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Guidelines

2011 JSDT Standard on the Management of Endotoxin Retentive Filter for Dialysis and Related Therapies

Ikuto Masakane,[1] Hideki Kawanishi,[1] Michio Mineshima,[1] Yoshiaki Takemoto,[1] Junji Uchino,[1] Taketoshi Hoshino,[1] Tadaaki Igoshi,[1] Hideki Hirakata,[2] and Tadao Akizawa[3]

[1]Working Group on the Development of the ETRF Management Standard, [2]Chairperson of the Committee of Scientific Academy, [3]President of the Japanese Society for Dialysis Therapy, The Japanese Society for Dialysis Therapy, Tokyo, Japan

The contamination of the fluids for dialysis therapy is an important factor that deteriorates the biocompatibility of HD and related therapies. In particular, microbiological contamination is considered to be an important factor that causes dialysis amyloidosis and malnutrition due to the strong physiological activity of endotoxin (ET) (1). High-performance dialyzers are more frequently used in Japan than in other countries; hence, dialysis facilities in Japan are particularly affected by the bacterial contamination of dialysis fluid when it occurs. Moreover, most of the dialysis facilities in Japan use central dialysis fluid delivery systems (CDDSs), which are considered to be less effective in suppressing microbiological contamination than systems used in other countries, which adopt single-patient dialysis machines. Although ET retentive filters (ETRFs) are indispensable for increasing the microbiological quality of dialysis fluid, they may be a source of contamination if not appropriately handled (2). Therefore, the installation of an ETRF, which is the final safety step in the purification of dialysis fluid, does not always guarantee the purity of the final fluid. Rather, it is important to manage the quality of dialysis fluid that passes through the ETRF. In 2008, the Japanese Society for Dialysis Therapy (JSDT) established the Microbiological Quality Standard for Dialysis Fluid, which merely states that users of ETRFs should handle them according to the manufacturer's instructions (3). To date, however, no consistent standard for handling ETRFs has been given by the Japan Medical Devices Manufacturers Association. ETRFs from different manufacturers have been deployed in various dialysis facilities using procedures at the discretion of each facility. Considering this situation, the JSDT has recommended that the management of ETRFs should be standardized and has formulated the JSDT original standard for managing ETRFs with the aim of supplying safe and stable dialysis fluid.

The Working Group on the Development of the ETRF Management Standard consisted of members of the JSDT Subcommittee of Scientific Academy for the Function and Efficiency of Blood Purification Therapy as well as representatives of the Japan Medical Devices Manufacturers Association and the Japan Association for Clinical Engineers. To the best of our knowledge, there are few reports with high-level evidence regarding ETRF management. Moreover, CDDSs are predominantly used in Japan and rarely used in the US and Europe, so that makes it extremely difficult to refer to overseas evidence and standards on ETRF management. Therefore, we summarized the opinion of experts based on the results of joint research with related societies, including the Japan Association for Clinical Engineers and the Japanese Society for Hemodiafiltration, as well as the standards presented by the Japan Medical Devices Manufacturers Association and the dialysis-related International Organization for Standardization (ISO) standards. We then formulated the ETRF Management Standard through a Consensus Conference at the 55th Annual Meeting of the JSDT.

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Address correspondence and reprint requests to Dr. Ikuto Masakane, Yabuki Hospital, 1-6-17 Honcho, Yamagata City, Yamagata 990-0885, Japan. Email: imasakan.aipod@seieig.or.jp

ETRF MANAGEMENT STANDARD

Chapter 1: Outline of ETRF management standard

1.1. Objective

The objective of this ETRF Management Standard is to ensure a regular supply of ultrapure dialysis fluid specified in the "Standard on Microbiological Management of Fluids for Hemodialysis and Related Therapies by the Japanese Society for Dialysis Therapy 2008" (3–5). This ETRF Management Standard will enable ETRF manufacturers to produce ETRFs with the required bacterial and ET retentive performance before shipping and will provide dialysis facilities with safe and effective methods for using and managing ETRFs.

1.2. Inclusions and exclusions

This ETRF Management Standard is applicable to ETRFs installed immediately before the dialyzer in the dialysis bedside console of CDDSs or single-patient dialysis machines. Therefore, large-capacity ETRFs installed along a production line of dialysis fluids for multiple patients are excluded. Moreover, ETRFs used as parts of dialysis machines that have been approved as medical devices are also not covered by this ETRF Management Standard because they should have been validated by the manufacturer.

