Article

Reflection about the hemodialysis water microbiological quality in Brazil

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Dialysis has been widely used in the treatment of patients with chronic kidney diseases and is considered a global public health issue. This treatment, which has changed the prognosis and quality of life in patients with chronic renal failure, can lead to complications that are often fatal. For this reason, there is a need for validation of alternative tests that favor the monitoring of treated water for dialysis in real-time to promote and prevent injuries to patients submitted to this procedure.

Keywords: Quality control. Dialysis. Water microbiological characteristics. Biological contamination.

INTRODUCTION
CHRONIC KIDNEY DISEASE AND DIALYSIS TREATMENT

Kidneys are organs situated on the posterior abdominal wall, positioned on either side of the spine, however, because of the location of the liver, the right kidney is smaller and it is situated a little lower than the left kidney. Each kidney is composed of approximately 800,000 to 1 million nephrons, its functional unit (Guyton, Hall, 2017; Koeppen, Stanton, 2017).

The kidneys exert great importance in the body, as they eliminate undesirable metabolism products, foreign chemicals, and toxins. They maintain the homeostasis by regulating the water balance and electrolytes, they also maintain the regulation of the blood pressure through the excretion of hormones or vasoactive substances such as renin, besides regulating the acid-base balance through the excretion of acids and promoting the regulation of body buffers, moreover they stimulate the production of erythrocytes by the excretion of erythropoietin in the circulation, supporting the formation of the bones by the vitamin D production and performing the glucose synthesis during prolonged fasting, in other words, they perform gluconeogenesis (Guyton, Hall, 2017; Pizzorno, 2015).

The progressive and irreversible loss of these functions added by renal injury results in chronic kidney disease. This disease has several stages being that renal dialysis or renal transplantation is recommended for chronic renal failure, the last stage, in which the kidneys are no longer able to maintain normality (Banasik, Copstead, 2018).

In this context, dialysis has been widely used, modifying the prognosis and quality of patients' life. The Scottish chemist Granham, in 1854, used the term

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"dialysis" for the first time by observing the separation of colloidal and crystalloid substances using semipermeable membrane constituted of vegetal material (Richet, 2001;Wisniak, 2013).

In the Netherlands, mid-1944, Kolff created the artificial kidney but only in 1945 the first successful dialysis was performed (Nakamoto, 2018). It was only in 1949 that the first hemodialysis was performed in Brazil, at the Hospital das Clínicas, in São Paulo, beginning the development of this technique, but it was only in the 1960s that hemodialysis was introduced as a therapy for patients with chronic renal failure (Gregório, 1996).

In Brazil, there are 758 dialysis centers with an active program for chronic dialysis, data from the last chronic dialysis survey conducted in 2017. There was an increase in the number of chronic dialysis centers compared to the previous survey in 2016, in which the number was 747. It is estimated that today, 126,583 patients are submitted to this treatment, an increase can be observed in relation to the previous census which was 122,825. Brazil has a prevalence of dialysis treatment of 610 patients per million population (pmp), being the Midwest region the most prevalent (Figure 1). In the analysis by state, Alagoas, Minas Gerais, and Federal District have the highest number of patients. There were 40,307 new patients, that is, an incidence rate of 194 pmp, the southeastern region has the highest incidence (Figure 2), but the state of Alagoas has the highest number of new patients, 340 pmp (Sesso *et al.*, 2017; Thomé *et al.*, 2019).
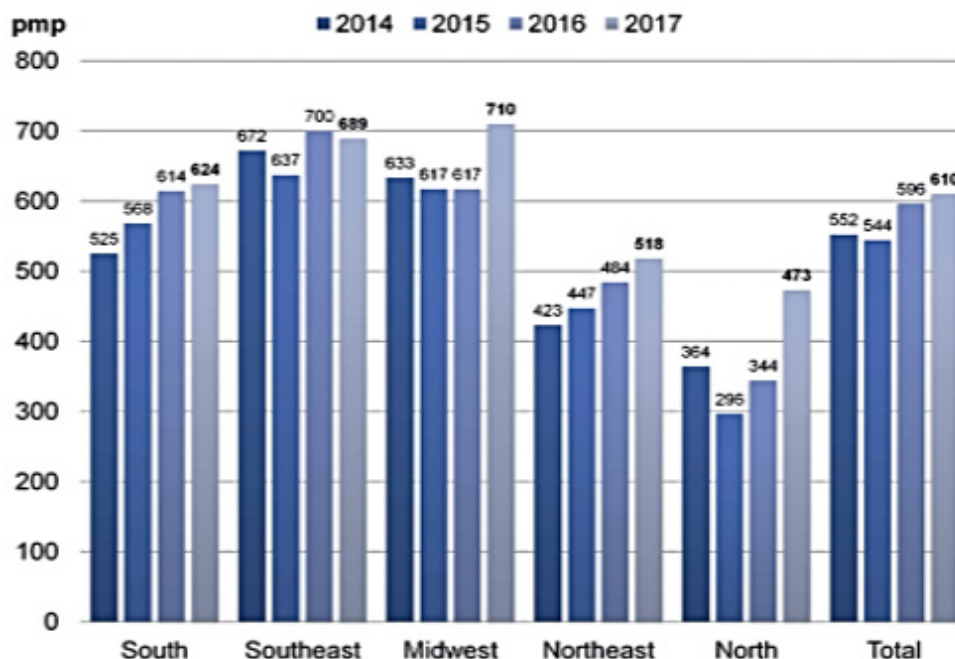

