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Standard on Microbiological Management of Fluids for Hemodialysis and Related Therapies by the Japanese Society for Dialysis Therapy 2008

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Abstract: The Committee of Scientific Academy of the Japanese Society for Dialysis Therapy (JSDT) proposes a new standard on microbiological management of fluids for hemodialysis and related therapies. This standard is within the scope of the International Organization for Standardization (ISO), which is currently under revision. This standard is to be applied to the central dialysis fluid delivery systems (CDDS), which are widely used in Japan. In this standard, microbiological qualities for dialysis water and dialysis fluids are clearly defined by endotoxin level and bacterial count. The qualities of dialysis fluids were classified into three levels: standard, ultrapure, and online prepared substitution fluid. In addition, the therapeutic application of each dialysis fluid is clarified. Since high-performance dialyzers are frequently used in Japan, the standard recommends that ultrapure dialysis fluid be used for all dialysis modalities at all dialysis facilities. It also recommends that the dialysis equipment safety management committee at each facility should validate the microbiological qualities of online prepared substitution fluid. **Key Words:** Bacteria, Central dialysis fluid delivery system, Endotoxin, Standard of fluid for hemodialysis.

The necessity of dialysis fluid purification has been discussed since the 1980s, primarily in Europe. The Japanese Society for Dialysis Therapy (JSDT) presented the Quality Standard of Microbiological Contaminants in Dialysis Fluid in 1995 (1) and revised it in 1998 (2) and 2005 (3). However, these Japanese standards required endotoxin level alone, and showed no clear criterion concerning bacterial count. Presently, the International Organization for Standardization (ISO) is preparing an international quality standard for dialysis fluid in which bacterial count is emphasized as the most important parameter for evaluating microbiological qualities of fluids for hemodialysis and related therapies (4). We propose a new quality standard for dialysis fluid that is in harmony with the ISO standards for microbiological management.

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MICROBIOLOGICAL QUALITY STANDARD FOR DIALYSIS FLUIDS

Attainment level

- Dialysis water (Reverse Osmosis [RO] Water)
  Bacteria: <100 CFU/mL
  Endotoxin: <0.050 EU/mL
- Standard dialysis fluid
  Bacteria: <100 CFU/mL
  Endotoxin: <0.050 EU/mL
- Ultrapure dialysis fluid
  Bacteria: <0.1 CFU/mL
  Endotoxin: <0.001 EU/mL (less than the detection limit)

Note: The action level shall be set depending on the quality condition of each facility, typically at 50% of the maximum allowable level, except for the endotoxin level of ultra-pure dialysis fluid.

- Online prepared substitution fluid
  Sterile and non-pyrogenic
  Bacteria: $<10^{-6}$ CFU/mL
  Endotoxin: <0.001 EU/mL (less than the detection limit)

Test for compliance

- Endotoxin:
  Limulus amoebocyte lysate (LAL) assay (gel-clot assay, spectrophotometric kinetic assay)
- Bacteria:
  Culture media: R2A (Reasoner's Agar No 2), TGEA (Tryptone Glucose Extract Agar) or equivalent
  Cultivation conditions: R2A and TGEA: 17–23°C for 7 days

Sampling sites

- Dialysis water: Outlet of RO equipment
- Dialysis fluid: Inlet line of dialyzer
  Note: Push and pull hemodiafiltration (HDF) systems should be tested on the outlet line of dialyzer.
- Online prepared substitution fluid: extraction site of substitution fluid

Sampling day

- Before the start of dialysis and after the maximal interval of dialysis (commonly Monday)

Frequency of monitoring

- Dialysis water: Every 3 months
  If the quality of each facility is not maintained to allowable levels, the monitoring frequency should be increased to once a month.
- Standard dialysis fluid: Monthly
  At least two machines are tested each month so that each machine is tested at least once per year.
- Ultrapure dialysis fluid: Monthly when used for conventional hemodialysis (including internal filtration enhanced hemodialysis)
  At least two machines are tested each month so that each machine is tested at least once per year.
- Ultrapure dialysis fluid for the preparation of online substitution fluid and the dialysis system for active use of back filtrate: Every 2 weeks until the system is validated
  After the quality is validated by the dialysis equipment safety management committee, monitor the same frequency as standard dialysis fluid.
- Online prepared substitution fluid: Monthly
  Endotoxin: Every 2 weeks until the system is validated. After the quality is validated by the dialysis equipment safety management committee, monitor monthly on every machine of dialysis fluid and substitution fluid.
  Bacteria: Sterility of 10^{-6} CFU/mL is impossible to detect. Dialysis fluid used for preparation of substitution fluid should be maintained to the quality of ultrapure dialysis fluid. Bacteria culture of dialysis fluid and substitution fluid should be conducted every two weeks until the system is validated. After the validation by the dialysis equipment safety management committee, monitor monthly, rotating among the machines so that at least two machines are tested each month and each machine is tested at least once per year.

