The Evolution of Technological Strategies in the Prevention of Dialysis Water Pollution: Sixteen Years’ Experience

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\textbf{Abstract}

\textbf{Aim:} This report attempts to illustrate the positive impact on the quality of dialysis water produced over a 16-year period through the progressive optimization of technological procedures. \textbf{Methods:} Fundamental steps included the following: elimination of polyvinyl chloride (PVC), periodical controls, introduction of stainless steel and/or polyethylene polymer and substitution of single-pass reverse osmosis (SRO) with double-pass reverse osmosis (DRO). Daily overnight automatic thermal disinfection of distribution piping rings represented the final step. \textbf{Results:} A dramatic improvement was observed in 645 water samples obtained from distribution piping. The measures applied resulted in a significant improvement of water quality, featuring levels of colony-forming units per milliliter ranging from 247.4 ± 393.7 in the presence of PVC and SRO to 14.1 ± 28.0 with stainless steel and DRO and 2.8 ± 3.2 with cross-linked polyethylene thermoplastic polymer and DRO (p < 0.01). \textbf{Conclusions:} Dialysis water should be viewed by nephrologists as a medicinal product, and every effort should be made to ensure a high-quality liquid.

\section*{Introduction}

In Europe, patients receiving hemodialysis undergo at least three treatment sessions a week. As standard hemodialysis treatment sessions last 4–6 h, individual patients are exposed to 15,000–20,000 liters of dialysis fluid yearly. In hemodiafiltration procedures, dialysis water is administered in the form of dialysate and infusate of up to 3,400–6,800 liters intravenously following single-step ultrafiltration of dialysis water [1]. An increasing tendency to use online hemodialysis procedures implies the potential presence in distribution rings of microbial, fungal and chemical substances which should be carefully monitored, as provided for in specific guidelines [2, 3]. Moreover, water should be tested by certified laboratories using specific analytical procedures, particularly in the case of microbiological controls, differing from those applied to test human blood and secretions [4]. A wide range of undesirable substances may originate from polluted water or infiltrations in the drinking water supply network. These are largely caused by human error through the contamination of waterworks following use of ineffective measures to reduce bacterial and/or chemical pollutants in surface water collected in artificial basins, or from the building of drinking water networks using obsolete or inappropriate materials. The majority of toxic contami-
nants derive from procedures applied in the treatment of municipal water, suggesting a potential unsuitability for direct use in hemodialysis. An unmonitored dialysis water treatment facility, an inefficient system for the distribution of dialysis water to monitors or infrequent disinfection constitute the most frequent causes of the presence of harmful or fatal substances, particularly hazardous when adopting online procedures lacking effective ultrafilters with intravenous administration. Thus, the main parameters to be applied in the treatment of water destined for use in hemodialysis procedures should be rigorously adhered to [4, 5].

As a premise, to illustrate the evolution of plants, a synthesis of the most recent ANSI/AAMI/ISO 23500 guidelines published in 2011 [2] is provided. The authors wish to underline that dialysis procedures used in the USA do not foresee administration to patients of the high number of intravenous infusions practiced in Europe when high convective methods such as hemodiafiltration are prescribed. Furthermore, double-pass reverse osmosis (DRO) with overnight thermal disinfection is referred to, although not described in detail, in the AAMI guidelines (point B 3.4.4) [2], although to date it is applied only scarcely throughout Italy and Europe.

Water used in hemodialysis applications is prepared according to a series of purification processes, the most common of which include [2, 5, 6] cartridge filters, softeners, carbon system dechlorination, reverse osmosis (RO) or deionization and distribution. Pretreatment procedures involve the use of a variety of cartridge filters, both in mains tap water purification processes and at the end of the pretreatment system to remove coarse particulate matter prior to purification and to protect RO membranes from damage from fine particles flushed from carbon beds. The performance of carbon beds is monitored on the basis of total chlorine content in outflow water from the carbon bed. Good-quality beds should be used to ensure an optimal chlorine concentration (0.5–1 ppm). The use of chlorination and dechlorination is not mandatory, although at times it is required. To prevent inadvertent exposure of patients to chloramine as the capacity of the carbon is exhausted, two carbon filters in series should be applied. However, inadequate chloramine removal may occur, and assays to detect the presence of chloramines and specific rapid water checks are mandatory [7].

