Microbiology of Water and Fluids for Hemodialysis

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In hemodialysis, huge amounts of water are used for diluting the concentrates to produce dialysis fluid. The water is produced on site by reverse osmosis units. The chemical and microbiological quality of the water is essential for dialysis patients. Reverse osmosis units produce water of acceptable chemical quality that can be kept throughout the water system. The microbiological water quality, on the other hand, does not depend on the reverse osmosis unit but on the maintenance of the whole water system. All over the world, dialysis units take water samples and send them to laboratories for cultivation and endotoxin tests. Depending on the method of microbiological analysis, the water may be judged to be very good even if in reality it is much worse and outside of standard recommendations. When standardizing the methods with adequate cultivation of water samples, the accuracy of the tests will be better, and as a result, dialysis units can use their resources for keeping the water systems in good shape, i.e. disinfect preventively and frequently and use less effort in collecting samples. This will benefit patients, who will receive a high-quality dialysis fluid, thus eliminating the effects of microbiological impacts such as increased levels of inflammation markers (e.g. C-reactive protein). In the situation of performing hemodiafiltration by producing the substitution fluid "on-line", it is even more important to have a sensitive method of microbiological verification to follow-up the hygienic quality. [*J Chin Med Assoc* 2008;71(5):223–229]

Key Words: cultivation technique, dialysis fluid, microbiology, "on-line", substitution fluid, water

Introduction

A dialysis patient who is treated 3 times a week with approximately 150 liters of dialysis fluid each time is exposed to 23,400 liters per year. The dialysis fluid consists of up to 99% of reverse osmosis water; in addition, chemicals are added, such as acids, salts and bicarbonate. An average-size dialysis unit produces more than 1,000,000 liters of dialysis fluid per year. Worldwide, the production of dialysis fluid is about 25,000,000,000 liters, which makes dialysis fluid one of the largest products by volume used in medicine.

There are about 28,500 dialysis units throughout the world. If 2 claims are raised—use of preventive disinfection strategy (frequent disinfection) and use of a sensitive microbiological cultivation technique as a verification tool—then only about 2,000 dialysis units are estimated to fulfill these.

Water used for dilution of hemodialysis concentrates has to meet official quality recommendations regarding microbiology and chemistry. Several recommendations/standards exist; in all of them, limits for microbiological quality are given. A number of different *cultivation techniques* are described. Unfortunately, they do not have the same efficiency. The differences can be 2–4 logarithms. Several ISO standards are currently in preparation in order to achieve better harmonization. New issues of ISO 13958 (concentrates),[1] ISO 13959 (water),[2] ISO 26722 (water equipment)[3] and a new ISO standard for dialysis fluid and substitution fluid[4] are in preparation.

Water quality is mentioned in all recommendations, but what about *water system quality*? Few microorganisms live in the water; most organisms will be found on surfaces but their presence is difficult to prove. There may be underestimation by up to 100,000 times when the total microbial content in a water system is calculated.[5]

Is it possible to secure good water quality at any time in such systems? The difficulties in performing adequate microbiological analysis make it obvious that there are large numbers of microbiological analytical records that have little to do with reality. In order to have quality assurance in water systems, it is necessary to have a good disinfection strategy. Disinfection not only includes the single disinfection procedure but, more importantly, the *frequency* of disinfection.

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In water systems that do not have a proactive disinfection strategy in place and a non-sensitive microbiological cultivation technique, there is risk of metabolite formation due to the large number of microbial cells that will be present, which may interfere with the human immune defense system. Water-borne microorganisms are generally very low-active with regard to endotoxins. A common water-borne organism like *Pseudomonas aeruginosa* requires 430,000 cells in suspension to give an activity of 1 EU/mL.[6] For this reason, it is not sufficient to just analyze endotoxins.

In many parts of the world, hemodiafiltration/hemofiltration (HDF/HF) "on-line" is becoming more frequent, which represents a more critical use of water. When performing HDF/HF on-line, i.e. preparing substitution fluid directly in the dialysis machine, high fluid quality is even more essential. Patients are exposed to as much as 100 liters directly into their blood during treatment. The substitution fluid is achieved by on-line filtration of water, dialysis fluid, and, as the last step, substitution fluid. To prepare sterile substitution fluid, the fluid must pass a sterile filter as the last step according to the definition in pharmacopoeias.

