

---

# Water treatment and monitor disinfection

Gianni CAPPELLI, Marco RICCARDI, Salvatore PERRONE, Moreno BONDI, Giulia LIGABUE, Alberto ALBERTAZZI

*Nephrology Dialysis and Renal Transplantation Unit, Department of Biomedical Sciences, University of Modena, Modena, Italy*

## Abstract

Water treatment system and dialysis monitors are susceptible to microbial contaminations and periodical disinfection procedures are mandatory to obtain results requested from international standards and guidelines. Several chemical germicides or some physical treatments are on the market validated by device manufacturer according to medical device directives. With time, interfering substances from dialysis device or water are able to modify disinfection efficiency. Simulating-use testing is not a common procedure to validate disinfectants and recent data document as biofilm represents the most important cause of disinfection inefficacy. Some international standards include tests in the presence of various interfering substances but their use is not widespread. When using a disinfectant, residue toxicity, material compatibility and potential risks for the staff also have to be considered. A quality assurance program has to be implemented to obtain adequate performances and to improve results on patients.

**Key words:** Hemodialysis, water, dialysate, microbial contamination, disinfection

---

## BACKGROUND

For a long time, the quality of water for dialysis and of dialysate have been neglected by many nephrologists<sup>1</sup> but today they have emerged as fundamental components of the dialysis procedure. They participate to the biocompatibility of the system<sup>2</sup> and, from more recent data, they represent contributory factors to the hypercytokinemia of dialysis patients with consequent chronic inflammation.<sup>3</sup> An amplifying effect is attributed to biofilm formation when water treatment system and monitors become contaminated with microbes because of inadequate maintenance.<sup>4</sup> As a result, disinfection procedures represent the possibility of avoiding the passage of endotoxins or even smaller bacterial fractions through the dialyzer membrane, thus significantly reducing the markers of microinflammation, such as C-reactive protein and interleukin-6. Disinfection assures water quality as a part of various

anti-inflammatory treatment strategies to improve outcome on these patients.

## STANDARDS, GUIDELINES AND DISINFECTION PROTOCOLS

The recent introduction of quality concepts into medicine has supported the definition of acceptable levels of contamination, both chemical and microbiological, in dialysis fluids. The Association for the Advancement of Medical Instrumentation (AAMI) standards have represented a worldwide reference for many years since 1980 and they have been recently updated.<sup>5,6</sup> In Europe most standards are defined by the European Pharmacopoeia<sup>7</sup> or suggested by national guidelines.<sup>8</sup> Table 1 reports the present microbiological standards. Of note is the definition of ultrapure dialysate. It represents one of the most important issues in dialysis since 2 decades. After the first demonstration of the potential clinical advantages, ultrapure dialysate has been accepted and routinely used from most nephrologists and nowadays it enters AAMI standards and EDTA guidelines being claimed as indicated for all type of hemodialysis treatments.<sup>8,9</sup> To assure adequate

---

Correspondence to: Prof. Gianni Cappelli, Nephrology Dialysis and Renal Transplantation Unit, University Hospital of Modena, Via Del Pozzo, 71, 41100 Modena, Italy. E-mail: cappelli@unimo.it

**Table 1** Standard for microbiological contamination in different dialysis fluids

	AAMI <sup>6</sup>	European pharmacopoeia <sup>7</sup>	EDTA-ERA guidelines <sup>8</sup>
Water for dialysis			
Bacteria (CFU/mL)	200 (action level at 50)	100	100
Endotoxin (EU/mL)	2 (action level at 1)	0.25	0.25
Concentrate			
Bacteria (CFU/mL)	200 (action level at 50)	—	—
Endotoxin (EU/mL)	2 (action level at 1)	0.5	—
Dialysate			
Bacteria (CFU/mL)	200 (action level at 50)	—	100
Endotoxin (EU/mL)	2 (action level at 1)	—	0.25
Ultrapure dialysate			
Bacteria (CFU/mL)	0.1	—	0.1
Endotoxin (EU/mL)	0.03	—	0.03
Dialysate for infusion			
Bacteria (CFU/mL)	< 10 <sup>6</sup>	< 10 <sup>6</sup>	< 10 <sup>6</sup>
Endotoxin (EU/mL)	< 0.03	0.25	< 0.03

CFU=colony forming units

quality from standard or ultrapure dialysate as well as from infusion solutions used in online treatments, contamination levels reported in Table 1 should be periodically checked while regular disinfection protocols are mandatory, both for water treatment system and monitors.

