

## Assessment and monitoring the quality of treated water and dialysate in a public hemodialysis center to prevent potential health risks

M.I. Mime<sup>1,3</sup>, S.Bellebia<sup>1</sup>, Z.Bengharez<sup>1\*</sup>, M.Louni<sup>2</sup>, K.Benrachedi<sup>3</sup>.

<sup>1</sup>Laboratory Advanced Materials and Physicochemistry for Environment and Health, DjillaliLiabes University, SidiBel Abbes 22000, Algeria

<sup>2</sup>Nephrology-Hemodialysis Department, Hospital of Thenia, Thenia 35005, Algeria

<sup>3</sup>Food Technology Research Laboratory, M'HamedBougara University, Boumerdes 35000, Algeria.

\*Corresponding author: E-mail: [dzbengharez@yahoo.fr](mailto:dzbengharez@yahoo.fr); Tel: +213 5 41 71 15 78;

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### ABSTRACT

*Abstract:* This study aimed to assess and monitor the physicochemical, bacteriological and endotoxin quality of dialysis water and dialysate used in a public HD center and to evaluate the performance of the unit to reject organic contaminants.

Samples were collected over two years period (October 2014 to April 2017) from different points of the hemodialysis chain. Physicochemical parameters, bacterial count and endotoxin levels in dialysis waters as well as in dialysate were measured according to international standard methods. The water treatment system used at the dialysis center is based on single reverse osmosis

The results of this study revealed the presence of chemical contaminants namely trihalomethanes (THMs), total concentration of THMs was found to be  $50.69 \pm 8.980$  and  $42.54 \pm 19.07$   $\mu\text{g/L}$  in treated water and in dialysate respectively and Chloroform was the major contributor to the THMs concentration. A peak of conductivity about 4-fold higher than the norm was recorded. Detection of low microbial perturbation in both feed and treated water as well as in dialysate due probably to inappropriate disinfection. Endotoxins were identified in 84% of treated water samples with values below the limit set by the European Pharmacopoeia ( $<0.25$  EU/mL). During the entire study period the performance of the dialysis unit for THM removal remained moderate and did not reach 50%.

More efforts are required regarding water quality management in the HD center for a successful HD therapy. Local guidelines on HD water quality should be developed and enforced.

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## I. Introduction

Chronic kidney disease (CKD) and end-stage renal disease (ESRD) are major public health concerns due to their prevalence and high incidence around the world. While in developed countries this disease affects mainly the elderly, in Africa and developing countries, it settles in young and active population, which represents a high burden for these countries. Recent estimates indicate that by the year 2030, more than 70 % of the patients with ESRD are from developing countries [1, 2].

In Algeria, according to the latest figures provided by the Algerian Society of Nephrology Dialysis and Transplantation during its 24<sup>th</sup> National Congress of Nephrology on 25<sup>th</sup> and 26<sup>th</sup> November 2017 at the International Conference Center of Algiers, the ESRD currently reaches 25,000 people with a prevalence of 600 pmp (patients per million population) and an incidence of 200 patients per year per million population. 92% of patients in ESRD are treated by hemodialysis (HD), 2% by peritoneal dialysis (PD) and only 6% by renal transplantation (RT). So hemodialysis remains the most used replacement therapy.

Water, the essential product in hemodialysis, is used to prepare the dialysate and disinfect the dialysis machines. About 120 liters of water are purified to ensure a hemodialysis session for each patient, nearly 400 L per week and 20 m<sup>3</sup> per year [3,4], so hemodialysis is the most demanding therapy in water resources. During dialysis, only a semi-permeable membrane separates treated water from patient's blood, this very close contact imposes a water with high quality which must meet the physicochemical and bacteriological compliance standards defined by the European Pharmacopoeia (EPH) [5] and the Association for the advancement of medical instrumentation (AAMI) [6]. However, today's methods for verification and monitoring water quality are time consuming and costly especially for endotoxins and microorganisms and consequently non-frequently performed, in particular at small dialysis centers, this can be at origin of serious health concerns for patients on hemodialysis.

In this context, the present study was undertaken to investigate the physicochemical, bacteriological and endotoxin quality of dialysis waters and dialysate used in the HD unit of public hospital of Thenia, center of Algeria over the period of October 2014 to April 2017.

