

# EDTNA/ERCA Guidelines: Technical Section

## 1 Quality assurance for dialysis-quality water and dialysis fluid

### 1.1 Guidelines for the control and monitoring of microbiological contamination in water for dialysis

#### 1.1.1 Scope

These guidelines apply to the control and monitoring of microbiological contamination in:

- water used for preparation of dialysis fluid in all settings, including patients' homes and acute care units
- water used for in-house production of liquid concentrates
- water used for reprocessing dialysers
- water used for preparation of ultrapure dialysis fluid by equipment fitted with additional point-of-use filtration

These guidelines should be taken into consideration when designing and modifying water treatment and distribution systems. Microbiological quality is affected by all components of the water treatment system, from the pre-treatment devices to the point of use. The suppliers of all components should ensure that their products are compatible with these guidelines. Where the complete installation is assembled using components from different suppliers, the renal unit staff must take overall responsibility for implementing the guidelines.

The microbiological quality of the water over the life of the system will depend on the maintenance. Responsibility for the specification and validation of the disinfection method and frequency may lie with a supplier or with the renal unit staff. This must be established before the system is put into use.

Although day-to-day management of the quality of the water used for dialysis is normally delegated to technical staff, ultimate responsibility for water quality may lie with the physician or pharmacist in charge of the dialysis service, or with a designated team, according to national regulations. To reflect this multidisciplinary process, these guidelines have been reviewed and approved by the European Society for Artificial Organs (ESAO) and by the European Renal Association/European Dialysis and Transplant Association (ERA-EDTA).

#### 1.1.2 Exclusions

These guidelines are not applicable to water used in the preparation of commercially available dialysis fluid or substitution fluid supplied in sterile bags.

#### 1.1.3 Applicable standards and guidelines

**Monograph 1167:1997 (corrected 2000, republished 2001) Haemodialysis solutions, concentrated, water for diluting.** *European Pharmacopoeia* Supplement 2001.

**Monograph 0128:2000: Haemodialysis, solutions for.** *European Pharmacopoeia*, Supplement 2001.

**Circulaire DGS/DH/AFSSAPS No 311 relative aux spécifications techniques et à la sécurité sanitaire de la pratique de l'hémofiltration et de l'hémodiafiltration en ligne dans les établissements de santé.** [Technical specifications and medical security of on-line haemofiltration and haemodiafiltration used in health care institutions] 7 June 2000 (*French*).

**Tillverkning och hantering av hemodialysvätskor och hemofiltrationsvätskor inom sjukvården.** [Manufacturing and handling of fluids for haemodialysis and haemofiltration in medical care] *Svensk läkemedelsstandard (SLS) 2001 (Swedish)*.

**ERA-EDTA European Best Practice Guidelines for Haemodialysis: IV. Dialysis fluid purity.** *Nephrology, Dialysis and Transplantation* 2002, Supplement (in press).

#### 1.1.4 Glossary

For the purpose of these guidelines, the following definitions apply:

**Biofilm:** A coating on surfaces consisting of microcolonies of bacteria embedded in a protective extracellular matrix. The matrix, a slimy material secreted by the cells, protects the bacteria from antibiotics and disinfectants.

**Colony forming units (CFU):** An organism capable of replicating to form a distinct, visible colony on a culture plate. In practice, a colony may be formed by a group of organisms.

**Dialysis fluid concentrate:** A solution of chemicals which, when appropriately diluted, produces the dialysis fluid (definition from IEC 60601-2-16 2.107). Concentrates may be purchased as ready solutions or as powder that must be dissolved before use. They are subject to the requirements of EP Monograph 0128:2000.

**Dialysis fluid:** A solution that is intended to exchange solutes and/or water with blood during haemodialysis or

haemodiafiltration (definition from IEC 60601-2-16 2.107). 'Dialysate' is commonly used in place of dialysis fluid, although this term should be reserved for contaminated fluid leaving the dialyser.

**Disinfection:** The destruction of micro-organisms by physical (e.g. heat or UV radiation) or chemical means. Disinfection is a less lethal process than sterilization, since it is expected to destroy most recognized pathogenic micro-organisms, but not necessarily all those present. Disinfection does not eliminate endotoxin or other bacterial 'by-products', such as exotoxins.

**Distribution system:** The pipework used to transport treated water to the dialysis machine, together with any associated tanks, pumps, filters, irradiation units etc. The connections to the dialysis machines also form a part of the distribution system.

**Endotoxin:** A group of pyrogenic substances (lipopolysaccharides) that form a structural part of the outer cell wall of gram-negative bacteria. Under stress or during proliferation, endotoxin can be released from the bacteria. Endotoxins belong to the group of cytokine inducing substances and can cause fever, rigors, hypotension (in some cases followed by shock), multi-organ failure and even death if they enter the circulation.

**Endotoxin units:** Endotoxin levels are measured in terms of biological activity as Endotoxin Units (in the US) or International Units per ml. The units are related to the activity of a standard *E. coli* endotoxin. In 1997, the American and European Pharmacopoeias adopted a harmonised standard so that the EU and IU are now identical.

**Exotoxin:** Proteins secreted by micro-organisms to destabilise the function of their host. Exotoxins are small molecules with very specific actions, such as blocking neural transmission or inhibiting protein synthesis. They may stimulate the body's immune system like endotoxins, but they cannot be detected by the LAL-test.