RO involves the use of high pressure to force water across a semipermeable membrane to form product water (permeate), thereby rejecting 90–99% of ionic contaminants and >95% of nonionic contaminants. Resin beads inside the tanks display a high affinity for bivalent cations (calcium and magnesium) present in feed water, releasing two sodium ions (monovalent) for each calcium or magnesium ion captured to prevent RO membranes from fouling due to calcium and magnesium salts. Softeners should be regenerated by exposing resin to a strong brine with a very high sodium content. Dechlorinators and softeners represent a favorable ‘pabulum’ for bacterial growth due to the impossibility of disinfecting with a specific product unless thorough rinsing is undertaken against the flow. An RO membrane is an effective barrier against microbiological contaminants, bacteria, viruses and endotoxins. However, currently DRO is probably the most effective form compared to single-pass RO (SRO), achieving the highest degree of contaminant removal. This procedure is particularly recommended for use in online hemodialytic methods with a high convective component [8]. Deionization uses ion exchange resins to remove ionic contaminants from water by exchanging hydrogen ions for cationic resins and hydroxyl ions for anionic resins. Mixed bed deionizers contain both cationic and anionic resins but do not remove microbiological contaminants. Deionizer performance should be closely monitored to avoid exhaustion, resulting in low-affinity binding of ions to the resin. This phenomenon, which may lead to a high content of contaminants in product water as the deionizer becomes exhausted [9], has been reported to cause acute, fatal fluoride toxicity in hemodialysis patients [10]. This method is virtually obsolete in Italy.

The treated water exits the central water treatment plant and is distributed to hemodialysis monitors through a distribution piping ring with an optimal diameter to avoid sluggish segments; the use of metals such as brass, aluminium or galvanized metal should be avoided, and the distribution system should be designed as a closed ring. The use of polyvinyl chloride (PVC) should be eliminated, and materials such as high corrosion resistance stainless steel (INOX AISI 316L), cross-linked polyethylene thermoplastic polymer (PEX) and polyvinylidene fluoride should be used to construct piping.

The pollutant potential of reservoir storage is frequently underestimated. Tanks should be opaque, made of plastic for foodstuffs and not located in a ‘stagnant’ corner of the circuit, but should guarantee a constant flow of water. Moreover, fundamental bacteriological and endotoxin controls should be implemented to ensure appropriate sampling and transportation procedures, inoculation of the culture medium with dialysis water and accurate interpretation of results, not only taking into ac-
count environmental mesophiles, but also testing for the presence of bacteria or mycetes [11].

Therefore, based on these premises, the present study was undertaken to assess the quality of water in 5 dialysis units in which numerous changes to disinfection and monitoring methods had been implemented over a 16-year period. Equipment used in the production and distribution of ultrapure dialysis water had also considerably improved, particularly in dialysis units adopting online convective procedures with production of high volumes of intravenous infusate, such as online hemodiafiltration techniques.

### Materials and Methods

#### Characteristics of the Units

Five dialysis units were tested over a period of 16 years and 3 months (195 months, from May 1996 to April 2012) for a total of 643 samples. On average, 3 samples per distribution ring were tested every 3 months in steady-state equipment. Chemical controls were performed twice yearly, as prescribed by ISO 13959. ISO standards 13958, 13959 and 11663, illustrated in the Italian guidelines, were used as a reference for microbiological and endotoxin values [5] (Table 1). The frequency and rate of tests were gradually decreased, in view of both the positive results yielded and budgetary restraints imposed by the Health Department. Microbiological sampling was undertaken in all units at the head, middle and end of the piping ring from the valve connecting the ring to the dialysis monitor pipeline. All out-of-range values were immediately retested.