Chapter 2: Normative references

The application of this ETRF Management Standard is based on the following references.

- Akiba T, Kawanishi H, Mineshima M, Masakane I, Tomo T, Kawasaki T, Nishizawa Y. 2008 JSDT Microbiological quality standard for dialysis fluids and standard for testing performance of blood purification devices. *J Jpn Soc Dial Ther* 2008; **41**: 159–167 (in Japanese). (3)
- Akiba T, Kawanishi H. [Supplementary article.] J Jpn Soc Dial Ther 2008. 41:457–9. (In Japanese) (4)
- Kawanishi H, Akiba T, Masakane I, Tomo T, Mineshima M, Kawasaki T, Hirakata H, Akizawa T. Standard on microbiological management of fluids for hemodialysis and related therapies by the Japanese Society for Dialysis Therapy 2008. *Ther Apher Dial* 2009; **13**: 161–166. (5)
- Akizawa T, Kawanishi H, Hirakata H, Minakuchi J, Kawasaki T. Recommendations for items required regarding artificial kidneys intended for online HDF and HF therapies and quality standard for online-prepared substitution fluids. *J Jpn Soc Dial Ther* 2009; **42**: 419–422 (in Japanese). (6)

Chapter 3: Terms and definitions

Terms and definitions are summarized in Table 1.

TABLE 1. *Terms and definitions*

CDDS	Central dialysis fluid delivery system
CFU	Colony forming unit (a unit indicating the number of bacterial colonies, CFU/mL)
EDTA	European Dialysis and Transplant Association
ET	Endotoxin
ETRF	Endotoxin retentive filter
HD	Hemodialysis
HDF	Hemodiafiltration
HF	Hemofiltration
ISO	International Organization for Standardization
JIS	Japanese Industrial Standard
JSDT	The Japanese Society for Dialysis Therapy
LRV	Logarithmic reduction value
R2A	Reasoner's Agar No. 2 (R2A medium, a type of agar medium for bacterial cultivation)
RO	Reverse osmosis
TGEA	Tryptone glucose extract agar (TGEA medium, a type of agar medium for bacterial cultivation)
UPD	Ultrapure dialysis fluid

Chapter 4: Summary of JSDT microbiological quality standard for dialysis fluids

This chapter explains the Standard on microbiological management of fluids for HD and related therapies by the Japanese Society for Dialysis Therapy 2008 (3–5) in order to help readers understand the ETRF Management Standard.

4.1. Microbiological quality management of dialysis fluids based on validation

The microbiological quality of dialysis fluids shall be managed using a validated system for producing dialysis fluids.

Rationale

Dialysis facilities also serve as the manufacturers of dialysis fluid and shall be responsible for ensuring the quality of dialysis fluid. Therefore, dialysis facilities must assign persons in charge of product quality. Each step in the purification process of dialysis fluid must be managed through a validated system to maintain quality.

4.2. Microbiological quality standard for dialysis fluids

Water and fluids used in dialysis therapies are categorized into the following four groups, each of which has to satisfy certain criteria in terms of bacterial count and ET level to maintain microbiological quality.

- Dialysis water

Bacteria: <100 colony forming units (CFU)/mL
ET: <0.050 EU/mL

- Standard dialysis fluid

Bacteria: <100 CFU/mL
ET: <0.050 EU/mL

- Ultrapure dialysis fluid

Bacteria: <0.1 CFU/mL
ET: <0.001 EU/mL (lower than the detection limit)

- Online-prepared substitution fluid

Sterile and nonpyrogenic
Bacteria: $<10^{-6}$ CFU/mL
ET: <0.001 EU/mL (lower than the detection limit).

Rationale

The action level (i.e. the contamination level at which steps should be taken to interrupt the trend toward higher, unacceptable levels) shall be set depending on the conditions of each facility. Typically, 50% of the maximum allowable level is recommended by the JSDT. However, the action level cannot be set for a bacterial count lower than 10^{-6} CFU/mL and an ET level lower than 0.001 EU/mL (lower than the detection limit). The standard values for the online-prepared substitution fluids are theoretical values obtained by validation. Therefore, the dialysis fluid immediately before the final ETRF must be the ultrapure dialysis fluid (UPD). An ETRF with logarithmic reduction values (LRVs) of 3 for ET and 7 for bacteria shall be installed in the final step of preparing substitution fluid to ensure that the fluid will have a bacterial count lower than 10^{-6} CFU/mL.

