reverse ultrafiltration at the downstream end of the blood compartment, so-called 'backfiltration'. This is because flow in the blood compartment is driven by higher pressure at the upstream end, compared to pressure downstream. This pressure difference also causes ultrafiltration of fluid from blood into the dialysis fluid compartment at the upstream end, balanced by reverse filtration in the opposite direction at the downstream end. Reverse ultrafiltration can enhance transfer of higher molecular weight toxins from dialysis fluid to blood across the membrane (Fig. 3.1). Standard dialysis fluid may contain up to 0.25 EU/mL and up to 2 L of dialysis fluid may enter the blood per hour due to backfiltration in high-flux dialysis. Assuming a 100-fold reduction of endotoxin concentration in the reverse filtrate due to absorption to the membrane, the dose of endotoxin to the patient would be 5 EU/h. That dose is a dose approximately 100 times lower than that thought to induce acute adverse effects (400 EU/h). Nevertheless, it is now recommended to use ultrapure dialysis fluid, which contains <100 CFU/L of bacteria and <0.03 EU/mL of endotoxin [5], for all modes of haemodialysis [8].

Adverse Effects of Microbial Contaminants

Acute Effects of Endotoxin

Drinking water supplies are treated to render all microorganisms non-viable (for example, by killing them with chlorine) so that they are not capable of causing infection. However, fragments of the killed bacteria remain in the water and drinking water can contain as much as 30 EU/mL of endotoxin. The gastrointestinal system will inactivate orally ingested endotoxins so that they do not cause inflammation. However, even small amounts of endotoxin will induce an inflammatory response if they enter the body by another route, such as injection. Studies with human volunteers have

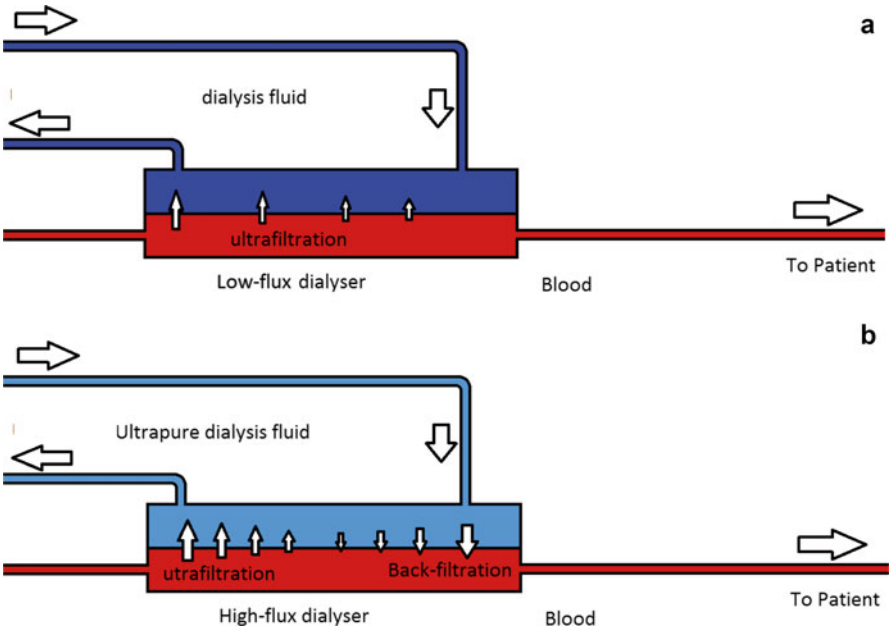

Fig. 3.1 The upper panel shows the difference in ultrafiltration between low-flux (**a**) and high-flux dialyser (**b**). With high-flux, there is back filtration from dialysis fluid to blood at the blood outlet end

shown that acute injection of endotoxin causes fever, hypotension and increases in leukocyte numbers and IL-6 concentrations. The minimum dose required to produce observable effects in 50 % of subjects was 4 EU/kg body weight. Informed by these studies, the FDA has set the maximum permissible mass of endotoxin which can be injected into the blood at 5 EU/kg. This is the amount of endotoxin present in approximately 0.2 mL of tap water. Because there are mechanisms to clear pyrogens from the blood, the time over which endotoxin is delivered is also important. Acute toxic effects of endotoxin (fever, hypotension) occur when endotoxin is delivered at a rate greater than 5 EU/kg/h [9]. For a patient weighing 80 kg, this would be 400 EU/h.

