On-line Preparation of Solutions for Dialysis: Practical Aspects Versus Safety and Regulations

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Abstract. On-line preparation, i.e., continuous mixing and immediate use, was introduced for dialysis fluid in 1964, and it contributed significantly to the expansion of dialysis therapy through simplified handling, improved microbiology, and enhanced efficiency. On-line prepared replacement solution for hemofiltration was shown to be clinically safe as early as 1978, but the implementation was delayed for 20 yr because of regulatory conservatism. On-line preparation of sterile and pyrogen-free solutions for infusion is based on the use of water and concentrates that contribute a minimum of microorganisms and are mixed and distributed in a hygienically designed and maintained flow path. Ultrafilters with known retention capacities are placed in strategic positions and dimensioned to remove bacteria and endotoxins, which gives a sterility assurance level of at least six magnitudes, as required by the Pharmacopoeia for sterile products. Microbiologic testing of the fluid should be applied when designing, validating, and troubleshooting on-line systems but not for routine quality control, because it only gives retrospective information. Quality assurance has to be built into a system and the way it is operated. On-line fluid preparation, when properly performed, is safe, simple, and cost-effective and enhances the efficiency as well as the biocompatibility of dialysis therapy.

The Role of Solutions in Dialysis

Blood purification by dialysis requires two essential components, a semipermeable membrane and a plasma-water–like solution. The fresh solution is either separated from blood, serving as a medium for diffusion, or mixed with blood for dilution and volume replacement in connection with ultrafiltration. In both cases, high demands are placed on the chemical composition and microbiologic quality of the solution. The concentration of electrolytes must be within narrow limits, and the microbiologic quality should be such that the fluid induces neither acute nor cumulative adverse effects.

The efficiency of any blood purification process depends on the volume of fluid that is used in the treatment, the exact relationship being determined by the quality of the solution. Only solutions of the highest microbiologic quality, i.e., sterile and pyrogen-free, can be used for convective transport to remove molecules of a wide range of sizes, because this requires mixing with blood. Fluid that is not sterile must be separated from blood by a membrane and thus can only be used for diffusive transport to remove mainly small solutes.

The volume and quality of fluid available for use in dialysis treatments is often limited by practical and economic considerations. On-line fluid preparation, which provides practically unlimited volumes of solution of appropriate chemical composition and quality, can remove these constraints and open the possibility for dialysis treatments to become more effective as regards blood purification and safer from a microbiologic point of view. On-line fluid preparation can be defined as continuous generation according to need and immediate use of a solution with desired composition and quality. The major alternative is batchwise preparation of solution followed by storage with preserved quality until the need for use arises. On-line fluid preparation facilitates the administration of dialysis treatments compared with the use of the corresponding volume of solution prepared in batches.

On-Line Preparation of Dialysis Fluid

A 4-h hemodialysis session requires at least 120 L of dialysis fluid, but the volume may be larger if the fluid flow rate is increased to enhance efficiency (“dialysis fluid” refers to the fresh fluid, and “dialysate” refers to the used fluid). In the early days of dialysis, the fluid was mixed by dissolving dry chemicals in large tanks, but since the mid-1960s, dialysis fluid has been prepared on-line by proportioning of water and a concentrated electrolyte solution (1). This development was fundamental for the expansion of dialysis, because it simplified the procedure. Furthermore, it increased treatment efficiency, because a single pass could now be used instead of recirculating dialysate (2). It also meant a great step forward in microbiologic quality, because bacterial growth was common in the tanks used for batch preparation and pyrogen reactions during dialysis were well-known phenomena (3). However, the chemical composition of each batch of dialysis fluid was easily checked before use, and a prerequisite for on-line preparation was that the composition could be continuously monitored and controlled; these requirements were met through conductivity measurements.

The first system for on-line preparation of dialysis fluid was a central piece of equipment that provided the same fluid for all
stations; however, single-patient dialysis machines that incorporated this function were soon developed, making it possible to individualize the electrolyte composition (4). When the disadvantages of using acetate as a buffer source became apparent to the dialysis community, those who wanted to use bicarbonate had to return to batch mixing until double-proportioning systems for two concentrates were developed (5). This led to serious microbiologic problems, because bacterial growth in bicarbonate-containing fluids, concentrated as and well as diluted, was prolific, and distribution pipes and fluid tanks became heavily contaminated (6). The introduction of single-patient dry powder cartridges for on-line preparation of bicarbonate concentrate improved the handling and microbiologic safety of dialysis fluid with bicarbonate (7).