4.3. Indications for dialysis systems based on quality of dialysis fluids

4.3.1. Standard dialysis fluid

- Standard dialysis fluid is the minimum requirement for dialysis therapies.

4.3.2. Ultrapure dialysis fluid

- Ultrapure dialysis fluid is used to prepare substitution fluid online.
- Ultrapure dialysis fluid is basically recommended to be used in all HD therapies, including dialysis systems that actively use dialysis fluid and back filtrate (e.g. fully automated dialysis systems), push-pull hemodiafiltration (HDF) systems, and internal-filtration-enhanced dialysis.

4.3.3. Online-prepared substitution fluid

- Online-prepared substitution fluid is used in online HDF/Hemofiltration (HF).

4.4. Standard for quality management of dialysis fluids

4.4.1. Test for compliance

- ET: Limulus amoebocyte lysate (LAL) assay (turbidimetric or colorimetric tests)
- Bacteria: Detection using culture media

Culture media: Basically, agar plate media, such as R2A or TGEA media, or media that have been confirmed to have an equivalent sensitivity

Cultivation conditions (R2A and TGEA): 17–23°C for 7 days

4.4.2. Sampling sites

- Dialysis water: RO equipment outlet
- Dialysis fluid: Dialyzer inlet line

Note: Dialyzer outlet line in the case of push-pull HDF systems

- Online-prepared substitution fluid: Substitution fluid line

4.4.3. Sampling dates

Sampling should be carried out before the start of dialysis performed after the maximal treatment interval (commonly Monday for patients treated on a Monday–Wednesday–Friday schedule).

4.4.4. Frequency of monitoring of ET and bacteria

- Dialysis water: Every 3 months (when the required values are satisfied)

Monitoring should be carried out once a month when the required values for dialysis water are not satisfied.

- Standard dialysis fluid: Monthly

Standard dialysis fluid in at least two dialysis machines should be monitored monthly in turn so that each machine is tested at least once a year.

- Ultrapure dialysis fluid used for conventional dialysis (including internal-filtration-enhanced dialysis): The same frequency as for standard dialysis fluid
- Ultrapure dialysis fluid used for preparing substitution fluid online or in dialysis systems actively using back filtrate: Every 2 weeks until the system stabilizes

After the system has been validated by the person in charge of preparing dialysis fluid, ultrapure dialysis fluid in at least two dialysis machines should be monitored monthly in turn so that each machine is tested at least once a year.

- Online-prepared substitution fluid:

ET: Every 2 weeks until the system stabilizes

After the system has been validated by the person in charge of preparing dialysis fluid, online-prepared substitution fluid from each dialysis machine should be monitored monthly.

Bacteria:

Dialysis fluid immediately before the final ETRF should maintain their microbiological quality at values for ultrapure dialysis fluid. The dialysis fluid immediately before the final ETRF and the online prepared substitution fluids in dialysis machines should be monitored every 2 weeks until the system stabilizes. After the system has been validated by the person in charge of preparing dialysis fluid, the above fluids in at least two dialysis machines should be monitored monthly in turn so that each machine is tested at least once a year.

Chapter 5: Definition of ETRFs and required performance

5.1. Endotoxin retentive filters generally refer to the filters used to remove ET and microorganisms from dialysis fluid for their purification. This ETRF Management Standard addresses the management of an ETRF installed immediately before the dialyzer in a bedside dialysis console.

5.2. An ETRF should maintain LRVs of at least 2 for ET and at least 4 for bacteria to obtain ultrapure dialysis fluid, assuming that the dialysis fluid before the ETRF maintains its microbiological quality at values for standard dialysis fluid specified in the JSDT Standard.

Rationale

In this ETRF Management Standard, ETRFs refer to the filters used to purify dialysis fluids and to prevent the passage of bacteria and ET. The JSDT Standard is consistent with the ISO Standard although there are differences in the definition of ETRFs (see Supplementary note A in Chapter 8). Note that ETRFs are defined in 5.1 of the ETRF Management Standard as follows: "ETRFs generally refer to the filters used to remove ET and microorganisms from dialysis fluids for their purification."