The hypotension caused by endotoxin injection is partly caused by vasodilatation and partly by transfer of fluid out of the vascular compartment due to altered capillary permeability and pressure. These changes are likely to be more serious during dialysis as they would oppose the vasoconstriction and vascular re-filling required to maintain blood pressure as fluid is removed by ultrafiltration.

Chronic Effects of Inflammation

Cardiovascular disease is now commonly thought to be caused or exacerbated by inflammation. The inflammation associated with cardiovascular disease is at a much lower level than would cause acute symptoms. Inflammatory markers have been associated with cardiovascular mortality in the general population and in dialysis patients.

Endothelial dysfunction induced by inflammation, macrophage migration into the arterial walls, and macrophage apoptosis are now seen as the key steps in the development of atherosclerotic plaques. Cardiovascular calcification is particularly common in dialysis patients and is shown to be associated with increased mortality. Cardiovascular calcification is now considered to be part of the inflammatory response [10].

Repeated hypotension during dialysis is very common. The hypotension induces cerebral and myocardial ischaemia. This contributes to organ dysfunction, including myocardial stunning which contributes to cardiac mortality. Inflammation is one of the causes of the hypotension [11].

Dialysis patients commonly exhibit a syndrome of malnutrition, inflammation and atherosclerosis (the MIA syndrome). Inflammatory cytokines cause both atherosclerosis and malnutrition which, in turn, cause further inflammation creating a self-perpetuating cycle associated with poor outcome [12].

In an observational study, patients dialysing in facilities with higher endotoxin levels had significantly increased mortality [13, 14]. Improvement in the microbiological quality of dialysis water has been shown to be associated with a reduction in inflammatory markers [15, 16] and an improvement in response to erythropoietin [17].

Achieving Fluid Quality for HDF

Taken together, the observations presented in the previous section highlight the importance of maintaining a high level of purity in the fluids used for HDF. That goal pursued at every stage of fluid preparation, from treatment of the incoming drinking water to generation of replacement fluid.

Water Treatment

Water treatment systems designed to produce water of the quality required for dialysis, including HDF, are generally obtained from specialized vendors, typically use a combination of relatively non-specific purification steps to reduce the level of all potential contaminants by a factor of about 100, and are almost always centred on reverse osmosis as the primary purification process. Optimum performance of the reverse osmosis unit is ensured by pre-treating the feed water to the unit. The types of pre-treatment will depend on the quality of the water entering the dialysis facility. For example, where the supply water is hard, the water treatment system should incorporate a softener to remove calcium and magnesium that would otherwise foul the reverse osmosis membranes and, where the water supply is disinfected with chlorine or chloramine, the water treatment system should include a means of removing those contaminants, such as carbon filtration.

The efficacy of each stage of the process is monitored by measuring the concentration of a single representative contaminant downstream of each stage: softener

function is tested by a hardness test or calcium ion-specific electrode; the efficacy of carbon filtration is tested by measuring the total chlorine concentration in the water exiting the carbon bed; and, the efficacy of reverse osmosis is tested by measuring the conductivity of the treated water. The water treatment system and its operation should incorporate sufficient redundancy and monitoring so that failure at a single point is detected and corrected while maintaining adequate water quality.

Maintaining the chemical purity of the water between the end of the water treatment system and the point at which it enters the HDF machine is ensured by constructing the water distribution system from inert materials, such as cross-linked polyethylene, Teflon, or stainless steel.