Particular interest was focused on the presence of trihalomethanes (THMs) and bacteria including endotoxins which constitute important water quality indexes for hemodialysis. A statistical treatment of the results and a comparison with the EPH and AAMI standards were carried out in order to conclude on the performance of the HD unit and the clinical repercussions that can be observed in the case of contamination of the dialysis waters.

## II. Materials and methods

### II.1. Study design

The study was performed from October 1, 2014 to April 30, 2017. It was conducted in the nephrology-hemodialysis service of the hospital of Thenia in Boumerdes city, center of Algeria.

### II.2. Characteristics of water treatment at the HD unit

The dialysis unit is supplied from the water distribution network of the hospital (municipal water or tap water). The water pretreatment system responds to a classic pattern with passing tap water through 10 µm particulate filters followed by a sand filter, two activated carbon filters, two softeners to remove Calcium and Magnesium and a microfilter 01µm. Then, Pretreated water undergoes a treatment by reverse osmosis (GAMBRO/ CWP 60, WRO 62) before feeding the dialysis machines. The reverse osmosis membrane is a modified polyamide thin film composite membrane. The material used in the water distribution system at the HD unit is polyvinylchloride (PVC). A schematic diagram of the dialysis water treatment system at the HD unit of Thenia is shown in **Figure 1**.

The station is operational since June 2013 and produces about 673.92 m<sup>3</sup> per year. 9 dialysis machines operate for eight hours a day (two sessions of four hours per day), so a capacity of 18 patients per day.

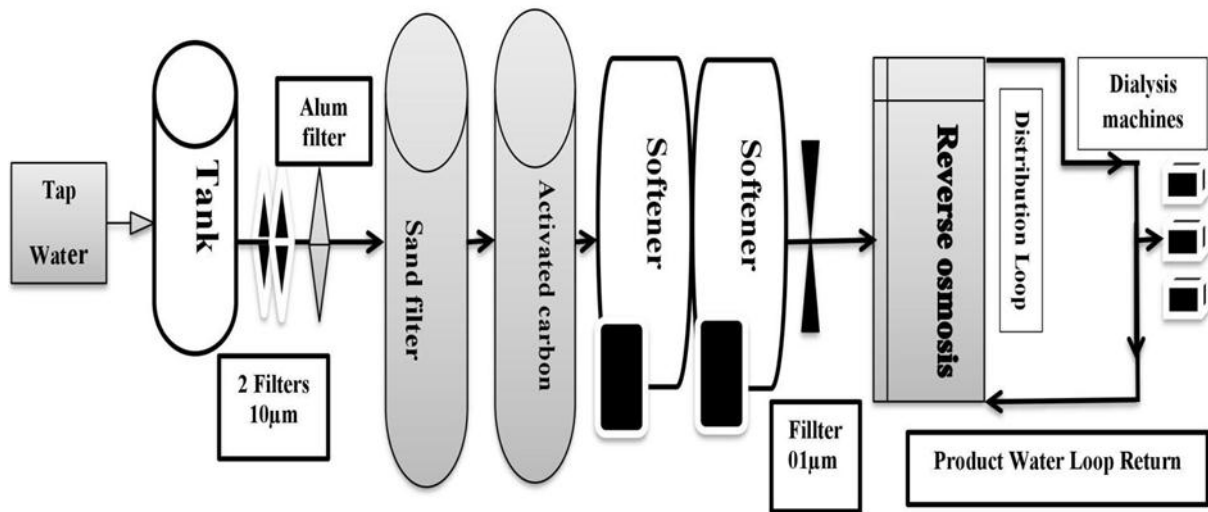


Figure 1: Schematic diagram of the dialysis water treatment system at the HD unit.

### II.3. Disinfection and management

Thermo-chemical disinfections by sodium hypochlorite of dialysis machines are routinely performed after each session. Citric acid monohydrate is used to disinfect the RO membrane every two weeks for 30 minutes.

### II.4. Sampling points

Three sampling points were selected, namely:

- Municipal water (tap water) that feeds the dialysis unit
- Treated water after reverse osmosis.
- The dialysate which constitutes the dialysis bath

All samples were collected during the study period at a frequency of 4 times a year and prepared according to the ISO 23500: 2011[7].

