Quality of Water for Haemodialysis and Dialysis Fluid
**FMC GUIDELINE**

**TITLE:** QUALITY OF WATER FOR HAEMODIALYSIS AND DIALYSIS FLUIDS  
**DOC. NO.:** C-CG-10-01  **REV.:** 00  **EFFECTIVE DATE:** 01.01.2001

**SCOPE OF GUIDELINE:**
This Guideline is valid for all European FMC Dialysis Centres.

**APPROVALS**

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Appendix: Overview of Water Quality Control in Tabular Form
**Scope**

This Guideline is valid for all European Dialysis Centres managed by FMC.

**Aim**

The aim of this guideline is to ensure, through FMC standard and preventive measures, that the patient treated with haemodialysis is not exposed to any unjustifiable risks and that neither acute reactions nor chronic damage are triggered off by the preparation of fluids and the treatment itself.

Therefore, this guideline defines the chemical and microbiological quality of water for the preparation of haemodialysis fluids and substitution fluids for haemo-(dia-) filtration dialysis fluids prepared from concentrates and water substitution fluids for HF and HDF (on-line)

According to the different treatment modes, graded limits are defined for the individual preparation steps.

Furthermore, in order to control and continuously prove the quality of the respective fluids a concept is outlined consisting of validation and routine analysis of the whole water treatment system starting from the quality control of the incoming feed water. The concept on the quality control of water treatment systems is a recommendation and should be carried out and kept to according to the local situation and the techniques/equipment installed (see Corporate Guideline C-CG-10-02: “Water Treatment Equipment”).

*Please note: recommendations are highlighted in italics*

Furthermore, a summary of this guideline (overview of water quality control) in tabular form can be found in the appendix of this guideline. In addition, the appendix of the guideline on water treatment equipment (C-CG-10-02/Rev.00) gives an overview on the minimum recommendations for sampling frequency dependent on sampling points.
Introduction

The chemical and microbiological contamination of dialysis fluids are serious problems in haemodialysis therapy and one of the causes might be the water used for the preparation of dialysis fluid. Obviously, the incoming feed (raw) water from municipal water supplies generally has not the sufficient quality for dialysis and it has to be purified with specifically designed water treatment devices to achieve the required quality. However, the composition and quality of incoming water vary widely depending on its source (ground water, surface water), its geographical origin and seasonal variations. Hence, the water treatment systems have to be adapted to the individual local situation in order to achieve a consistent quality of water. Nevertheless, such water treatment systems may invoke additional hazards if malfunction or user error occur. Moreover, different treatment steps as well as the order of those steps may lead to severe chemical and microbiological contamination. Based on this knowledge routine monitoring of water quality has to be implemented.

During haemodialysis each patient is exposed to approximately 250 – 600 litres of water per week and hence, each patient is exposed to the potential risk of chemical or microbiological contamination. If water purity is inadequate toxins may diffuse non-selectively across the dialysis membrane directly into the blood stream of the dialysis patient. Moreover, end stage renal disease patients are no longer able to excrete distinct toxins via their kidneys. The extent of exposure together with the non-selective absorption and incapability of urinary excretion places the dialysis patient at much higher risk to water-borne contamination than the healthy population. Therefore, the chemical and microbiological quality of the water used for dialysis is essential if an additional health risk to those patients is to be avoided.

Strict quality standards for dialysis water are demanded, especially when today’s dialysis practice with the use of bicarbonate dialysis, high-flux dialysis with highly permeable membranes and on-line methods are considered. However, poor chemical and microbiological quality of water and dialysis fluids is widespread and has been demonstrated in multicentre studies conducted in the US, Sweden, Germany, Canada, Japan, Greece and Austria. Moreover, patient reactions such as headache, nausea, vomiting, cramps or haemolysis related to chemical impurity of water as well as outbreak of infections related to microbial contamination are still more or less frequently described.