In this ETRF Management Standard, it is assumed that facilities have already attained the allowable levels for dialysis water and standard dialysis fluid (i.e. bacterial count < 100 CFU/mL and ET level < 0.050 EU/mL), as determined by the JSDT and given in 4.2. Therefore, the ETRF installed in the final line should produce dialysis fluids with allowable levels for ultrapure dialysis fluid (i.e. bacterial count < 0.1 CFU/mL and ET level < 0.001 EU/mL [lower than the detection limit]). Considering the safety factor, the required performance of ETRF is specified in 5.2 ("An ETRF should maintain LRVs of at least 2 for ET and at least 4 for bacteria to obtain ultrapure dialysis fluid, assuming that the dialysis fluid before the ETRF maintains its microbiological quality at values for standard dialysis fluid specified in the JSDT Standard."). ETRFs have been developed to separate ET and bacteria from dialysis fluids and remove these contaminants based on the principles of filtration and adsorption or adherence. Therefore, the evaluation of ETRF performance should be based on conventional separation principles of ultrafiltration and microfiltration. In addition to separation capability, the durability of ETRFs should also be taken into consideration.

Chapter 6: Methods for determining ETRF performance

6. Voluntary Standard Established by Japan Medical Devices Manufacturers Association

6.1. The performance of ETRFs should be tested in terms of their capability to remove bacteria and ET.

6.2. *Brevundimonas diminuta* should be used in the bacterial test. Culture conditions should be the same as those specified in JIS K 3823. The Japanese Pharmacopoeia Reference Standard ET or certified ET should be used in the ET test.

6.3. Bacterial and ET test samples should be prepared so that they have a bacterial count of at least 10^6 CFU/mL and an ET level of at least 10 EU/mL, respectively.

6.4. The retentive performance of an ETRF should be expressed as LRV, which is given as an integer (e.g. "LRV of at least 00").

Rationale

The methods for determining bacterial and ET retentive performance of ultrafiltration modules are specified in JIS (bacteria, JIS K 3823 (7); ET, JIS K 3824 (8)). Following these standards, the methods for determining the bacterial and ET retentive performance of ETRFs are summarized in the Voluntary Standards by the Japan Medical Devices Manufacturers Association and were published on its website in July 2010 (see Supplementary note B in Chapter 8).

Brevundimonas diminuta (ATCC 19146 or NBRC 14213) should be used in the bacterial test. Culture conditions for the bacterial test solutions prepared before the test and the solution sampled in the test should be the same as those specified in JIS K 3823. ET used in the test should be the Japanese Pharmacopoeia Reference Standard ET or commercially available ET certificated using the Reference Standard ET.

For both the bacterial and ET tests, test solutions should be prepared by diluting the original concentrated solutions to the predetermined concentration.

The performance of an ETRF is expressed as LRV. For bacteria, LRV is given by the common logarithm of the ratio of the bacterial count in a 1000-mL test

solution to that in a 1000-mL filtrate. For ET, LRV is given by the common logarithm of the ratio of the ET level in a test solution to that in a filtrate. LRV should be expressed as an integer for both bacteria and ET.

LRV is highly dependent on the bacterial count or ET level in the test solution, as is clear from the equation for calculating LRV. Therefore, it is meaningless to compare the performance of ETRFs on the basis of the calculated LRV. However, LRV can be used as an index that indicates whether the ETRF has a certain level of performance. Specifically, the bacterial count and ET level in a test solution are specified so that the LRVs are at least 8 for bacteria and at least 3 for ET, considering slight variations in the procedure used to prepare test solutions.

Chapter 7: Standard for handling ETRFs

7.1. Installation of ETRFs

7.1.1. Manufacturers' instructions for the ETRF installation shall be observed.

7.1.2. To obtain ultrapure dialysis fluid sample, the dialysis fluid at the inlet of the ETRF should be maintained within the allowable level for standard dialysis fluid specified in the JSDT Standard.

Rationale

As of December 2010, several types of ETRF with different performances and usages were available in Japan (9). Users must read through the manufacturer's instructions for each product because information differs among products for various aspects including installation, disinfection agents, and duration of use. Notes on the fundamental rules of ETRF installation are added below.