Indications for dialysis system based on the quality of dialysis fluids

- Standard dialysis fluid
  Minimum requirement for dialysis therapy
- Ultrapure dialysis fluid[#]
  Dialysis fluid for the preparation of online substitution fluid
  Dialysis system for active use of back filtrate (e.g. fully automated dialysis system (5))
  Push and pull HDF system
  Internal filtration enhanced dialysis (IFEHD)*
- Online prepared substitution fluid
  Online HDF/online hemofiltration (HF)

#Ultrapure dialysis fluid is desirable for all dialysis modalities.

*Estimated filtration rate in IFEHD ≥ 35 mL/min (3).

Standard of endotoxin retentive filter (ETRF)

- ETRF should meet the requirement of the Japan Medical Devices Manufactures Association.
- The user should follow the user manual of each ETRF.
- Exchange times should meet the manufacture's standard for each ETRF.
- If the manufacture's standard does not indicate the exchange time, each facility should validate the performance of ETRF. The validated data should be reported to and confirmed by the dialysis equipment safety management committee.

Safety assurance programs

The dialysis fluids and apparatus must be managed according to an appropriate manual; therefore, the persons responsible for the safety management of medical equipment must validate the dialysis apparatus at their facilities. They should then take the following measures:

1. Establishment of a curriculum for education and training of dialysis operators.
2. Preparation and assurance of the availability of a manual for dialysis fluid management.
3. Management records and measurement records must be prepared and preserved similar to clinical records. The related documents must be preserved for three years from the date of preparation.
4. For the management of the dialysis apparatus and water quality of dialysis fluids, a dialysis equipment safety management committee must be established under the person responsible for the safety management of medical equipment to perform the following activities:
   - A management plan for dialysis equipment and water treatment apparatus must be prepared. Appropriate maintenance work must be performed. Reports must be preserved.
   - Seminars for staff members to promote the appropriate use of dialysis equipment must be arranged.
   - Related medical information must be collected by a single entity. Delivery of the information to staff members must be assured. Accident-related information must be reported immediately to the committee.
5. Online prepared substitution fluids can be used only after validation by the dialysis equipment safety management committee. The committee shall provide written approval prior to use.

THEORETICAL BACKGROUND OF DIALYSIS FLUID PURIFICATION

Standard on bacteriological quality of water and dialysis fluids in Japan

The JSDT established the microbiological quality standard for conventional hemodialysis in 1995 (1). The standard was revised for the domestic approval of Gambro's online HDF apparatus (AK100-Ultra) in 1998 (2). The JSDT revised the standard for endotoxin levels to address IFEHD in 2005 (3). Water quality standards concerning online HDF were proposed in 1994 by the Kyushu Society for HDF (6), and have been used as the basis for all subsequent standards.

The 2005 JSDT standard (3) required an endotoxin level <0.050 EU/mL (desirable level to be attained: less than detection limit), even in conventional hemodialysis, and an endotoxin level less than detection limit as an indispensable condition for both dialysis fluid and substitution fluid for online HDF. Also, an endotoxin level of <0.010 EU/mL (desirable level to be attained: less than detection limit) was required in IFEHD (estimated internal filtration rate ≥ 35 mL/min). Thus, more rigid standards concerning the endotoxin level have been imposed in Japan compared to the standards of the Association for the Advancement of Medical Instrumentation (AAMI) and ISO. Regarding the bacterial count, however, the 1995 JSDT standard (1) required a level of <100 CFU/mL for dialysis fluid. This criterion has not been revised since, and the JSDT standards in 1998 (2) merely mentioned 1×10^{-3} CFU/mL for online prepared substitution fluids.

There have been various reports and reviews regarding the necessity of dialysis fluid purification. In addition, the adverse effects of microbiological contamination and dissociation between the bacterial count and endotoxin level have become widely recognized. Further, very low levels of contamination have been reported to impair biocompatibility (7–11). Acceptable endotoxin levels assure the safety of the dialysis fluid. The presence of bacteria suggests a risk for the subsequent spread of contamination. The measurement of endotoxin compensates for the long culture time required for the bacterial test. Therefore, simultaneous evaluation of the bacteria count and endotoxin level is necessary for the assessment of microbiological contamination.