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

Chemical Contaminants

The degree of chemical contaminants is subject to local and seasonal variations. The main contaminants are inorganic salts, but also organic substances of natural (tannin, lignine) or of agricultural origin (pesticides, nitrogen-compounds) or industrial pollution (aromatic hydrocarbons). The main sources of these chemicals are supplementation of drinking water with chemicals by the municipal authorities (e.g. aluminium sulphate, chlorine, chloramine or fluoride), migration from the water piping system (e.g. copper, zinc, lead), disinfectants from routine disinfection of water treatment system (chlorine, hypochlorite, peracetic acid).

The most important chemical contaminants can be divided into five groups: ions also found in standard dialysis fluids (Ca, K, Na, Mg), trace elements (aluminium, copper, silver, zinc, cadmium, arsenic, mercury, lead, silver, iron, selenium, chromium, silicon, barium), organic substances (pesticides and aromatic hydrocompounds such as benzene), disinfectants and preservatives (formaldehyde, sodium hypochlorite, hydrogen peroxide, chloramines, free chlorine, peracetic acid), group of N-compounds (nitrate, nitrite, nitrosamines), sulphates and fluorides

In general, the chemical contaminants may cause acute and chronic complications during dialysis. Some of them interfere with maintenance of body homeostasis, cell membrane potential or multiple enzyme activities. Others are toxic when present in the human body in relatively low concentrations. Furthermore, even extremely high or low concentrations of some chemicals, especially the electrolytes present in the dialysis fluid, can be physiologically unsafe. High magnesium and calcium content, for instance, leads to “hard water syndrome” and nausea, hypertension, headache, confusion, seizure or progressive lethargy. Other contaminants like heavy metals may accumulate in the body and produce various toxic side effects such as haemolysis or nervous system disorders. Aluminium overload for instance may cause anaemia, encephalopathy and osteopathy. However, the relatively wide range of side effects from inadequate purity of water will not be discussed in this guideline (for an overview, please see).

Microbiological Contaminants

Naturally occurring water-born filamentous fungi, yeasts, bacteria and fragments may pollute water used for haemodialysis. Commonly found micro-organisms are gram-negative bacteria or non-tuberculous mycobacteria. In addition, biologically active substances released by living bacteria or products of bacterial
lysis can be found in water. Due to their ability to induce fever in humans these derivatives are termed pyrogens. The most important pyrogen in dialysis is the cell-wall component of gram-negative bacteria called endotoxin or lipopolysaccharide (LPS), which is released during bacterial lysis.

In general, the microbiological quality of dialysis fluids is highly influenced by the hygienic status of preparation and delivery systems. However, micro-organisms are able to colonize solid surfaces and give rise to sessile communities called biofilms. These biofilms, - potentially being formed in the whole water system and tubing of dialysis machines - in turn, are the main source of re-contamination resulting in high levels of living germs and their products in dialysis water, liquid bicarbonate concentrates, and the final dialysis fluids.

Originally this appears to be of minor clinical relevance as the likelihood of whole bacteria to penetrate dialysis membrane is very small. However, their products (e.g. pyrogens) such as endotoxins are able to penetrate the dialysis membrane by diffusion and stimulate blood monocytes to produce cytokines. Due to their heterogeneous nature, it is impossible to completely abolish dialysis penetration by these so-called cytokine-inducing substances.

Several in vitro studies have shown that the adsorption capacity of a membrane to cytokine-inducing substances is much more important for the prevention of cytokine induction than solely the pore size of the membrane. Therefore, low-flux dialysis does not necessarily lead to higher microbiological safety than high-flux dialysis or on-line treatment modalities.

Similarly to chemical pollution also the microbiological contaminants may cause acute and chronic complications during dialysis. A typical acute consequence is a pyrogenic reaction accompanied by fever, chills, nausea, vomiting, hypotension, myalgias and headache. If cytokine-inducing substances, e.g. endotoxins, invade human subjects, high plasma levels of cytokines, fever and hypotension are induced within a couple of hours. Use of ultrapure dialysis fluid (as defined in chapter 6.3) can decrease the incidence of pyrogenic reactions in the hemodialysis population significantly.