In both standard and online methods, dialysis monitors were fitted with two ultrafilters certified to lower colony-forming units (CFU) $>10^{-7}$.

Throughout the 5 units involved, following withdrawal of PVC, the mean distribution of online hemodiafiltration was approximately 65% (ca. 104 hemodialysis patients).

Subsequent to a variable period ranging between 65 and 84 months, all PVC piping was withdrawn; additionally, throughout this period it was not possible to perform LAL tests.

### Table 1. Microbiological controls and range values

<table>
<thead>
<tr>
<th></th>
<th>Tap water</th>
<th>Refined water after osmosis (start and end of ring piping)</th>
<th>Final dialysate for standard hemodialysis</th>
<th>Nonpyrogenic dialysate for hemodialysis/ hemodiafiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>maximum allowable level</td>
<td>frequency</td>
<td>maximum allowable level</td>
<td>frequency</td>
</tr>
<tr>
<td>Bacteria at 22° C CFU/ml</td>
<td>100</td>
<td>every 6 months</td>
<td>100</td>
<td>monthly</td>
</tr>
<tr>
<td>Mycetes at 22° C CFU/ml</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>every 6 months</td>
</tr>
<tr>
<td>Endotoxins, EU/ml</td>
<td>–</td>
<td>–</td>
<td>0.25</td>
<td>monthly</td>
</tr>
</tbody>
</table>

Unit 1. A self-care center providing for 6 outpatient positions located in a seaside area. After 65 months of using PVC and SRO, stainless steel AISI 316L was implemented as a replacement. DRO (CWP WRO 131/132 ROHH Gambro®) is scheduled to be installed in the near future.

Unit 2. A hospital unit located in a mountain zone, which was recently increased from 14 to 17 positions. The unit was previously managed by a neighboring Health Department and was taken over by ourselves in 2006; highly polluted, obsolete PVC was immediately replaced with PEX. In 2010, the unit was moved within the hospital, and Cleanpex® and DRO with thermal disinfection were installed.

Unit 3. An outpatient medical unit purpose-developed in 1996. After 66 months, PVC was replaced with stainless steel AISI 316L; 70 months later, the latter was replaced with SRO and DRO with thermal disinfection.

Unit 4. A hospital unit located in a seaside town, which was recently increased from 9 to 16 positions. The unit was previously managed by a neighboring Health Department and was taken over by ourselves in 1998. PVC was eliminated 18 months later and replaced with stainless steel AISI 316L with SRO; after 122 months, the unit was moved within the hospital, and Cleanpex and DRO with thermal disinfection were installed.

Unit 5. A self-care unit located at sea level purpose-developed in 1999 and providing for 11 positions. After 84 months, PVC and SRO were withdrawn and replaced with Cleanpex and DRO with thermal disinfection.

### Pretreatment Procedures

Hard water should undergo treatment with double water softeners. Softeners are sized in grains of capacity and are invariably regenerated on a routine daily basis with concentrated sodium chloride solution (brine). This method was applied throughout the observation study.

Another fundamental step is dechlorination. In each unit, tanks containing approximately 400 liters of granular activated carbon which had never been regenerated were used. Throughout the first 5 years of operation, the carbon beds had not been replaced, although in the last 7 years, granular activated carbon had been changed on a yearly basis, and carbon tanks were subjected to daily backwashing. Softeners and dechlorinators cannot be disinfected as commonly applied disinfection products may damage their material beds. The amount of softeners used in the units in-
vestigated was invariably overestimated, in order to guarantee an effective softening of inflow water featuring >1,000 mS/cm.