The maximum allowable levels of microbiological contaminants in water used to prepare replacement fluid for HDF are set by the manufacturer of the HDF equipment [18, 19] and are generally the same as those recommended for standard hemodialysis [4]. A well-functioning water treatment system for dialysis should produce water free of any microbial contaminants as it emerges from the reverse osmosis unit and enters the treated water distribution system. Some authorities recommend the use of two-stage reverse osmosis to prevent microbial contaminants from entering the treated water distribution system; however, available evidence suggests it could be unnecessary to do so [20].

Since the water treatment system produces water that is also free of chlorine or chloramine, any bacteria that do gain entry to the treated water distribution system can colonize the distribution system and contaminate the water entering the dialysis machine. Thus, to achieve high-quality dialysis fluid it is important to focus on preventing bacterial entry and controlling bacterial growth within the system. Bacteria can gain entry to the system via contaminated ports or connectors. Bacteria can also proliferate in the complex fluid pathway of the dialysis machine. In particular, *Pseudomonas* species of bacteria thrive in the low-nutrient, low light, room-temperature aqueous environment of the dialysis water system. *Pseudomonas sp.* can secrete a protein- and polysaccharide-containing slime which facilitates adherence of colonies to the internal surfaces of the piping. That biofilm will shed microbial fragments and occasional viable organisms into the dialysis water, especially after disinfection.

Minimizing the development of biofilm and routinely achieving low levels of microbial contaminants in the water entering the HDF machine depends on both the design and maintenance of the water distribution system. Some key design features related to minimizing biofilm growth are summarized in Table 3.3. For biofilm control, the water distribution system is best configured as direct feed, where the water leaving the reverse osmosis unit flows directly to the HDF machines with any surplus being returned to the inlet of the reverse osmosis unit. Direct feed systems are often impractical, however, and in many situations an indirect feed system incorporating a storage tank will be necessary, either to deal with fluctuating demands for water or to enable the pressure in the distribution system to be boosted. No matter which configuration is used, the water distribution system should be fabricated from a material that allows disinfection with hot water or water containing ozone.

Table 3.3 Design strategies for minimizing microbiological contamination in the preparation of dialysis fluid

Design feature	Benefit
The final stage in the water treatment system should provide a barrier against microbiological contaminants; for example, reverse osmosis or an endotoxin-retentive filter	Prevents entry of bacteria which proliferate in the water treatment system following removal of disinfectants from the potable water supply
Reverse osmosis membranes should be of the hygienic or "full-fit" type	Bacteria can bypass the brine seals used in older types of membrane module
The water distribution system should be configured as the shortest possible loop without branches or dead-ends	Avoids stagnant areas that disinfectants have difficulty reaching
A direct feed water distribution system should be used where practical	Avoids the use of a storage tank where water can be semi-stagnant around the periphery and which can be difficult to disinfect
For direct feed systems, a means of preventing retrograde flow from the inlet line to the reverse osmosis system to the returning treated water distribution loop	Prevents untreated water from entering the treated water distribution system if there is a transient fluctuation in pressure
If a water storage tank is used (indirect feed system), it should have the smallest practical volume and have an easily implemented means of disinfection	Maximizes fluid turn-over in the tank and minimizes stagnation
The water distribution system should be constructed of materials compatible with disinfection by hot water or water containing ozone	Disinfection with hot water or water containing ozone allows daily disinfection, which is impractical with chemical disinfectants
There should be an easily implemented method of disinfecting the inlet water line to the HDF machine	This line is not disinfected when the HDF machine is disinfected and is a common site of biofilm formation
Dry powder cartridges should be used for the preparation of bicarbonate concentrate	Avoids the need to store batches of bicarbonate concentrate which are susceptible to proliferation of haloduric organisms
Connectors should be designed to resist contamination and disinfected regularly	Avoids contamination of the dialysis fluid as it enters the dialyser

