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Chapter

# The Dialyzer as the Last Line of Protection against Endotoxins

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## Abstract

When dialysis fluid is contaminated with endotoxins, the dialyzer membrane is often referred to as the last line of protection to prevent endotoxins from entering the patient's blood. However, a quantifiable requirement for this endotoxin retention property of the membrane has not yet been defined. The ANSI/AAMI/ISO 23500 standard series provides the framework for the microbiological quality of dialysis water, concentrates, and dialysis fluid, and defines the limit value for the non-pyrogenic endotoxin dose. After defining the boundary conditions of the endotoxin loading of the membrane by dialysis fluid and the patient's non-pyrogenic endotoxin dose, quantifiable requirements for the endotoxin retention properties of a membrane, expressed as a dimensionless logarithmic retention value (LRV), were developed in this work. Based on standard dialysis fluid quality, the LRV should minimally be two for a protein-coated membrane after contact with patient blood and minimally be one for a protein-free pristine membrane during online priming before contact with patient blood. This work also presents the critical factors for endotoxin retention tests and shows that the defined LRV values are reached by membranes in modern dialyzers.

**Keywords:** dialysis, endotoxins, water quality requirements, membranes, endotoxin retention, pyrogenicity

## 1. Introduction

In hemodialysis treatment of end-stage renal disease, the patient's blood is separated from dialysis fluid by a semipermeable membrane in the dialyzer. In cases of microbiological contamination of dialysis fluid, it contains pyrogenic substances, such as endotoxins, which would cause fever in the patient if they entered the patient's blood. However, the permeability of the membrane for endotoxins is limited. Therefore, the dialyzer membrane is often called the last line of protection against endotoxins when the microbiological quality of dialysis fluids is discussed. The simplicity of this visualization makes it easy to believe that the underlying concept would have been understood. However, comprehensive and quantitative evaluations and specific definitions of this concept are missing or remain vague. Different situations during a dialysis treatment need to be evaluated in a differentiated manner to account for specific contamination levels, membrane conditions, and fluid flow circumstances. Especially, trends of

using online prepared fluid during online priming, online bolus, and online rinse-back need to be examined, since that fluid is filtered across the dialyzer membrane before it is infused into the patient. A verifiable quantitative requirement for the dialyzer membrane to serve as the last line of protection against endotoxins has not yet been defined. This work develops a proposal of how such a requirement could look like.

## 2. What are endotoxins and can endotoxin retention requirements be inferred from other dialyzer properties?

Endotoxins are mainly lipopolysaccharides (LPS) originating as fragments of bacterial cell membranes. The dominant bacterial source is gram-negative bacteria, which possess an LPS-rich outer membrane. A chemical feature of LPS is their amphiphilic character, which means the molecules combine hydrophilic and hydrophobic parts. This amphiphilic character enables the LPS to form supramolecular structures and biological entities, such as micelles, vesicles, or cell membranes. In those structures, the hydrophobic lipid parts bind together to avoid interaction with water while the hydrophilic polysaccharide parts are exposed to the hydrophilic aqueous environment. This chemical behavior takes place in dialysis fluid and aqueous solutions, in general. More on endotoxins can be found in work done by Williams [1] and Bishop [2].

Dialyzers are primarily designed to remove water and uremic toxins from a patient's blood, and design specifications typically address those requirements. Chemically, uremic toxins are distinctly different from LPS molecules. Uremic toxins are small molecules or proteins that accumulate in the patient's blood when kidney function is impaired. Uremic toxins, even though diverse in chemical nature, are commonly water-soluble in most cases; therefore, do not form larger, aggregated structures in blood plasma water. Water-insoluble uremic toxins bind to albumin, which makes them water-soluble in the albumin-bound form, and they are then called protein-bound uremic toxins.

Dialyzer specifications, such as clearance, sieving coefficient, or ultrafiltration coefficient, as defined and required by technical standards, such as ISO 8637-1 [3], specify the ability to remove water or uremic toxins. Due to the distinct differences in chemical nature and chemical environment of LPS vs. uremic toxins, endotoxin retention properties cannot be simply inferred from specifications describing uremic toxin removal but rather need to be described and investigated in their specific way.

## 3. How can endotoxin retention properties be characterized and measured?

To measure endotoxin retention properties of a membrane, the concentrations on both sides of the membrane need to be put into relation. The dimensionless ratio of concentrations on the feed side and the filtrate side of the membrane is a practicable relation. Since feed and filtrate concentration typically differ by a few orders of magnitude, it is convenient to transform the ratio as a decadic logarithm into a more practical dimension called logarithmic retention value (LRV).

$$LRV = 10\log\frac{C(Feed)}{C(Filtrate)} \tag{1}$$

It is important to understand that the LRV is not a universally constant intrinsic property of the membrane but rather depends on the conditions under which it has been measured. For a meaningful interpretation of the LRV, it is critical that experimental conditions under which the LRV was measured match the context in which the LRV is to be applied. The fundamental experimental parameters that need to be taken into account are:

- *The concentration of endotoxin on the feed side*. The LRV is a dimensionless number and could be measured in theory at any level of endotoxin concentration. However, the formation of supramolecular structures by LPS depends on the LPS concentration. Since the specific nature of the supramolecular structure may impact the retention properties, and the absolute endotoxin concentration needs to be considered.