**LAL (Limulus Amoebocyte Lysate) test:** An assay used to detect endotoxins, which exploits the immune response of the horse shoe crab (*Limulus polyphemus*).

**Microbiological contamination:** Contamination with any form of micro-organism (e.g. bacteria, yeast, fungi and algae) and/or with the by-products of living or dead organisms such as endotoxins and exotoxins, such as microcystin, which is derived from blue-green algae.

**Peptidoglycans:** These pyrogenic substances are found in the cell walls of gram-positive bacteria in much higher quantities than in gram-negative bacteria. Peptidoglycans cannot be detected by the LAL-test but they can be assayed using Silkworm Larvae Reagent. Due to their ability to induce cytokines from blood cells they also can be analysed quantitatively by measuring the release of IL-1Ra (Interleukin-1-receptor-antagonist) in whole blood.

**Point-of-use filtration:** Filtration of the water and dialysis fluid at the dialysis machine to produce ultrapure dialysis fluid. Most current systems pass the dialysis fluid through at least two filters.

**Pyrogen:** A substance that can cause fever (elevation of body temperature to  $> 37.8$  °C). Symptoms of a pyrogenic reaction may include chills, rigors, nausea, vomiting and hypotension.

**Substitution (replacement) fluid:** Very highly purified fluid produced from ultrapure dialysis fluid. Substitution fluid, which can be infused directly into the blood, must be sterile and non-pyrogenic. Guidelines on monitoring the quality of substitution fluid are given in Circulaire DGS/DH/AFSSAPS No 311 and SLS 2001 (see 1.1.3). The effects of treatment mode and patient size on allowable endotoxin levels are discussed in reference 1.

**Ultrapure dialysis fluid:** Highly purified dialysis fluid that can be used in place of conventional dialysis fluid. The definition of ultrapure dialysis fluid varies and probably reflects practical detection limits. The specification for ultrapure water used in the ERA/EDTA Guidelines, i.e.  $< 0.1$  CFU/ml and  $< 0.03$  IU/ml, is often used as a specification for ultrapure dialysis fluid. EDTNA/ERCA accept this specification, on the basis that ultrapure dialysis fluid must be further purified to produce substitution fluid.

**Water treatment system:** The entire system, including purification devices, pumps, monitoring systems and distribution systems, from the incoming water supply to the point of use.

### 1.1.5 Introduction

The ERA-EDTA Best Practice Guidelines for Dialysis Fluid Purity specify that water for the production of dialysis fluid should meet or exceed the requirements of the European Pharmacopoeia. References 2 to 6 of this document review the clinical arguments for the use of high quality dialysis fluid, a more extensive discussion and bibliography is provided in the ERA-EDTA Guidelines.

EP Monograph 1167 requires that water used for diluting concentrated haemodialysis solutions should have a total viable aerobic count of less than 100 CFU/ml and that the concentration of endotoxin should be less than 0.25 IU/ml.

The European Pharmacopoeia monograph for solutions for haemodialysis (EP 0128) does not give a clear requirement for the level of endotoxin in prepared dialysis fluid. The manufacturer of a concentrated solution must ensure that the solution has an endotoxin level of less than 0.5 IU/ml at the dilution used for dialysis. This could be interpreted as a limit of 1.25 IU/ml for bicarbonate dialysis fluid (where two concentrates are used), i.e. 0.5 IU/ml from each diluted concentrate plus 0.25 IU/ml from the water. This limit has not been widely adopted and, in practice, a much lower level

of contamination is easy to achieve so there is no reason why the dialysis fluid should not comply with the requirements of EP 1167 for microbiological contamination.

Lower levels for viable organisms and endotoxin in the water and/or dialysis fluid may be specified locally. Where ultrapure fluid is required, the limits will normally be < 0.1 CFU/ml and < 0.03 IU/ml.

This document provides practical guidance to help dialysis units produce water for dialysis that meets or exceeds the requirements for microbiological contamination in EP 1167, and for monitoring the quality of water and dialysis fluid. Although the guidelines are primarily intended for in-centre dialysis, the principles apply to home haemodialysis and self-care units, but the frequency of monitoring may have to be adapted.

The EP does not provide limits or suggest tests for other bacterial 'by-products', such as exotoxins or peptidoglycans. The Silkworm Larvae Reagent Assay (7) can be used to check for peptidoglycans if a substantial proportion of the bacteria in the water or dialysis fluid are gram positive. The growth of all micro-organisms should be minimised to reduce the levels of known and unknown harmful contaminants generated by living and dead organisms. Minimisation of bacterial growth is possible with good quality assurance (see Appendix).

For regulatory purposes, it is necessary to specify an 'acceptable' level of contamination, but there is mounting evidence to suggest that exposure to relatively low levels of endotoxin causes chronic inflammation and contributes to amyloidosis (8,9). There is increasing evidence linking inflammation to cardiovascular disease in the general public but the effect of inflammation on rates of mortality and morbidity in haemodialysis patients is still under debate. To be certain that the health of patients is not being compromised, dialysis units should aim to minimise their exposure to any detectable level of endotoxin.

Ideally, a water treatment and distribution system installed today should be designed to meet the requirements that will be in place in 7 to 10 years time. Whilst it is not possible to predict what the standard for dialysis-quality water will be in the future, it is likely that the maximum allowed levels of bacteria and endotoxin will be reduced. The ERA-EDTA guidelines advise dialysis units to work towards supplying ultrapure fluid routinely for all dialysis modalities.