As a matter of fact disinfection should be scheduled to prevent rather than to eliminate bacteria and biofilm.

Different liquid chemical germicides or physical disinfectant techniques are commercially available. Table 2 reports the most common disinfectants used in dialysis and their effects in terms of tolerability on piping materials. Chemical and physical germicides may damage the devices or lead to deterioration of the materials, adversely affecting the safety and effectiveness of the device. Disinfectant action may result in surface cracking or pitting,

making the piping more difficult to clean or more prone to maintenance needs with increased costs.

Optimal disinfectant agent is supposed to obtain not only disinfection but also a descaling and a cleaning effect. To help in obtaining all these effects it is a common procedure to intermittently add to disinfectant some descaling agent. Acetic acid, citric acid, acetohydroxy acid, lactic acid, sodium carbonate represent most commonly used products and depending on the agent this is used alone or in mixed combination with chemical or heat disinfection.

## DISINFECTANT VALIDATION

Most germicides used for dialysis equipment are derived from substances with a well documented antibacterial

**Table 2** The most common disinfectants used for water treatment system and monitors and their compatibility with piping material (Adapted from AAMI Standard and Recommended Practices. AAMI/RD52:2004. *Dialysate for Haemodialysis*. Arlington, VA, USA: Association for the Advancement of Medical Instrumentation; 2004)

	Water treatment system	Monitors	Compatibility <sup>a</sup>
Physical			
Ultraviolet irradiators	X	—	nr
Hot water (>80 °C)	X	X	PVDF, PEX, SS
Chemical			
Hypochlorites	X	X	PVC, PVDF, PEX, PP, PE
Peracetic acid	X	X	PVC, PVDF, PEX, PP, PE, ABS
Chlorine dioxide	—	X	PVC, PVDF, PEX, PP, PE
Formaldehyde	X	—	PVC, PVDF, PEX, PP, PE, SS
Ozone	X	—	PVC (low concentration), PVDF, SS

ABS = acrylonitrile butadiene styrene; PEX = cross-linked polyethylene; PE = polyethylene; PP = polypropylene; PVC = polyvinylchloride; PVDF = polyvinylidene fluoride; SS = stainless steel; nr = not reported; X = applicable; — = not applicable.

effect and with a long tradition of use in hospital disinfection. The criteria for choosing from several compounds are based not only on general antibacterial activity but mainly on specific items: time contact dependent activity, toxicity from residues, handling problems, biocompatibility, stability, material/device compatibility. A validation process is derived from these parameters to indicate a disinfectant agent for use on dialysis water system treatment or monitors.

In the U.S.A., up to some years ago, the Food and Drug Administration (FDA) would consider liquid chemical germicides to be accessories to the devices they were used to process and regulated them in the same class as the primary device. Subsequently, FDA defined 3 types of liquid chemical germicides for medical devices: sterilant/high-level disinfectant, intermediate level disinfectant, and low level disinfectant and from a regulatory perspective divided these products into 2 categories: (i) high-level disinfectants for processing critical and semicritical devices and (ii) general purpose disinfectants (including intermediate level disinfectants and low level disinfectants) for processing noncritical devices and medical equipment surfaces. More recently, in 2002, the FDA reviewed disinfectants specifically used in dialysis to process dialysate delivery systems and water purification systems. It was recognized that, even if liquid chemical germicides do not meet the definition of sterilants/high-level disinfectants, they can have an impact on the exposure of dialysis patients to bacterial toxins. The final decision was that to provide reasonable assurance of safety and effectiveness, manufacturers had to submit premarket notification and receive clearance before marketing a disinfectant agent for dialysis. Disinfectants are now considered as accessories to the dialysate delivery systems and water treatment systems, which are both class II devices, and therefore are in the same class II classification.<sup>10</sup> Recent AAMI standards underline these requirements and note, at the same time, that day-to-day microbial dialysate quality is under the control of the healthcare professionals who deliver dialysis therapy.<sup>6</sup> In Europe water treatment system and dialysis monitor are as well regulated by medical devices and CE mark application directives. Disinfection is a part of the maintenance procedure validated by the device's manufacturer. Once the device has entered a clinic with a prolonged routine use, many critical variables as difficulty in cleaning, materials modification, pH, temperature and hardness variations from incoming water, various bacterial strains presence, and biofilm presence may limit contact and effectiveness of disinfection. These variables represent modification of initial validation process and induction