Theoretical background of standard dialysis fluid

In the 1980s the interleukin hypothesis was proposed by the group of Henderson and Shaldon (12). Subsequent research demonstrated the risk of contamination of dialysis fluid, as well as bioincompatibility of the dialyzer, as causes of long-term complications to dialysis patients such as amyloidosis. This hypothesis was then evolved by Stenvinkel, who established the concept of the malnutrition-inflammation-atherosclerosis syndrome, which are predominant causes of death in dialysis patients (13).

The ISO has proposed an endotoxin level <0.500 EU/mL and a bacterial count <100 CFU/mL as the standard for dialysis fluid (4). In Japan, where more than 95% of the dialyzers used are high-performance membrane types (level II or higher according to the 2006 criteria by the Japanese Ministry of Health, Labor and Welfare) (14), endotoxin levels of <0.050 EU/mL and bacterial counts <100 CFU/mL were adopted as the quality standard for dialysis fluid. This endotoxin standard has been achieved at 89% of the facilities that responded to a JSDT statistical survey at the end of 2006, and so it is appropriate as a criterion (15).

Theoretical background of ultrapure dialysis fluid

The term "ultrapure dialysis fluid" was first used in the report by Baz et al. (7). In this report, a reduction

in the rate of dialysis induced amyloidosis by "dialysis fluid purification" was shown. The present definition of ultrapure dialysis fluid (endotoxin < detection limit, bacteria < 0.1 CFU/mL) is a conceptual water quality standard required of dialysis fluid immediately before the last ETRF to guarantee an endotoxin level below the detection limit and a bacterial count $<10^{-6}$ CFU/mL (16), which are considered to be equivalent to sterility. This standard is required for online prepared substitution fluid for online HDF. Recently, however, ultrapure dialysis fluid has become necessary even when a high-performance dialyzer is used as contamination of very low levels of endotoxin or ultra-microparticles, such as bacterial DNA, has been shown to induce inflammatory reactions (17).

According to the JSDT survey in 2006, 84 and 96% of the samples met the above criteria concerning endotoxin and bacteria, respectively, even without an ETRF (15). Since the logarithmic reduction value (LRV) of ETRFs widely used today is 3 for endotoxin and 7 for bacteria, ultrapure dialysis fluid could be theoretically obtained through the use of an ETRF based on the result of JSDT survey (15). However, the contamination of dialysis fluid is occasionally observed, even when using an ETRF, so attention to the safe and effective installation of an ETRF should be promoted. With the fulfillment of all the above conditions, it is considered possible to prepare ultrapure dialysis fluid at all dialysis facilities in Japan; therefore, in this standard, we recommend the use of ultrapure dialysis fluid for all dialysis modalities.

Theoretical background of online prepared substitution fluid

The current standards for the endotoxin level of online prepared substitution fluid is <0.001 EU/mL (detection limit) and that of bacterial count is $<10^{-6}$ CFU/mL. These are nearly in agreement with the criteria of the European Best Practice Guidelines (18), AAMI (19), and the proposed ISO standard (4). A bacterial count of 10^{-6} CFU/mL is recognized as equivalent to sterility. If one is to prove sterility by the actual measurement of bacteria, more than 1 ton of substitution fluid would have to be analyzed, which is absolutely impractical, so this is a theoretical value that can be attained by validation. Ledebo (20) proposed that the quality of dialysis fluid before the last ETRF should be ultrapure dialysis fluid (<0.1 CFU/mL). Even if unexpected 100-fold (10^2) contamination occurs before the last ETRF, the LRV of 7 for bacteria could guarantee 10^{-6} quality. Weber et al. (21) performed challenge tests of reused ETRFs and leak tests prior to each online HDF therapy session, and concluded the safety of the online prepared substitution fluid in their system. In Japan, online prepared substitution fluid in the Central Dialysis Fluid Delivery System (CDDS) is prepared by a membrane filter sterilization process using multiple use ETRFs. The theoretical grounds for the assurance of the quality of online prepared substitution fluids are basically the same as those presented in the above two articles; however, as online HDF systems used in Japan today consist of parts manufactured by various manufacturers, they are not integrated by the manufacturer. On the other hand the above two systems are integrated by Gambro and Fresenius, respectively; therefore, those who prepare dialysis fluids in Japan must guarantee the quality of ultrapure dialysis fluids prepared with their equipment and strictly observe the replacement period of ETRF as indicated by its manufacturer.