Reverse Osmosis
Thin-film SRO membranes are made from polyamide spiral wound around a permeate-collecting tube. This material is compatible with peracetic acid, used in disinfection. SRO with Kosmed KSL® in polyamide has been abandoned over the last 3 years and substituted with DRO. The new CWP WRO 131/132 ROHH Gambro equipment with ISO 14021 (using polyamide membranes) adopted latterly is provided with a fully automated system of thermal disinfection that no longer requires chemical disinfection and features an integrated system for the thermal disinfection of the membranes and distribution ring. Thermal disinfection of DRO systems provides an automated daily thermal disinfection of the dialysis room distribution circuit using water heated to 90/95 °C, to prevent the formation of biofilms. The heated disinfection unit, dialysis room distribution circuit using water heated to 90/95 °C, systems provides an automated daily thermal disinfection of the membranes and distribution ring. Thermal disinfection of DRO systems provides an automated daily thermal disinfection of the dialysis room distribution circuit using water heated to 90/95 °C, to prevent the formation of biofilms. The heated disinfection unit, integrated into the DRO plant, is programmed to heat water from 63 to 90/95 °C at a specified time of day, towards the end of the dialysis session, with the possibility of varying programming for each day of the week. The circulation of hot water in the dialysis room distribution circuit occurs at the end of the dialysis session and continues throughout the night until shortly before the start of the morning session when piping temperatures return to safer levels. In addition to providing correct operating parameters, the integrated computer in the DRO system records data for the last three operating sessions and elaborates data as required, in the form of text, synoptic diagram or a graph.

Two-stage chemical disinfection of the distribution ring downstream of the dialysis monitors was carried out on a monthly basis, using peracetic acid for 2 h and chloroxidant agent for an additional 2 h followed by accurate rinsing. Chemical disinfection was abandoned following the introduction of thermal disinfection in 4 centers.

The connection between the distribution ring and monitors remains a highly critical and considerably underestimated aspect. Use of a mesh tube, which constitutes an ideal pabulum for the growth of bacteria, mycetes and algae, has been definitively prohibited. Swabs obtained from the inside of mesh tubes after a period of approximately 15 days yielded significant results with a microbiological load >10,000 CFU/cm² [pers. observation]. Mesh tubes were subsequently replaced at all kidney positions by large extendable PEX springs suitable for monthly disinfection using peracetic acid and/or chloroxidant. Use of these methods at no time resulted in corrosion and/or decoloration of stainless steel AISI 316L.

Microbiological and Endotoxin Controls
Sampling was carried out according to a series of procedures aimed at obtaining a small aliquot of water for analysis. Depending on the test to be performed, the volume of water required for testing should be established prior to sampling. Bottles and vials used to collect samples for microbiological analysis should never be washed at the time of sampling. In addition to exposing recipients to possible contamination, rinsing would remove traces of sodium thiosulfate present in the tap. Water should be left to run long enough to eliminate disinfectants prior to sampling.

Taps should be cleaned and disinfected prior to sampling; both taps and the inside of collars should be cleaned, and residues, dust, mucilage, detergents, disinfectants and other substances capable of affecting the outcome of microbiological analysis should be removed. A solution of sodium hypochlorite or similar types of disinfectant should be used in cleansing; 10% solutions of commercially available sodium hypochlorite or sodium dichloroisocyanurate may be used. Due to the corrosive nature of these products, particular caution should be applied. Fatty deposits should be removed by rubbing with isopropyl alcohol. The disinfectant solution should be left on the taps for 2–3 min and all traces of the product subsequently removed. During sampling procedures, contamination should be avoided and the quality of the water sample maintained. In addition to the mandatory cleansing and disinfection procedures, metal taps may also be flame gouged. However, if the latter procedure is performed in a perfunctory manner, it will not produce the desired effect on potential microbial contamination. As previously, the tap should be left to run for 2–3 min and flow left untouched during sample collection. On collection, open the sterile bottle taking care not to touch the inside of the cap or bottle neck, which will come into direct contact with the sample, and close immediately after sampling. Particular care should be taken not to overfill the bottle to enhance effective lab homogenization of the sample at the time of testing.

Transportation
During transportation and storage of samples for microbiological testing, the representative nature of samples should be ensured by preventing regrowth of micro-organisms. As far as possible, alterations which may at times be inevitable in a small aliquot of water stored in a closed container should be kept to a minimum. The sample should be stored away from light (both ultraviolet and visible) and high temperatures, and appropriate conditions of hygiene should be applied during transportation. From the time of sampling to arrival in the lab, all samples should be stored at a temperature of below 10 °C, with the optimum range of 2–8 °C being recommended. Samples must be inseninated within 2 h.