Ultrafiltration of dialysis fluids is an effective technique for the production of ultrapure dialysis fluids. Most of the retention capacity of dialysis fluid filters is based on adsorptive mechanisms which may exhaust during operation if highly contaminated dialysis fluid is filtered. Ultrafiltration of dialysis fluid, therefore, cannot compensate for inadequate hygienic conditions upstream of the filter. Instead, dialysis fluid filtration should be considered as a polishing procedure and part of a comprehensive hygiene concept. With such an approach dialysis fluid (before filtration) can be obtained with appropriate microbiological qualities.

More important than the generation of acute reactions is the impact of cytokine-inducing substances on the long-term well-being of hemodialysis patients. Known long-term effects such as impaired immunodeficiency state or altered erythropoietin response are believed to be related to a chronic cytokine induction, which may
be linked, at least partly, to microbiological contamination of dialysis fluid. Clinical studies revealed that even minute amounts of cytokine-inducing substances are perceived by the patients' immune system without causing any acute clinical symptoms\textsuperscript{xiii}. Several lines of evidence suggest that permanent stimulation leads to various chronic complications such as dialysis-related amyloidosis, muscle wasting, progressive loss of bone mass, immunodysfunction, and cardiovascular disease\textsuperscript{xiv, xv, xvi, xvii, xviii}. Since infections and cardiovascular disease are major causes of mortality, it is tempting to speculate whether systemic cytokine induction due to poor microbiological dialysis fluid quality increases mortality in the hemodialysis population as well\textsuperscript{xix, xx}. 

Concept of Water Quality Control

In order to assure the quality of water certain standards have to be defined and the main process as well as the results of the evaluation of water quality have to be documented. Routine risk analyses have to be performed and potential adaptations of the purification process have to be considered. The process of water quality control consists of validation, re-validation and routine analysis.

Definitions

The Validation is the confirmation by examination and provision of objective evidence that the particular requirement for a specific intended use can be consistently fulfilled.

Within the Re-validation the evidence base is confirmed.

Validation (See Procedure C-CP-10-01/Rev.00)

The validation records the status and the characteristics of the whole water treatment system including the technical details and the quality of incoming feed water as well as the quality of water during the purification process. Sample points are defined based on the design of the entire system. Samples are taken and analysed in order to identify malfunctions and areas with a potentially high risk of contamination. Finally, a protocol for routine analysis is defined according to the results of validation. It has to cover at least the different sampling points, the frequency of sampling, the type of analysis and potential actions in case of quality deviations. Based on this protocol the routine analysis is designed and established.

Operating water treatment systems are inspected retrospectively in order to meet the requirements of validation. Data mandatory for validation which are not available are completed supplementarily.

Routine Analysis and Re-validation (See Instruction C-CI-10-01/Rev.00)

The routine analysis has to be performed based on the protocol established during validation. It comprises of regular monitoring of critical areas which are representative for the whole treatment system. In defined intervals samples are taken and analysed. If the results of water analysis (chemical or microbiological) reveal a decline in water quality, a re-analysis has to be performed followed by necessary actions in order to improve the water quality. If necessary, the protocol has to be adapted. In case of any system changes or after opening any areas of the water treatment system, a re-analysis has to be performed, where appropriate, and the protocol has to be adapted accordingly. A re-validation is required once or preferably twice per year. It is based on the retrospective analysis of routine results (trend analysis) plus the prospective analysis of additional samples as defined in Table 5 (chapter 7.2), according to the results of re-validation, the routine plan has to be adapted.
**Relationship with water supplier**

The attention of the water supplier should be drawn to the fact that the feed water delivered to a dialysis centre requires a distinctly defined water quality. In order to evaluate the source and quality as well as the quality stability and changes of contaminants/additives of feed water from the water supplier, the water supplier is asked to provide the respective information in a questionnaire (see “Questionnaire for Municipal Water Supplier”). *The first evaluation of feed water is performed during the validation. A re-evaluation is recommended once, preferably twice per year (see Tab. 5). In addition, any changes of water source or contaminants/additives affecting water quality are reported immediately to the dialysis centres by the water supplier. After a detailed risk analysis potential actions are initiated to further ensure the adequate water quality. Every step of the evaluation as well as any changes have to be documented and the protocol is adapted accordingly.*
Quality Standards

The chemical and microbiological quality of dialysis water, concentrates, dialysis fluids and substitution fluids for haemo-(dia-)filtration (substitute) should comply with the European Pharmacopoeia (Eu.Ph.: Monography 0128\textsuperscript{xxi}, 1167\textsuperscript{xxii} and 0861\textsuperscript{xxiii}), or if not regulated here, with the Association for the Advancement of Medical Instrumentation (AAMI, USA)\textsuperscript{xxiv} and the European Norm (Draft: EN 13867)\textsuperscript{xxv} or, if more stringent, with regional or local standards.