- *The concentration of endotoxin on the filtrate side*: In cases of strong LPS retention by a membrane, the concentration on the filtrate side might drop below a detectable concentration level. In such cases, the concentration cannot be assumed as zero. A practical approach for such results is to calculate the LRV using the lower detection limit of the applied assay and report LRV as > the obtained number.

- *Fluid flow conditions*: The transport of solutes across a membrane follows two different mass transport mechanisms: diffusion and convection. Especially, in cross-flow filtration settings, the filtration fraction must be defined carefully because the filtered fluid determines the amount of LPS convectively transported toward the membrane. The fluid flow direction must also be considered. When testing endotoxin retention in the dialysis fluid, the relevant flow direction is from dialysate to the blood compartment, which is the opposite of the ultrafiltration flow direction in hemodialysis.

- *Total endotoxin amount*: The total amount of endotoxins in the feed solution is calculated by multiplying endotoxin concentration with a fluid volume of the feed solution. It plays a role because the mechanism of endotoxin retention by a dialyzer membrane cannot be unambiguously and uniformly described for every membrane. The retention mechanism is likely based on two effects: one is size exclusion of larger LPS aggregates, and the other one is adsorption of LPS molecules by either hydrophobic interaction between the hydrophobic part of the LPS molecule and hydrophobic patches of the membrane or by electrostatic interaction between the negatively charged part of LPS and positive charges of the membrane. Total endotoxin amounts in a test should not be chosen too low and potential saturation of adsorption sites needs to be taken into account.

- *The electrolyte composition of the test fluid*: LPS molecules carry negative charges from phosphate groups in the molecule. These negative charges lead to attractive or repulsive electrostatic forces and impact the formation of supramolecular structures. Especially, double-charged ions, such as $Ca^{2+}$ or $Mg^{2+}$, in the test fluid can shield the charges in the LPS molecules and influence the formation of the supramolecular structures and LPS retention by the membrane.

- *The temperature of the test fluid*: The temperature of the test fluid affects the formation of supramolecular LPS structures and has a strong impact on diffusion rates.

- *The bacterial source from which the LPS in the test was prepared*: LPS molecules are not uniform, they contain a variable polysaccharide chain, which can differ between bacterial species. This may impact the formation of supramolecular structures and affect how LPS are retained by membranes. What guiding principle can be used to select the bacterial source of LPS? One approach is to select the bacteria species, that is, the most abundant type in microbial contamination. In microbiological studies of dialysis fluid quality [4, 5], the water-borne *Pseudomonas aeruginosa* was the most abundant type of bacteria species found in the dialysis fluid. In cases where the Limulus amebocyte lysate (LAL) assay is used, the selection of LPS can be guided by considering pyrogenic potency. Pyrogenic potency can be measured by testing in rabbits. To avoid animal testing, the LAL assay can be used instead, and manufacturers of the assays match the assay response to a specific LPS preparation using the pyrogenic potency of the rabbit test. An LPS preparation with particular potency in LAL assays can be obtained for LPS, for example, by *Escherichia coli* strains.

- *Absence or presence of a protein layer on the membrane*: In experimental studies, it was observed that a protein layer on the membrane, which forms after the initial contact of the membrane with protein-rich fluids, such as blood plasma, changes endotoxin retention properties compared to a protein-free pristine membrane [6, 7]. Proteins on the membrane are likely to impact pores size, and may also affect surface chemistry, which would account for a modified LPS adsorption to the membrane.

## 4. Which factors determine endotoxin concentration in dialysis fluid?

The current requirements of dialysis fluid are set out in the ANSI/AAMI/ISO 23500 standards series on the preparation and quality management of fluids for hemodialysis and related therapies. There are two potential sources of endotoxins, water and concentrates, and there is the option to use ultrafilters for endotoxin removal. The water system provides water to the monitor in accordance with the provisions of ANSI/AAMI/ISO 23500-3 (formerly ISO 13959) [8], which defines maximum endotoxin concentration as 0.25 EU/ml for water for dialysis. Concentrates to prepare dialysis fluid to follow the provisions of ANSI/AAMI/ISO 23500-4 (formerly ISO 13958) [9]. Endotoxin concentrations in concentrates must be at a level that allows the preparation of standard dialysis fluid with a maximum of 0.5 EU/ml from concentrate and water for dialysis. The maximum endotoxin concentration for standard dialysis fluid is governed by ANSI/AAMI/ISO 23500-5 (formerly ISO 11663) [10]. If an ultrafilter is used to filtrate the prepared dialysis fluid, ultrapure dialysis fluid can be prepared with a maximum allowed endotoxin concentration of 0.03 EU/ml.