of conditions under which germicide will fail. Hard water and 5% albumin are commonly referenced as examples of inorganic and organic challenges, respectively, to be used in simulated worst-case-use testing during validation process. At this moment, although most disinfectant agents used in water treatment system and inside hemodialysis machines have proven efficacy in killing planktonic bacteria, few of them have been tested for biofilm activity. From literature it has been demonstrated a concentration-dependent effect from bleach on biofilm removal<sup>11</sup> and, with different disinfectants, a bacterial regrowing after some hours from a disinfection performed in the presence of biofilm on piping.<sup>12,13</sup> At the same time chemical or heat disinfections show a reduced effect on endotoxins removal when devices are heavily contaminated and biofilm is present.<sup>11,12</sup> Only autoclaving is able to obtain biofilm removal with an almost complete (>99.9%) bacteria and endotoxins reduction.<sup>11,14</sup> Unfortunately this method is limited to 1 model of dialysis monitor and application of steam disinfection to water treatment plant results in a high-initial cost followed by a still high maintenance.

The Association Francaise de Normalisation (AFNOR) has set several standards for determining bactericidal activity of a disinfectant, depending on intended use, and simulating 'in vitro' clean and dirty conditions.<sup>15</sup> Based on standard recommended for high-level disinfection of medical instrumentation, specific standards could be suggested for dialysis equipment. Based on AFNOR standards, Table 3 reports suggested standards description as well as considered interfering substances and specific test requirements. Some of these standards have been adopted as European standards from CEN (European Committee for Standardization). In Europe, following medical device directives for dialysis-related devices, the problem of assuring disinfection has caused movements at regulatory level on germicidal validation but inquiry on a recent database from France reports only 3 products as validated according to some AFNOR standards.<sup>16</sup>

## PROBLEMS FROM CHEMICAL GERMICIDES

Germicide residues that could remain associated with devices following disinfection may be toxic and may pose a risk to patients and users. The residues may be active or inert ingredients as well as their by-products or neutralizers. The remaining amount of residue may vary depending upon the conditions of use of the germicide, the specific component materials, and the methods used to rinse the device itself. Therefore, it is important that the

Table 3 Example of standards applicable to germicides validation for dialysis device