Quality standard of dialysis water

In this standard, an endotoxin level of <0.050 EU/mL and a bacterial count of <100 CFU/mL were set as criteria for the microbiological contamination of dialysis water (RO water). This is the strictest criteria in the world. RO produces the highest reduction rates of both chemical and biological contaminants in dialysis fluid preparations. The contamination level of RO treated water, which is defined by the supply water contamination level and the leak rate of RO membrane, is the most important determinant of contamination in dialysis fluid. Biological contamination of dechlorinated RO water in the RO product water tank can cause secondary contamination downstream. Measures for suppressing contamination of the RO module and RO tank are extremely important, hence strict contamination criteria is applied in Japan. Also, to prevent secondary contamination in a CDDS, quality evaluation of dialysis fluid is required at the outlet of the CDDS as well.

Method for bacterial detection

This standard recommends the use of R2A, TGEA agar plate medium or similar media, which should be verified to show comparable sensitivity. This is similar to the ISO draft (4). The number of samples to be cultured depends on the degree of contamination. A sample volume of 100 mL or more is necessary to accurately demonstrate contamination of ultrapure dialysis fluid (<0.1 CFU/mL).

Validation concept

Validation is a concept for the assurance of system compatibility and product quality, and includes the

validation of the following: the manufacturing and quality control methods, including the system design and equipment of the manufacturing facility, manufacturing procedure and processes, etc. Confirmed results must yield acceptable limits, and this must be documented in writing (22). In Japan, CDDS are used in many dialysis facilities, and dialysis fluids are prepared through multiple processes performed by various apparatuses connected in series. These apparatuses are selected and arranged by each dialysis facility. In such a manufacturing system, a validation concept is necessary for the process management and product quality assurance. A dialysis facility functions as a dialysis fluid manufacturing facility and is responsible for the final quality of the dialysis fluid. Therefore, a person responsible for manufacturing and managers of various processes must be appointed for dialysis fluid purification in the same manner as at manufacturing facilities.

At a dialysis facility that is also a manufacturer of dialysis fluid, the following requirements must be established as validation processes for the dialysis fluid purification procedure. If the results of comprehensive evaluation of these processes meet the requirements, the dialysis fluid preparation system is considered to have been validated.

- Intended quality (purity standard of dialysis fluid)
- Validity of equipment, preparation processes, products, and plan of the preparation method (system design of apparatuses)
- Confirmation of the designs of the equipment, processes and preparation methods, and their completion as designed (installation qualification)
- Confirmation of the utility of the equipment, processes, and methods to achieve the intended objective (operational qualification)
- Testing of the system under the conditions of actual preparation and confirmation of the operation of the system as designed (performance qualification) (periodic evaluation of the quality of supply water, RO water, and dialysis fluid)
- Guarantee the disinfection of the interior of the system during non-use periods.

Clinical effects of purified dialysis fluid

The most notable clinical effect of dialysis fluid purification is the improvement in anemia. Sitter et al. reported significant decreases in interluekin-6 and C-reactive protein levels and an associated improvement in the response to recombinant human erythropoietin after replacement of the standard dialysis fluid (bacterial count: 85 CFU/mL) with ultrapure dialysis fluid (bacterial count <0.1 CFU/mL) (8). Such a phenomenon has been reported by many facilities and is presently considered to be the clearest benefit of dialysis fluid purification. Retention of residual kidney function (9,10), prevention of dialysis amyloidosis (7), and a decrease in advanced glycation end-products (11) have also been reported; however, the effects on atherosclerosis or the survival rate have not been reported because of the necessity of long-term follow-up and the presence of many confounding factors. Also, most of the studies to date have been small-scale observational studies, and the evidence level of their results has been low. Large-scale comparative studies are necessary in the future.

CONCLUSION

The JSDT has published new quality standard of dialysis water and dialysis fluids for hemodialysis and related therapies. This standard is similar to the criteria of the ISO, which is currently being considered for revision. This standard is aimed to be applied to CDDS, which is widely used in Japan. Since high-performance dialyzers are frequently used for most patients in Japan, the standard recommends the use of ultrapure dialysis fluid for all dialysis modalities at all dialysis facilities. This standard also requires the use of online prepared substitution fluid with validation by the dialysis equipment safety management committee at each facility. This standard would help protect hemodialysis patients from the adverse effects arising from microbial contaminants and can contribute immensely to long-term positive patient outcomes.

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