Although on-line preparation makes it possible to profile the dialysis fluid composition—i.e., to introduce variations during the treatment according to a selected pattern—such changes of the major electrolytes, sodium and bicarbonate, have in the past involved accompanying changes also of the minor electrolytes. This linkage can now be avoided through the latest innovation in this field, the separation of the electrolytes in the nonbicarbonate concentrate, the A-concentrate, into a dry sodium chloride cartridge and a small bag with the remaining minor electrolytes (8). The general trend for dialysis concentrates is to remove water and separate the different electrolytes, to allow facilitated distribution, storage, and handling and more individualization of the final fluid composition. Recent development in the area of batch-blended dialysis fluid is mainly for a specific purpose, namely simplified systems for short dialysis treatments at home. For general dialysis applications, on-line preparation of the dialysis fluid is widely superior in handling, flexibility, and microbiologic quality.

**On-Line Preparation of Sterile Solutions for Dialysis**

Dialysis therapies with a significant amount of convective solute removal require the use of sterile fluids to substitute for the excess volume ultrafiltered from blood. The fluid serves as a plasma water replacement and has a composition similar to that of dialysis fluid. In fact, if dialysis fluid were sterile, it could serve as an infusion solution. With this vision, Henderson started his development work to produce sterile, pyrogen-free electrolyte solutions by ultrafiltration (9). He showed that bacteria and pyrogens could be effectively removed from dialysis fluid by filtration through a membrane that was freely permeable to water and electrolytes but restricted the passage of intact bacteria and pyrogens. The clinical safety of using this fluid as a replacement solution in hemofiltration was demonstrated by Henderson and colleagues as early as 1978 (10), and his results were later reproduced by European investigators (11,12). It was clear that the quality of the on-line prepared infusion solution depended on the integrity of the ultrafilter membrane, and attempts were made to verify the membrane quality continuously during fluid preparation. Shaldon (13) developed a loop with blue dextran and a photocell that would detect leakage of the dextran through the ultrafilter membrane and so indicate pinholes. This had to be abandoned when it was found that the dextran contained pyrogens that contaminated the fluid. Instead, a rigorous quality control was introduced in production with pressure testing of each ultrafilter to detect pinholes (Figure 1).

The on-line pioneers felt assured about the safety of the system and the process for on-line fluid preparation and continued the use in their clinics (14), but the widespread application of on-line prepared infusion solution was delayed for many years, mainly because of a lack of regulatory recognition. With the introduction of the Medical Device Directive in the European Union in 1995, all products required for on-line fluid preparation—i.e., machines, ultrafilters and concentrates—were classified as medical devices. As a consequence, manufacturers can affix the approval sign, the CE mark, for on-line fluid preparation to their products once they have fulfilled the applicable requirements (15). As part of these requirements, the products have to be shown to be safe under the indicated conditions of operation. Thus, it is important that users of on-line systems are familiar with the documentation provided by the manufacturer.

**Sterilization and Sterility**

The main reason for the long delay by regulatory authorities to approve on-line preparation of sterile fluids was the lack of rules in the pharmacologic rule book, the *Pharmacopoeia*, that apply to this form of sterilization. The *Pharmacopoeia* recognizes mainly the traditional form of sterilization of fluids, steam sterilization by autoclaving (16,17). Fluid is packed in 1- to 2-L bags, and thousands of bags are exposed to a certain pressure and temperature for a fixed time. The process conditions are controlled, and, at the end, samples are taken for microbiologic testing. Confirmation of sterility is obtained

![Figure 1. Quality control of a production batch of polyamide ultrafilters (U8000S, Gambro), showing the pressure drop of individual filters when subjected to 1.6 bar. All ultrafilters are pressure tested, and filters with a pressure drop >0.3 bar are rejected. Courtesy of M. Pirner, Gambro Dialysatoren, Hechingen, Germany.](image-url)
after 7 to 14 d, at which time the products are released. The *Pharmacopoeia* uses the term “sterility assurance level” to denote the degree of assurance with which a certain process renders sterility, and a sterility assurance level of one million is usually required. This means that, in a batch consisting of one million items, only one item may be contaminated for the entire batch to be considered sterile.