Standards on process (physical) parameters (Tab. 2) should comply with the instructions of the manufacturer for the individual treatment steps of the water purification plant and the dialysis machines as well as with medical requirements.

Quality of Feed Water

The chemical and microbiological purity of feed water should comply with the national, or if more stringent, with local standards for drinking water. The water treatment system has to be designed, taking into consideration the local situation, in order to produce water for dialysis in the recommended quality as follows below.

Chemical Quality of Dialysis Water (RO-Permeate)

Table 1 summarises standards for the chemical quality of dialysis water. It refers to water treated by reverse osmosis (dialysis water or RO-permeate) used for the preparation of dialysis fluids out of concentrates. The final composition of dialysis fluids and substitutes is not subject of this guideline. However, it is regulated in the European Pharmacopoeia which should be complied with. Furthermore, the quality of water achieved after each purification step has to correspond with purity limits given by the manufacturer.

Microbiological Quality of Dialysis Water, Concentrates and Dialysis fluids

Table 3 summarises the microbiological standards to be met. It refers to dialysis water (permeate) as well as dialysis fluid and all reprocessing fluids.

To assure the defined microbiological quality it is essential to determine microbial counts and endotoxin concentration on a regular basis. Samples should be aseptically collected from representative points and analysed immediately according to methods recommended in the European Pharmacopoeia (see chapter 7.4).

Based on the growing wealth of data on the clinical consequences of inadequate microbiological dialysis fluid quality, the use of ultrapure dialysis fluid is advised regardless of the treatment modality.
The prerequisite to perform on-line techniques, such as on-line HF and on-line HDF, is an additional filtration of ultrapure fluids. This double filtration of dialysis fluid results in sterile substitute, which contains less than 0.03 IU/ml of endotoxin.
### Tab. 1: FMC Standard for Chemical Quality of Dialysis Water (RO-Permeate)

<table>
<thead>
<tr>
<th>CONTAMINANTS</th>
<th>Max. Conc. Level (mg/l) AAMI</th>
<th>Eu.Ph.</th>
<th>FMC</th>
<th>CONTAMINANTS</th>
<th>Max. Conc. Level (mg/l) AAMI</th>
<th>Eu.Ph.</th>
<th>FMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Chromium</td>
<td>0.014</td>
<td>------</td>
<td>0.014</td>
</tr>
<tr>
<td>Magnesium</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>Lead</td>
<td>0.005</td>
<td>------</td>
<td>0.005</td>
</tr>
<tr>
<td>Potassium</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>Zinc</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium</td>
<td>70</td>
<td>50</td>
<td>50</td>
<td>Mercury</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>Barium</td>
<td>0.1</td>
<td>------</td>
<td>0.1</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.1</td>
<td>------</td>
<td>0.1</td>
<td>Arsenic</td>
<td>0.005</td>
<td>------</td>
<td>0.005</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Selenium</td>
<td>0.09</td>
<td>------</td>
<td>0.01</td>
</tr>
<tr>
<td>Sulphate</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>Silica</td>
<td>-------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.01</td>
<td>0.01</td>
<td>0.005</td>
<td>Ammonium</td>
<td>------</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Copper</td>
<td>0.1</td>
<td>------</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

### Tab. 2: Standard for Process (physical) Parameters

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Standard according to</th>
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<tr>
<td>pH</td>
<td>1. Manufacturer’s instructions for the individual process steps of the water purification plant and for the dialysis machines.</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>Hardness</td>
<td>2. Medical requirements</td>
</tr>
<tr>
<td>Conductivity</td>
<td></td>
</tr>
<tr>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Pressures</td>
<td></td>
</tr>
<tr>
<td>Flows</td>
<td></td>
</tr>
</tbody>
</table>