AFNOR reference	CEN reference	Disinfectant type	Evaluated activity	Strain	Result
NF T72-152	NF EN 1040	Chemical disinfectant	<i>In vitro</i> antibacterial basic activity under defined conditions ( <i>Mycobacterium</i> not considered)	<i>Pseudomonas aeruginosa</i> CIP 103-467 Staphylococcus aureus CIP 483	Reduction of at least 10 <sup>5</sup> times the initial selected strains with 60'/45'/30'/15'/5'/1' of contact time at 20 °C
NF T72-201		Liquid germicide	Antifungal (not on <i>Aspergillus</i> )	<i>Absidia corymbifera</i> IP 1129-75 <i>Candida albicans</i> CIP 1180-79 <i>Cladosporium cladosporioides</i> IP 1232-80 <i>Penicillium verrucosum</i> varcyclopium IP 1231-80	Reduction of at least 10 <sup>4</sup> times the initial selected strains with 15' of contact time at 32 °C
NF T72-202	NF EN 1275	Chemical disinfectant	Complete antifungal activity	<i>Aspergillus niger</i> IP 1431-83 <i>Candida albicans</i> IP 4872 or <i>Candida albicans</i> IP 4872 alone	Reduction of at least 10 <sup>4</sup> times the initial selected strains with 60'/30'/15'/5' of contact time at 20 °C
NF T72-180		Liquid germicide	Antiviral activity	<i>Adenovirus human</i> type 5 Enterovirus polio 1 Orthopoxvirus	Reduction of at least 10 <sup>4</sup> times the initial selected strain with 60'/30'/15' of contact time at 32/20 °C
NF T72-230		Liquid germicide	Sporicidal activity (Dilution-neutralization method)	<i>Bacillus cereus</i> CIP 7-803 <i>Bacillus subtilis</i> var. <i>niger</i> CIP 7-718 Clostridium sporogenes 51 CIP 7-939	Reduction of at least 10 <sup>5</sup> times the initial selected strains with 60'/5' of contact time at 75/20 °C
NF T72-231		Liquid germicide	Sporicidal activity (Membrane filtration method)	<i>Bacillus cereus</i> CIP 7-803 <i>Bacillus subtilis</i> var. <i>niger</i> CIP 7-718 Clostridium sporogenes 51 CIP 7-939	Reduction of at least 10 <sup>5</sup> times the initial selected strains with 60'/5' of contact time at 75/20 °C
NF T72-170		Liquid germicide	<i>In vitro</i> antibacterial basic activity ( <i>Mycobacterium</i> not considered) Presence of interfering conditions (Dilution-neutralization method)	<i>Enterococcus hirae</i> CIP 5-855 <i>Escherichia coli</i> CIP 54-127 <i>Pseudomonas aeruginosa</i> CIP A-22 <i>Staphylococcus aureus</i> CIP 53-154	Reduction of at least 10 <sup>5</sup> times the initial selected strains with 60'/45'/30'/15'/5'/1' of contact time at 32/20 °C. Interference from 1% or 3% albumin, 1% yeast extract, 30 °F or 60 °F water hardness
NF T72-171		Liquid germicide	<i>In vitro</i> antibacterial basic activity ( <i>Mycobacterium</i> not considered) Presence of interfering conditions (Membrane filtration method)	<i>Enterococcus hirae</i> CIP 5-855 <i>Escherichia coli</i> CIP 54-127 <i>Pseudomonas aeruginosa</i> CIP A-22 <i>Staphylococcus aureus</i> CIP 53-154	Reduction of at least 10 <sup>5</sup> times the initial selected strains with 60'/45'/30'/15'/5'/1' of contact time at 32/20 °C. Interference from 1% or 3% albumin, 1% yeast extract, 30 °F or 60 °F water hardness

residues are analyzed and quantified to avoid the potential health risks to patients.

In addition, the staff is exposed to the germicide solution while repeatedly processing devices with the germicide. Therefore, the potential health risks that the germicide solution poses to the staff from handling the solution also should be assessed and adequate preventive measures should be used.

To avoid problems from chemicals use and to simplify treatments, some physical treatments have entered dialysis units mainly for disinfection of water treatment system. Ultraviolet (UV) irradiators, ozone generators, as well as hot water or steam generators are progressively increasing in use. The goal would be to completely substitute chemicals to avoid most problems and contribute to reduce impact on environmental pollution.

Ultraviolet emission has to be carefully controlled as it is lamp frequency dependent and decreasing with time of use. Ultraviolet lights are effective in killing bacteria when a low-pressure mercury lamp emits UV at a wavelength of 254 nm and assures a radiant energy dose of 30 mWs/cm<sup>2</sup>. The effect on endotoxins removal is quite low and therefore its use is limited to the pre-treatment section of the water plant. Ozone is quite effective in killing bacteria and degrading endotoxins with efficacy being concentration and time dependent. It is generated from air oxygen and injected in the water of the system. A concentration of 0.2–0.5 mg/L with a 10-min contact time is needed to completely kill bacteria, spores and virus, whereas longer time and higher concentrations are necessary for biofilm removal. Ozone production should be monitored and its level in ambient air should also be periodically checked to enter contamination air standard of 0.1 ppm. Hot water (>85 °C) is used for distribution loop and monitor disinfection. Prescribed temperature degrees and contact time should also be periodically controlled and temperature level has to be controlled at the most distant place from central heater. In some water treatment system hot water is rinsed from distribution loop to the monitors, allowing a simultaneous monitor disinfection. Ozone and heat water may degrade or damage some plastic material (Table 2) of the water system or monitor and attention has to be reserved to the use of resistant materials.