FIGURE 1 - Estimated prevalence of dialysis patients in Brazil, by region in the period of 2014-2017 (SOURCE: Adapted from Thomé *et al.*, 2019).
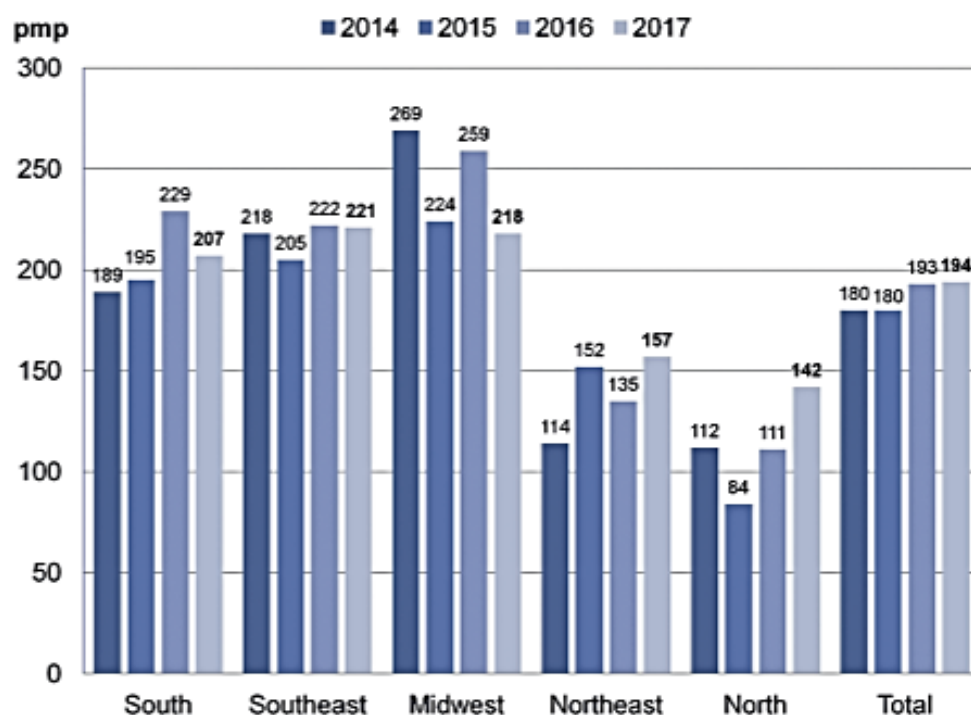
FIGURE 2 - Estimated incidence of dialysis patients in Brazil, by region in the period of 2014-2017 (SOURCE: Adapted from Thomé *et al.*, 2019).

The dialytic treatment consists in to remove the excess of water and solutes through a semipermeable membrane. There are two types of dialysis treatments: peritoneal dialysis and hemodialysis (Vadakedath, Kandi, 2017). In the chronic dialysis survey conducted in 2017, 91.8% of dialysis patients underwent conventional hemodialysis, 1.3% underwent frequent hemodialysis (less than 4 times per week) and 6.9% were under peritoneal dialysis (Thomé *et al.*, 2019).

In peritoneal dialysis, the peritoneum acts as a membrane that will separate the dialysis solution (dialysate) from the peritoneal capillaries, the water, and solutes exchanges occur by diffusion, ultrafiltration, and absorption simultaneously, draining the solution will be eliminated from the body toxins and water excess. The dialysis solution is industrially packaged in transparent and flexible plastic bags, which are available in volumes of 1.5 to 3 liters (Daugirdas, Blake, Ing, 2016; Vadakedath, Kandi, 2017).

At hemodialysis, patients are exposed weekly to 360 liters of water, a considerably larger volume when compared to a person's usual consumption of 14 liters per week (Agar, Perkins, Heaf, 2019). Conventionally or according to the clinical evaluation of each patient, the weekly treatment may consist of three hemodialysis sessions of approximately 4 hours duration, making a total of 12 hours weekly (Okada *et al.*, 2001).

The hemodialysis machine performs the mixing of the electrolyte concentrate with the treated water, resulting in the dialysis solution, which is sent to the dialyzer where the blood is exposed to the dialysis solution through the semipermeable membranes, providing substances exchanges between the blood and the dialysis solution. In each session, approximately 120 to 200 liters of purified water will be in contact with the patient's blood (Daugirdas, Blake, Ing, 2016).

TREATED WATER FOR HEMODIALYSIS

Low molecular weight contaminants present in the dialysis solution may cross the filtration membrane reaching the bloodstream and causing severe complications to the patient, for this reason, this solution must be chemicaly and microbiologicaly pure for an

extended time, due to the large amount of solution which the patient is exposed to during the treatment (Pontoriero *et al.*, 2003; Penne *et al.*, 2009; Ramirez, 2009).

Because it is an industrialized product, the dialysis concentrate, in powder or solution, is subject to strict quality control and to the vigilance of regulatory agencies, whereas the quality of the water used for hemodialysis is the responsibility of the dialysis unit (Daugirdas, Blake, Ing, 2016). Figure 3 shows a treatment system for dialysis water, which reflects the prevalence of water treatment systems. However, some centers adopt systems with some modifications, such as double reverse osmosis. In a study comparing dialysis centers, it was observed that the water quality in the one with single reverse osmosis was not inferior to the one with double reverse osmosis (Penne *et al.*, 2009).
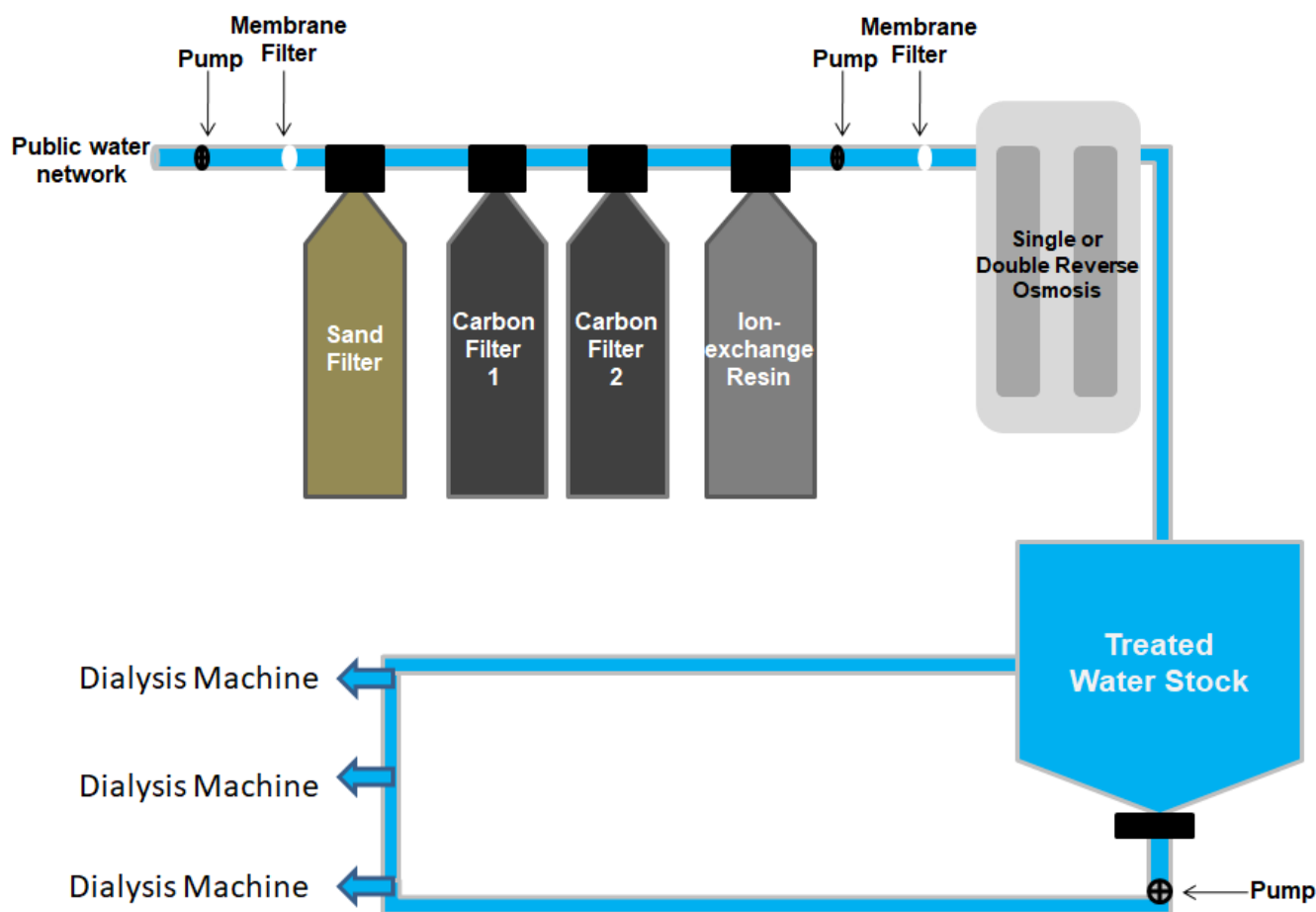