### Tab. 3: FMC Standard for Microbiological Quality

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>Microbial Counts (CFU/ml)</th>
<th>Endotoxin (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAMI</td>
<td>SPh</td>
</tr>
<tr>
<td>Dialysis water (RO-Permeate)</td>
<td>≤ 200</td>
<td>≤ 100*</td>
</tr>
<tr>
<td>Bicarbonate concentrate</td>
<td>≤ 100</td>
<td>≤ 100*</td>
</tr>
<tr>
<td>Acid concentrate</td>
<td>≤ 100</td>
<td>≤ 100*</td>
</tr>
<tr>
<td>Dialysis fluid (unfiltered)</td>
<td>≤ 2000</td>
<td>≤ 100*</td>
</tr>
<tr>
<td>Ultrapure (1 x filtered) dialysis fluid</td>
<td>≤ 1</td>
<td>≤ 1</td>
</tr>
<tr>
<td>Substitute (2 x filtered dialysis fluid)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

of those ≤ 10 CFU/ml yeast and mould
# when concentrates are appropriately diluted with dialysis water (ready to use dilution)

AAMI: Association for the Advancement of Medical Instruments, USA
SPh: Swedish Pharmacopoeia 1997
RKI: Robert Koch Institute, Germany
EuPh: European Pharmacopoeia 1997
EN = European Norm (prEN 13867)

xxiv
xxvi
xxvii
xxviii
xxix
Standards for Quality Control

**Number of Samples and Sampling Points** (see Table 4)

In the validation (first evaluation) the minimum number of samples to be analysed are the feed water entering the water treatment system (which with respect to quality does not necessarily correspond to the feed water delivered to the centre by the water supplier because of internal pipelines).

*the pre-reverse osmosis (softened) water,*
*the RO-permeate (dialysis water),*
*the concentrates (central delivery system only) and*
*the dialysis fluids*

The plan of routine sampling is developed according to the results of the validation (sampling plan). The number of samples and sampling points have to be chosen depending on the expected water quality at the respective area / component of the whole system.

The minimum number of samples to be analysed **routinely** are

*the RO-permeate,*
*the concentrates and*
*the dialysis fluids*

so that each machine is checked at least once per year (if samples are taken quarterly, 25% of the machines alternately, in case of contamination or if the frequency of usage is low more samples have to be taken). Additional samples are required during re-validation beyond the retrospective analysis of routine results (trend analysis) in order to evaluate potential risks and to adapt the routine plan accordingly (see 5.3 and Table 5). In case of special events such as

*patient reactions,*
*any system changes,*
*opening of the water treatment and distribution system (e.g. repairs, maintenance),*
*after disinfection,*
*additional samples have to be analysed beside the routine evaluation (see chapter 8).*

**Tab. 4: Sampling Points**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sampling point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed water</td>
<td>directly at water inflow</td>
</tr>
<tr>
<td>Pre-reverse osmosis (softened) water</td>
<td>before reverse osmosis</td>
</tr>
<tr>
<td>RO-Permeate (dialysis water)</td>
<td>before first dialysis machine and return-flow, after last dialysis machine and in the middle of the water distribution loop at the permeate inflow tube (rotate around different machine points)</td>
</tr>
<tr>
<td>Concentrates (centrally delivered)</td>
<td>at the transition of the utility line to the dialysis machines or at special sampling points at fore-running and back-flow.</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>- at least at two points of the delivery system (inflow, back-</td>
</tr>
</tbody>
</table>
Acid concentrate - one sampling point (back-flow) due to lower risk of contamination

Dialysis fluid (unfiltered & ultrapure) directly before the dialyser

Substitute (2 x filtered dialysis fluid) directly after second filtration at the outflow tube.

### Analysis Parameters

Table 5 outlines analysis parameters per single samples during validation and routine.