Some *Pharmacopoeia* also permit sterile filtration by use of validated filters and verification of filter integrity before and after the sterilizing procedure (17). This filtration technique cannot be classified as on-line preparation, because it requires the filtration to be applied batchwise and the filtered fluid to be aseptically packaged and stored until the filter integrity is proved, usually by microbiologic testing of the fluid. Aseptic processing is not an accepted form of sterilization of large fluid volumes, and, although it could probably be safely performed industrially, it should not be practiced in a hospital setting (18).

Thus, it is neither safe nor practical to delay the use of ultrafiltered, aseptically packaged fluid until some microbiologic test has been performed. Fluid testing can only give a retrospective assurance of safety, which is acceptable when designing and validating a system but not for quality control in routine use. Instead, an assurance of quality must be built into an on-line system and the way it is operated.

**Critical Components for On-Line Fluid Preparation**

**The Water.** Water for dialysis makes up ~95% of the final volume of all solutions used in dialysis and therefore contributes most of the bioburden that needs to be removed. Standard water for dialysis, according to European and US guidelines, may contain 100 to 200 colony-forming units (CFU)/ml and 0.25 to 2 endotoxin units (EU)/ml. One step of controlled ultrafiltration can make the water ultrapure, which, according to a common definition, means <0.1 CFU/ml and <0.03 EU/ml when the appropriate detection methods are used (19,20). Ultrapure water can also be prepared in a well-functioning central water plant by double reverse osmosis, but this quality is difficult to maintain during passage through the distribution system unless biofilm formation is prevented by frequent prophylactic disinfection (21).

**The Concentrates and the Flow Path in the Machine.** The dialysis concentrates used for on-line preparation should be labeled for this purpose, because they should add a minimum of contaminants to the ultrapure water. The acid concentrate is usually no problem—the high salt content prevents bacterial growth—whereas the bicarbonate component could be highly contaminated, especially if it has been prepared by a central delivery system (22). It is recommended that bicarbonate concentrate freshly prepared from a powder cartridge be used. Furthermore, the flow path in the dialysis machine must be hygienically designed and maintained, and it is again important to prevent the formation of biofilm in dead ends and low-flow areas (23,24). Daily prophylactic disinfection is necessary to prevent bacteria from colonizing the attractive interior of the dialysis machine.

**The Ultrafilters.** The retention of bacteria and endotoxin by ultrafilters is based on two mechanisms, size exclusion and adsorption. The membrane, which is usually polyamide- or polysulfone-based, is highly permeable to water and solutes up to a molecular weight of 30 to 40 kD, but intact bacterial cells and large cell wall components cannot pass through the pores (25–27). The pore size distribution and integrity of the membrane in each individual filter should be tested at manufacture by a pressure-holding test that clearly identifies filters that do not meet the specification (Figure 1). The adsorptive capacity of the membrane is related to the choice of polymer blend, in which hydrophobic components are important for the retention of similarly hydrophobic bacterial products. Many pyrogenic bacterial products are below the size of the pores, so the adsorptive capacity is vital for the preparation of nonpyrogenic fluids (28).

The *Pharmacopoeia* requires ultrafilters designated for sterile filtration to have a logarithmic reduction value ≥7 for bacteria when a prescribed test procedure is used. Ultrafilters for on-line systems should further have logarithmic reduction value ≥4 for endotoxin. These retention properties should also remain valid after a given number of disinfection cycles with defined agents (29). Still, when inserted into the flow path of the dialysis machines, ultrafilters are exposed to varying levels of microbiologic contaminants and particulate matter, high pressures, strong cleaning agents, and high temperatures, which can affect both the pore size and the adsorption sites (30). The rational approach to estimating a safe lifetime for an ultrafilter is to calculate the potential load of bacterial cells and endotoxin hitting the filter surface under prevailing operating conditions and set the filter lifetime so as not to exceed this exposure while still achieving the necessary sterility assurance level. For additional safety, the final filter, in which the ultrapure dialysis fluid is converted into sterile substitution solution, should be for a single use. This filter arrangement comes closest to fulfilling the requirements of the *Pharmacopoeia*, considering that no functional test that shows exhaustion of adsorption sites is available.