FIGURE 3 - Dialysis water treatment scheme.
The water first passes through a membrane filter and follow to a sand sediment filter. Then it passes through two activated carbon filters, subsequently, the water passes through the ion exchange resin (softener or deionizer depending on the objective). Then the water passes through another membrane filter, finally, it passes by reverse osmosis (which can be single or double). The treated water is stored in clean tanks and then distributed to the point of use.

First, the water passes through a membrane filter and a sand sediment filter, both to eliminate particles. Then the water goes through two activated carbon filters, in which chlorine and chloramine are held and organic contaminants are reduced. Subsequently, the water passes through the ion exchange resin, a system that removes ions, softener if the objective is to eliminate cations, or deionizer if the objective is to eliminate cations

and anions. At this point, another membrane filter is required to remove any remaining particles (Riella, 2018; Daugirdas, Blake, Ing, 2016).

Finally, the water passes through reverse osmosis, which acts as a barrier against bacteria and endotoxins. The treated water is stored in clean tanks and then distributed to the point of use by the water distribution system (Pontoriero *et al.*, 2003; Riella, 2018; Daugirdas, Blake, Ing, 2016).

Different contaminants, eventually found in the water treated for dialysis, such as heterotrophic bacteria, endotoxins, and chemical substances may occasionally trigger several complications, being manifested by signs and symptoms such as chills, nausea, headache, fever, hemolysis, sepsis and even death (Coulliette, Arduino, 2013).

Microbiological and biological contamination of treated water for dialysis

Bacteria and their degradation products like endotoxins are often found as contaminants in treated water for dialysis, eventually, protozoa, viruses, and fungi can also be found (Pontoriero *et al.,* 2003). Gram-negative bacteria and nontuberculous mycobacteria are the most frequently found as contaminants, there is also the possibility that other types of microorganisms, such as cyanobacteria, increase the risk associated with hemodialytic treatment (Silva *et al.*, 1996; Lima *et al.*, 2005; Gueguim *et al.*, 2016).

In 1996, in Caruaru, Pernambuco State, an incident considered as the "Tragedy of Hemodialysis" occurred, with the death of approximately 60 people, and the quality of the water used to filter patients' blood was indicated as the cause of the deaths. Furthermore, it was concluded that people were dialyzed by microcystin which was released from cyanobacteria when chlorine was added to the tank truck. At the hemodialysis clinic, the water passed through treatment, which had no reverse osmosis. This tragic situation consisted of an important mark for actions of sanitary regulations and inspection in Brazil (Azevedo *et al.*, 2002).

Also, in 1996, an outbreak of bacteremia occurred in a Hemodialysis Center in Campinas, in the state of São Paulo. After this episode, water and dialysate samples were collected from different sites of the hemodialysis system. At the first collection, 80% of the samples presented counts of *Pseudomonas aeruginosa* and *Burkholderia cepacia*, both gram-negative bacteria, whereas in the second collection 100% of samples showed counts of both bacteria (Pisani *et al.*, 2000).

In a collect realized by the Sanitary Surveillance of Piracicaba, São Paulo State, in 2003, from two hospitals named A and B, 200 samples of treated dialysis water were analyzed. Unit A showed yeast, *Pseudomonas aeruginosa* and heterotrophic bacteria above 200 colony forming units (CFU) / mL in 5, 14 and 52 samples, respectively for each of the contaminants. While unit B showed yeast, *Pseudomonas aeruginosa* and heterotrophic bacteria above 200 CFU / mL in 20, 5 and 36 of the samples, respectively for each of the contaminants (Simões, Pires, 2004).

In the city of Recife, State of Pernambuco, three strains of *Burkholderia cepacia* were isolated from both dialysis treated water samples collected from various sites of the water system and from the patients' blood, both of which were collected during an outbreak of bacteremia in 2001. Samples collected after reverse osmosis showed a much higher bacterial count than the samples collected before passing through it, suggesting possible bacterial colonization of reverse osmosis membrane. After cleaning the water system and replacing the membrane, the outbreak ceased (Magalhães *et al.*, 2003).

In the past, the water distribution system for the point of use was made by long, large-diameter polyvinyl chloride (PVC) pipes, thereby reducing water flow, resulting in increased bacterial contamination. Nowadays, tubes with smaller diameters and made from other materials such as stainless steel, polyvinylidene fluoride (PVDF) and cross-linked polyethylene (PEX) are preferable because they are smoother materials, which prevents microbial adhesion and facilitates disinfection. Blind spots, areas of stagnation and reserve tanks should also be avoided, as they are potential sources of contamination (Pontoriero *et al.*, 2003; Silva *et al.*, 1996).

To prevent contamination in this system, a routine of disinfection of pipes, tanks and dialysis machines is of fundamental importance (Silva *et al.*, 1996). Chemical

agents like peracetic acid and hypochlorite, heat and ozone are widely used for the disinfection of water for dialysis treatment systems. Disinfection that encompasses the whole system, performed at least once a month, can prevent the formation of biofilms, but once present in the system its removal is very difficult becoming a source of constant contamination (Pontoriero *et al.*, 2003; Montanari *et al.*, 2009).