Tighter control of microbiological contamination can only be achieved where the water distribution system is designed for very frequent automated disinfection. Frequent disinfection may be costly, but it will improve the water quality. Frequent monitoring to check for problems in a badly designed system is also costly, but testing does not improve quality (see Appendix). The extra cost of installing a system with automated daily heat disinfection is minimal when calculated as a cost per treatment over the lifetime of the plant.

## **1.1.6 Guidelines**

### **1.1.6.1 Design and disinfection**

**Water treatment systems should be designed to be capable of producing water that meets the requirements for dialysis with a sustainable level of maintenance.**

The water treatment system should be designed to minimise the formation of biofilm. Pipework with a smooth surface that resists corrosion and does not crack with age should be used for the distribution system. Internal ridges and/or grooves at the joints in the pipework should be minimised. Dead spaces and regions with low flow should be eliminated where possible. Where dead-end connectors to dialysis machines are used, they should be as short as possible.

Storage tanks in the distribution system provide a low flow zone where biofilm can form easily and should be avoided where possible. If treated water must be stored, the tank(s) should be designed to minimise bacterial growth and facilitate routine disinfection. An option to switch to direct feed by bypassing the tank is recommended.

The water treatment system should be designed to allow routine disinfection of the entire system, including the distribution pipework. An appropriate disinfection programme should be established when the system is installed or modified (see Appendix). The schedule may be based on information available for similar installations. Where no information is available, the reverse osmosis system and distribution pipework should be disinfected at least once a month.

The method of disinfection (heat or chemical) should be validated for use with the materials used in the distribution system. The disinfection programme should include the connections to the dialysis machines.

**Rationale:** Biofilm is the cause of persistent bacterial contamination of water and dialysis fluid (10). Connection and disconnection of dialysis machines and other devices mean that contamination of the distribution system with environmental bacteria cannot be avoided. If allowed to proliferate, the bacteria will form a biofilm. An established biofilm can be difficult, if not impossible to remove (11,12) [Evidence].

### **1.1.6.2 Frequency of microbiological testing**

**Water used for haemodialysis should be tested routinely to verify the efficacy of the disinfection programme, and to ensure that the water meets the requirements of the European Pharmacopoeia (total viable count < 100 CFU/ml and endotoxin level < 0.25 IU/ml) or the local specifications for purity.**

Microbiological testing should be used to monitor the efficacy of the disinfection programme. When a water treatment system and/or distribution system is installed, the microbiological quality of the treated water should be checked at least once a week as part of the procedure for validating the disinfection programme. After validation, microbiological testing should be carried out at least once a month.

Trends in the test results should be monitored carefully, so testing must be frequent enough to pick up changes. If the trends show that the disinfection programme is not adequately controlling the proliferation of bacteria, the interval between disinfections should be reduced. Action should be initiated to reduce contamination before the allowed limits are reached (e.g. when the bacteria level exceeds 25 CFU/ml or the endotoxin level exceeds 0.125 IU/ml).

If a biofilm has become established, the test results should be treated with caution (see 1.1.8). The bacterial counts will drop after disinfection but may rebound soon after. High endotoxin levels and the appearance of fungi and yeasts in the water also indicate the presence of biofilm. Swabs taken from internal surfaces of the pipework can be cultured to confirm that a biofilm has formed. It may be possible to control the biofilm with extended disinfection times and/or more aggressive cleaning agents. If the levels of microbiological contamination remain high, the cause should be determined and the components responsible replaced.

**Rationale:** Good manufacturing, laboratory and clinical practices separate monitoring from control methods. In this case, the control of bacteria levels is the disinfection that takes place at regular intervals. Microbiological testing is the independent monitoring method [Opinion].

### 1.1.6.3 Sampling location, timing and techniques

**Samples should be taken from the distribution system at the time and location expected to give the highest level of contamination, using a port designed for taking microbiological samples, and tested within 6 hours.**

Microbiological contamination may vary with the consumption of water. Where treated water is stored, the levels may be higher following non-dialysis days. Samples should be taken at a consistent time, which should be when the levels are expected to be high.

A study of the variation of contamination with time should be carried out in order to establish the most appropriate sampling schedule. It may be necessary to repeat this study if the system is modified or the pattern of water consumption changes.

Samples should be taken from the point(s) expected to have the highest bacterial load, normally the end of the distribution loop, or the last machine point in a dead-end system, where the flow is lowest. If there are dead-end connections to the dialysis machines, samples should also be taken from the tubing to the machine. A sampling port designed for collecting samples for microbiological testing (e.g. an in-line septum port allowing mid-stream samples to be taken with a syringe) should be installed at this location. The manufacturer of the port should provide a validated procedure for taking samples.

If samples are taken from taps, the tap should be flame sterilised or chemically disinfected (e.g. by soaking with 70% ethanol or 80-90% isopropyl alcohol and allowing the alcohol to evaporate). When the tap is opened, at least 2 litres of water should be run off at a high flow before taking the sample. The sample should be taken without touching the tap as any movement of the valve may release biofilm.

Samples of dialysis fluid should also be taken from ports designed for collecting samples for microbiological testing, ideally a septum port in the line between the machine and the dialyser.