The arrangement of ultrafilters in a generalized on-line system is shown in Figure 2. The first filter is a water filter that makes it possible to use standard water for dialysis. The second filter prepares ultrapure dialysis fluid. Both of these filters are integrated in the flow path and can be used for a certain time, usually 2 to 3 mo, depending on the operating conditions. A portion of the ultrapure dialysis fluid, as much as is required for the therapy in question, undergoes a further step of ultrafiltration in a sterile ultrafilter and is converted into sterile and pyrogen-free infusion solution to be used for volume replacement in hemodiafiltration (HDF) and hemofiltration and as rinsing solution (29).

**Validating an On-Line Fluid Preparation System**

The *Pharmacopoeia* states that sterility can not be guaranteed by testing: it has to be assured by the application of a suitable validated production process, and manufacturers of on-line systems have to perform such validation to gain approval for their systems (31–33). Such a validation of a European on-line system, the AK 100/200 ULTRA (Gambro AB, Lund, Sweden), was performed in a US clinic under conditions...
designed to fulfill the requirements of authorities. The system design and operation are described in detail elsewhere (29) and are illustrated schematically in Figure 2. Operation was strictly in accordance with the manufacturer’s instructions regarding disinfection and filter exchange. Fluid was generated under in vitro conditions, and two mock HDF treatments were performed each day for 4 wk. At the end of each treatment day, fluid samples were taken from five positions that represented critical locations along the flow path (Figure 2, Table 1). The volume of each sample was adjusted to the expected degree of contamination. The sample volume from the final position, 30 L, corresponds to the volume infused in a patient in a typical HDF treatment. Sample volumes up to 1 ml were directly spread on agar plates, but for larger samples the entire volume was passed through a 0.22-μm bacteria filter, the filter disc being placed on plates with tryptone glucose extract agar and incubated at 22 °C for 7 d. Samples for endotoxin testing were 1 ml each, and the LAL endotoxin gel clot method, with a sensitivity of 0.03 EU/ml, was used. The result of the system validation shows the gradual removal of bacteria and endotoxin as the fluid passes the different filters (Table 1) (34). The dialysis fluid in position 4 was always ultrapure, and the final fluid contained no CFU in the 30 L tested and was negative for endotoxin.

This validation provided the authorities with sufficient evidence of the safety of the fluid to allow a clinical trial. It was accepted that the fluid quality could not be verified before the fluid was used, but, to include some component of fluid testing in the clinical trial, it was decided to sample the fluid in position 4—i.e., the ultrafiltered dialysis fluid—immediately after each HDF treatment. This position was selected because it is the only position where it is practical as well as meaningful to take a sample. The second ultrafilter, which is disinfected with the system and used for 1 to 2 mo, is the most critical component. The final filter, by virtue of being quality-controlled with logarithmic reduction value ≥7 and for a single

### Table 1. Microbiologic validation of the quality of fluids for dialysis prepared by an on-line system, the AK 100/200 ULTRA (Gambro). For system description, see reference (28). For results, see reference (33).

<table>
<thead>
<tr>
<th>Position</th>
<th>Location</th>
<th>Type of Fluid</th>
<th>Sample Volume</th>
<th>Positive for Bacteria</th>
<th>Positive for Endotoxin</th>
<th>Microbiologic Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water inlet to machine</td>
<td>Water</td>
<td>0.2 ml</td>
<td>18/20</td>
<td>7/20</td>
<td>Water for dialysis</td>
</tr>
<tr>
<td>2</td>
<td>After 1st ultrafilter</td>
<td>Filtered water</td>
<td>1000 ml</td>
<td>12/20</td>
<td>0/20</td>
<td>Ultrapure water</td>
</tr>
<tr>
<td>3</td>
<td>Before 2nd ultrafilter</td>
<td>Dialysis fluid</td>
<td>1000 ml</td>
<td>9/20</td>
<td>0/20</td>
<td>Dialysis fluid</td>
</tr>
<tr>
<td>4</td>
<td>After 2nd ultrafilter</td>
<td>Filtered dialysis fluid</td>
<td>1000 ml</td>
<td>0/20</td>
<td>0/20</td>
<td>Ultrapure dialysis fluid</td>
</tr>
<tr>
<td>5</td>
<td>After 3rd ultrafilter</td>
<td>Infusion solution</td>
<td>2 × 15 L</td>
<td>0/40</td>
<td>0/20</td>
<td>Sterile and pyrogen-free solution</td>
</tr>
</tbody>
</table>