1. There are two filtration methods: inside-out and outside-in filtration. The manufacturer's instructions for ETRF installation shall be followed.
2. Some ETRFs may only be connected to the dialysis machine with special holders or a special connecting device produced by the manufacturer. ETRFs shall be installed in accordance with the manufacturer's instructions.

Currently, commercially available ETRFs have LRVs of at least 3 for ET and at least 7 for bacteria (9). Therefore, when an ETRF is installed and the LRVs for standard dialysis fluid specified in the 2008 JSDT Standard (5) (i.e. bacterial count < 100 CFU/mL and ET level < 0.050 EU/mL) are maintained at the inlet of the ETRF, it is possible to achieve the allowable levels for ultrapure dialysis fluid (i.e. bacterial count < 0.1 CFU/mL and ET level < 0.001 EU/mL). Basically, these values are recommended at the inlet line of the dialyzer after filtration for all HD therapies in accordance with the abovementioned Standard.

7.2. Cleaning and disinfection of ETRFs

7.2.1. Dialysis machines shall be cleaned and disinfected following the methods recommended by the manufacturer.

7.2.2. The inside and outside of hollow fibers shall be cleaned and disinfected during the cleaning and disinfection of ETRFs.

7.2.3. When cleaning and disinfection methods other than those recommended by the manufacturer are used, the durability of ETRFs shall be tested at each facility to confirm their safety.

Rationale

The performance of ETRFs is affected by factors related to cleaning and disinfection, such as the type of cleaning and disinfection agents, concentration, and the duration of disinfection. Hence, users must adopt the cleaning and disinfection methods recommended by the manufacturer. The manufacturer measures the LRVs of ETRFs that have been cleaned and disinfected by the recommended methods in durability tests. The recommended duration of use is based on an appropriate safety margin, thus guaranteeing the ETRF safety.

Because ETRFs are generally used for dead-end filtration, the bacterial count and ET level in the filtered fluid may become high when bacteria and ET accumulate on the membrane surface. Therefore, ETRFs should be disinfected and flushed to deactivate and remove bacteria and ET. Both sides of hollow fibers in ETRF should be flushed with a sufficient amount of water at a high flow rate during cleaning and disinfection.

When cleaning and disinfection methods other than those recommended by the manufacturer are adopted, the Dialysis Equipment Safety Management Committee shall be responsible for the safety of ETRFs. To ensure ETRF safety, test results on the persistence of cleaning and disinfection agents and the physical and chemical resistance of ETRFs to the agents should be taken into consideration, including the LRVs obtained in a durability test. It is recommended that cleaning and disinfection methods should be assessed by the Dialysis Equipment Safety Management Committee in consultation with the manufacturers of cleaning and disinfection agents and ETRFs.

7.3. Duration of ETRF use

7.3.1. The duration of ETRF use recommended by the manufacturer shall be observed when the dialysis

fluid flowing into the ETRF cannot be maintained within the allowable levels for standard dialysis fluids as specified in the JSDT Microbiological Quality Standard for Dialysis Fluids.

7.3.2. The duration of ETRF use should be determined by the Dialysis Equipment Safety Management Committee when the standard dialysis fluid falls within allowable levels.

Rationale

Endotoxin retentive filter manufacturers carry out durability tests on ETRFs and recommend the duration of their use by considering an appropriate safety margin that guarantees the safety of ETRFs. However, leakage due to enlarged pores on the membrane cannot be detected in a leakage test, which is the sole monitoring method for ETRFs. Therefore, the duration of use recommended by the manufacturer must be observed when dialysis fluid at the inlet of the ETRF cannot be maintained within the allowable levels for standard dialysis fluids (ET level < 0.05 EU/mL, bacterial count < 100 CFU/mL).

When facilities that maintain at least the allowable levels for standard dialysis fluids at the inlet of ETRF use ETRFs longer than recommended by the manufacturer, the Dialysis Equipment Safety Management Committee shall be responsible for the safety of the ETRFs. To ensure ETRF safety, it is recommended that facilities not only maintain the required LRVs by performing a durability test under their own conditions of ETRF use but also examine the duration of use in consultation with the manufacturer with consideration for the physical and chemical durability of materials used for the membranes and module cases.