### Chemical and Process Parameters

During **validation** all samples – as defined in chapter 7.1 - should be analysed on the chemical parameters summarised in Table 1. In addition, process parameters (Table 2) such as conductivity, temperature, pressures and flows have to be evaluated. In general, the single parameters to be analysed depend on the quality of feed water (source of water, type of routine additives, kind of pipe line materials) and on the type / components of the whole water treatment system. Additional and different parameters may have to be evaluated in order to assess the whole system and the adequate quality of water.

**Routine** analysis – depending on the results of validation – have to at least cover the conductivity, hardness, pH, temperature, pressures and flows. In addition, common local contaminants such as free chlorine, chloramines and trace elements have to be considered and included in the routine analysis. At least once, preferably twice per year, a complete chemical analysis of feed water has to be obtained. For feed water delivered to the centre the chemical analysis has to be obtained from the water supplier (see 5.4); for the feed water entering the water treatment system samples have to be taken internally (Tab. 4 and 5).

### Microbiological Parameters

During **validation** all samples – as defined in chapter 7.1 - have to be analysed on microbial counts (viable cells) including yeast / mould and endotoxins in order to identify areas of higher risk of contamination.

During **routine** analysis – depending on the results of the first analysis (validation) – samples of less critical areas as well as analysis of yeast and mould may be excluded.

### Tab. 5: Samples to be Analysed in Validation and Routine

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>VALIDATION &amp; RE-VALIDATION</th>
<th>ROUTINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHEMICAL (Tab. 1)</td>
<td>PROCESS (Tab. 2)</td>
</tr>
<tr>
<td>Feed water</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Softened water</td>
<td>(X)</td>
<td>X</td>
</tr>
<tr>
<td>Permeate</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Concentrates</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Acid concentrate</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dialysis fluid (unfiltered)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ultrapure dialysis fluid</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Substitute (2 x filtered)</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

CFU: Colony forming units of micro-organisms;  
LAL: Limulus-Amoeocyte-Lysate assay for endotoxins

X mandatory, (X) optional  
* water supplier and dialysis centre  
+ all machines  
+ selected parameters  
+ including yeast & mould
Frequency of Sampling (for Standard see Table 6)

A validation (first evaluation) has to be performed for all newly installed water purification systems. During routine operation the frequency of sampling has to depend on the results of validation. Critical areas have to be checked more frequently (e.g. dead end water distribution systems) than less critical areas (e.g. closed ring systems with continuous flow). During routine operation a comprehensive chemical analysis should be conducted at least once per year, preferably twice per year (re-validation). Measurement of levels of specific local contaminants (aluminium, iron etc.) should be performed more frequently. However, chemical and most of the process parameters such as conductivity, hardness, flows and pressures should be checked daily. If available on-line tests offered as part of single treatment devices can be used to test some of these parameters.

Depending on the microbial quality of water evaluated during validation the frequency of sampling should be in intervals of 1 – 3 months. However, more frequent sampling is recommended for the first months after validation. The frequency can be lowered if stable and adequate results are achieved for 4 consecutive measurements. In case of quality deterioration the frequency of sampling has to be increased and appropriate actions have to be initiated. However, more critical sampling points (e.g. bicarbonate concentrates) have to be evaluated more frequently than less critical ones (acidic concentrates).

After each opening of the water treatment and distribution system a disinfection has to be performed and microbial samples have to be analysed. The timing of sampling has to be chosen in order to detect any contamination. Therefore, it should not be performed directly after disinfection and at least 15 minutes after the start of operation.