For endotoxin tests, the sample containers must be sterile and endotoxin-free, and made from material that does not adsorb endotoxin. Polystyrene does not normally adsorb endotoxin, while polypropylene should be avoided. Advice on containers should be sought from the laboratory carrying out the LAL tests or from the suppliers of LAL reagent.

Ideally, water samples should be tested immediately for bacteria and endotoxin. If the samples cannot be tested immediately they should be kept cool (< 10 °C), but not frozen. Where testing has to be delayed for more than 6 hours, the storage conditions should be validated. If necessary, dialysis units should consider training staff to test samples in-house. The LAL gel-clot test can be carried out relatively easily in a dialysis unit. The suppliers of the LAL reagent will normally provide training in the testing and validation procedures. Any in-house procedure for culturing bacteria should be validated by an accredited Microbiology Laboratory.

**Rationale:** Variation of microbiological contamination with consumption is more likely to occur in systems with storage tanks. It is important to establish the normal variability in levels to avoid misinterpreting results [Opinion].

Samples taken from taps may have poor reproducibility. In an EDTNA/ERCA study, several units measured higher endotoxin levels in samples taken from taps in the distribution circuit than from septum ports in the tubing to the dialysis machine (13). If samples must be stored, they should be kept cool to stabilise the level of bacteria. Repeated freezing can reduce the measurable endotoxin level. If it is necessary to transport samples on dry ice, the storage conditions should be validated [Opinion].

#### 1.1.6.4 Techniques for culturing water borne micro-organisms

**A culturing technique that is proven to give a good recovery of bacteria from purified water should be used. Levels of fungi and yeast should be monitored periodically using an appropriate technique.**

Bacteria levels in water and dialysis fluid should be monitored using a culturing technique that is proven to give good recovery of bacteria from clean water.

The general technique for “microbiological examination of non-sterile product” described in the European Pharmacopoeia (Section 2.6.12) is not appropriate for dialysis water. The evidence suggests that a low nutrient agar, such as Tryptone Glucose Extract Agar or Reasoner’s 2A, should be used (14-16) and that samples should be incubated for at least 7 days at room temperature (17). These conditions have been shown to give good recovery for most environmental bacteria found in purified water. Some species are better adapted for growth at a higher temperature and/or on richer media, but the long incubation time will allow most of these to grow.

If local regulations require testing for specific organisms (e.g. *Pseudomonas aeruginosa*) with a specially adapted agar, a separate test should be conducted.

The inoculation volume should be chosen according to the allowed limit and the expected count. When testing to the requirements of EP 1167, a spread or pour plate inoculated with 1 to 5 ml of water is recommended. If the water is highly contaminated it can be diluted with water for injection to give a count. When testing ultrapure water, a sample of at least 100 mls should be filtered using a sterile 0.2 micron filter. The filter should then be cultured using the conditions above.

The number of colony forming units should be counted in a consistent way. Careful visual inspection under bright lighting is sufficient.

The European Pharmacopoeia does not give a specific limit for fungi and yeasts, as they are grouped in the total viable aerobic count. The Svensk läkemedelsstandard 2001 specifies a limit of 10 CFU/ml.

To monitor levels of fungi and yeasts, a large sample volume is normally required because these organisms adhere well to surfaces and appear at very low levels in the flowing water. The sample should be filtered through a 0.2 micron filter. Fungi and yeasts need more nutrients than bacteria and grow better on a richer agar (such as Malt Extract Agar or Sabouraud’s Dextrose Agar). Incubation should be for 7 days at room temperature. It can be of interest to incubate plates for longer as some organisms, such as fungi, may take more than 7 days to grow. Testing for yeasts and fungi may be less frequent than for bacteria where levels of these organisms are consistently very low. They should not appear at all in a well-maintained system and are rarely seen in any system in the first year or so of operation.

**Rationale:** Comparisons of different test methods indicate that the recovery of bacteria from water used for dialysis is highest when a low nutrient agar, a long incubation time and a low temperature is used [Evidence].

The Swedish and French Standards listed in 1.1.3, and the ERA-EDTA Best Practice Guidelines, recommend testing for bacteria by incubating on Tryptone Glucose Extract Agar (TGEA) or Reasoner’s 2A at 20-22 °C. The Swedish Standard recommends incubation for 5 days, the other documents prefer 7 days. The 7 day incubation is generally more convenient (as counts over the weekend can be avoided) and gives better recovery.

The above method should be regarded as the standard for water. Additional methods that are in current use, e.g. Cled agar or blood agar at 35-37 °C for at least 5 days, may be of interest but should not replace the standard. New techniques that give comparable or better recovery with a shorter incubation time must be validated against the standard method [Evidence and opinion].

#### 1.1.6.5 Measuring endotoxin levels in water and dialysis fluid

**Endotoxin levels should be measured using Limulus Amoebocyte Lysate (LAL) by the gel-clot, turbidimetric or chromogenic method, or an equivalent test.**

Bacterial endotoxin levels should be monitored using the Limulus Amoebocyte Lysate (LAL) assay, as recommended in the European Pharmacopoeia (Section 2.6.14). All the established techniques (gel-clot, turbidimetric and chromogenic) for LAL testing can be used for testing water for haemodialysis. Alternative techniques may be used provided they have been validated at endotoxin levels of 0.25 IU/ml or below.