### II.5. Physical and chemical analysis

Water and dialysate samples were assayed for the following parameters:

Color was measured by platinum-cobalt method, pH was determined with pH meter MA5730 Iskra, conductivity was measured using a multiparameter HANNA HI 2030. Concentrations of Sodium ( $\text{Na}^+$ ), Calcium ( $\text{Ca}^{2+}$ ), Potassium ( $\text{K}^+$ ), Magnesium ( $\text{Mg}^{2+}$ ), Aluminum ( $\text{Al}^{3+}$ ), Iron ( $\text{Fe}^{2+}$ ), Copper ( $\text{Cu}^{2+}$ ) and Mercury ( $\text{Hg}^{2+}$ ) were analyzed by flame atomic absorption spectrophotometry (ThermoSolaar M Series) while Ammonium ( $\text{NH}_4^+$ ) and Sulfate ( $\text{SO}_4^{2-}$ ) were assayed by UV-Visible spectrophotometry (UV/VIS Jasco V-530). Chloride concentration was measured using titrimetric method and fluoride ions by potentiometry using fluoride selective ion electrode (ELIT 8221F-41936).

THMs (Chloroform  $\text{CHCl}_3$ , Dichlorobromomethane  $\text{CHBrCl}_2$ , Dibromochloromethane  $\text{CHClBr}_2$  and Bromoform  $\text{CHBr}_3$ ) were analyzed by gas chromatography (Perkin-Elmer, model Clarus 680MS).

### II.6. Microbiological Analysis

Detection of total microbial counts (TMCs) at 22 and 37°C, total coliforms (TC), *Escherichia coli* (*E. Coli*) and faecal streptococci was performed for the same sampling points according to the international standard methods (ISO): TC and *E. Coli* by membrane filtration technique [8], TMCs by incorporation into the medium [9] and faecal streptococci according to ISO 7899-2: 2000 [10].

### II.7. Endotoxin Analysis

Despite the treatment of drinking water used to prepare dialysate, Gram-negative bacteria can multiply rapidly in pure water without any nutrients and resist to disinfectants [11], it is the case of endotoxins. Lipopolysaccharide endotoxin (LPS) is the main lipid component of the outer membrane of Gram-negative bacteria and is released into the surrounding medium during bacterial proliferation and more when the bacterium dies [12]. The toxicity of endotoxin is associated with the lipid component (Lipid A) and immunogenicity is associated with the polysaccharide components. At a frequency of one sample per quarter, endotoxins were analyzed by the gelation method LAL (Limulus amoebocyte lysate) [13] for treated water samples at the start of the distribution loop.

### II.8. Statistical analysis

Statistical analysis was performed using the software STATISTICA 7.0. Quantitative variables were recorded as mean values  $\pm$  standard deviation. Data were considered to be statistically significant if  $P < 0.05$  at 95% confidence interval.

### III. Results and discussion

The physicochemical characteristics of dialysis fluids are presented in **Table 1**. The concentration of THMs and the efficiency of water treatment system to remove them (estimated by the % rejection ratio) are summarized in **Table 2**. The results of bacteriological and endotoxin analyzes appear in **Tables 3 and 4** respectively.

Water for hemodialysis (treated water) represents 97% of the dialysate's composition, its quality is thus the determining factor of the dialysate quality and is one of the most important aspects of ensuring safe and effective delivery of hemodialysis to patients with end-stage renal disease.

Based on the obtained results, excepting conductivity, all physicochemical parameters of

treated water used to make up dialysate solutions were found to be below the maximum limits specified by both EPh and AAMI guidelines. A large difference of some ions concentrations was observed between water samples collected before and after treatment, this is the case of Ca ( $p < 0.001$ ) Mg ( $p = 0.01$ ), Na ( $p = 0.035$ ), K ( $p < 0.001$ ),  $\text{NH}_4^+$  ( $p = 0.005$ ),  $\text{Cl}^-$  ( $p = 0.003$ ),  $\text{F}^-$  ( $p < 0.001$ ),  $\text{SO}_4^{2-}$  ( $p < 0.001$ ) and Al ( $p = 0.008$ ) which proved the efficiency of the HD water treatment system in eliminating ions from water.