### Tab. 6: Standard of Sampling Frequency

<table>
<thead>
<tr>
<th>Quality Control</th>
<th>FREQUENCY</th>
<th>Parameters and Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation</td>
<td>Once for the whole water</td>
<td>all chemical, process and microbial parameters - in general before the start of operation of the whole system and/or significant system changes</td>
</tr>
<tr>
<td></td>
<td>treatment system</td>
<td></td>
</tr>
<tr>
<td>Re-validation</td>
<td>Once or twice per year</td>
<td>all chemical, process and microbial parameters based on trend analysis of routine measurements</td>
</tr>
<tr>
<td>Routine (Routine plan according to</td>
<td>1 - 3 months intervals</td>
<td>viable micro-organisms and endotoxins</td>
</tr>
<tr>
<td>validation/re-validation)</td>
<td>Monthly</td>
<td>local chemical contaminants, others on local demands</td>
</tr>
<tr>
<td></td>
<td>Daily</td>
<td>process parameters such as conductivity, hardness etc</td>
</tr>
<tr>
<td>Adaptation during routine with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>respect to microbial control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. results out of limit</td>
<td>Increase</td>
<td>Introduce corrective actions (see chapter 8)</td>
</tr>
<tr>
<td>2. results meet standard &lt; 4 times</td>
<td>Constant</td>
<td>keep current frequency for the respective parameter</td>
</tr>
<tr>
<td>consecutively</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. results meet standard &gt; 3 times</td>
<td>Constant or decrease</td>
<td>keep or lower frequency for the respective parameter, but not below minimum recommendation</td>
</tr>
<tr>
<td>consecutively</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample Collection**

Samples have to be taken under standardised conditions by trained personnel only. Instructions for handling, storage and transport of samples have to be taken into consideration depending on the individual parameters to be analysed and on the analytical method to be applied.

**Methods of Analysis**

The results of any chemical or microbial analysis depend heavily on the method used. Therefore, standardised methods are required in order to achieve reproducible results and to allow a reliable assessment of water and dialysis fluid quality. In general the analytical methods recommended by the European Pharmacopoeia should be applied. If more stringent other methods complying with
local standards should be used. All methods and devices used have to be validated and revalidated regularly. Each new method has to be validated before initiation.

Analysis of Chemical and Process Parameters

The following table summarises common methods recommended for the analysis of chemical contaminants.

<table>
<thead>
<tr>
<th>Contaminants</th>
<th>Methods of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Flame Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Flame Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Potassium</td>
<td>Flame Photometry</td>
</tr>
<tr>
<td>Sodium</td>
<td>Flame Photometry</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Molecular Photoluminescence</td>
</tr>
<tr>
<td>Chloride</td>
<td>Ion Chromatography</td>
</tr>
<tr>
<td>Free Chlorine</td>
<td>Calorimetry</td>
</tr>
<tr>
<td>Chloramines</td>
<td>Calorimetry</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Molecular Photoluminescence</td>
</tr>
<tr>
<td>Sulphate</td>
<td>Ion Chromatography</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Copper</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Chromium</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Lead</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Zinc</td>
<td>Flame Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Mercury</td>
<td>Cold Vapour Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Barium</td>
<td>Flame Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Silver</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization) / Flame Atomic Absorption Spectroscopy / Furnace Atomic Absorption (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Emissions Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Selenium</td>
<td>Furnace Atomic Absorption Spectroscopy (Electrothermal Atomization)</td>
</tr>
<tr>
<td>Silica</td>
<td>Molecular Photoluminescence</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Molecular Photoluminescence</td>
</tr>
</tbody>
</table>

In daily clinical routine process or chemical parameters such as conductivity or resistance can be evaluated by on-line methods integrated as functioning test for certain devices. Such devices have to be validated regularly. Other methods such as the peroxide test for disinfectants or dippaddle cassettes for bacterial growth are recommended for “quick checks” in the daily routine only. They do not replace validated analytical methods.

Microbiological Analysis

The microbiological tests recommended are microbial growth and endotoxin levels. The samples have to be collected under sterile and pyrogen-free conditions in order to avoid falsely positive results.
Viable micro-organisms (microbial growth) should be assayed by the membrane filter method with subsequent culturing in plates: 10ml of the sample are filtered through a sterile membrane with a pore size < 0.45µm in order to retain micro-organisms effectively. This filter is transferred under sterile conditions onto a culture dish containing the appropriate medium. Even tough different kind of media such as Tryptone Glucose Extract Agar and Reasoners 2A are currently under discussion, the media as proposed by the European Pharmacopoeia are recommended (see Table 7).