All that has been said about water and water systems is also valid for dialysis fluid and central concentrate systems, both central bicarbonate concentrate systems and central acid concentrate systems. Special microorganisms colonize these kinds of systems and, consequently, special cultivation techniques should be used. It is important to see fluid handling in the dialysis unit as a system where all components are of importance and will influence the final quality.

Official Recommendations

The number of different official recommendations for microbiological water quality is increasing. Both national authorities as well as international organizations publish recommendations (Table 1).[2,7–19] The most widespread recommendation is currently the *AAMI RD 52* (2004) (published by the Association for the Advancement of Medical Instrumentation).[9] This is often used and referenced in areas where no local recommendations exist.

A problem is that different recommendations refer to different cultivation techniques. This means that even if the limit given in numbers is the same, the interpretation is not the same due to the different cultivation techniques used.

Microbiological Cultivation Technique

Microbiological cultivation technique is the combination of cultivation medium, incubation temperature and incubation time. Different cultivation techniques differ in their ability to show growth. The principle is: good general cultivation medium, low incubation temperature (similar to the system), and long incubation time (to make it possible for slow-growing species to form

Table 1. Cultivation techniques

National and international standards	Year issued	Cultivation technique		
		Cultivation medium	Incubation temperature (°C)	Incubation time
AAMI	1982[7]	TSA	35	48 hr
Ph Eur, 2nd ed.	1992[8]	TSA	30–35	5 d
ISO 13959	2002[2]	TSA	35	48 hr
AAMI RD52	2004[9]	TSA	35	48 hr
Ph Eur, 4th ed.	2002[10]	R2A	30–35	5 d
Sweden LS	1990–2007[11]	TGEA	22	> 5 d
Germany	1998–2005[12]	TGEA	17–23	7 d
France Circ 311	2000[13]	TGEA	17–23	7 d
EDTNA	2001[14]	TGEA	17–23	7 d
EDTA	2001[15]	TGEA	17–23	7 d
Italy	2005[16]	TGEA	17–23	7 d
Denmark	2006[17]	TGEA	17–23	7 d
Norway	2007[18]	TGEA	17–23	7 d
ISO/DIS 13959 (draft)	2007[19]	TGEA	17–23	7 d

AAMI = Association for the Advancement of Medical Instrumentation; Ph Eur = European Pharmacopoeia; ISO = International Organization for Standardization; EDTNA = European Dialysis and Transplant Nurse Association; EDTA = European Dialysis and Transplant Association; TSA = tryptone soy agar; TGEA = tryptone glucose extract agar.

visible colonies).[20] If a poor cultivation technique is used, there is the risk of having a much worse situation in reality than that shown by the analysis results.[21] This may have a practical impact on decisions for disinfection frequency. In more critical applications such as the production of substitution fluid, the issue of microbiological quality control prior to the final filter is more important as microbiological testing is part of the quality assurance for production of a fluid that is infused.[22]

Fluids in Dialysis

Water

The national and international standards for water are shown in Table 2.[2,9,11,12,14,15,17–19,21,23–26] The microbiological content of water seldom originates in the fluid phase, i.e. the water, but on surfaces in the system.[5] However, water transports microbial material to dialysis machines. The microbial material includes microbial cells, endotoxins, and microbial metabolites formed during growth in the system. Microbial cells and even endotoxins may be removed by ultrafiltration. Microbial metabolites formed can include as many as 5,000 different types of molecules, which are, in principle, not possible to remove by filtration. What is formed in the water system will be transported into the dialysis machine and end up in the dialysis fluid. The presence of metabolites may "tease" the human immune defense system.[27,28] If the water system is contaminated, it will affect the rest of the system.

Concentrates

The national and international standards for acid and basic concentrates are shown in Table 3.[1,9,11,21,29]

Acid concentrates

Acid concentrates are frequently distributed in central acid systems. In these systems, there are pipes that over time will be contaminated. Microbial growth is difficult to prove as wrong cultivation techniques are used and surface growth occurs. The cultivation technique must be adapted to the concentrate used as there are several compositions of acid concentrates.