7.4. Monitoring and durability test of ETRFs

7.4.1. When ETRFs are used under conditions other than those recommended by the manufacturer, their durability shall be tested at each facility to confirm their safety.

7.4.2. The durability test of ETRFs at each facility shall be performed using randomly selected samples at the time of exchanging ETRFs.

7.4.3. The durability test of ETRFs at each facility shall be performed by following the method described in 6.1. When ET in raw water at each facility is used for test solutions, the solutions should be prepared to have an ET level of at least 10 EU/mL.

7.4.4. ETRFs used to obtain ultrapure dialysis fluid shall satisfy the requirements described in 5.2.

Rationale

When facilities use disinfection agents and methods other than those recommended by the manufacturer, the durability of ETRFs must be tested at each facility. Hence, a method that can be continuously adopted at minimal cost is preferable. Although the reference standard ET used in the test of ETRF performance originates from *Escherichia coli*, the bacteria contained in dialysis fluids at facilities are mostly *Pseudomonas* species. Therefore, a durability test using ET from raw water at each facility may be possible; this is cost-effective and can be easily performed routinely. Note that the aggregation state and level of ET in this method may be different from those in the test method described in Chapter 6.

7.5. Records of ETRF management

7.5.1. When ETRFs are used, the cleaning and disinfection information shall be recorded including the type, concentration, frequency, duration and temperature of each cleaning and disinfection agent used, as well as other necessary factors. These records should be kept for at least 3 years.

Rationale

Central dialysis fluid delivery systems account for the majority of dialysis systems in Japan. This ETRF Management Standard targets the ETRFs installed immediately before the dialyzer in bedside dialysis consoles of CDDSs and single-patient dialysis machines. Hence, it is necessary to record data related to the entire cleaning and disinfection system, such as the type, concentration, frequency, duration and temperature of each cleaning and disinfection agent used, as well as data on the management of ETRFs (i.e. ETRF Lot No., installation date, and disposal date). These data and related documents must be kept for at least 3 years after their preparation or one year after the expiration of the ETRFs in accordance with the 2008 JSDT Microbiological Quality Standard for Dialysis Fluids. However, according to the regulations of the Japanese Medical Service Law, hospitals are obliged to follow a 2-year retention period for records related to diagnosis, such as test records, images, and surgical findings other than clinical records. Persons in charge of managing the safety of dialysis fluids should keep and manage the records and report them to the Dialysis Equipment Safety Management Committee.

Chapter 8: Supplementary notes

A. Rationale for development of ETRF Management Standard

A1. History of ultrapure dialysis fluid and ETRF development

Since the report by Baz et al. (10) in 1991, it has been recognized that bacterial contamination of dialysis

fluid is a cause of not only dialysis hypotension but also long-term complications such as dialysis-related amyloidosis. They installed three ultrafiltration filters to produce ultrapure dialysis fluid, thus succeeding in markedly delaying the onset of dialysis-related amyloidosis. Since then, dialysis researchers have recognized the importance of preventing the bacterial contamination of dialysis fluid and the necessity of ETRFs for the effective elimination of contaminants.

Baz et al. (10) referred to the dialysis fluid that passed through the three ETRFs as UPD but did not accurately define the quality required for UPD. In contrast, Ledebo et al. (11) determined the theoretical assurance level for online-prepared substitution fluids to be 10^{-6} CFU/mL during the course of development of online HDF therapy. To achieve this assurance level, they specified the ET level and bacterial count required for the dialysis fluid before the final ETRF to be lower than the detection limit and 10^{-1} CFU/mL, respectively, which were defined as the allowable levels for ultrapure dialysis fluid. Because conventional ETRFs usually have LRVs of 3 for ET and 7 for bacteria, the assurance level of bacteria for online-prepared substitution fluids becomes 10^{-8} CFU/mL, and the quality of the replaced fluids can be theoretically maintained even when an unexpected contamination occurs. Since then, the definition of UPD has been established as a dialysis fluid with an ET level lower than the detection limit and a bacterial count lower than 10^{-1} CFU/mL. However, these assurance levels were considered as merely conceptual requirements for preparing online substitution fluids. Ultrapure dialysis fluid was recognized as an important requirement for dialysis fluids only after the EDTA guidelines were published in 2002 (12). Ultrapure dialysis fluid was also mentioned in the JSDT Microbiological Quality Standard for Dialysis Fluids (3–5). Ledebo et al. assumed that the final ETRF used for preparing online substitution fluid would be disposable and would constantly maintain LRVs for ET and bacteria equivalent to those of a new ETRF. Nevertheless, ETRFs used to prepare ultrapure dialysis fluid are reused in all countries, thus requiring an appropriate ETRF management standard to regulate the preparation of ultrapure dialysis fluid.