Bacteria can be found in two ways, isolated as independent cells that float in liquid (planktonic) or in agglomerated communities (benthic) adhering to a solid surface called biofilms, being that 99% of the bacteria present in nature are in the form of biofilms. By definition, these are polymer matrices containing bacterial agglomerates and even multilayer fungi linked together by exopolysaccharides (EPS), produced by bacteria. EPS also provides the adhesion of the biofilm to the surface of a solid, in most cases immersed in an aqueous solution (Norf, Arndt, Weitere, 2009; Tortora, Funke, Case, 2016).

There are numerous difficulties caused by biofilms, their presence in undue places can cause serious damages such as pipe clogging due to their accumulation (Tortora, Funke, Case, 2016). Biofilms are the cause of the limitation of water sampling where the bacteria collected are benthic rather than planktonic (Sandle, 2015).

Biofilm is a bacteria virulence factor because of its ability to adhere strongly to surfaces. Virulence factor is a strategy that increases the bacteria's ability to promote infections. Besides, within the biofilm, the microorganisms are protected from disinfectants, body defenses and antibiotics through the expression of specific genes (Pontoriero *et al.*, 2003; Trabulsi, Alterthum, 2015; Singh *et al.*, 2017).

Infectious processes can be considered as the main cause of morbidity and mortality in dialysis patients, along with pyrogenic reactions, due to the endotoxins present during the inadequate treatment of dialysis water (Roth, Jarvis, 2000).

Endotoxins are present in gram-negative bacteria, which have an outer membrane constituted of lipoprotein, phospholipid, and lipopolysaccharide (LPS). The lipid portion of LPS, called lipid A, confers the toxicity to it when released during the lysis of the bacterium after its death, it may also be released during bacterial multiplication (Trabulsi, Alterthum, 2015; Tortora, Funke, Case, 2016).

In the human body, endotoxins stimulate cytokines released by macrophages, they are IL-1, IL-6, and TNF-α, which stimulate fever in the hypothalamus. Substances that cause fever are called pyrogens. There are two types of pyrogens: endogenous and exogenous. Exogenous pyrogens are substances that are foreign to the body, such as endotoxins, which, when entering the body, activate endogenous pyrogens such as IL-1, IL-6, and TNF-α (Carvalho, 2002; Trabulsi, Alterthum, 2015).

Dialysis water samples collected in the city of São Luís, Maranhão State, in 2005, in three hospitals, named A, B, and C, presented endotoxins in 100% of the pre-treatment samples, and 33.33% in those collected after treatment. Regarding the bacterial analysis in hospital B strains of *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Alcaligenes xylosoxidans* and *Stenotrophomonas maltophilia*, all gram-negative bacteria, were isolated. In hospital C, strains of *Burkholderia cepacia*, *Ralstonia pickettii* and *Flavimonas oryzihabitans*, all Gram-negative bacteria were identified (Lima *et al.*, 2005).

A study performed in the state of Mato Grosso do Sul examined water analysis reports from the hemodialysis service for the period of 2012 - 2013, and, 1% to 3% of the samples had total coliforms, 1% to 7% were above the allowed by legislation for heterotrophic bacteria and 6% were above the allowed for endotoxins. The analysis also showed that 1% of the samples were contaminated by *Escherichia coli* and 1% by *Pseudomonas aeruginosa*. As the collection was realized about 15 days after the cleaning and disinfection of the water treatment system, this showed that the cleaning procedure was not effective (Tristão, 2014).

Water samples were collected monthly in a survey conducted between 2015 and 2016 in nine dialysis wards in hospitals in Italy, in which the pipes underwent monthly disinfection with peracetic acid (0.5%). All samples had endotoxin levels less than 0.03 endotoxin unit (EU) / mL, a level well below the maximum allowed, and absence of fungi, but two of the nine dialysis wards had bacterial counts. It should be noted that in one of the wards, *Burkholderia cepacia* strain was isolated and

in the other ward strain of *Pseudomonas aeruginosa*. To cease the contamination, a disinfection process was performed with peracetic acid (2%), and sodium hypochlorite (2%) followed by washing with water (Totaro *et al.*, 2017).

Current legislation on dialysis water in Brazil

The criteria used for the evaluation of dialysis water arose through the awareness of competent authorities regarding the potential risk to which patients undergoing treatment were exposed (Faria, 2011).

The Resolution of the Collegiate Board of Directors (RDC) nº 33/2008 provides for the technical regulation for planning, programming, elaboration, evaluation, and approval of Water Treatment and Distribution Systems for Hemodialysis in the Brazilian health regulatory agency (Anvisa, 2008).

RDC nº 11/2014 provides for the Good Practice requirements for Dialysis Services, it applies to all dialysis services, whether public, private, philanthropic, civilian, or military, including those performing teaching and researching activities. It also determines that the samples for microbiological analyses are collected at least monthly at the point of return of the distribution loop and in one of the points in the processing room. In addition, it determines that the water microbiological quality has to be verified whenever there are pyrogenic manifestations, bacteremia or suspicion of sepsis in patients on dialysis. This RDC nº 11/2014 establishes the water quality standard for dialysis, with the biological and microbiological attributes being highlighted in Table 1 (Anvisa, 2014).

TABLE I - Biological and microbiological quality standard for dialysis water

Component	Maximum Allowable Value
Heterotrophic bacteria count	100 UFC*/mL
Total Coliforms	Absence in 100mL
Endotoxins	0,25 EU**/mL

* Colony-forming unit; **Endotoxin unit
Source: adapted from Anvisa, 2014.

The International Organization for Standardization (ISO) in its standard nº 13959:2014 provides for hemodialysis water and related therapies, specifying as a quality standard a total of microbiological count less than 100 CFU / mL or less if so, provided in local legislation. It also specified levels of endotoxins below of 0.25UE / mL or equally minor if so, provided by local legislation (International Organization for Standardization, 2014).

CONVENTIONAL AND ALTERNATIVE MICROBIOLOGICAL METHODS

Heterotrophic bacteria enumeration

It can be considered as an initial mark of the microbiological tests when in the mid of 1610 and, in 1665 the microscopes were invented by Galileo Galilei and Van Leeuwenhoek respectively. Although Leeuwenhoek was probably not the first to observe bacteria and protozoa, he was the first to make reports with drawings and descriptions of his observations. Among these, in 1675, he described living things present in water (Dias, 2018; Pelczar, Reid, Chan, 1980).