In this work, the assumption is made that dialysis fluid of at least standard dialysis fluid quality per ANSI/AAMI/ISO 23500-5 [10] is provided by the dialysis monitor, and maximum endotoxin concentration is at 0.5 EU/ml even if no ultrafilter is used or in case of failure of the ultrafilter.

## 5. What is the total endotoxin amount the dialyzer membrane can be exposed to on its dialysate side?

The total endotoxin amount the dialyzer membrane can be exposed to depends on two variables: endotoxin concentration and fluid volume passing or crossing the

membrane. In the section above, the assumption of a maximum endotoxin concentration of 0.5 EU/ml was made, because standard dialysis fluid quality per ANSI/AAMI/ISO 23500-5 [10] was assumed in any case. In order to consider the fluid volume passing through or crossing a dialyzer membrane as well as the resulting total amount of endotoxins, various phases and situations during a dialysis treatment need to be differentiated: priming, treatment, bolus, and rinse-back. In the following considerations, only configurations in which online prepared fluid is infused into the patient by crossing the dialyzer membrane are included. Configurations using saline bags or online prepared fluid bypassing the dialyzer membrane are excluded in this work because the dialyzer membrane does not act as a last endotoxin barrier in such situations. Relevant configurations, where the dialyzer acts as the last line of protection against endotoxins are:

- *Online priming with dialysate infusion*: Online priming is used to rinse and fill the dialyzer with online-prepared dialysis fluid. The volume for filling and rinsing is assumed to be up to 3 L. During online priming, the protein-free pristine dialyzer membrane can be exposed to a maximum endotoxin amount of 3000 ml × 0.5 EU/ml = 1500 EU. However, the largest portion of the priming fluid will be discarded, and the patient will not be exposed to the complete priming volume. In cases of "wet" patient connection, (i.e., arterial and venous access are connected simultaneously) the fluid contained within the blood side of the extracorporeal circuit is infused into the patient, which is typically not more than 500 ml. For example, the blood compartment volume of a Polyflux 210 H dialyzer is 125 ml [11], and the fill volume of a single-needle blood line BL 40 SN is 279 ml [12], which sums up to 404 ml.

- *Online bolus with fluid infusion*: Online bolus is used to infuse a specific amount of fluid into the patient, for example, for blood pressure stabilization. Bolus volume can be assumed to be not more than 500 ml per hour. The total maximum endotoxin amount that a protein-coated membrane can be exposed to is 500 ml × 0.5 EU/ml = 250 EU for standard dialysis fluid.

- *Backfiltration and backdiffusion* are physical effects produced by the pressure conditions of the fluidic circuit and the hydraulic permeability of the membrane. The amount of backfiltration is hard to measure or calculate and difficult to predict. It has been calculated and experimentally measured to be in the range of 30–50 ml/min [13] and can be assumed to not exceed 100 ml/min at which dialysis fluid is filtered from dialysate to the blood side of the dialyzer. As a worst-case estimate the maximum endotoxin amount passing the membrane during dialysis treatment can be estimated irrespective of the amount of backfiltration by the total amount of dialysis fluid passing through the dialyzer. Assuming a maximum dialysate flow rate of 800 ml/min of standard quality dialysis fluid with 0.5 EU/ml, the dialyzer would be exposed to a total amount of 800 ml/min × 60 min × 0.5 EU/ml = 24000 EU per hour. If the blood flow rate is lower than the dialysate flow rate, the LPS transfer is physically limited by the blood flow rate, irrespective of whether diffusion or convection is the dominant mass transfer mechanism. Assuming a maximum blood flow rate of 600 ml/min, the maximum amount of endotoxins that could pass through the dialyzer membrane is 600/800 x 24000 EU = 18000 EU per hour.

- *Online rinse-back with fluid infusion*: Rinse-back is the reinfusion of the blood in the blood compartment of the extracorporeal circuit to the patient after

treatment. The volume of rinse-back is limited by the volume of the blood pathway of the extracorporeal circuit and can be assumed to be not more than 500 ml. The total maximum endotoxin amount that the protein-coated membrane may be exposed to by rinse-back fluid with standard dialysis fluid quality is 500 ml × 0.5 EU/ml = 250 EU.

## 6. How much endotoxin could a patient tolerate without developing a pyrogenic reaction?

Various endotoxin dose limits have been developed to prevent pyrogenic reactions during and after medical treatments. The European Pharmacopeia [14] defines a limit of 5 EU/kg body weight per hour for intravenously administered drugs, which is also referenced in ISO 23500-1 [15] as the minimum dose that produces fever and is therefore applied in this work to develop a requirement for the endotoxin retention properties of the dialyzer membrane as the last line of protection against endotoxins. During a 1-hour dialysis session, the upper limit of the endotoxin dose for a patient of 50 kg body weight would be 5 EU/kg/h × 50 kg × 1 h = 250 EU. The body weight was taken from ICH Q3(R8) Guideline [16], which defines 50 kg as "relatively low body weight," to allow some extra safety margin.