Concentrates

While preparation of water meeting the specifications of the manufacturer of the HDF machine is a necessary requirement for HDF, it is not sufficient to ensure trouble-free treatments. The concentrates used to prepare the dialysis fluid from which the replacement fluid is generated must also be of high microbiological quality. Here, the concern is with the bicarbonate-containing concentrate since the pH of the acid concentrate is sufficiently low to prevent microbial growth. Bicarbonate-containing concentrates provide a good growth medium for haloduric organisms [21] and preparation of batches of bicarbonate-containing concentrate from water

and powder at the dialysis facility, and distribution of that concentrate to individual HDF machines, can be an important source of contamination. The most effective means of overcoming that risk is to use powder cartridges designed for use with a particular HDF machine to prepare bicarbonate-containing concentrate on-line at the point of use [22]. Systems that prepare bicarbonate-containing concentrate online for multiple HDF machines have been developed, but to date their use appears to be restricted to Japan [23].

Dialysis Fluid

A properly designed and managed dialysis system will produce dialysis fluid which meets the ISO standards shown in Tables 3.1 and 3.2. That fluid will contain less than 0.25 EU/mL of endotoxin. A patient can be exposed to up 50 L/h of the fluid, which could contain 12,500 EU endotoxin. Fortunately the dialysis membrane is a barrier to the endotoxin, preventing most of it from entering the blood. Nonetheless, many believe standard dialysis fluid should be subjected to filtration though an ultrafilter, usually fitted as part of the dialysis machine, to render the dialysis fluid ultrapure; that is, with a maximum endotoxin concentration of less than 0.03 EU/mL and a total exposure of less than 1,500 EU. While ultrafilters effectively reduce endotoxin concentrations by a factor of at least 100, they may not efficiently remove smaller microbiological contaminants such as DNA fragments [24, 25]. Therefore, a system should not rely on ultrafilters, alone, to maintain fluid quality. It is still necessary to keep bacterial growth within the system to a minimum so that the fluid upstream of the filter conforms to standard quality.

The last point at which the dialysis fluid can be contaminated is where it enters the dialyser. Standard Hansen connectors can be difficult to clean and disinfect and the use of connectors purposefully designed to minimize contamination [19, 26] is preferred.

In post-dilution HDF, approximately 5 L of dialysis fluid is ultrafiltered from the blood each hour. This could be increased up to 20 L/h in pre-dilution HDF. This 5–20 L/h of ultrafiltrate is balanced by infusing a similar volume of fluid directly into the blood.

The pressure difference across the dialyser membrane required to drive that ultrafiltration, effectively prevents the back-filtration which would occur in high-flux dialysis.

However, to avoid infusing more than 400 EU/h of endotoxin, the infused fluid needs to contain less than 0.02 EU/mL of endotoxin. This is below the limit of detection for many endotoxin assays. Thus, current on-line HDF machines use a validated process based on two stages of filtration. The first stage generates ultrapure dialysis fluid, with endotoxin <0.03 EU/mL. The second stage subjects the ultrapure fluid to further ultrafiltration by a device which has been shown to reduce endotoxin levels by a factor of at least 100. It is not possible to verify that the infusion fluid produced by this two-step process is of the required purity. Instead, the process must have been

validated to show that an adequate quality of the infusion fluid is produced every time the machine is used. The validated process is, in part, controlled and monitored automatically by the dialysis machine. However, the process also requires actions by the machine operator and it is imperative that these actions are preformed strictly in accordance with the manufacturer's instructions for use of the machine.

In post-dilution HDF, the infusate is pumped into the venous bubble trap which is under high pressure. In case of leaks or failure of the infusate pump, there is a risk of blood entering the infusate line, potentially contaminating the upstream fluid pathway which is shared between patients. This could allow transfer of blood-borne viruses between patients if there was a simultaneous failure of the disinfection process. To reduce this risk, there is a non-return valve in the infusate line. In HDF, the uncontrolled, unmonitored back-filtration of high-flux dialysis is replaced by controlled and monitored infusion through the infusate filter. The infusion rate is driven by the infusate pump (Fig. 3.2). The safety of HDF depends on the integrity and sterility of the filter, non-return valves and infusate fluid pathway.