Analytical Procedures
In agreement with microbiologists, the authors opted to extend tests to a greater spectrum of micro-organisms frequently detected in previous studies, particularly as the hydrogeographic situation in Sardinia is almost exclusively characterized by the presence of surface water collected in artificial basins. Accordingly, and in view of the high temperatures registered during summer months, the Italian Department of Health has not ruled out the potential development of harmful bacteria, including cyanobacteria [12], resulting in the release of cyanotoxins. Preparations are therefore currently being made to assemble procedures for use in detecting the latter.

In the authors’ opinion, microbiological testing procedures should not only assess the presence of environmental mesophiles and mycetes but should also address the issue of detecting specific pathogens [13–17]. Samples were obtained as far away as possible from the most recent point of disinfection according to a rotation schedule between at least three locations situated at the beginning, centre and end of the ring; culture dishes were read as provided for in standard guidelines.

Total Coliforms
An aliquot (100 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration char-
acteristics corresponding to a nominal pore size of 0.45 μm. Place the membrane over M-Endo agar LES medium and incubate at 36 ± 1°C for 18–24 h. Red-colored colonies with a metallic sheen that grow over a 24-hour period, generally accompanied by a dark brick-red coloring in the area beneath the membrane, are deemed to be coliform bacteria (presumed coliforms). Tests should be carried out on colonies aged no more than 24 h. Tests should then be undertaken to verify coliform status; the presence of the cytochrome oxidase enzyme on all or at least a representative number of typical colonies should be assessed, and biochemical identification tests should be performed. Tests should be carried out on colonies aged no more than 24 h.

Enterococci
An aliquot (100 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration characteristics corresponding to a nominal pore size of 0.45 μm. Place the membrane over Slanetz and Bartley agar medium and incubate at 36 ± 1°C for 40–48 h. Following incubation, colonies ranging from pink to dark red and brown (in the center or throughout the colony) are deemed typical (presumed enterococci). Tests should be undertaken to confirm enterococcus status; the presence of the catalase enzyme and the esculin hydrolysis test should be undertaken on all or at least a representative number of typical colonies, and biochemical identification tests should be performed. Tests should be carried out on colonies aged no more than 24 h.

_Pseudomonas aeruginosa_
An aliquot (250 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration characteristics corresponding to a nominal pore size of 0.45 μm. Place the membrane over the pseudomonas agar/CN medium and incubate at 36 ± 1°C for 40–48 h. After 2 ± 2 and 44 ± 4 h, assess the growth of typical colonies on isolation medium. _Pseudomonas aeruginosa_ may develop as greenish-blue colonies that produce pyocyanin or fluorescent or reddish-brown colonies. Count all characteristic colonies (presumed _P. aeruginosa_). Tests should then be undertaken to confirm _P. aeruginosa_ status; place under a Wood’s lamp, and following observation of all or at least a representative number of typical colonies, biochemical identification tests should be performed. Tests should be carried out on colonies aged no more than 24 h.

_Clostridium perfringens_
An aliquot (100 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration characteristics corresponding to a nominal pore size of 0.45 μm. Place the membrane over tryptose sulphite cycloserine agar medium, and cover the membrane completely with 5–6 ml of liquid culture medium at a temperature of 50 ± 5°C. Leave to set and incubate at a temperature of 44 ± 1°C for 21 ± 3 h under anaerobic conditions. Micro-organisms belonging to the _Clostridium perfringens_ species form black or grey/yellowish-brown colonies on the tryptose sulphite cycloserine agar medium, conveying a slightly darker shade above or below the filtering membrane. Count all typical colonies and treat as presumed _C. perfringens_. Isolate colonies on two parallel blood agar plates and incubate one plate under anaerobic conditions and the other under aerobic conditions at 36 ± 1°C for 21 ± 3 h. Following incubation, examine plates to check the presence or absence of growth. _C. perfringens_ colonies typically produce areas of clear hemolysis on culture medium. Undertake confirmation tests only on colonies growing on blood agar under anaerobic conditions, and subsequently perform biochemical identification tests. Tests should be carried out on colonies aged no more than 24 h.