## 7. How much endotoxin must a dialyzer be able to retain?

Two boundary conditions were defined in the previous sections: the endotoxin load on the dialysate side and the non-pyrogenic dose on the blood side. The dialyzer membrane must reduce the endotoxin load on the dialysate side to a non-pyrogenic level on the blood side. With the help of these two boundary conditions, a minimum LRV can be calculated for a dialysis membrane. Since protein-free pristine membranes are different from protein-coated membranes, two requirements can be developed for either case. In the first step, the requirement for a protein-coated membrane will be developed. In the second step, the requirement for the pristine membrane is derived. In the following calculations, the use of standard dialysis fluid will be assumed as worst-case, and the accumulated effects of online bolus, backfiltration/backdiffusion, and online rinse-back are concentrated into 1-hour treatment.

**Figure 1** shows the dose calculation for the 1-hour treatment case using standard dialysis fluid of 0.5 EU/ml. Fluid volumes for online bolus and rinse-back are assumed to be 500 ml each. The volume assumed for backfiltration or backdiffusion was derived from a blood flow rate of 600 ml/min being the limiting factor for endotoxin transfer irrespective of the mass transport mechanism; therefore, the volume is 600 ml/min × 60 min = 36 L. The endotoxin amount at each step is calculated as 0.5 EU/ml × fluid volume. This results in 250 EU, 18000 EU, and 250 EU for online bolus, backfiltration/backdiffusion, and online rinse-back, respectively, producing a total of 18500 EU in 1 hour. The minimum LRV (rounded up to 1 significant digit) for the protein-coated membrane to reduce 18500 EU below 250 EU is LRV 2. The dose threshold of 250 EU was calculated using the limits of 5 EU/kg/hour for a 50 kg person as 5 EU/kg/hour x 50 kg × 1 hour = 250 EU. A protein-coated membrane with an LRV of 2 reduces the endotoxin load of 18500 EU on the dialysate side to 185 EU on the blood side.

**Figure 2** shows in summarized form the dose calculation when combining the 1-hour treatment case as described above with the online-priming situation with a pristine

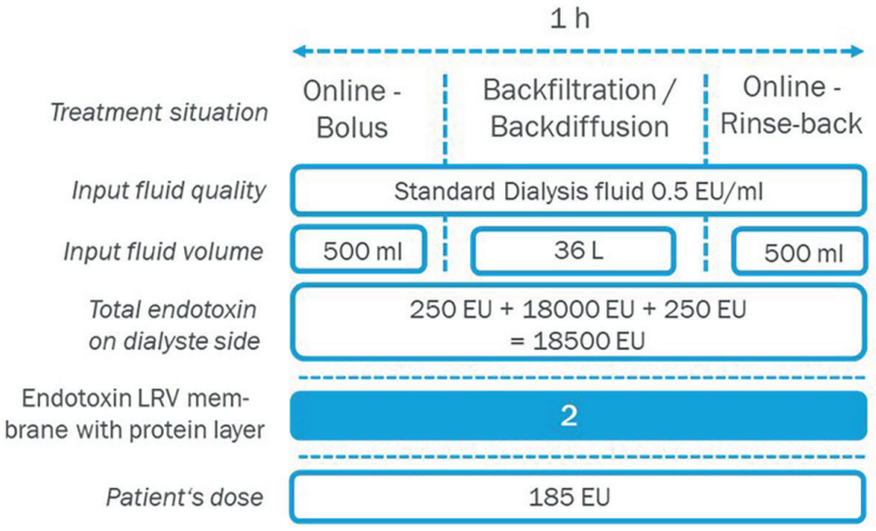


**Figure 1.**
*Calculation of endotoxin load on the feed side, LRV, and endotoxin dose on the filtrate (patient) side for treatment cases, where a protein-coated membrane, reduces the transfer of endotoxins from the standard dialysis fluid.*