One approach to ensuring this is to replace the valve, infusate filter and the entire fluid pathway downstream of the filter with new, sterile packaged components each treatment (Fig. 3.2, top). In this case, the burden of quality control is shifted partly from user to the manufacturer of the packaged components. The infusate line, valve and filter can be integrated into the blood line so there are no connectors in the sterile fluid pathway.

In an alternative approach (Fig. 3.2, bottom), the infusate filter and part of the sterile fluid pathway is disinfected and tested before each treatment. The disinfection process uses measurable physical conditions (e.g. low pH, high temperature). The dialysis machine software monitors the fluid flow and conditions during the disinfection process. The integrity of the fluid pathway, including valve and filter, is tested automatically by the system by monitoring pressure under defined flow conditions. The filter membrane integrity can be tested by allowing air to enter the filter on one side of the membrane (pressure holding test). An intact wet filter membrane is impervious to air. The machine control software will not allow treatment unless the tests have been passed and the system has been adequately disinfected. The disinfection and testing process can be combined with the testing and disinfection of ultrapure dialysis fluid pathway. In this approach, there is a connector in the sterile part of the infusate line, downstream of the filter. The connector design and operation must minimize the chance of introducing contaminants at this point. This approach has the advantage of reduced cost, as the infusate filter is used multiple times. Disadvantages of this approach include increased complexity of equipment and operation.

System Maintenance

Since the potential sources of contamination of dialysis fluids are so diverse and depend on multiple procedures and equipment, each fluid preparation system faces its own unique challenges. No matter how well the water treatment and