Mycetes
An aliquot (100 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration characteristics corresponding to a nominal pore size of 0.45 μm. Place the membrane over Sabouraud dextrose agar medium and incubate at 22–25°C for 3 ± 5 days. Recognition of fungal species is a complex issue requiring lengthy observation and proven experience. The aspect of colonies varies according to the type of substrate (natural or artificial), and colonies develop on the basis of numerous factors. To identify yeasts, sample from the center of the colony and smear onto a glass slide in a drop of sterile distilled water. View under a microscope, preferably under contrast and at 20× or 40× magnification to identify pseudomycelium and spores; if necessary, perform commercially available miniaturized biochemical identification tests. Use the direct observation technique to view filamentous fungi under the microscope. Carefully attach a piece of transparent sticky tape to the surface of the colony to be examined; transfer the tape with the sticky part facing downwards onto a glass slide containing a drop of lactophenol blue solution and cover with a glass cover slip; observe at 100× to enhance distinguishing of fructiferous bodies [18].

**Counting of Colonies at 22–37°C**
Analytical procedures undertaken to assess the number of micro-organisms at 37 and 22°C are identical for both parameters, comprising the agar inclusion technique. Seed 1 ml of the sample onto the bottom of a Petri dish. Pour approximately 15 ml of isolation substrate maintained in a liquid state at a temperature of 45 ± 1°C onto the dishes containing the inoculum.

Mix thoroughly by rotating the dishes backwards and forwards to facilitate a complete mixing of culture medium and sample. Leave to set and incubate one dish at 36 ± 1°C for 40–48 h and the other at 22 ± 1°C for 64–72 h.

**Counting at 22°C (Mesophiles)**
An aliquot (100 ml) of the sample collected should be filtered through a 47-mm cellulose ester membrane with filtration characteristics corresponding to a nominal pore size of 0.22–0.45 μm. Place the membrane on the agar medium and incubate at 20–23°C for 7 days.

**Endotoxin Tests**
Endotoxin tests are carried out both on osmotic water as monitors are detached [19] and at random initial-intermediate and end points in the ring. Spectrophotometric methods are used (Endosafe®-PTS™ Charles River®) with gel clotting at 37°C. Acceptable limits of detection on ring detachment are <0.05, and <0.01 for online infusion liquid. A training course in the use of this piece of equipment is required; the machine should be calibrated by the manufacturers on a yearly basis.

Indispensable requisites for online hemodiafiltration are as follows: 0 CFU/ml for bacteria and mycetes and <0.01 for endotoxins. This method should not be used if the requisites are lacking.
Results

The results of the strategies implemented are illustrated in Table 2. The use of improved technologies and methods, together with a constant increase in online procedures, has resulted in a progressive amelioration of procedures and safer treatment for patients. The changes made by the authors focused on four main aspects: (1) progression from sporadic maintenance and disinfection of plants to scrupulous periodic controls and disinfection geared to seasonal chemical and microbiological variations; (2) regular chemophysical, microbiological and endotoxinic monitoring of plants in conjunction with a highly specialized lab which does not test for mesophiles at 22°C but at 37°C for frequently pathogenic bacteria commonly detected in summer months (e.g. *Escherichia coli*, *P. aeruginosa*); (3) evolution from SRO to DRO with daily thermal disinfection, and (4) gradual replacement of PVC and related problematic issues with stainless steel INOX 316L and/or PEX. Table 2 should therefore be interpreted bearing in mind not only the switch to PVC and other types of ring piping, but also the considerable rise in the number of kidney positions in dialysis units in recent years. No pathogenic germs were detected in the dialysis units investigated.