The simplest form of LAL assay is the gel-clot test, which can be performed in the dialysis unit, provided staff have received the necessary training. It gives a pass-fail result, which is sufficient for testing water with a stable endotoxin level, but it can be made semi-quantitative by diluting the sample with endotoxin-free water.

The kinetic LAL tests (chromogenic, turbidimetric) measure the quantity of endotoxin in each sample directly, provided it is within the calibrated range. The kinetic tests are not often used in dialysis units as the equipment required is expensive and has significant operating overheads.

Dialysis fluid should be tested routinely for endotoxin to monitor the efficacy of the dialysis machine disinfection procedure. If the endotoxin levels in the dialysis fluid are significantly higher than in the water feeding the machine, the

disinfection schedule or the method used should be modified. If all machines are subject to the same procedures then a representative sample of machines can be tested each month.

Dialysis fluid samples should also be tested if a patient experiences a pyrogenic reaction during dialysis. A gel-clot test can be performed in about 1 hour to clarify the origin of the reaction.

When the LAL test is used to monitor endotoxin in dialysis fluid, the sample should be diluted at least 1:1 with endotoxin-free water to prevent interference with the clotting mechanism due to the bicarbonate (13). Note that LAL test works over a limited pH range so that it cannot be used to test the acid component of the dialysis fluid.

**Rationale:** From a clinical viewpoint, it is more important to test water and dialysis fluid for endotoxin than for bacteria, as bacteria do not pass through an intact dialyser membrane. There is still controversy over the transmembrane passage of bacteria-derived substances, but smaller bacterial endotoxins have been shown to cross all types of membrane (2,18-21). Hydrophobic high-flux membranes (such as polysulphone) can adsorb low molecular weight endotoxins (22), but the backfiltration zone that usually occurs in high flux dialysers could allow these molecules to pass into the blood if there is any damage to the membrane [Evidence].

The LAL assay is the method recommended for endotoxin testing by the European Pharmacopoeia, and is widely used to test non-pyrogenic products in the pharmaceutical industry. However, staff should be aware that the LAL assay will not detect all pyrogenic substances derived from bacteria (7,23).

### 1.1.7 Outcome measures

The implementation of this guideline should be audited by reviewing the record of bacterial counts and endotoxin measurements over time. The record should show planned test dates, actual test dates and the results obtained. Adherence to the disinfection schedule should also be audited.

If routine measurements of pre and post dialysis body temperature and/or markers of inflammation such as C-reactive protein (CRP) are available for unselected groups of patients, these should be audited periodically to look for trends that may be related to water quality. Non-routine measurements, and measurements made only on selected ('at-risk') patients must not be used for this purpose.

The dialysis unit should have documented procedures that come into effect when the European Pharmacopoeia (or local) limits for contamination are exceeded. The ERA-EDTA Best Practice Guidelines state that the procedures should include temporary closure of the dialysis unit when the safe limits for contaminants are exceeded. The content of these procedures must be reviewed regularly. The ability of staff to implement the procedures effectively should also be checked.

### 1.1.8 References

1. Nystrand R. **Endotoxin and "on line" production of substitution fluid in haemodiafiltration and haemofiltration.** EDTNA/ERCA Journal 2002; Jul; 28 (3).  
*Dr Nystrand describes how endotoxin levels are controlled and monitored in substitution fluid. He also presents a simple method for calculating the allowed endotoxin levels based on pharmaceutical standards, patient size and treatment mode.*
2. Mion CM, Canaud B, Garred LJ, Stec F, Nguyen QV. **Sterile and pyrogen-free bicarbonate dialysate: a necessity for hemodialysis today.** Advanced Nephrology 1990; 19: 275-314.  
*Although somewhat dated, this visionary paper provides an excellent overview of the arguments for using cold sterilisation to produce pyrogen-free dialysis fluid. The sections describing the formation of metabolites and degradation products from different types of organism, and the transfer of these toxins into the blood, are particularly interesting.*
3. Brunet P, Berland Y. **Water quality and complications of haemodialysis.** Nephrology, Dialysis and Transplantation 2000; 15: 578-580.  
*A brief review of the clinical complications of exposure to microbiological contamination, including amyloidosis, atherosclerosis and malnutrition from which the authors conclude that the level of bacteria and endotoxin in dialysis fluid should be < 100 CFU/L and < 0.01 EU/ml.*
4. Bonnie-Schorn E, Grassmann A, Uhlenbusch-Körwer I, Weber C, Vienken J. **Clinical consequences of microbial contamination.** In "Water Quality in Haemodialysis", Lengerich: Pabst Science Publishers 1998; 69-79.  
*This overview of the clinical consequences microbiological contamination of dialysis water and dialysis fluid is part of an excellent textbook for technicians and other renal care staff.*
5. Lonnemann G. **Should ultra-pure dialysate be mandatory?** Nephrology, Dialysis and Transplantation 2000; 15 (1): 55-59.  
*Dr Lonnemann describes acute and long-term effects of haemodialysis with contaminated dialysis fluid. He recommends maintaining bacteria levels at less than 100 CFU/ml in dialysis fluid and mentions the need for guidelines for sampling and culturing dialysis fluid. The paper concludes that 'the use of pyrogen-free dialysis fluid should become mandatory as soon as further studies reveal that dialysis-associated diseases can be ameliorated or prevented by the use of ultra-pure dialysate'.*