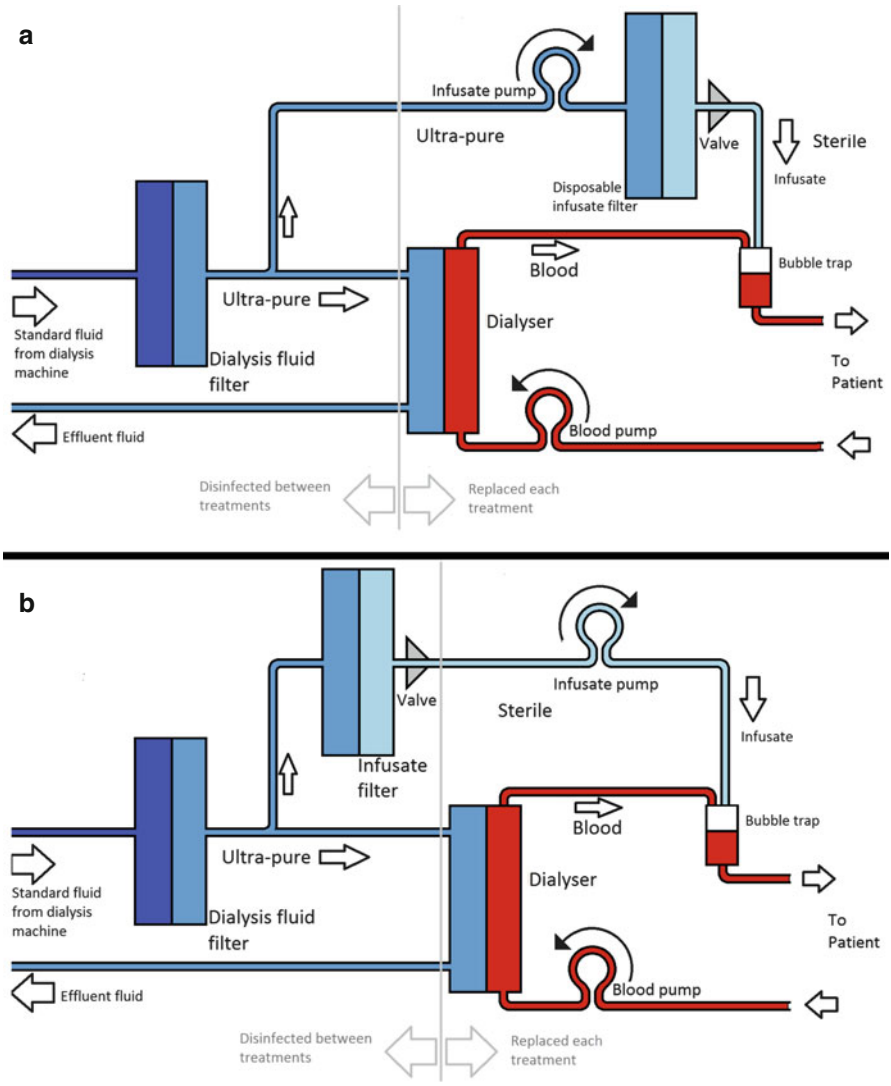

Fig. 3.2 (**a**, **b**) Two designs for HDF

distribution systems are designed, their performance, particularly with regard to microbiological purity, will deteriorate over time unless they are well maintained. The medical director of the dialysis unit is ultimately responsible for the quality of the fluids to which the patients are exposed and it is her or his responsibility to ensure that a maintenance program capable of maintaining the desired quality of the fluids delivered to the patient is established and executed. Guidance on how to achieve this goal has been published as an adjunct to the fluid quality standards [8].

To be successful, a maintenance program must be forward-looking and instituted from first use of the fluid handling systems. The primary goal of the maintenance program is to maintain the systems in such a way that fluid quality is sustained as a routine; it should not be thought of as a means of responding to unacceptable monitoring results. Development of an environment that routinely delivers fluids of the desired quality proceeds through a number of steps [27] (Table 3.4).

The first step should be an initial qualification of the entire fluid handling system. While the initial qualification is best done immediately following installation of a system, it can also be done on an existing system at the time the decision is made to implement an HDF program. The purpose of the initial qualification is to document the system in written form, confirm that there is a written maintenance program for the system, and verify that the staff has received appropriate training on the operation and maintenance of the system. In the case of new systems, it is the time to verify that the system has been installed in accordance with its specifications. Responsibility for the various facets of the operation of the system should be clearly defined at this stage and the qualification should be approved by the person at the dialysis facility with overall responsibility for fluid production. For new systems, that approval often establishes the point at which responsibility for the fluid production systems passes from the vendors who provide and install those systems to the persons with day-to-day responsibility for operation of the dialysis facility. It is important that the disinfection program be initiated as soon as the integrity of the system has been established by pressure testing using filtered air, not fluids which may contaminate the system's internal surfaces.

Table 3.4 Steps in establishing stable fluid production systems

Step	Purpose	Includes
Initial qualification	Define the fluid handling systems and its management	System documentation (Flow diagrams, operating and maintenance manuals)
		Determination of proper installation
		Evidence of proper training
Operational qualification	Demonstration that systems operate as specified	Demonstration that systems operate over intended range of operation
		Demonstration that safety systems operate as intended
		Demonstration that fluid quality specifications are met
Performance qualification	Demonstration that performance is stable over time under routine operating conditions	Intensive monitoring of fluid quality
Routine monitoring	Demonstrate ongoing compliance with fluid quality specifications	Regular monitoring of system components and fluid quality
		Trend analysis of performance data

The initial qualification should be followed by an operational qualification to verify that the system operates as specified, including fluid quality, safety systems and maintenance procedures.

Once the fluid systems have been demonstrated to perform as specified, a period of intensive monitoring should follow to demonstrate that fluid quality is routinely maintained under normal operating conditions. In assessing microbiological quality, it is important to remember that initial negative cultures can be misleading since it can take several weeks before biofilm in the dialysis water distribution system matures to the point that it sheds bacteria into the water.