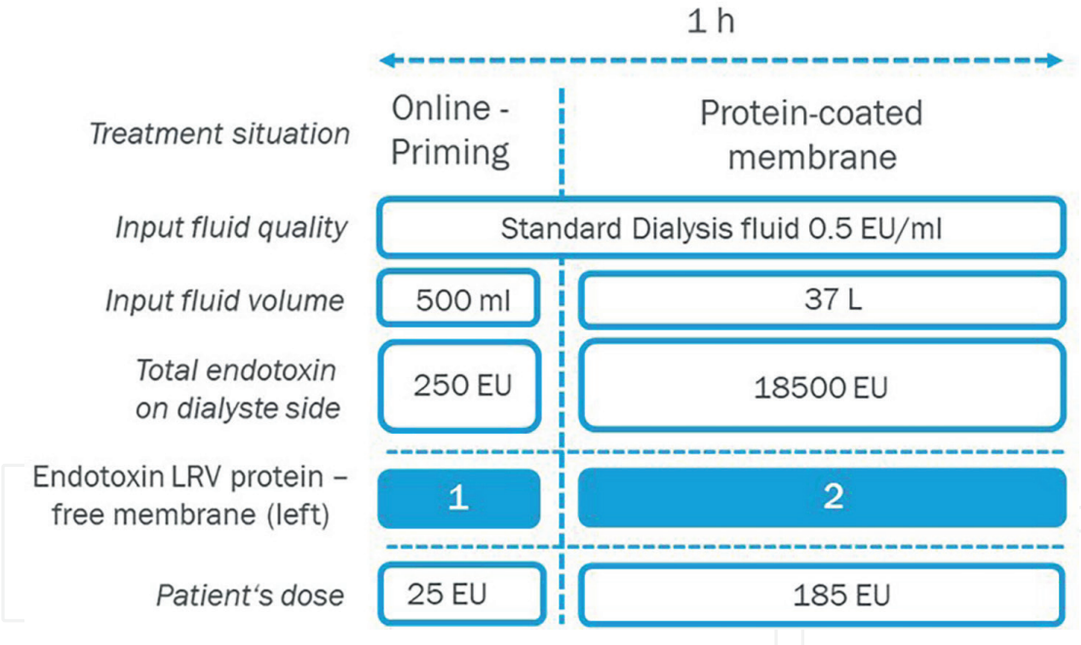


**Figure 2.**
*Calculation of endotoxin load on the feed side, LRV, and endotoxin dose on the filtrate (patient) side for treatment cases, where a protein-coated membrane (right side) and a pristine membrane (left side), reduces the transfer of endotoxins from the standard dialysis fluid.*

membrane. The volume of online-priming fluid infused into the patient was assumed to be 500 ml. The endotoxin amount of online priming calculates as 0.5 EU/ml x 500 ml = 250 EU. The dose threshold of 65 EU for online priming can be calculated as the total dose threshold of 250 EU (calculated with the limit of 5 EU/kg/hour for a 50 kg person) minus 185 EU covering online bolus, backfiltration/backdiffusion, and online rinse-back. The minimum LRV (rounded to one significant digit) for the pristine membrane to reduce 250 EU below 65 EU is LRV 1. A pristine membrane with an LRV of 1 reduces the endotoxin load of 250 EU on the dialysate side to 25 EU on the blood side.

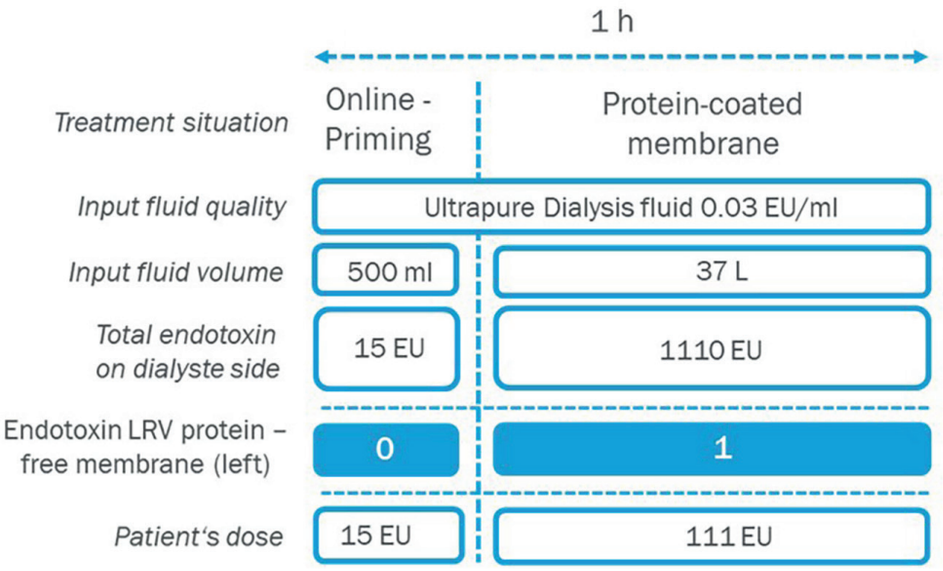


**Figure 3.**
*Calculation of endotoxin load on the feed side, LRV, and endotoxin dose on the filtrate (patient) side for treatment cases, where a protein-coated membrane (right side) and a pristine membrane (left side), reduces the transfer of endotoxins from ultrapure dialysis fluid.*

**Figure 3** shows a dose calculation for a 1-hour treatment case analogous to the calculations shown in **Figure 2**. The only difference is that the use of ultrapure dialysis fluid of 0.03 EU/ml was assumed instead of standard dialysis fluid. The minimum LRV of the protein-coated membrane would need to be LRV 1 in order to reduce 1110 EU to 111 EU. The pristine membrane would not need to have endotoxin retention properties at all during online priming. The total endotoxin dose of 126 EU (111 EU + 15 EU= 126 EU) would be below the endotoxin dose limit of 250 EU (5 EU/kg/hour for a 50 kg patient).