*Figure 2. Schematic flow diagram of on-line fluid preparation. Standard water for dialysis is ultrafiltered to become ultrapure (<0.1 colony-forming units/ml and <0.03 endotoxin units/ml). Mixing with high-quality concentrate components in a hygienically designed and maintained flow path generates dialysis fluid that, after ultrafiltration, becomes ultrapure. This solution is used as dialysis fluid in hemodialysis (HD) and hemodiafiltration (HDF). The final conversion of ultrapure dialysis fluid into sterile infusion solution takes place in a sterile, quality-controlled ultrafilter. This solution is used for infusion in HDF and hemofiltration (HF). Numbers refer to sampling positions described in the text and in Table 1. RO, reverse osmosis; A, acid concentrate; B, bicarbonate cartridge.*
use, will always yield a sterile fluid when provided with ultrapure fluid. The clinical trial showed the fluid in position 4 to contain 0 CFU/1000 ml and <0.03 EU/ml, confirming that it was indeed ultrapure (35). Blood cultures taken from the patients at the end of each treatment month, before the stationary ultrafilters were replaced, were all negative, and no patient adverse reactions were seen.

Microbiologic Fluid Testing and Maintenance of On-Line Systems

Once an on-line system has been shown to generate solutions of appropriate quality, microbiologic testing of the fluid needs only to be performed at specific times. The water for dialysis should be tested according to the protocol of the unit, usually monthly. The on-line prepared fluid could be tested every 1 to 2 mo just before the stationary ultrafilters are replaced, which gives a good indication of the performance of the system, provided that an appropriate sample volume is taken and sensitive culturing techniques are used (19,36,37). Other reasons for fluid testing could be changes of routines or staffing and, of course, suspected problems. The handling of fluid samples and bacteria filters is highly sensitive to secondary contamination, and false-positive results are often encountered. Identification of the bacteria may therefore be necessary to differentiate between waterborne primary contaminants and airborne or human secondary infections (14).

Microbiologic fluid testing improves the quality only if it leads to corrective action as a consequence of an unsatisfactory result (38). Rather than excessive fluid testing, which could give a feeling of false security, efforts should be spent on adhering to disinfection protocols and increasing the general microbiologic awareness among the staff. It is important that the procedures for hygienic maintenance and day-to-day handling of on-line systems are realistically designed to ensure that they are properly implemented.

Conclusions

The introduction of on-line prepared fluid in a dialysis unit provides new possibilities to perform convective therapies for patients undergoing acute as well as chronic dialysis, without being limited by the volume or composition of the substitution solution. Compared with performing these therapies in the traditional way, it means simplified routines, because the handling of heavy fluid-filled bags is eliminated. The third area to benefit is economic, because the total cost of on-line prepared fluid is considerably less than that of commercially prepared solutions for volume replacement. Finally, the use of on-line prepared fluid means improved fluid quality, which may lead to a better outcome, because the inflammatory stimulus from bacterial products in dialysis solutions is associated with several long-term complications in patients undergoing dialysis (20,39).

It is possible to design and operate systems that continuously prepare high-quality fluids for dialysis, ultrapure dialysis fluid, as well as sterile solution for substitution and rinsing in practically unlimited quantities. The fluid quality is safeguarded by strict adherence to the use of products and operating conditions described by the manufacturers and approved by authorities. Confidence in the process is necessary because there is no way to test the microbiologic quality of the fluid before it is used.

However, it is important to realize how different a biologic contaminant is from a chemical substance. It is not sufficient to lower the concentration of bacteria to a level that seems safe at a certain moment, because logarithmic growth will restore the numbers to previous magnitudes within a short time. Instead, the number of bacteria has to be reduced to such levels that the probability of existence meets the definition of sterility, i.e., there is a sterility assurance level of six magnitudes.

On-line fluid preparation, when properly performed, is safe, simple, and cost-effective and may contribute to an improved outcome in patients undergoing dialysis. It thus fulfills several requirements that can be placed on new dialysis techniques. In a recent opinion poll among nephrologists, 62% of the respondents, or >1500, considered on-line preparation of sterile infusion solution to be a valuable new technique, and few saw any risk with it (40). It is time for on-line preparation of sterile fluids to become integrated into everyday practice of dialysis units, just as on-line preparation of dialysis fluid was 35 yr ago.

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References