Acid concentrates in canisters (5–10 L) represent no microbial growth problem. Acid concentrates may contain some endotoxins as there is normally no ultrafiltration of concentrate in production. The *European Pharmacopoeia* allows 0.5 EU/mL when the concentrate is diluted to user concentration.[29] This means

Table 2. Water

National and international standards	Year issued	Microorganisms, CFU/mL	Endotoxins, EU/mL	Note
Germany Robert Koch Institute	1996[23]	100	–	*E. coli* < 1 CFU/100 mL *P. aeruginosa* < 1 CFU/100 mL
ISO 13959	1998[24]	10^2	5	
JSDT	1998[25]	–*	0.25	*No cultivation
ISO 13959	2002[2]	10^2	1 – (5)	
EDTNA	2001[14]	$< 10^2$	< 0.25	
EDTA	2001[15]	$< 10^2$	< 0.25	
AAMI RD 52	2004[9]	200 (alert 50)	2 (alert 1)	
Denmark Danish Society of Nephrology (draft)	2004[17]	0.1	0.05	
Italy	2005[19]	$< 10^2$	< 0.25	*P. aeruginosa* < 1 CFU/250 mL
Germany Hygiene Guideline	2005[12]	$< 10^2$	< 0.25	
Ph Eur, 5th ed.	2005[26]			
USP XXX	2007[21]	100	2	
Norway Norwegian Society of Nephrology (draft)	2007[18]	100	< 0.25	
Sweden SLS	2007[11]	$< 10^{2\dagger}$	< 0.25	†Of which $< 10^1$ fungi and/or yeast
ISO/DIS 13959 (draft)	2007[19]	100	0.25	

ISO = International Organization for Standardization; JSDT = Japanese Society for Dialysis Therapy; EDTNA = European Dialysis and Transplant Nurse Association; EDTA = European Dialysis and Transplant Association; AAMI = Association for the Advancement of Medical Instrumentation; RD = Renal Disease and Detoxification Committee; Ph Eur = European Pharmacopoeia; USP = United States Pharmacopoeia; SLS = Svensk Läkemedels Standard (Swedish drug book).

Table 3. Concentrates (acid and basic)

National and international standards	Year issued	Microorganisms, CFU/mL	Endotoxins, EU/mL
ISO 13958	2002[1]	–	0.5*
AAMI RD 52	2004[9]	Limits for diluted concentrate same as for dialysis fluid 200 (alert 50)	Limits for diluted concentrate same as for dialysis fluid 2 (alert 1)
USP XXX	2007[21]	–	–
Ph Eur, 5th ed.	2005[29]	–	<0.5*
SLS	2007[11]	–	<0.5*

**Diluted to user concentration. ISO = International Organization for Standardization; AAMI = Association for the Advancement of Medical Instrumentation; RD = Renal Disease and Detoxification Committee; USP = United States Pharmacopoeia; Ph Eur = European Pharmacopoeia; SLS = Svensk Läkemedels Standard (Swedish drug book).*

that the "concentrated" concentrate may contain up to 17.5 EU/mL in the case of a 1:34 dilution.

Bicarbonate concentrate

Basic concentrate in fluid form is a solution with about 8.4% $NaHCO_3$. Distributed in canisters (5–10 L), the solution seldom has a microbiological problem. However, when bicarbonate concentrate is distributed in a central system with piping, growth occurs in the pipes. Microbiological analysis of bicarbonate concentrate mostly just shows microflora from the water used in production. If an adjusted microbiological medium is used, tryptone glucose extract agar, with the addition of 4% $NaHCO_3$, a specialized microflora will appear.[30] The best alternative from a microbiological standpoint is to use $NaHCO_3$ in a dry powder form.

Bicarbonate concentrate may contain some endotoxins as there is normally no ultrafiltration of concentrate in production. The *European Pharmacopoeia* allows 0.5 EU/mL when the concentrate is diluted to user concentration.[29] This means that the "concentrated" concentrate may contain up to 17.5 EU/mL in the case of a 1:34 dilution.

Dialysis fluid

Dialysis fluid (Table 4)[4,9,11–16,25] can be tested using the same cultivation technique as used for water.[20] Most endotoxin reagents will function in dialysis fluid as well, but the technique used must be validated in order to verify the functionality of the test set-up. The microbiological quality of dialysis fluid is, in practice, determined by the quality of the water used as dialysis fluid consists of 95–99% water. In some countries, standard hemodialysis machines are equipped with ultrafilters to improve the microbiological quality of dialysis fluid.