Discussion

The island of Sardinia features a typically Mediterranean climate with subtropical characteristics during the summer months. However, in recent decades, rainy periods meeting the needs for water supply have alternated with frequently lengthy periods of severe drought, the last of which was in 2001. This situation led to severe and often unforeseen consequences for the Department of Nephrology, created largely by the fact that the Municipal Authorities were forced to implement drastic measures and supply water only on alternate days. This resulted in reduced pressure throughout the water supply networks, frequently old and leaking, and infiltration of polluted water; indeed, town and rural water supply networks are frequently situated in the vicinity of water tables that may be polluted by sewage and fecal waste. Moreover, it should be underlined that in Italy the use of underground water tables in the supply of water to hospitals is permitted by law. The authors’ experience in 5 territorial dialysis units, based on microbiological results obtained at several points throughout the central plant and piping ring, has led to the substitution of materials and procedures used previously, resulting in vast improvements in the microbiological/endotoxinic quality of water. Specifically, it has been demonstrated how, in the presence of materials other than PVC, regular disinfection and frequent controls (even monthly) represent a fundamental step forward in achieving safe levels of CFU [20]. The use of PVC should be discarded, particularly in view of its unsuitability for use with thermal disinfection methods and potentially cancerogenic status [21]. Overnight thermal disinfection achieved by means of biosmosis resulted in a dramatic decrease in microbial load [7], which was more significant following the use of PEX compared to INOX in construction of the distribution ring. In the authors’ opinion, this difference may be associated with the diameter of the piping; a smaller diameter decreased the possibility of stagnant areas, creating a less suitable habitat.

<table>
<thead>
<tr>
<th>PVC/SRO</th>
<th>INOX/SRO</th>
<th>INOX/DRO</th>
<th>PEX/SRO</th>
<th>PEX/DRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of technical posts checked (number of centers)</td>
<td>38 (4)</td>
<td>27 (3)</td>
<td>28 (2)</td>
<td>14 (1)</td>
</tr>
<tr>
<td>Observation period, months</td>
<td>58.2 ± 24.4</td>
<td>96.6 ± 21.2</td>
<td>19.0 ± 7.0</td>
<td>52</td>
</tr>
<tr>
<td>Total trimestral controls, n</td>
<td>233</td>
<td>290</td>
<td>38</td>
<td>52</td>
</tr>
<tr>
<td>Bacteria, CFU/ml</td>
<td>247.4 ± 393.7</td>
<td>150.5 ± 235.5</td>
<td>14.1 ± 28.0</td>
<td>175.5 ± 365</td>
</tr>
<tr>
<td>Mycetes, CFU/ml</td>
<td>14.9 ± 26.35</td>
<td>7.7 ± 12.2</td>
<td>1.2 ± 0.4</td>
<td>15.5 ± 29.4</td>
</tr>
<tr>
<td>Cost/year, EUR</td>
<td>52,425</td>
<td>65,250</td>
<td>11,400</td>
<td>15,600</td>
</tr>
<tr>
<td>Endotoxins, EU/ml</td>
<td>&lt;0.03 ± 0.05</td>
<td>&lt;0.03 ± 0.01</td>
<td>&lt;0.02 ± 0.005</td>
<td>&lt;0.04 ± 0.02</td>
</tr>
</tbody>
</table>

PVC was abandoned and replaced in 3 centers with INOX distribution ring piping and in 1 center with PEX piping.
for biofilm growth. It is fundamental that pressure along the walls and the velocity of fluid shear stress on the smaller inner surfaces are higher than those recorded in larger-sized sections; in other words, a higher water pressure and velocity enhance filling of the circuit and ensure against the formation of concealed areas where bacteria and hyphae may flourish.

Moreover, frequent thermal disinfection does not rapidly prevent the formation of bacterial biofilm and hyphae along inner surfaces. Therefore, in the light of experience gained in units operating with PVC rings, the authors discarded this material entirely.