Once it is clear from the initial qualification phases that the systems are stable and can produce fluid meeting the requirements of the HDF machine manufacturers under normal operating conditions and with the specified maintenance program, ongoing routine monitoring should be performed to ensure continued compliance with the fluid quality requirements. Monitoring data should be subjected to trend analysis to provide advanced information on any changes in system performance so that changes to correct problems can be made prospectively rather than in a reactive manner. This approach is particularly important for the microbiological quality of the fluid since it can be difficult to remove biofilm once it matures.

While the tendency for biofilm formation can be lessened by attention to the design of the distribution system, consistently achieving low levels of microbial contaminants in the water delivered to the HDF machine requires frequent disinfection of all the fluid pathways to minimize biofilm formation. Because biofilm is extremely difficult to eradicate once it has become established, it is key that the disinfection program be designed to prevent biofilm formation rather than eliminate biofilm once it has formed. The preferred means of disinfection is by hot water or water containing ozone. These methods are preferred because, in the case of hot water, there are no residuals or, in the case of ozone, residuals with a very short lifespan. That lack of residuals allows disinfection to be carried out on a daily basis if needed. In contrast, disinfection with chemicals, such as sodium hypochlorite, leaves residuals that require extensive rinsing to remove, thereby limiting disinfection generally to no more than once per week.

An effective ultrafilter will reduce the endotoxin concentration by a factor of at least 100. To remain effective, the system must be properly maintained and operated, since there is the potential for contamination to enter the fluid through the solute additions, connectors, filter membrane defects or bacterial growth downstream of the filter. The filter must be disinfected by a compatible disinfectant and changed at regular intervals as specified by the manufacturer. To reduce the risk of contaminants passing through the filter membrane defects, redundant filters in series may be employed. In an alternative approach, the filter integrity is tested automatically before each use by the system. A typical membrane integrity test would require the filter to hold a pressure difference across the membrane when air has been allowed to enter on one side. An intact membrane should be impervious to air when wet.

Teaching Points

- During online hemodiafiltration, up to 20 L/h of dialysis fluid can be infused directly into the bloodstream.
- This infusion fluid must be free of chemical contaminants, sterile and pyrogen-free.
- This infusion fluid must be prepared with a dialysis machine that has been validated to produce sterile and pyrogen-free fluid.
- The user of the machine is responsible to ensure the machine manufacturer's instructions are followed, including providing the dialysis machine with water and concentrates that meet the specifications as set by the machine manufacturer.
- Properly designed systems for water treatment and distribution, and concentrate preparation are central to produce sterile and pyrogen-free infusion fluid, free of contaminants.
- Water treatment and distribution systems must be subject to rigourous quality control including maintenance practices designed to prevent contamination of the dialysis and infusion fluids.

References

1. Ledebo I. On-line preparation of solutions for dialysis: practical aspects versus safety and regulations. J Am Soc Nephrol. 2002;13:S78–83.
2. Ward RA. Water processing for hemodialysis. A historical perspective. Semin Dial. 1997;10:26–31.
3. World Health Organization. Guidelines for drinking water quality. 4th ed. WHO press, Geneva, Switzerland. 2011.
4. International Organization for Standardization. Water for haemodialysis and related therapies (ISO 13959:2014). Geneva: International Organization for Standardization; 2014.
5. International Organization for Standardization. Quality of dialysis fluid for haemodialysis and related therapies (ISO 11663:2014). Geneva: International Organization for Standardization; 2014.
6. Handelman GJ, Megdal PA, Handelman SK. Bacterial DNA in water and dialysate: detection and significance for patient outcomes. Blood Purif. 2009;27:81–5.
7. Henrie M, Ford C, Andersen M, et al. In vitro assessment of dialysis membrane as an endotoxin transfer barrier: geometry, morphology, and permeability. Artif Organs. 2008;32:701–10.
8. International Organization for Standardization. Guidance for the preparation and quality management of fluids for haemodialysis and related therapies (ISO 23500:2014). Geneva: International Organization for Standardization; 2014.
9. Williams KL. Endotoxins: pyrogens, LAL testing and depyrogenation. 2nd ed. New York: Marcel Dekker Inc; 2001.
10. New SE, Aikawa E. Cardiovascular calcification: an inflammatory disease. Circ J. 2011;75:1305–13.
11. McIntyre CW, Harrison LE, Eldehni MT, et al. Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol. 2011;6:133–41.
12. Yao Q, Axelsson J, Heimburger O, et al. Systemic inflammation in dialysis patients with end-stage renal disease: causes and consequences. Minerva Urol Nefrol. 2004;56:237–48.