## 8. What should a requirement for the endotoxin barrier property of a dialyzer membrane look like?

The calculations above show different endotoxin retention requirements for standard dialysis fluid and ultrapure dialysis fluid. Although the use of ultrapure dialysis fluid is recommended and it is not mandatory, and in the case of the absence or failure of an ultrafilter, only standard fluid quality can be assumed. Consequently, a requirement must orient itself to the use of standard dialysis fluid quality.

For standard dialysis fluid, a dialyzer membrane must have a minimum endotoxin retention capacity measured as an LRV of 2 after exposure to blood plasma and the formation of a protein layer, and it must have a minimum LRV of 1 in its protein-free pristine form.

Is this a realistic requirement for current, state-of-the-art dialyzer membranes? The LRVs of dialyzer membranes with a protein layer are well documented under treatment conditions. Polyflux L (low flux), Revaclear (high flux), and Theranova (medium cut-off) dialyzers were tested in various experimental configurations using mixed filtrates of *Pseudomonas aeruginosa* and *Pelomononas saccharophila* [17], lysates of *Pseudomonas aeruginosa* and isolated LPS from *Escherichia coli* [18]. In all cases, the LRV was above 2 [18]. Experimental data in the perspective of this

work is not available for pristine membranes. An experimental study is described in the sections below to provide data to answer the question if the minimum LRV requirement of LRV 1 is realistic for state-of-the-art dialyzer membranes before protein contact.

## 9. Experimental study to determine endotoxin LRV of a protein-free membrane under online-priming test conditions

Test articles were high flux dialyzers Polyflux 210H and Revaclear 500, as well as medium cut-off dialyzer Theranova 500. The products were taken from regular manufacturing with standard sterilization and within their specified shelf-life. For each type of dialyzer, the test items were taken from three separate production lots. Revaclear 500, Theranova 500, and Polyflux 210H are the products with the largest membrane area of their respective product family. In this study, the product with the largest membrane area in each product family was chosen because it provides the largest interface between the dialysate and the blood side compartment. An experimental pre-study under online-priming test conditions did not indicate an impact by the membrane area between 1.4 $m^2$ and 2.1 $m^2$ on endotoxin retention properties and, if adsorptive retention was assumed, a saturation of adsorptive capacity was not observed for smaller and larger membrane areas. Under this presumption, the items selected for testing in this study can be considered to be representative under online-priming conditions for versions with smaller membrane area down to 1.4 $m^2$ in their respective product family.

The sample number was defined to be six (6). The sample number definition was not based on a formal statistical approach. Previous studies had shown 95% confidence intervals within a range of ± 10% of the mean LRV for a similar experimental design using six samples.

The test system had two parts. The first part comprised sample generation, and the second part the sample analysis.

Sample generation was done in a benchtop experiment of filtration (**Figure 4**). Fluid from a challenge solution made of endotoxin-contaminated bicarbonate-based dialysis fluid was pumped by a peristaltic pump across a dialyzer membrane. The fluid flow direction in the dialyzer was from dialysate to blood side. The filtrate was collected on the blood side for subsequent analysis.

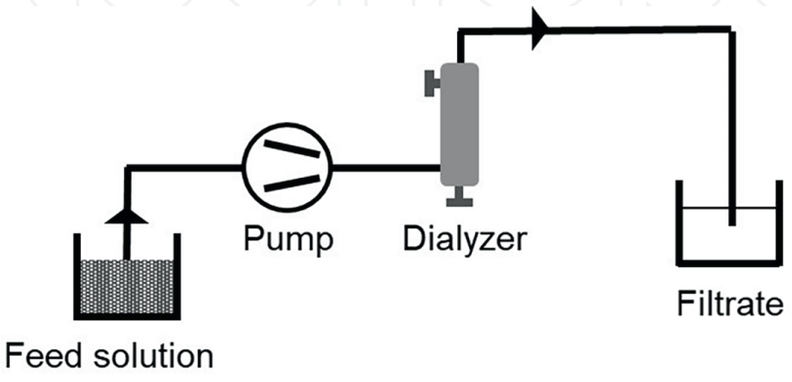


**Figure 4.**
*Schematic drawing of the filtration setup. A feed solution containing a defined amount of endotoxin is pumped through the dialyzer using a peristaltic pump. Flow direction is from dialysate to blood compartment. Unused ports of the dialyzer were blocked. The filtrate was collected on the blood side.*