The conductivity was significantly reduced after treatment (from 469.63 to 16.325  $\mu\text{S}/\text{cm}$ ) but not sufficiently to achieve the set limits, it was ranged from a minimum of 6.79  $\mu\text{S}/\text{cm}$  to a maximum of 27.9  $\mu\text{S}/\text{cm}$  with an average of  $16.33 \pm 6.02$   $\mu\text{S}/\text{cm}$ , about 4-fold higher than the standard established by the European Pharmacopoeia (4  $\mu\text{S}/\text{cm}$ ). However, the conductivity of the different dialysate samples, measured at the outlet of the dialyzer, varies between a minimum of 12300  $\mu\text{S}/\text{cm}$  and a maximum of 15620  $\mu\text{S}/\text{cm}$  with an average of  $14\,038.75 \pm 1\,303.86$   $\mu\text{S}/\text{cm}$ , these values are remained within the recommended range of 12 to 16  $\text{mS}/\text{cm}$  for dialysate quality [14].

*Table 1: Mean values of chemical parameters detected at different points of sampling.*

PARAMETERS	SAMPLINGS			LIMITS		
	Tap water	HD water	Dialysate	Tapwater <sup>a</sup>	HD W <sup>b</sup>	HD W <sup>c</sup>
Color, Co/Pt	1.50 $\pm$ 0.535	0.1 $\pm$ 00	7.31 $\pm$ 1.308	15	0	-
Temperature, °C	19.07 $\pm$ 2.982	18.975 $\pm$ 2.330	34.25 $\pm$ 1.092	25	20	-
pH	7.53 $\pm$ 0.343	6.841 $\pm$ 0.901	7.7 $\pm$ 0.315	[6.5 – 9]	[4.4-7]	-
Conductivity, $\mu\text{S}/\text{cm}$	469.63 $\pm$ 36.91	16.325 $\pm$ 6.021	14038.75 $\pm$ 1303.8	2800	4	-
Total hardness, mg /L	210 $\pm$ 48.391	4 $\pm$ 5.555	259 $\pm$ 102.964	500	-	-
Calcium, mg /L	75.11 $\pm$ 39.960	0.875 $\pm$ 1.458	65.21 $\pm$ 41.092	200	2	2
Magnesium, mg /L	7.52 $\pm$ 6.325	0.588 $\pm$ 1.561	17.047 $\pm$ 12.684	150	2	4
Sodium, mg /L	8.54 $\pm$ 8.503	5.447 $\pm$ 8.677	2134.91 $\pm$ 1097.30	200	50	70
Potassium, mg /L	1.48 $\pm$ 0.848	00 $\pm$ 00	43.46 $\pm$ 30.533	12	2	8
Chlorides, mg /L	138.46 $\pm$ 87.61	13.265 $\pm$ 7.84	3569.9 $\pm$ 1134.92	500	50	-
Ammonium, mg/L	0.0717 $\pm$ 0.037	0.0206 $\pm$ 0.023	0.01737 $\pm$ 0.0137	0.5	0.2	-
Sulfate, mg/L	38.373 $\pm$ 10.31	12.727 $\pm$ 7.892	10.274 $\pm$ 7.351	400	50	100
Fluoride, mg /L	0.0663 $\pm$ 0.018	0.017 $\pm$ 0.0114	0.0083 $\pm$ 0.00109	1.5	0.2	0.2
Aluminium, mg /L	0.04 $\pm$ 0.024	0.008 $\pm$ 0.009	0.01 $\pm$ 0.006	0.2	0.01	0.01
Iron, mg/L	0.0185 $\pm$ 0.005	0.016 $\pm$ 0.004	0.01 $\pm$ 0.009	0.3	0.1	0.1
Copper, mg /L	0.01 $\pm$ 0.031	00 $\pm$ 00	00 $\pm$ 00	2	0.1	0.1
Mercury, mg /L	00 $\pm$ 00	00 $\pm$ 00	00 $\pm$ 00	0.006	0.001	0.0002

<sup>a</sup>Algerian standards for tap water. Official Journal N°13 (2014)

<sup>b</sup>European Pharmacopoeia standards for HD water(8<sup>th</sup>edition 2014)

<sup>c</sup>ANSI/AAMI/ISO 23500:2011

During the whole study period, the presence of trihalomethane compounds were highlighted in all sampling points (**Table2**), Chloroform was detected in treated water and in dialysate to average concentrations of  $19.61 \pm 16.08 \mu\text{g} / \text{L}$  and  $19.19 \pm 17.27 \mu\text{g}/\text{L}$  respectively and Dichlorobromomethane at levels of  $14.09 \pm 5.430 \mu\text{g} / \text{L}$  and  $9.230 \pm 2.145 \mu\text{g}/\text{L}$  for the same samples while Dibromochloromethane and Bromoform were present at lower concentrations. Similar results regarding the presence of THMs, in particular Chloroform, were obtained in antecedent works [15-18], the authors explained this fact by excessive water chlorination.