Although, German scientists had observed the growth of colonies in boiled potatoes, which characterized the practice of microbial cultivation and the development of culture media. It was Koch, who initiated the cultivation of microorganisms in a solid medium. He named as agar the substance extracted from algae that could solidify at room temperature. Richard Petri developed a glass plate for depositing the culture medium (Jay, 2001; Pelczar, Reid, Chan, 1996).

After 200 years of the discovery of living beings in water by Leeuwenhoek, Louis Pasteur, Robert Koch, Theovald Smith and a few other scientists associated microscopic beings with diseases. Joseph Lister, in 1878, obtained the first pure cultures of bacteria employing serial dilutions in liquid medium (Pelczar, Reid, Chan, 1980).

At the end of the 19th century, cultivation techniques were adopted to analyze the quality of potable water. For *E. coli* analysis and other coliforms, culture broth, through the most probable number (MPN), became the main method, as well Koch's agar medium or solid medium for the total count, both of which have gone

through few modifications. In 1950 the use of membrane filters for bacterial enumeration was introduced (Pelczar, Reid, Chan, 1996; Sartory, Watkins, 1999).

MNP is simple but requires a longer time of analysis (up to 5 days), whereas in membrane filtration presumptive results are available after 3-5 days of incubation (Anvisa, 2019). As for the solid medium, one of the options consists of plaque counting agar (PCA), media poor in nutrients and unsuitable for bacteria recovery that is already stressed in water. Nutritionally weaker culture media may also be used, for example, because they can recover a larger number of bacteria, but not the entire viable population (Sartory, Watkins, 1999). Reasoner's 2A Agar (R2A), is an example of nutritionally weaker culture media and is the most recommended for water microbiological analysis (USP, 2017).

Currently, there are many conventional microbiological methods, such as plate method, membrane filtration and multiple tubes by the MNP process (Anvisa, 2019). Although they are simple, efficient and economical, they have some limitations: low selectivity of the culture medium, variability of the biological response and late results in the detection of microorganisms, compromising the time to determinate preventive measures to reduce patients' injuries (Anvisa, 2019).

In an attempt to minimize these limitations, alternative microbiological methods have been developed to provide a higher level of quality to the tests, greater sensitivity, and faster results, allowing corrective actions to be taken early (Anvisa, 2019).

Bacterial Endotoxins

Theodor Billroth, in 1865, used the term pyrogen to refer to substances that caused fever (Kikkert, Groot, Aarden, 2008; Medical Staff Conference, 1978). Richard Pfeiffer's studies on cholera in 1892, which were encouraged by Robert Koch, consecrate him as the father of endotoxin for his discovery (Rietschel, Cavaillon, 2003).

In 1942, the pyrogen test by *in vivo* method was added in the American Pharmacopoeia in its twelfth edition (Mc Closky *et al.*, 1943). Since its inclusion, this test has been widely used to evaluate the contamination

by pyrogens in parenteral drugs (Kikkert, Groot, Aarden, 2008). However, only in 1976, this test was included in the Brazilian Pharmacopoeia (Navega *et al.*, 2015).

The test is based on the measurement of the rabbit's febrile response after intravenous injection of the test solution, and the interpretation of the results is used to characterize the biological control (Anvisa, 2019). There are, however, some limitations, such as animal management, biological variability, and ethical issues. These aspects encouraged researchers to develop alternative methods for the *in vivo* test of pyrogens (Kikkert, Groot, Aarden, 2008). In their research, Levin and Bang (1964), quoted by Kikkert, Groot, and Aarden (2008), observed that *Escherichia coli* endotoxin caused clotting in the hemolymph of the crab *Limulus polyphemus*.

The Limulus Amebocyte Lysate (LAL) test, was added in the American Pharmacopoeia in 1980, whereas it was included in the Brazilian Pharmacopoeia only in 1996 (Farmacopéia, 1996; USP, 1980). There are two types of LAL tests, the first is the semi-quantitative coagulation test, which is based on gel formation; the second one is the photometric, a quantitative test, which can be divided into chromogenic method that is based on color development, and turbidimetric method which is based on turbidity development (Anvisa, 2019).

However, LAL tests have some limitations such as the variability of the analyst technique, and the error inherent to the instruments used, compromising the analyzes quality, in this context alternative methods that eliminate these limitations are desirable (Anvisa, 2019; Charles River, 2017; Lemgruber *et al.*,2011).

Alternative Microbiological Methods

Alternative microbiological methods are desirable when it is sought to overcome the limitations of conventional methods. In different compendia, alternative methods are classified into qualitative, quantitative or identification (Anvisa, 2019; PDA, 2013; USP, 2017).

The Brazilian Pharmacopoeia highlights the main methods: viability-based, growth-based, and cellular component-based (Anvisa, 2019). The main types of alternative microbiological methods and their respective technologies are described in table II.

TABLE II - Main types of alternative microbiological methods and their respective technologies

Methods		Technologies				
Growth-based		Electrochemical Methods	Bioluminescence	Gas production or consumption detection	Use of chromogenic substrates	
Viability-based		Solid phase cytometry	Flow cytometry	Direct epifluorescence		
Cellular component-based	Phenotypic	Immunological	Fatty Acid Profile	Fourier Transform-Infrared	Mass spectrometry	Biochemical Assays Based on Physiological Reactions
	Genotypic	Nucleic Acid Amplification	Fingerprint			

Source: Adapted from Anvisa, 2019.

Despite the importance of using rapid alternative methods to provide not only the possibility of corrective actions in real-time but also greater patient safety, few studies are being conducted to demonstrate their applicability in the field of dialysis treated water analysis (Anvisa, 2019). Riepl *et al.* (2011) evaluated the applicability of solid-phase cytometry, revealed by epifluorescence microscopy, and observed a high correlation between the conventional and the alternative method.

The solid-phase cytometry application in dialysis water microbiological analysis is promising because, besides being reliable, it is a fast method, since the analysis time is around three hours, and its use is suggested for monitoring not only the water quality but also the dialysis fluid (Canaud, 2011; Riepl *et al.*, 2011).

Cytometry, besides being a fast method, also allows the detection of viable non-cultivable microorganisms. These microorganisms are responsible for results divergences between the laboratory experiments and real water microbiota. They are unable to divide and form colonies, even though they have an active metabolic mechanism and remain alive. Many bacteria, especially gram-negative, have this ability. Mycobacterium species can be highlighted as potential VNC (Anvisa, 2019; Joux, Lebaron, 2000; Díaz *et al.*, 2010; Sandle, 2015).