Tab. 7: Culture of Micro-organisms

<table>
<thead>
<tr>
<th>Microbial growth of</th>
<th>Culture Medium</th>
<th>Incubation Time</th>
<th>Culture Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>CASO-agar *</td>
<td>5 days</td>
<td>30 – 35 °C</td>
</tr>
<tr>
<td>Yeasts/moulds</td>
<td>Sabouraud-agar**</td>
<td>5 days</td>
<td>20 – 25 °C</td>
</tr>
</tbody>
</table>

**CASO-agar culture medium:**
15.0 g casein-peptone (pancreas-hydrolysate), 5.0 g soy-peptone (papain-hydrolysate), 5.0 g sodium chloride, 15.0 g agar, 1000 ml deionised or distilled water. The solution is sterilised by autoclaving for 15 min. at 121 °C. After sterilisation the pH has to be 7.3 ± 0.2.

**Sabouraud-agar culture medium:**
10.0 g meat- and casein-peptone, 40.0 g glucose-monohydrate, 15.0 g agar, 1000 ml deionised or distilled water. The solution is sterilised by autoclaving for 15 min. at 121 °C. Before sterilisation the medium is supplemented with 50 mg chloramphenicol per litre. After sterilisation the pH has to be 5.6 ± 0.2.

At the end of incubation the colonies growing on the surface of the membrane are counted. The number of colonies should be less than 100 per plate. However, if the count is between 10 - 50 CFU/ml take another sample or carry out a disinfection, if the count is greater than 50 CFU/ml a disinfection should be initiated. If higher numbers are expected, the sample volume should be reduced and/or the sample should be diluted accordingly and immediately carry out a disinfection, do not wait a further 5 days for the results. Each sample should be cultured in duplicate and the result expressed as mean.

Alternatively, in case of high germ counts spread plate technique or pour plate techniques can be used. The results are expressed as colony forming units per millilitre (CFU/ml), separately per culture medium.

The concentration of endotoxins should be measured quantitatively by kinetic Limulus-Amoebocyte-Lysate (LAL)- assay. The test should be turbidimetric or preferably chromogenic with a sensitivity of 0.001 IU/ml. According to the European Pharmacopoeia a batch specific validation of the LAL-test has to be performed before initiation of the measurement. The samples are evaluated on substances which might catalyse or inhibit the LAL-reaction. Furthermore, a representative and linear standard curve has to be guaranteed.
External Laboratory

External laboratories can be engaged to perform the chemical or microbiological analysis. However, they have to certify that the methods as well as the devices used comply with the present requirements and that those methods and devices are validated and revalidated regularly.

Cleaning and Disinfection

Cleaning and disinfection of the water treatment and water distribution system has to be scheduled according to the microbial quality of water assessed during validation and routine analysis. Adequate disinfectants have to be chosen under consideration of manufacturer’s recommendations. A plan for routine disinfection has to be outlined. It also has to consider regular maintenance of the treatment devices based on individual requirements as proposed by the manufacturer. For further details please see Corporate Guideline C-CG-09-01/Rev00: “Water Treatment Equipment”.

Corrective and Preventive Action

The results of each routine analysis have to be evaluated in order to detect any deviations from the defined quality standards. In case of any deviations the source of that deviation has to be identified immediately and a risk analysis has to be performed by a qualified and authorised person in order to judge potential hazards for the patients. Depending on the kind (e.g. bacterial, chemical), amount (concentration level) and source of contamination immediate actions such as disinfections and/or installation of additional filters in case of microbial contamination as well as additional and more frequent sampling have to be initiated.

In general, during validation potential weak points of the local water treatment system e.g. with respect to contamination or operation, have to be identified. As a worst case situation these weak points as well as other generally known risks have to be considered in the form of corrective (immediate) and preventive actions in the plan for routine analysis.

The corrective actions describe operations/procedures to be initiated if any incident occurs. Preventive actions are measures implemented to avoid the occurrence or re-occurrence of any incident.

Documentation

As part of the quality concept of FMC, a detailed documentation of every single manipulation of water purification is mandatory. Sampling points, the sampling itself, results of analysis, cleaning and disinfection as well as every system change have to be documented in the appropriate forms.
References


Draft of the European Norm: Concentrates for haemodialysis and related therapies 2000 (prEN 13867).