| Test setting | Set value | Justification |
|---|---|---|
| Composition of feed fluid. | Bicarbonate-based dialysis fluid prepared from<br>dialysis concentrate D200 (MTN Neubrandenburg, Code 20000060401)<br>Acid concentrate D204 (MTN Neubrandenburg, 20400100001, 1.75 mM $Ca^{2+}$. 0.5 mM $Mg^{2+}$ in final fluid).<br>Reverse osmosis water.<br>Before adding endotoxins, the dialysis fluid was sterile filtered using a U9000 plus Ultrafilter (Baxter) and checked for pH 7.1–7.5 | Bicarbonate dialysis fluid is a state-of-the-art dialysis fluid. |
| Bacterial endotoxin from two different sources. | 1. *Eschericha coli* strain O55:B5 (Lonza, Code 00193783)<br>2. *Pseudomonas aeruginosa* (Sigma-Aldrich, Code L9143) | *Escherichia coli* O55:B5 is an endotoxin, which is traceable to a defined reference standard material; therefore, suitable to provide accurate endotoxin concentrations [1].<br>*Pseudomonas aeruginosa* is a common water-borne organism and can be found in dialysis fluid; therefore, represents clinical conditions [4, 5]. |
| Bacterial endotoxin concentration in feed fluid. | The bacterial endotoxin concentration must be > 0.5 EU/ml; the targeted set value was 2 EU/ml. | 0.5 EU/ml is the maximum allowed endotoxin concentration in standard dialysis fluid according to ANSI/AAMI/ISO 23500-5 [10] and therefore the minimum concentration in this study to represent worst-case clinical conditions. |
| Volume of feed solution | 3000 ml[1] | 3000 ml was assumed as maximum rinse volume and is considered as worst-case in this study because it results in the largest possible endotoxin amount. |
| Fluid temperature | 37°C +/− 2°C | 37°C +/- 2°C was assumed as clinically relevant temperature range of the priming fluid. |
| Fluid flow rate | 200 ml/min +/− 10% | 200 ml/min was assumed as maximum priming flow rate. No impact by flow rate between 100–500 ml/min was observed in a feasibility study. Nevertheless, 200 ml/min is considered to be worst-case, as it results in a minimum time that the endotoxin interacts with the membrane, which minimizes potential interactions; +/− 10% was assumed typical flow accuracy. |
| Sampling at two sampling points at end of sample generation. | 1. Sample of about 2 ml was taken from the filtrate at the end of filtration.<br>2. Sample of about 1 ml was taken from the feed solution at the end of the filtration. | Sample at the end of filtration represents the dialysis fluid that remains in the extracorporeal circuit and could be infused into the patient in clinical practice. A sample from the challenge solution was taken for LRV calculation. |

[1]*Volumetric measurement accuracy range within the accuracy range of the scale of suitable measurement cylinders.*

**Table 1.**
*Sample generation test settings and justification.*

| Filter type | Sample # | LRV (*P. aer*) | LRV (*E. coli*) |
|---|---|---|---|
| Revaclear 500 | 1 | 2.6 | >2.8 |
| | 2 | >2.5 | 2.6 |
| | 3 | >2.6 | >2.6 |
| | 4 | >2.4 | >2.5 |
| | 5 | >2.7 | >2.7 |
| | 6 | >2.5 | >2.6 |
| Polyflux 210H | 1 | >2.5 | >2.6 |
| | 2 | >2.6 | >2.6 |
| | 3 | >2.3 | >2.6 |
| | 4 | >2.0 | >2.5 |
| | 5 | >2.2 | >2.7 |
| | 6 | >2.4 | >2.6 |
| Theranova 500 | 1 | 2.5 | >2.8 |
| | 2 | 2.3 | 2.5 |
| | 3 | >2.7 | >2.6 |
| | 4 | >2.7 | >2.5 |
| | 5 | 2.3 | >2.7 |
| | 6 | >2.4 | >2.6 |

**Table 2.**
*Results of the experimental study. LRV was measured using the protein-free pristine membrane under online-priming conditions. LRV is shown for Pseudomonas aeruginosa (P. aer) and Escherichia coli (E. coli). When LRV was calculated using the lower limit of detection of the LAL assay (0.005 EU/ml) values are shown as " > ."*

**Table 1** identifies and specifies the critical settings and provides justification for the selected value.

The challenge solution was filtered directly through the dry dialyzer to simulate the clinical priming process.

A portion of each feed sample was diluted 10x with bicarbonate-based dialysis fluid. A diluted sample was needed in certain cases to bring the endotoxin concentration within the working range of the endotoxin assay. The endotoxin concentration was determined using Limulus amebocyte lysate (LAL) assay in accordance with the manufacturer's instruction and validated using local work instruction

The results obtained are shown in **Table 2**.

In conclusion, all dialyzers tested met the proposed LRV requirement of a minimal LRV of 1, for both types of endotoxin, LPS from *Pseudomonas aeruginosa* and *Escherichia coli* under conditions of online priming with dialysate infusion.