Cellular component-based techniques as 16S rDNA PCR analysis is also a promising technique because it is independent of culture and therefore able to detect non-cultivable viable microorganisms (Anvisa, 2019; Gomila *et al.*, 2010; Gomila, Ramirez, Lalucat, 2007).

MONITORING THE QUALITY OF TREATED WATER FOR DIALYSIS

Monitoring intends to reduce the risks caused by microorganisms, providing safety to patients undergoing hemodialysis treatment (Jasson *et al.*, 2010; Riepl *et al.*, 2011). Additionally, it allows directing preventive measures promptly to avoid damages to the patient's health (Figueras, Borrego, 2010; Nazemi *et al.*, 2016).

According to the capacity of the water system and its control indicators, it is important to establish alert and action limits (Clontz, 2009). An alert limit is understood as a signal, that is, a value that, if exceeded, shows that the process has deviated from its normality, thus corrective actions may or may not be necessary. On the other hand, limit action, if exceeded, indicates that the process deviated from normality, requiring corrective actions (Pinto, Kaneko, Pinto, 2015).

Therefore, when sample contamination levels reach the alert limit it is desirable that corrective actions, such as water system disinfection, are carried out to prevent contamination from reaching the action level (Coulliette, Arduino, 2013). The action limit definition is inherent to each unit characteristics, generally it corresponds to 50% of the maximum value established by the current legislation (Kawanishi *et al.*, 2009).

In 1999, the State of São Paulo, in partnership with the Adolfo Lutz Laboratory, implemented the Treated Water Monitoring Program for Hemodialysis. The results obtained during the years of this program execution indicate that the State Dialysis Units have implemented actions to ensure compliance with the water quality standards used in dialysis procedures (Buzzo *et al.*, 2010).

In an article published in 2018 about the monitoring program in the state of São Paulo, the authors conclude that the program still contributes to the improvement of the treated water for dialysis quality, as it can be seen in Figure 4 the percentage of unsatisfactory samples is small. Also in Figure 4, it can be observed that from the first period studied to the last one, there was an increase of unsatisfactory samples, which the authors justify being due to the RDC update in 2014, which set stricter limits and therefore resulted in the need for adjustment in the treatment system by the dialysis units (Buzzo *et al.*, 2018).

After determining the implementation of sanitary surveillance measures in the State of Rio de Janeiro, it was observed an improvement in the quality of the water treated for dialysis during the monitoring program in the period of 2006 - 2007 (Ramirez, 2009). However, in the monitoring realized in the state of Bahia results obtained were in disagreement with the recommended in 31% of the hemodialysis therapy units evaluated (Costa, 2012).
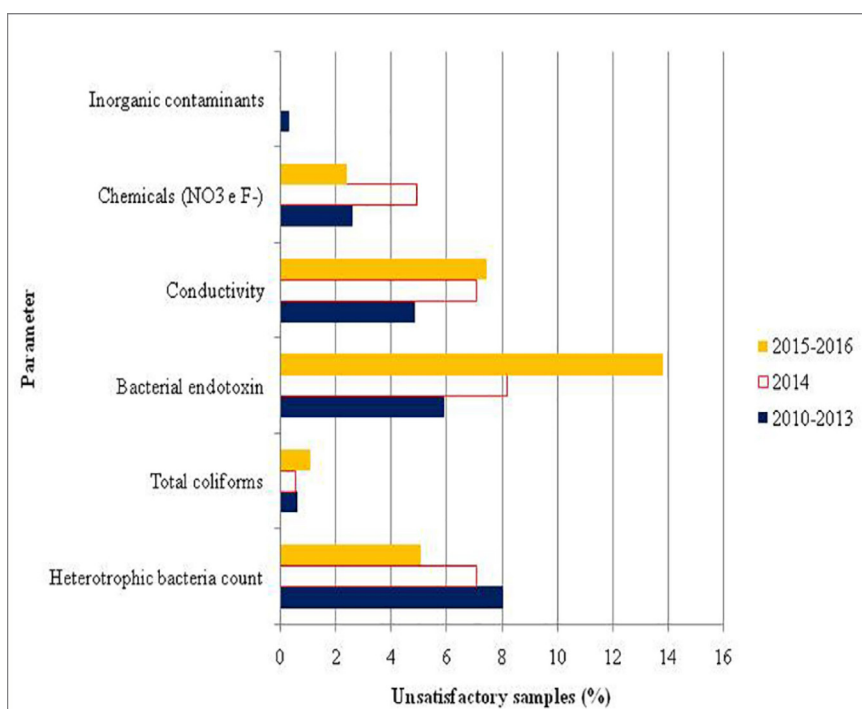

FIGURE 4 - Frequency of unsatisfactory results determined at the initial sample collection as a function of the analyzed parameter (Source: Buzzo *et al.,* 2018).

CONCLUSIONS

This literature review leads us to reflect on the need to implement monitoring of treated water for dialysis at a national level through appropriate analytical methods that provide results on time allowing corrective actions to be performed immediately because during this therapy the patient needs a large volume of water within the quality standards in order to provide safety to the patient.

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REFERENCES

Agar J, Perkins A, Heaf J. Home Hemodialysis: Infrastructure, Water, and Machines in the Home. [citad 2019 Ago 16] Available from: http://www.ishd.org/8-home-hemodialysis-infrastructure-water-and-machines-in-the-home/

Agência Nacional de Vigilância Sanitária, Brasil (Anvisa). Resolução nº 33, de 3 de junho de 2008. Regulamento Técnico para planejamento, programação, elaboração, avaliação e aprovação dos sistemas de tratamento e distribuição de água para hemodiálise no sistema nacional de vigilância sanitária. Diário Oficial da União, jun. 2008.

Agência Nacional de Vigilância Sanitária, Brasil (Anvisa). Farmacopéia brasileira. 6. ed. Brasília, 2019. v. 1.

Agência Nacional de Vigilância Sanitária, Brasil (Anvisa). Resolução nº 11, de 13 março 2014. Requisitos de Boas Práticas de Funcionamento para os Serviços de Diálise e dá outras providências. Diário Oficial União, 14 mar. 2014.

Azevedo SMFO, Camichael WW, Jochimsen EM, Rinehart KL, Lau S, Shaw GR, et al. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. Toxicology. 2002;181-182:441-446.

Banasik JL, Copstead LEC. Pathophysiology. 7. ed. Maryland Heights: Elsevier, 2018.

Buzzo ML, Bugno A, Almodovar A AB, Kira CS, Carvalho FH, Souza A, et al., A importância de programas de monitoramento da qualidade da água para diálise na segurança dos pacientes. Rev Inst Adolfo Lutz. 2010;69(1):1-6.