## QUALITY ASSURANCE PROGRAM

Most dialysis monitors include possibility of choosing between isolate chemical or heat disinfection or to combine both. A further option is to include monitors in the daily disinfection of the water treatment system, as with hot water. The different options are manufacturer vali-

dated but microbiological controls should be performed to confirm efficacy of scheduled protocol disinfections. Monitoring levels of bacteria and endotoxins helps to demonstrate that the disinfection program is effective, not to indicate when disinfection should be performed. Bacterial growing should be prevented to avoid biofouling and microbial fractions passage to the patient through dialyzer membrane. The institution of a quality assurance program in dialysis units includes not only a generic definition of periodical monitoring but also the creation of a log sheet to register disinfection executions, obtained results and actions performed for inadequate results. Operators should be trained in the use of the equipment and periodic audits should be performed to test compliance with predefined protocols.

## REFERENCES

- 1 Bommer J, Ritz E. Water quality: A neglected problem in hemodialysis. *Nephron*. 1987; **46**:1.
- 2 Cappelli G. Dialysate contribution to bio-incompatibility in hemodialysis. *Contemp Dial Nephrol*. 1991; **12**:20–22.
- 3 Yao Q, Axelsson J, Heimburger O, Stenvinkel P, Lindholm B. Systemic inflammation in dialysis patients with end stage renal disease: Causes and consequences. *Minerva Urol Nefrol*. 2004; **56**:237–248.
- 4 Cappelli G, Tetta C, Canaud B. Is biofilm a cause of silent chronic inflammation in hemodialysis patients?: A fascinating working hypothesis. *Nephrol Dial Transplant*. 2005; **2**:266–270. Epub 2005 Jan 12.
- 5 AAMI Standard and Recommended Practices. AAMI/RD62:2001. *Water Treatment Equipment for Hemodialysis Applications*. VA, U.S.A.: Association for the Advancement of Medical Instrumentation; 2001.
- 6 AAMI Standard and Recommended Practices. AAMI/RD52:2004. *Dialysate for Haemodialysis*. Arlington, VA, U.S.A.: Association for the Advancement of Medical Instrumentation; 2004.
- 7 European Pharmacopoeia 4th ed. *Haemodialysis solutions, concentrated, water for diluting*. Monograph 1167:2002. Council of Europe, Strasbourg, 2002.
- 8 European best practice guidelines for haemodialysis (part 1) Section IV: Dialysis fluid purity. *Nephrol Dial Transplant*. 2002; **17**(Suppl 7):45–62.
- 9 Mion CM, Canaud B, Garred LJ, Stec F, Nguyen QV. Sterile and pyrogen free bicarbonate dialysate: A necessity for hemodialysis today. *Adv Nephrol Necker Hosp*. 1990; **19**:275–314.
- 10 Center for Device and Radiological Health: *Regulatory Status of Disinfectants Used to Process Dialysate Delivery Systems and Water Purification Systems for Hemodialysis; Guidance for Industry and FDA*. Document 1419. CDRH, Rockville, MD, U.S.A., August 30, 2002.

- 11 Marion-Ferey K, Pasmore M, Stoodley P, Wilson S, Husson GP, Costerton JW Biofilm removal from silicone tubing: An assessment of the efficacy of dialysis machine decontamination procedures using an in vitro model. *J Hosp Infection*. 2003; **53**:64–71.
- 12 Cappelli G, Sereni L, Scialoja MG, et al. Effects of biofilm formation on haemodialysis monitor disinfection. *Nephrol Dial Transplant*. 2003; **18**:2105–2111.
- 13 Cappelli G, Ricardi M, Perrone S, et al. Disinfection on dialysis monitors and biofilm impairment. *Blood Purif*. 2004; **22**:385. (Abstract 12).
- 14 Cappelli G, Di Felice A, Perrone S, et al. Miro-Clav: Assessment of disinfection efficacy. *Nephrol Dial Transplant*. 2000; **15**:A200.
- 15 Association Francaise de Normalisation (AFNOR): Disinfectants and antiseptic. [www.afnor.fr](http://www.afnor.fr)
- 16 *Produits Hygiene Base*. <http://Prodhybase.univ-lyon1.fr>