The application of meticulously implemented periodic thermal and chemical disinfection procedures should be further supported by additional checks and immediate correction of any 'pathological' areas detected by microbiological results. Accordingly, a specialized team should be appointed to carry out controls and periodic and non-scheduled maintenance following the finding of microbiological levels exceeding established safety limits.

Irrespective of the material used in construction of the distribution ring, the connection valves to dialysis monitors should be of stainless steel AISI 316L, in view of the potential liability of the connection and increased risk of stagnant areas at this level; this option results in a decreased probability of microbial adhesions. In all the processes described, chemical, physical and bacteriological tests should be undertaken by a certified laboratory. The authors maintain that standard procedures should be established in conjunction with an institution specializing in environmental water testing; moreover, labs should possess a working knowledge of the equipment used and ensure that methods described in the leading guidelines in the field are adhered to. A synergic cooperation between the dialysis unit staff, the maintenance team and the lab is mandatory, with purpose-trained specialized technicians performing sampling throughout all units and providing for correct transportation to the testing lab.

Systems should be monitored continuously and the following taken into account [22]: (1) on application of chemical disinfection alone, solutions capable of removing both bacterial biofilms and potential mineral scales that may protect bacteria and/or enhance their survival should be used every 30 days, and (2) daily overnight thermal disinfection should be implemented, and additional monthly or twice monthly disinfection of the osmosis membranes and ring should be undertaken as described above. The authors decided to reduce the interval established by the Italian guidelines for microbiological controls from 4 to 3 months [5] in view of the wide range of seasonal variations. Based on the results obtained, in an attempt to limit the high costs involved, bacteriological controls will comprise the following: one testing of drinking water supply, frequently stored in water tanks, one postsoftener testing, one postdechlorinator testing, two instances of pre- and post-DRO monitoring and testing at three random points of the ring (head/intermediate/end) and one testing of recirculating water for a total of 9 tests per unit. The increased safety has been felt in the estimated costs of operations and transfers of technicians and bacteriological tests for the 5 dialysis units, as shown in table 2.

The experience gained has led the authors to progressively undertake alterations aimed at optimizing the microbiological quality of dialysis water. Weak points may be represented by an excessive inner diameter of piping, both in PVC and INOX, resulting in pipes that are disproportionate to water flow and volume; the definitive abolishment of PVC and mesh tubes connecting the ring to the monitors is fundamental.

In contrast, the strong points that emerged from the present study focused largely on the use of PEX. However, very few literature reports published to date have focused on the efficacy of the latter material in preventing the adhesion of bacterial biofilms. Stainless steel INOX AISI 316L is a long-lasting but rather costly material; however, its longevity may enhance amortization of costs. When using stainless steel, assembly and soldering should be undertaken by specialized technicians to guarantee a sealed connection between the various segments. Studies undertaken with stainless steel yielded perfectly satisfactory results; however, the authors underline the suitability of PEX circuits, which are devoid of seams and, according to the quality of water, provide for a scheduled replacement of the circuit every 7–10 years. The importance of applying correctly sized piping and an antireflux gooseneck drainage spout should be emphasized.

### Conclusion

Over a 16-year period, the dynamic procedures set up have demonstrated that DRO should be viewed as a necessary investment aimed at establishing a safer microbiological profile and producing a positive impact on microinflammation in dialysis patients, particularly in the case of increased infusion volumes during online hemodiafiltration procedures [23]. Likewise, daily overnight thermal disinfection procedures have proved at times to be more effective than frequent chemical disinfection. By stating
this, the authors do not intend to imply that this type of disinfection is an obligatory option, although implementa-
tion of DRO should be seen as an essential ‘standard’. Spe-
cialists in the field may choose between thermal and/or
chemical disinfection and establish a treatment schedule
on the basis of microbiological and endotoxinic character-
tistics of the water, even feasibly combining daily thermal
treatment with chemical disinfection every 2 or 3 months.
Finally, it has become increasingly clear that staff op-
erating in nephrology units should possess a strong, mo-
tivated cultural synergic background to aid them in view-
ing dialysis water as a medicinal product.

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