Despite the lack of standards for organic compounds such as chlorination by-products in the European Pharmacopoeia guidelines and those of the Association for the Advancement of Medical Instrumentation (AAMI) for dialysis fluid, their presence constitutes a permanent danger for hemodialysis patients because of the ease with which they pass into the blood compartment, indeed Poli and al [16] showed that the affinity of Trichloroethylene (TCE) for blood was about four times greater than its affinity for water.

Taking into account the tolerable daily intake established by WHO (2005) [19] for Chloroform:  $\text{TDI} = 15 \mu\text{g} / \text{kg} / \text{day}$  (for a daily water consumption of 2 liters and an individual body

weight of 64 kg), the tolerable weekly quantity is estimated at:  $15 \mu\text{g} / \text{kg} \times 64 \text{ kg} \times 7\text{d} = 6720 \mu\text{g}$ , thus a dialysis water containing a mean chloroform concentration of  $19.61 \mu\text{g}/\text{L}$  (**Table2**) corresponds to a weekly exposure of 7844  $\mu\text{g}$  of  $\text{CHCl}_3$ , 1.2 times higher than the tolerable dose. If at this time, there are no published papers reporting a causal relationship between THM exposure and the development of cancer [20-22], hemodialysis remains a therapy that overexposes patients to THMs, particularly Chloroform.

Furthermore, the performance of the dialysis unit for THM removal remained moderate in all our experiments, the % rejection ratio did not reach 50% (**Table2**), it was 44.08 % for Dibromochloromethane, 37.54% for Dichlorobromomethane and was only 10.33% for Bromoform. These results reflect either a saturation of activated carbon filters supposed to ensure effective removal of THM in the pretreatment step; in this case carbon filters should be changed for optimal operation, or an insufficient performance of the reverse osmosis membrane. Several factors affect the decline in performance, the most important of them is the lifetime of membrane which is generally limited to 3–5 years [23] or 5–7 years [24]. The RO membrane used at the HD center was two years old when beginning our study.

**Table 2:** Mean values of THM detected at different points of sampling and their rejection rates (%) from water treatment chain.

Trihalomethanes	Tap water	HD water	Dialysate	Rejection%	p-value
Chloroform, $\mu\text{g}/\text{L}$	$29.96 \pm 20.45$	$19.61 \pm 16.08$	$19.19 \pm 17.27$	34.54	0.0179
Dichlorobromomethane, $\mu\text{g}/\text{L}$	$22.38 \pm 8.290$	$14.09 \pm 5.430$	$9.230 \pm 2.145$	37.04	0.0456
Dibromochloromethane, $\mu\text{g}/\text{L}$	$14.65 \pm 6.690$	$8.192 \pm 1.991$	$7.627 \pm 2.457$	44.08	0.0361
Bromoform, $\mu\text{g}/\text{L}$	$8.305 \pm 4.960$	$7.447 \pm 7.292$	$6.501 \pm 6.385$	10.33	0.1364
Total THMs, $\mu\text{g}/\text{L}$	$74.84 \pm 10.29$	$50.69 \pm 8.980$	$42.54 \pm 19.07$	32.27	< 0.001

Microbiological monitoring of the different samples used to prepare the dialysis bath revealed low microbial perturbation during the entire study period (**Table3**), in drinking water that supplies the dialysis unit of Thenia, TMCs at 22 and 37°C were  $21 \pm 20,79$  to  $2 \pm 3,30 \text{ CFU} / \text{mL}$  respectively. HD samples showed mean TMCs at 22 and 37°C of  $9 \pm 7.746$  and  $1.6 \pm 2.32 \text{ CFU}/\text{mL}$ . In dialysate samples TMCs at 22 and 37°C were  $7.4 \pm 9.252$  and  $1.4 \pm 2.37 \text{ CFU}/\text{mL}$ . The low viable counts detected in this study indicate that the microbiological quality of HD water and dialysate was well below the stated AMMI limits [6] and the European Pharmacopoeia