Buzzo ML, Silva FPL, Hilinski EG, Bugno A. Effectiveness of the monitoring program for ensuring the quality of water treated for dialysis in the state of São Paulo. Braz J Nephrol. 2018;40(4):344-350.

Canaud B, Rapid assessment of microbiological purity of dialysis water: the promise of solid-phase cytometry assessment and the epifluorescence microscopy method. Nephrol Dial Transplant. 2011;26:3426-3428.

Carvalho AR. Mecanismo da Febre.[Completion course work's Monography]. Brasília: Faculdade de Ciências da Saúde, Centro Universitário de Brasília; 2002.

Charles River Endosafe®-PTS™. [citad 2017 May 4]. Available from: https://www.criver.com/products-services/qc-microbial-solutions/endotoxin-testing/endotoxin-testing-systems/endosafe-nexgen-pts?region=3621

Clontz L. Microbial limit and Bioburden Tests: validation approaches and global requirements. 2. ed. Boca Raton: CRC Press, 2009.

Costa JSC. Proposta de monitoramento da qualidade da água utilizada no tratamento hemodialítico no estado da Bahia. [Master's dissertation]. Bahia: Instituto de Saúde Coletiva, Universidade Federal da Bahia; 2012.

Coulliette AD, Arduino MJ. Hemodialysis and Water Quality. Semin Dial. 2013;26(4):427-438.

Daugirdas JT, Blake P G, Ing TS. Manual de diálise. 5. ed. Rio de Janeiro: Guanabara Koogan, 2016.

Dias IS. A história do surgimento da microbiologia: fatos marcantes. Rio de Janeiro: Instituto de Microbiologia Paulo de Goés, Universidade Federal do Rio de Janeiro, 2018. [citad 2018 May 6]. Available from: http://www.microbiologia.ufrj.br/portal/index.php/pt/destaques/novidades-sobre-a-micro/384-a-historia-do-surgimento-da-microbiologia-fatos-marcantes

Díaz M, Herrero M, García LA, Quirós Q. Application of flow cytometry to industrial microbial bioprocesses. Biochem Eng J. 2010;48:385-407.

Faria PGS. Alternativas para reaproveitamento do rejeito do tratamento de água em clínica de hemodiálise. [Completion course work's Monography]. Paraná: Departamento Acadêmico de Construção Civil, Universidade Tecnológica Federal do Paraná; 2011.

Figueras MJ, Borrego JJ. New Perspectives in Monitoring Drinking Water Microbial Quality. Int. J. Environ. Res. Public Health. 2010;7:4179-4202.

Farmacopéia Brasileira. 4 ed. São Paulo: Atheneu, 1996. pt. 1.

Gomila M, Gascó J, Gil J, Bernabeu R, Iñigo V, Lalucat J. A molecular microbial ecology approach to studyinghemodialysis water and fluid. Kidney Int. 2010;70:1567-1576.

Gomila M, Ramirez A, Lalucat J. Diversity of environmental mycobacterium isolates from hemodialysis water as shown by a multigene sequencing approach. Appl Environ Microbiol. 2007;73(12):3787–3797.

Gregório MC. Ponto de Vista Histórico: Centro de Nefrologia da região Sudeste do Brasil. J Bras Nefrol. 1996;18(3): 221-235.

Gueguim C, Nga N, Folefack FK, Ragon A, Kamga HG. Microbiological Analysis of Hemodialysis Water at the University Teaching Hospital of Yaounde, Cameroon. Am J Biomed Life Sci. 2016;4(6):81-86.

Guyton AC, Hall JE. Tratado de fisiologia médica. 13. ed. Rio de Janeiro: Saunders Elsevier, 2017.

International Organization for Standardization. ISO 13959:2014: Water for haemodialysis and related therapies. Geneva: ISO; 2014. 16 p.

Jasson V, Jacxsens L, Luning P, Rajkovic A, Uyttendaele M. Alternative microbial methods: an overview and selection criteria. Food Microbiol. 2010;27:710-730.

Jay V The Legacy of Robert Koch. Arch Pathol Lab Med. 2001;125(9):1148-1149.

Kawanishi H, Akiba T, Masakane I, Tomo T, Mineshima M, Kawasaki T, et al. Standard on Microbiological Management of Fluids for Hemodialysis and Related Therapies by the Japanese Society for Dialysis Therapy 2008. Ther Apher Dial. 2009;13(20):161-166.

Joux F, Lebaron, P. Use of fluorescent probes to asses physiollogical funtions of bacteria at single-cell level. Microbes Infect. 2000;2(12):1523-1535.

Kikkert R, Groot ER, Aarden L A. Cytokine induction by pyrogens: Comparison of whole blood, mononuclear cells, and TLR-transfectants. J Immunol Methods. 2008;336:45-55.

Koeppen BM, Stanton BA. Berne & Levy: Fisiologia. 7. ed. Rio de Janeiro: Elsevier, 2017.

Lemgruber APM, Presgrave OAF, Presgrave RF, Alves EN, Silva RS, Gimenes I, et al. Effect of micropipette inaccuracy on performance of spectrophotometric assay for bacteria endotoxin dosage (chromogenic LAL) employed in products subjected to sanitary surveillance. Rev Inst Adolfo Lutz. 2011;70(3):368-72.

Lima JRO, Marques SG, Gonçalves AG, Salgado Filho N, Nunes PC, Silva HS, et al., Microbiological analysis of water from hemodialysis services in São Luis, Maranhão, Brazil. Braz J Microbiol. 2005;36:103-108.

Mc Closky W, Price CW, Van Winkle WJ, Welch H, Calvery HO. Results of the first U.S.P. collaborative study of pyrogens. J Am Pharm Assoc. 1943;32(3):69-73.

Magalhães M, Doherty C, Govan JRW, Vandamme P. Polyclonal outbreak of Burkholderia cepacia complex bacteraemia in haemodialysis patients. J Hosp Infect. 2003;54:120-123.

Medical Staff Conference. Drug fever. West J. Med., 1978; 129: 321-326. [citad 2018 May 7]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1238351/pdf/westjmed00254-0079.pdf

Montanari LB, Sartori FG, Cardoso MJO, Varo SD, Pires RH, Leite CQF, et al. Microbiological contamination of a hemodialysis center water distribution system. Rev Inst Med Trop S Paulo. 2009;51(1):37-43.

Nakamoto S. Reflections on My Lifetime Teacher: Dr. Willem J. Kolff. Artif Organs. 2018;42(2):115-126.