6. Ward RA. **Ultrapure dialysate: a desirable and achievable goal for routine hemodialysis.** *Seminars in Dialysis* 2000; 13 (6): 378-380.  
*This paper gives the case for using ultrapure dialysis fluid and shows that there are no longer any real technological barriers preventing renal units from producing ultrapure fluid.*
7. Tsuchida K, Takemoto Y, Yamagami S, Edney H, Niwa M, Tsuchiya M, Kishimoto T, Shaldon S. **Detection of peptidoglycan and endotoxin in dialysate, using silkworm larvae plasma and limulus ameocyte lysate methods.** *Nephron* 1997; 75 (4): 438-443.  
*The authors used an assay based on silkworm larvae plasma (SLP) reagent to measure muramyl dipeptide (MDP), the biologically active constituent of peptidoglycan, which is a fragment of both the gram-positive and gram-negative bacterial cell wall. This pyrogen is not detected by the LAL assay. The authors not only showed that MDP is present in some dialysis fluid samples, but that cytokine production was 5-10 times higher where MDP and endotoxin coexisted than where either MDP or ET existed alone.*
8. Schömig M, Eisenhardt A, Ritz E. **The microinflammatory state of uremia.** *Blood Purification* 2000; 18: 327-332.  
*An easy-to-follow review of the links between inflammation, malnutrition and cardiovascular mortality. This paper shows the striking relationship between high levels of C-reactive protein (a measure of inflammation) and poor survival. The authors list poor quality water as amongst the causes of inflammation in HD patients.*
9. Lonnemann G. **Chronic inflammation in hemodialysis: the role of contaminated dialysate.** *Blood Purification* 2000; 18 (3): 214-223.  
*A review of recent experimental and clinical data suggesting that the use of pyrogen-free dialysis fluid improves the state of chronic inflammation, as indicated by decreased plasma levels of C-reactive protein in chronic haemodialysis patients.*
10. Cappelli G, Ballestri M, Perrone S, Ciuffreda A, Inguaggiato P, Albertazzi A. **Biofilms invade nephrology: effects in hemodialysis.** *Blood Purification* 2000; 18 (3): 224-230.  
*Describes how bacteria attach to surfaces and aggregate in a biopolymer matrix to form a biofilm which produces slime that renders the chemical products for disinfection and descaling procedures ineffective.*
11. Stickler D. **Biofilms.** *Current Opinions in Microbiology* 1999; Jun 2 (3): 270-275.  
*A review of the war against biofilms, though not specific to dialysis. Bacteria in biofilms are up to 500 times more resistant to antimicrobials than free bacteria. The author points out that the most effective method for removing mature biofilms is mechanical cleaning, which is not usually an option in a dialysis unit.*  
*Another good general review of biofilms by JW Costerton and PS Stewart was published in Scientific American in July 2001. This includes a picture of undamaged bacteria in the interior of a biofilm after 60 minutes of exposure to bleach. The authors are based at the "Center for Biofilm Engineering" at Montana State University. Still images and videos of biofilms can be viewed at the center's web site (<http://www.microbelibrary.org>, select "Visual Resources" and search the library using "Biofilm" as a Keyword).*
12. Meiller TF, Depaola LG, Kelley JI, Baqui AA, Turng BF, Falkler WA. **Dental unit waterlines: biofilms, disinfection and recurrence.** *Journal of American Dental Association* 1999; Jan 130 (1): 65-72.  
*Much of the literature on biofilms comes from the dental community where the transmission of microbial pathogens to patients from biofilm within dental unit waterlines is a concern. These trials demonstrated that whilst chemical disinfectants reduce levels of micro-organisms in the effluent water, the biofilm was not destroyed even with periodic treatments, and the waterlines were rapidly recolonized.*
13. Lindley EJ, Lopot F, Harrington M, Elseviers MM. **Treatment of water for dialysis – A European survey.** *EDTNA/ERCA Journal* 2000; Oct 26 (4): 34-40.  
*A report on the Collaborative Research Programme survey of water treatment practice which included a standard endotoxin test. In 6 of 9 centres where the distribution system had an endotoxin level > 0.125 IU/ml, the sample from the septum port in the machine tubing was negative, suggesting that the system samples were contaminated with biofilm from the tap.*  
*The study also showed that the dialysis fluid could not be tested for endotoxin using the gel-clot test without dilution as the electrolytes interfere with the clotting process.*
14. van der Linde K, Lim BT, Rondeel JM, Antonissen LP, de Jong GM. **Improved bacteriological surveillance of haemodialysis fluids: a comparison between Tryptic soy agar and Reasoner's 2A media.** *Nephrology, Dialysis and Transplantation* 1999; Oct 14 (10): 2433-2437.  
*This study compared TSA and R2A using 229 samples of water and dialysis fluid. 0.1 ml spread plates were incubated for 10 days of incubation at 25 °C. Reasoner's 2A gave significantly higher colony counts than Tryptic Soy Agar for both water and dialysis fluid.*
15. Ledebø I, Nystrand R. **Defining the microbiological quality of dialysis fluid.** *Artificial Organs* 1999; Jan 23 (1): 37-43.  
*This study showed a 10-100 fold increase in recovery of bacteria from dialysis fluid incubated on Tryptone Glucose Extract Agar (TGEA) at 20 °C for 5 days compared with incubation on Tryptic Soy Agar at 37 °C for 2 days.*