guidelines [5] (<100 CFU/mL). The mean content of total coliforms was  $2.6 \pm 2.84 \text{ CFU} / 100 \text{ mL}$ , the count of TC decreased to  $1.2 \pm 1.93 \text{ CFU} / 100 \text{ mL}$  in treated water and then increased to  $1.6 \pm 3.24 \text{ CFU} / 100 \text{ mL}$  in dialysate. Faecal coliforms (*E. coli*) were detected in treated water at level less than 1 colony per 100 mL and streptococci at  $1.5 \pm 2.37 / 100 \text{ mL}$ . These quantities remain very low and may be associated with inappropriate disinfection. The literature [25-30] reported that bacteria are often detected in dialysis waters and health risks occur at high enough concentrations.

**Table 3: Mains values of microbiological parameters**

PARAMETERS		SAMPLINGS				LIMITS	
		Tap water	HD water	Dialysate	Tapwater <sup>a</sup>	HD W <sup>b</sup>	HD W <sup>c</sup>
TMCs /CFU mL <sup>-1</sup>	22°C	21± 20.79	9± 7.746	7.4± 9.252	<100	<100	<100
TMCs /CFU mL <sup>-1</sup>	37°C	2± 3.30	1.6± 2.32	1.4± 2.37	-	-	-
TC /100mL		2.6± 2.84	1.2± 1.932	1.6± 3.24	0	-	-
<i>E. Coli</i> /100 mL		0	0.2± 0.632	0.1± 0.316	0	-	-
Faecalstreptococci /100mL		1.4± 1.65	1.5± 2.37	0	0	-	-

<sup>a</sup>Algerian standards for tap water. Official Journal N°13 (2014)

<sup>b</sup>European Pharmacopoeia standards for HD water (8<sup>th</sup>edition 2014)

<sup>c</sup>ANSI/AAMI/ISO 23500:2011

Excepting one sampling, bacterial endotoxins in all treated water samples were less than 0.25 EU / mL (**Table4**), a compliance rate of 84% with Eph and AAMI standards for endotoxin was estimated. However, clinical studies confirmed the occurrence of chronic pyrogenic and inflammatory reactions in hemodialysis patients despite the acceptable level of bacterial contamination in dialysis waters [31, 32], others concluded that endotoxin contaminants identified in small amounts and insufficient to produce febrile reactions could reduce patients' response to erythropoietin therapy and compromise

their health [33]. Another study [34] conducted in Japan showed that the rate of all-cause mortality increased about 28% among hemodialysis patients when the level of endotoxins in dialysis fluids was  $\geq 0.1$  EU/mL. Considering the debilitated immune system of ESRD patients, the presence of bacteria and/or endotoxin even at low levels could harm patients. Improving disinfection protocols and frequency of microbiological controls are of extreme urgency to eradicate the microbial burden and improve the outcome of hemodialysis patients.

**Table 4: Results of endotoxin analyzes in treated water samples**

	Measure1	Measure2	Measure3	Measure 4	Measure 5	Measure 6
<b>Endotoxin concentration, EU / mL</b>	<0.25	>0.25	<0.25	< 0.25	<0.25	< 0.25
<b>Conformity*</b>	-	+	+	+	+	+

\*EPh Standard for endotoxin < 0.25 EU / ml

#### IV. Conclusion

The prevalence of end stage renal disease continues to increase significantly in Algeria especially in patients requiring hemodialysis replacement therapy which represents a major public health problem and a heavy burden both for society and at the individual level.

Patients with end-stage renal disease treated by hemodialysis are sensitive to any abnormal variation on the quality of dialysis fluids (treated water and dialysate), the success of this therapy requires dialysis according to international standards.

The results recorded in this study highlighted the presence of chemical contaminants in dialysis waters namely Trihalomethanes and in particular chloroform, a conductivity drift of 4 times the European Pharmacopoeia standard, the detection although at low concentrations of bacteria including endotoxins, exposing patients to potential health risks. Our study points out that at the present time there are no Algerian guidelines on HD water quality.

Given these findings, it would be essential to regularly monitor the physicochemical and microbiological parameters of dialysis fluids and to ensure more effective water disinfection procedures in order to reduce the risk of contaminants for hemodialysis patients and to guarantee an optimum safety and a better quality of life.

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