Navega ECA, Silva CC, Presgrave OF, Almeida AS, Delgado IF, Mattos KA. Métodos alternativos ao uso de animais para a detecção de pirogênio: oportunidades e desafios no controle da qualidade de produtos biológicos. Arch Vet Sci. 2015;20(4):71-81.

Nazemi S, Mirzaii M, Yaslianifard S, Darban-Sarokhalil D, Khoramrooz SS, Norozi P, et al. Microbiological qualification of air, water and dialysate in a haemodialysis centre: a new focus on Legionella spp. Iran J Microbiol. 2016;8(4):219-225.

Norf H, Arndt H, Weitere M. Responses of Biofilm-Dwelling Ciliate Communities to Planktonic and Benthic Resource Enrichment. Microb Ecol. 2009;57:687-700.

Okada S, Katagiri K, Kumazaki T, Yokoyama H. Safety of gadolinium contrast agent inhemodialysis patients. Acta Radiol. 2001;42:339-341.

Parenteral drug association (PDA). Evaluation, validation and implementation of alternative and rapid microbiological methods. Bethesda: Parenteral Drug Association, 2013. 53 p. (Technical Report, n. 33).

Pelczar M, Reid R, Chan ECS. Microbiologia. São Paulo: McGraw-Hill, 1980. v. 1.

Pelczar M, Reid R, Chan ECS. Microbiologia. 2. ed. São Paulo: McGraw-Hill, 1996. v. 1.

Penne EL, Visser L, van den Dorpel MA, van der Weerd NC, Mazairac AHA, van Jaarsveld BC, et al. Microbiological quality and quality control of purified water and ultrapure dialysis fluids for online hemodiafiltration in routine clinical practice. Kidney Int. 2009;76(6):665-72.

Pinto TJA, Kaneko TM, Pinto AF. Controle biológico de qualidade de produtos farmacêuticos, correlatos e cosméticos. 4. ed. São Paulo: Manole, 2015.

Pisani B, Simões M, Prandi MAG, Rocha MMM, Gonçalves CR, Vaz TMI, et al. Surto de bacteriemia por Pseudomonas aeruginosa na unidade de hemodiálise de um hospital de Campinas, São Paulo, Brasil. Rev. Inst. Adolfo Lutz. 2000;59(1/2):51-56.

Pizzorno J. The Kidney Dysfunction Epidemic, Part 1: Causes. Integr Med. 2015;14(6):8-13.

Pontoriero G, Pozzoni P, Andrulli S, Locatelli F. The quality of dialysis water. Nephrol Dial Transplant. 2003;18 Suppl 7:vii21–vii25.

Ramirez SS. Água para hemodiálise no estado do Rio de Janeiro: uma avaliação dos dados gerados pelo programa de monitoramento da qualidade nos anos de 2006-2007. [Master's dissertation]. Rio de Janeiro: Instituto Nacional e Controle de Qualidade em Saúde, Fundação Oswaldo Cruz. 2009.

Richet G. The osmotic pressure of the urine — from Dutrochet to Korányi, atrans-European interdisciplinary epic. Nephrol Dial Transplant. 2001;16:420-424.

Riella MC. Princípios de nefrologia e distúrbios hidroeletrolíticos. 6. ed. Rio de Janeiro: Guanabara Koogan, 2018.

Riepl M, Schauer S, Knetsch S, Holzhammer E, Farnleitner AH, Sommer R, et al. Applicability of solid-phase cytometry and epifluorescence microscopy for rapid assessment of the microbiological quality of dialysis water. Nephrol Dial Transplant. 2011;26(11):3640-3645.

Rietschel ET, Cavaillon JM. Richard Pfeiffer and Alexandre Besredka: creators of the concept of endotoxin and anti-endotoxin. Microbes Infect. 2003;5:1407-1414.

Roth VR, Jarvis WR. Outbreaks of infection and/or pyrogenic reactions in dialysis patients. Semin Dial. 2000;13(2):92-96.

Sandle T. Characterizing the microbiota of a pharmaceutical water system-A metadata study. SOJ. Microbiol Infect Dis. 2015;3(2):1-8.

Sartory DP, Watkins J. Conventional culture for water quality assessment: is there a future? J Appl Microbiol. 1999;85:2258-2338.

Silva AMM, Martins CTB, Ferraboli R, Jorgetti V, Junior JER. Revisão/Atualização em Diálise: Água para hemodiálise. J Bras Nefrol. 1996;18(2):180-188.

Simões M, Pires MFC. Água de diálise: ocorrência de leveduras, *Pseudomonas aeruginosa* e bactérias heterotróficas. Rev Inst Adolfo Lutz. 2004;62(2):224-231.

Singh S, Singh SK, Chowdhury I, Singh R. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. Open Microbiol J. 2017;11:53-62.

Sesso RC, Lopes AA, Thomé FS, Lugon JR, Martins CT. Inquérito Brasileiro de Diálise Crônica 2016. J Bras Nefrol. 2017;39(3):261-266.

Thomé FS, Sesso RC, Lopes AA, Lugon JR, Martins CT. Brazilian chronic dialysis survey 2017. Braz J Nephrol. 2019; [Ahead of Print]. [citad 2018 June 6]. Available from: http://www.scielo.br/pdf/jbn/2019nahead/2175-8239-jbn-2018-0178.pdf

Tortora GJ, Funke BR, Case CL. Microbiologia. 12. ed. Porto Alegre: Artmed, 2016.

Totaro M, Casini B, Valentini P, Miccoli M, Giorgi S, Porretta A, et al. Evaluation and control of microbial and chemicalcontamination in dialysis water plants of Italiannephrology wards. J Hosp Infect. 2017;97:169-174.

Trabulsi LR, Alterthum F. Microbiologia. 6. ed. São Paulo: Atheneu, 2015.

Tristão AMV. Qualidade da água nas clínicas de hemodiálise no estado de Mato Grosso do Sul. [Master's dissertation]. Mato Grosso do Sul: Programa de Pós Graduação em Saúde e Desenvolvimento na Região Centro Oeste, Universidade Federal de Mato Grosso do Sul; 2014.

United States Pharmacopeia (USP). 20.ed. Rockville:The United States Pharmacopeia Convention, 1980.

United States Pharmacopeia (USP). 40. ed. Rockville: The United States Pharmacopeia Convention, 2017.

Vadakedath S, Kandi V, Dialysis: A Review of the MechanismsUnderlying Complications in theManagement of Chronic Renal Failure. Cureus. 2017;9(8):e1603.

Wisniak J. Thomas Graham. II. Contributions to diffusion of gases and liquids, colloids, dialysis, and osmosis. Educ Quím. 2013;24(núm. extraord. 2):506-515.