13. Hasegawa T, Nakai S, Masakane I, et al. Dialysis fluid endotoxin level and mortality in maintenance hemodialysis: a nationwide cohort study. Am J Kidney Dis. 2015. doi:10.1053/j.ajkd.2014.12.009.
14. Susantitaphong P, Riella C, Jaber BL. Effect of ultrapure dialysate on markers of inflammation, oxidative stress, nutrition and anemia parameters: a meta-analysis. Nephrol Dial Transplant. 2013;28:438–46.
15. Kwan BC, Chow KM, Ma TK, et al. Effect of using ultrapure dialysate for hemodialysis on the level of circulating bacterial fragment in renal failure patients. Nephron Clin Pract. 2013;123:246–53.
16. Arizono K, Nomura K, Motoyama T, et al. Use of ultrapure dialysate in reduction of chronic inflammation during hemodialysis. Blood Purif. 2004;22:26–9.
17. Sitter T, Bergner A, Schiffl H. Dialysate related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients. Nephrol Dial Transplant. 2000;15:1207–11.
18. Ledebo I. On-line haemodiafiltration: technique and therapy. Adv Ren Replace Ther. 1999;6:195–208.
19. Polaschegg H-D, Roy T. Technical aspects of online hemodiafiltration. In: Ronco C, Canaud B, Aljama P, editors. Hemodiafiltration, Contrib Nephrol, vol. 158. Basel: Karger; 2007. p. 68–79.
20. Penne EL, Visser L, van den Dorpel MA. Microbiological quality and quality control of purified water and ultrapure dialysis fluids for online hemodiafiltration in routine clinical practice. Kidney Int. 2009;76:665–72.
21. Ebben JP, Hirsch DN, Luehmann DA, et al. Microbiologic contamination of liquid bicarbonate concentrate for hemodialysis. Trans Am Soc Artif Intern Organs. 1987;33:269–73.
22. Delin K, Attman PO, Dahlberg M. A clinical test of a new device for on-line preparation of dialysis fluid from bicarbonate powder: the Gambro BiCart. Dial Transplant. 1988;17:468–82.
23. Koda Y, Mineshima M. Advances and advantages in recent central dialysis fluid delivery system. Blood Purif. 2009;27 Suppl 1:23–7.
24. Schindler R, Beck W, Deppisch R, et al. Short bacterial DNA fragments: detection in dialysate and induction of cytokines. J Am Soc Nephrol. 2004;15:3207–14.
25. Glorieux G, Hulko M, Speidel R, et al. Looking beyond endotoxin: a comparative study of pyrogen retention by ultrafilters used for the preparation of sterile dialysis fluid. Sci Rep. 2014;4:6390.
26. Kawanishi H, Moriisha M, Sato T, et al. Fully automated dialysis system based on the central dialysis fluid delivery system. Blood Purif. 2009;27 Suppl 1:56–63.
27. Ward RA. Quality management of dialysis fluid for online convective therapies. In: Kawanishi H, Yamashita AC, editors. Hemodiafiltration – a new era, Contrib Nephrol, vol. 169. Basel: Karger; 2010. p. 1–11.