16. Harding GB, Pass T, Million C, Wright R, DeJarnette J, Klein E. **Bacterial contamination of hemodialysis center water and dialysate: are current assays adequate?** *Artificial Organs* 1989; Apr 13 (2): 155-159.  
*Compared Tryptic Soy Agar, Plate Count Agar and Reasoner's 2A and found that R2A had a strong selectivity for water-borne bacteria.*
17. Pass T, Wright R, Sharp B, Harding GB. **Culture of dialysis fluids on nutrient-rich media for short periods at elevated temperatures underestimate microbial contamination.** *Blood Purification* 1996; 14 (2): 136-145.  
*Compared counts at 37 °C for 48 hours and at 23 °C for 48, 72, 168 hours using Standard Methods Agar, Reasoner's 2A and nutrient-rich Tryptic Soy Agar. Increased colony counts over time occurred for all three fluids. Counts were greater on the lower nutrient agars. The 7 day, room temperature cultivation gave up to 104 times the count for standard 48-hour TSA cultures.*
18. Urena P, Herbelin A, Zingraff J, Lair M, Man NK, Descamps-Latscha B, Druke T. **Permeability of cellulosic and non-cellulosic membranes to endotoxin subunits and cytokine production during in-vitro haemodialysis.** *Nephrology, Dialysis and Transplantation* 1992; 7: 16-28.  
*This study showed that radiolabelled endotoxin in the dialysis fluid could transfer into the blood with cellulosic, polyacrylonitrile and polysulphone membranes.*
19. Laude-Sharp M, Caroff M, Simard L, Pusineri C, Kazatchkine MD, Haeffner-Cavaillon N. **Induction of IL-1 during hemodialysis: transmembrane passage of intact endotoxins (LPS).** *Kidney International* 1990; Dec 38 (6): 1089-1094.  
*This study demonstrated that intact bacterial lipopolysaccharide (LPS) molecules can cross cuprophane, AN69 and polysulfone membranes under simulated dialysis conditions. 40 to 70% of the LPS originally present in the dialysis fluid was adsorbed onto the AN69 and polysulphone membranes while the cuprophane membrane adsorbed very little LPS.*
20. Lonnemann G, Behme TC, Lenzner B, Floege J, Schulze M, Colton CK, Koch KM, Shaldon S. **Permeability of dialyzer membranes to TNF alpha-inducing substances derived from water bacteria.** *Kidney International* 1992; Jul 42 (1): 61-68.  
*The authors used a sterile filtrate of Pseudomonas maltophilia grown from standard bicarbonate dialysis fluid was used to test the permeability of various dialyzer membranes (regenerated cellulose, cellulose triacetate, polyacrylonitrile, polysulfone and polyamide) to TNF alpha-inducing bacterial substances. The transfer of TNF-inducing activity to the blood side of the dialyzer was most rapid for the low-flux regenerated cellulose dialyzer and lowest for the polysulphone and polyamide dialyzers. The authors suggest that the synthetic membranes may adsorb the bacteria-derived substances.*
21. Pereira BJ, Snodgrass BR, Hogan PJ, King AJ. **Diffusive and convective transfer of cytokine-inducing bacterial products across hemodialysis membranes.** *Kidney International* 1995; Feb 47 (2): 603-610.  
*In this in-vitro study, bicarbonate dialysis fluid contaminated with Pseudomonas maltophilia filtrate was circulated through the dialysis fluid compartment of polyamide and hemophan dialyzers whole human blood in the blood side. Cytokine production in the blood due to the passage of bacterial toxins was found to lower in the high-flux polyamide dialyzer compared to the cellulosic hemophan dialyzer.*
22. Weber C, Linsberger I, rafiee-Tehrani M, Falkenhagen D. **Permeability and adsorption capacity of dialysis membranes to lipid A.** *Int J Artif Organs* 1997; 20(3): 144-152.  
*This paper describes the controversy over the transmembrane passage of bacteria-derived substances and describes an in-vitro study in which dialyzer membranes were challenged with low and high molecular weight endotoxin (lipid A from E. Coli and lipopolysaccharides from Pseudomonas aeruginosa). The results showed that polysulphone and polyamide membranes have a much higher adsorption capacity for lipid A and LPS than the cellulosic membranes. In this study, good rejection of endotoxin was also observed with hydrophilic membranes and the authors attribute this to aggregation of the molecules.*
23. Bonnie-Schorn E, Grassmann A, Uhlenbusch-Körwer I, Weber C, Vienken J. **Clinical consequences of microbial contamination.** In "Water Quality in Haemodialysis", Lengerich: Pabst Science Publishers 1998; 29-34.  
*A review of the methods for detecting pyrogens and other biologically active substances. For more details about this book see reference 4.*

### 1.1.9 Appendix: Quality assurance or Quality control?

**Quality assurance (QA)**, applied to production of water for dialysis, means designing and maintaining the entire system for the production and distribution of purified water to a standard that ensures that the water meets or exceeds the requirements of the European Pharmacopoeia. This will include preventing the proliferation of bacteria. In the context of quality assurance, bacteria levels are monitored for the purpose of audit only.