Ultrapure dialysis fluid

The term *ultrapure* was coined for dialysis fluid prior to the final filter in dialysis machines intended for on-line production of substitution fluid in HDF/HF, and is defined as < 0.1 CFU/mL and < 0.03 EU/mL.[31] Ultrapure dialysis fluid (Table 5)[4,9,11,13–15,17,18,32,33] is produced by ultrafiltration of dialysis fluid in dialysis machines intended to be used for on-line production of substitution fluid. The use of ultrapure dialysis fluid has been discussed for more general applications.[34,35] Even in recommendations for fluid quality, suggestions are given for more widespread use of ultrapure dialysis fluid, for instance, for high-flux dialysis.[17]

Substitution fluid

Substitution fluid is used in HDF/HF. In some dialysis units, up to 25% of treatments are done with these techniques. The possibility of using certain dialysis machines to produce substitution fluid on site and on-line allows large volumes to be used, up to 100 liters per session.[22]

The microbiological quality differs in different recommendations (Table 6).[4,9,11–16,18,32,33,36] The expression "10^{-6} CFU/mL" for sterility is not adequate and cannot be substantiated. The correct expression for sterile is sterility assurance level (SAL) = 6.

The word *sterile* is used in some recommendations. In order to achieve a sterile fluid, one obvious condition must be fulfilled: the equipment (in this case, the final filter and line downstream of the filter) must be sterile.

Substitution fluid has a monograph in the 5th edition of the *European Pharmacopoeia* and is a drug.[36] In this monograph, it is not stated how the fluid is to be produced. Prior to the introduction of the on-line technique, small volumes were used and, hence, the allowed endotoxin concentration was relatively high—0.25 EU/mL. Using the drug definition makes the substitution fluid interesting regarding endotoxin. As an intravenous drug, a maximum of 5 EU per kilogram of bodyweight per hour is allowed.[37] For a person who weighs 70 kg, this equates to 350 EU/hour. In Table 7, examples are shown that clearly illustrate that

Table 4. Dialysis fluid

National and international standards	Year issued	Microorganisms, CFU/mL	Endotoxins, EU/mL
JSDT	1998[25]	100	0.1
EDTNA	2001[14]	$<10^2$	<0.25
EDTA	2001[15]	$<10^2$	<0.25
France Circ 311	2001[13]	$<10^2$	<0.25
AAMI RD 52	2004[9]	200 (alert 50)	2 (alert 1)
Germany Hygiene Guideline	2005[12]	$<10^2$	<0.25
Italy	2005[16]	$<10^2$	<0.25
SLS	2007[11]	$<10^2$	<0.25
ISO/DIS 11663 (draft)	2008[4]	100 (alert 50)	0.5

JSDT = Japanese Society for Dialysis Therapy; EDTNA = European Dialysis and Transplant Nurse Association; EDTA = European Dialysis and Transplant Association; AAMI = Association for the Advancement of Medical Instrumentation; RD = Renal Disease and Detoxification Committee; SLS = Svensk Läkemedels Standard (Swedish drug book); ISO/DIS = International Organization for Standardization/Draft International Standard.

Table 5. Ultrapure dialysis fluid prior to last filter for hemodiafiltration "on-line"

National and international standards	Year issued	Microorganisms, CFU/mL	Endotoxins, EU/mL
EDTNA	2001[14]	<0.1	<0.03
EDTA	2001[15]	<0.1	<0.03
France Circ 311	2001[13]	<0.1	<0.25
The Netherlands DGN	2003[32]	<0.1	<0.03
AAMI RD 52	2004[9]	0.1	0.03
Denmark Danish Society of Nephrology (draft)	2004[17]	<0.1	<0.05
France Circ 52	2007[33]	<0.1	<0.05
Norway Norwegian Society of Nephrology (draft)	2007[18]	<0.1	<0.025
SLS	2008[11]	<0.1	<0.1
ISO/DIS 11663 (draft)	2008[4]	<0.1	<0.03

EDTNA = European Dialysis and Transplant Nurse Association; EDTA = European Dialysis and Transplant Association; DGN = Dialyse Groep Nederland; AAMI = Association for the Advancement of Medical Instrumentation; RD = Renal Disease and Detoxification Committee; SLS = Svensk Läkemedels Standard (Swedish drug book); ISO/DIS = International Organization for Standardization/Draft International Standard.

when the limits[11,32,33] were established, the use of large volumes was not taken into consideration.

System Quality

Microbial growth in fluid systems occurs on the inner surfaces of the systems. In the water system of a dialysis unit, the clean side of reverse osmosis membranes, distribution piping, inlet lines to dialysis machines and the dialysis machines themselves are included. A water system is large: there are several square meters of surface of reverse osmosis membranes, 100–300 meters of distribution piping, and normally around 50–100 meters of inlet lines. All these areas must be kept at a good hygienic level, which in principle means no growth in the system.