## 10. Discussion and conclusion

This work aimed to develop and propose a measurable requirement to define the retention properties of the dialyzer being the last line of protection against endotoxins potentially present in the dialysis fluid. By setting out the boundary conditions of the endotoxin load on the feed side and the tolerable endotoxin dose on the patient

side, a requirement could be defined. The requirement employed the concept of the dimensionless logarithmic retention value (LRV). Since protein-free pristine membranes and protein-coated membranes show different endotoxin retention properties, two requirements were developed, one for each case. The requirement for protein-coated membranes is a minimum LRV of 2, and the requirement for protein-free pristine membranes is a minimum LRV of 1. These requirements are based on the assumption that standard dialysis fluid per ISO 23500-5 [10] can be provided at any time by controlling the water system, the concentrates, and potential ultrafilters in the mixing unit (dialysis monitor). The development of the requirements considered various treatment conditions, where fluid can cross the dialyzer membrane from dialysate to blood side or endotoxins could pass by diffusion from dialysis fluid into the patient's blood. During the development of the requirements, worst-case considerations were employed when specific values were selected from a potential range. Maximum endotoxin concentrations and fluid volumes were assumed—to represent the highest possible endotoxin load—while on the patient side a relatively low body weight was selected. When backfiltration and backdiffusion during a dialysis treatment were taken into account, the hypothetical transfer of all endotoxins across the membrane was considered, which is probably an overestimation to some degree, because a large amount of endotoxins will probably just bypass the membrane in the dialysis fluid stream due to diffusion rate limitations. The proposed endotoxin requirements are realistic for state-of-the-art dialyzers; reference literature supported this for protein-coated membranes [17, 18], and experimental data were presented for protein-free pristine membranes. Even though standard dialysis fluid can be used to perform dialysis therapy according to ISO 23500-1 [15], it also stated that "standard dialysis fluid shall be regarded as the minimum acceptable quality. Ultrapure dialysis fluid is a step forward in improving biocompatibility, reducing inflammation, and preventing dialysis-related complications."

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## Conflict of interest

All authors are employed by Gambro Dialysatoren GmbH (Affiliate of Baxter International Inc.).

## Legal disclaimer

The manufacturer of the tested dialyzers does not extend any representation or warranty (expressed or implied) regarding the dialyzers that go beyond the respective instructions for use or product labels of the dialyzers. The manufacturer of the dialyzers shall in no event be held liable for the suitability of the dialyzers beyond the label information.

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## References

[1] Williams KL. Endotoxins: Pyrogens, LAL Testing and Depyrogenation (Drugs and the Pharmaceutical Sciences, # 167). 3rd ed. Boca Raton, USA: Taylor & Francis Inc

[2] Bishop RE. Fundamentals of endotoxin structure and function. Contributions to Microbiology. 2005;**12**:1-27

[3] ISO 8637-1. Extracorporeal Systems for Blood Purification — Part 1: Haemodialysers, Haemodiafilters, Haemofilters and Haemoconcentrators. Geneva, Switzerland. 2017

[4] Vanholder R et al. Endotoxin transfer through dialysis membranes: Small- versus large-pore membranes. Nephrology, Dialysis, Transplantation. 1992;**7**(4):333-339

[5] Lonnemann G. Assessment of the quality of dialysate. Nephrology, Dialysis, Transplantation. 1998;**13** (Suppl. 5):17-20

[6] Lonnemann G et al. The role of plasma coating on the permeation of cytokine-inducing substances through dialyser membranes. Nephrology, Dialysis, Transplantation. 1995;**10**(2):207-211

[7] Schindler R et al. Blood components influence cytokine induction by bacterial substances. Blood Purification. 2001;**19**(4):380-387

[8] ANSI/AAMI/ISO 23500-3. Preparation and Quality Management of Fluids for Haemodialysis and Related Therapies—Part 3: Water for Haemodialysis and Related Therapies. Arlington, USA. 2019

[9] ANSI/AAMI/ISO 23500-4. Preparation and Quality Management of Fluids for Haemodialysis and Related Therapies—Part 4: Concentrates for Haemodialysis and Related Therapies. Arlington, USA. 2019

[10] ANSI/AAMI/ISO 23500-5. Preparation and Quality Management of Fluids for Haemodialysis and Related Therapies—Part 5: Quality of Dialysis Fluid for Haemodialysis and Related Therapies. Arlington, USA. 2019

[11] Baxter Polyflux H Dialzer Brochure (GLBL/MG3/18-0020e - 2019

[12] Baxter Novaline bloodline tubing system Brochure (GBU-RC60-210001 v5.0 – February 2022)

[13] Lorenzin A et al. Quantification of internal filtration in hollow fiber hemodialyzers with medium cut-off membrane. Blood Purification. 2018;**46**:196-204

[14] European Pharmacopoeia 5.0 2.6.14. Bacterial Endotoxins. Strasbourg: Council of Europe

[15] ANSI/AAMI/ISO 23500-1. Preparation and Quality Management of Fluids for Haemodialysis and Related Therapies—Part 1: General Requirements. Arlington, USA. 2019

[16] ICH Harmonised Guideline - Impurities: Guideline for Residual Solvents Q3C(R8) Current Step 4 version dated 22 April 2021

[17] Schepers E et al. Assessment of the association between increasing membrane pore size and endotoxin permeability using a novel experimental dialysis simulation set-up. BMC Nephrology. 2018;**19**(1):1. DOI: 10.1186/s12882-017-0808-y

[18] Hulko M et al. Pyrogen retention: Comparison of the novel medium cut-off (MCO) membrane with other dialyser membranes. Scientific Reports. 2019;**9**(1):6791. DOI: 10.1038/s41598-019-43161-2