QA is provided by modern water distribution systems with high quality pipework, minimal dead space and automated heat disinfection. Daily or twice weekly disinfection is required to prevent bacteria growth on the surfaces of the distribution system. In most units this is feasible only with an automated disinfection procedure that is easy for dialysis unit personnel to use. Heat disinfection is amenable to automation and can be left unattended. It also eliminates the need to handle chemicals and carries no risk for the patients as all the dialysis machines have high temperature alarms.

**Internal quality control (QC)** involves the analysing of samples to find out if they meet the criteria for acceptability. If a sample fails the QC test, appropriate action is taken. When monitoring water for dialysis, QC measurements showing a increasing trend in counts may be used to adapt the maintenance schedule, for example by initiating a disinfection.

Most water distribution systems in current use were designed with no provision for routine disinfection or for cold chemical disinfection at long intervals. With such systems, bacteria are allowed to proliferate and QC tests are required to check that the water quality is acceptable. When contamination exceeds the allowed limits, the bacteria must be destroyed. The disinfection processes required are time consuming (and have to be undertaken outside normal operating hours) and are not without risk to the staff and patients.

These systems are difficult to keep clean because bacterial biofilm can build up during the interval between disinfections, and because the disinfectant cannot penetrate biofilm and destroy bacteria that are deeply embedded. They are also very difficult to monitor effectively because bacteria levels obtained by culturing the viable organisms in a mid-stream sample gives a very limited indication of the number of bacteria in the system.

Even with the best incubation techniques, there will be some bacteria that do not grow and some colony forming units that contain more than one viable organism. And even if the culture technique could recover every organism in the flowing water, it cannot show the extent to which the distribution system is colonised. To understand this, it is necessary to gain some insight into the life of micro-organisms and the difficult job of the microbiologist.

Nutrients are mainly organic molecules with electrical charges that are attracted to surfaces. Bacteria will try to adhere to surfaces to be where their food is – the ones that fall off are the losers. Species that adhere well will rarely be seen in the flowing water. Pieces of biofilm that come unstuck will contain many cells, but will appear as '1 CFU'. So the microbiologist performing the QC tests sees only the colonies formed from the pieces of biofilm that have been lost from the surface that will grow in his cultivation conditions. This is a tiny fraction (perhaps 1/100000) of the actual number in the system.

Internal QC can be used to keep the measured level of microbiological contamination below the EP limits it relies on the ability of staff to keep the growth of bacteria under control. Over the lifetime of a system this will inevitably become more difficult. With the QA approach, there is no build-up of resistant biofilm, so there is no reason why the system should not continue to produce high quality water as long as the maintenance schedule is followed.

#### **1.1.10 Acknowledgements**

This document was prepared by the Research Board and the Technicians Special Interest Group of EDTNA/ERCA with advice from Dr Rolf Nystrand, Microbiology Advisor to EDTNA/ERCA.

A panel of experts, including representatives from industry and regulatory bodies, as well technical and clinical staff from dialysis units in Europe and North America, provided comments and criticism as the document passed through three revisions during 2000-01.

EDTNA/ERCA thanks all those involved for their help in producing a document that will help our members improve the quality of the care they provide to dialysis patients throughout the world, especially:

**Matthew J. Arduino**, Environmental and Applied Microbiology Section, Centers for Disease Control & Prevention, USA; **Bernard Canaud**, Department of Nephrology, Lapeyronie Hospital, Montpellier, France, President-elect of the French Dialysis Society and Expert member of AFFSAPS on behalf of the *European Renal Association/European Dialysis and Transplant Association*; **Jim Cooper**, Charles River Endosafe, Charleston, USA; **Jean-Yves De Vos**, Werken Glorieux, Ronse, Belgium; **David J Evans**, Fresenius Medical Care GmbH, Huthwaite, UK; **LeRoy J Fischbach**, Minntech Corporation, Minneapolis, USA and **AAMI Renal Disease and Detoxification Committee Co-Chair\***; **Alois Gorke**, Kreis-krankenhaus, Murnau, Germany; **Roger Greenwood**, Renal Unit, Lister Hospital, Stevenage, UK; **Maurice Harrington**, Renal Unit, Hope Hospital, Salford, UK; **Dan Jonsson**, Gambro, Lund, Sweden; **Stephan Krietemayer**, Lauer Membran Wassertechnik, Germany; **Elizabeth Lindley**, Dept of Renal Medicine, St James's University Hospital, Leeds, UK; **Franta Lopot**, Dept of Internal Medicine, Charles University Medical School, Prague, Czech Republic; **Thomas Ludewig**, TÜV Product Service, Munich, Germany; **Ian Morgan**, Renal Unit, King's College Hospital, London, UK; **Rolf Nystrand**, Bio-TeQ Nystrand Consulting, Malmö, Sweden; **Hans Polaschegg**, Medical Devices Consultant, Köstenberg, Austria; **Bernd Stegmayr**, Nephrology Division, Norrlands Universitets Sjukhus, Umeå, Sweden on behalf of the *European Society for Artificial Organs*; **André Stragier**, Clinique Saint-Luc, Brussels, Belgium; **James Tattersall**, Basildon District Hospital, UK; **Hans Träger**, Werner GmbH, Leverkusen, Germany; **Richard A. Ward**, University of Louisville, Kentucky, USA, and **AAMI Water for Hemodialysis Working Group Chair\***

\* Input from Lee Fischbach and Dick Ward was in their role as individuals and should not be seen as representing the views of AAMI.