If surface growth (biofilm) occurs in a system, the content of microorganisms in the system is much higher than what the microbiological water analysis indicates.[5]

An analysis result of 5–10 CFU/mL in a system, which is the target for regular disinfection, is already a clear indication that somewhere in the system, microbial growth is in progress.

Disinfection

Disinfection must be preventative and frequent to give a situation where the dialysis unit is in control of the

Table 6. Substitution fluid "on-line"

National and international standards	Year issued	Microorganisms, CFU/mL	Endotoxins, EU/mL
EDTNA	2001[14]	$<10^{-6}$	<0.25
EDTA	2001[15]	$<10^{-6}$	<0.25
France Circ 311	2001[13]	No microorganisms	<0.05
The Netherlands DGN	2003[32]	$<10^{-6}$	<0.03
AAMI RD 52	2004[9]	$<10^{-6}$	<0.03
Ph Eur, 5th ed.	2005[36]	Sterile	<0.25
Germany Hygiene Guideline	2005[12]	Sterile	Non-pyrogenic
Italy	2005[16]	$<10^{2}$	<0.25
France Circ 52	2007[33]	No microorganisms	<0.05
Norway Norwegian Society of Nephrology (draft)	2007[18]	Sterile	<0.025
SLS	2007[11]	Sterile	<0.1
ISO/DIS 11663 (draft)	2008[4]	Sterile	Non-pyrogenic

EDTNA = European Dialysis and Transplant Nurse Association; EDTA = European Dialysis and Transplant Association; DGN = Dialyse Groep Nederland; AAMI = Association for the Advancement of Medical Instrumentation; RD = Renal Disease and Detoxification Committee; Ph Eur = European Pharmacopoeia; SLS = Svensk Läkemedels Standard (Swedish drug book); ISO/DIS = International Organization for Standardization/Draft International Standard.

Table 7. Substitution fluid, endotoxin

Mode of treatment	Substitution fluid volume, L	Treatment time, hr	L/hr	Maximum endotoxin concentration, EU/mL
Hemodiafiltration	20	4	4	0.07
Hemofiltration	70	4	17.5	0.02
Hemofiltration	150	6	25	0.014

hygiene quality.[38] When a strict strategy of preventative disinfection is followed, it is possible to maintain microbiological activity at low levels for a long time. Several dialysis water systems were analyzed for 6–14.5 years and found to have 0.1 to <1 CFU/mL for microorganisms and <0.03 EU/mL for endotoxins. The disinfection protocol used was twice-a-week chemical disinfection of reverse osmosis membranes and daily heat disinfection of distribution piping and inlet lines.[38] It should be noted that all disinfection procedures used in dialysis are compromises. There are technical limitations for high concentrations of chemicals and high temperatures, and time is limited. To overcome this, an additional parameter is introduced, the *frequency* of disinfection.

Modern Methods for Microbiological Analysis

Since the start of microbiological cultivation, attempts have been made to come up with methods that can produce rapid results. The most promising technique is based on DNA. However, at present, DNA techniques are only useful when searching for specific organisms. For mixed environmental microflora, DNA techniques are not yet applicable.[39]

Discussion

All fluids used in dialysis are listed in various recommendations concerning microbiological quality and are also targets for microbiological sampling and analysis. Now it starts to be interesting, as a certain value, for instance the limit 100 CFU/mL, is not the same in practice when different microbiological cultivation techniques are compared. The number 100 CFU/mL analyzed using tryptone glucose extract agar with 7 days of incubation at 17–23°C will correspond to about 0.1–1 CFU/mL if the method had been tryptone soy agar with 48 hours of incubation at 35°C. Pass et al[40] found an even higher deviation between

different cultivation techniques. It is obvious that a problem arises when an insensitive method of verification of microbiological quality is used. In fact, the choice of microbiological cultivation technique is the most important decision as the outcome of analysis may be used to determine disinfection intervals and strategy and thereby influence the hygiene quality of the system. The use of an insensitive cultivation technique will show low growth and result in long intervals between disinfections based on the cultivation analysis.

In official recommendations, no claims for disinfection are highlighted, but the focus is on microbiological analysis. The opposite should apply! Recommendations for disinfection are more important since quality comes from action and not verification analysis.

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