A2. Requirement of ETRF Management Standard for CDDSs

Central dialysis fluid delivery systems generally consist of devices fabricated by one or multiple manufacturers and have been considered to insufficiently suppress microbiological contamination. With this background, the JSDT started investigating the status of bacterial contamination of dialysis fluids at the end of 2006. The results revealed that 89% of the surveyed dialysis facilities attained an ET level of less than 50 EU/mL in dialysis fluid, indicating that the quality of dialysis fluid in CDDSs in Japan was very high on a global scale (2). However, only 27.3% of the surveyed dialysis facilities carried out a test to determine the ET level at least monthly, which was recommended in the new ISO standard under preparation at that time. Moreover, only 37.1% of the dialysis facilities carried out a test to determine the bacterial count, and the test was carried out less than once a month. However, these results have improved over time. In the 2006 survey, ETRFs were also investigated. ETRFs were used in 78.5% of dialysis facilities, and 53.4% of the dialysis bedside consoles across Japan were equipped with ETRFs. The ET level and bacterial count in dialysis fluid for facilities that had installed ETRFs tended to be lower than those for facilities not using ETRFs, indicating the effectiveness of ETRFs for purifying dialysis fluid. However, some facilities not using ETRFs also reported an ET level lower than the detection limit and a bacterial count lower than 0.1 CFU/mL, whereas contamination was observed in some facilities using ETRFs (2). These results indicated that the ETRFs themselves might be the origin of dialysis fluid contamination at some facilities, requiring an appropriate ETRF management standard.

A3. Opinions of JSDT on ETRFs

The JSDT has presented its opinions on ETRFs through reports and recommendations from the Committee of Scientific Academy as listed below.

1. 2008 JSDT Microbiological Quality Standard for Dialysis Fluids and Standard for Testing Performance of Blood Purification Devices. (3–5)

ETRFs that conform to the draft standard for testing ETRF performance specified by the Japan Medical Devices Manufacturers Association shall be used. The usage and exchange time determined by each manufacturer shall be followed. Otherwise, each facility shall validate the performance and durability of ETRFs, keep records, and regularly report them to the persons in charge of medical equipment safety management. In the 2008 Standard, the microbiological quality of dialysis fluids is specified as follows.

- Dialysis water
  Bacteria: <100 CFU/mL
  ET: <0.050 EU/mL
- Standard dialysis fluid
  Bacteria: <100 CFU/mL
  ET: <0.050 EU/mL

- Ultrapure dialysis fluid
  Bacteria: <0.1 CFU/mL
  ET: <0.001 EU/mL (lower than the detection limit)
- Online-prepared substitution fluid

Sterile and nonpyrogenic
Bacteria: $<10^{-6}$ CFU/mL
ET: <0.001 EU/mL (lower than the detection limit)

Currently, generally used ETRFs have LRVs of at least 3 for ET and 7 for bacteria (9). Therefore, when the allowable levels for dialysis water are maintained, ultrapure dialysis fluid can be theoretically achieved by installing an ETRF. In this case, online substitution fluid can be prepared by installing the final ETRF.

2. Recommendations for Required Items regarding Artificial Kidneys Intended for Online HDF and HF Therapies and Quality Standard for Online-Prepared Substitution Fluids (3–5)

Endotoxin retentive filters are defined as filters used to purify dialysis water and dialysis fluids. ETRFs are also used to prepare substitution fluid online. Before use, the LRVs of the ETRF must be confirmed by determining the bacterial retentive performance using the method described in JIS K 3823 (7) and the ET retentive performance using the method described in JIS K 3824 (8).

A4. Opinions of ISO on ETRFs

The ISO has presented its opinions on ETRFs in ISO 23500 (13) as summarized below.

1. ISO 23500 3.22 endotoxin-retentive filter (Definition of ETRF)

Membrane filter used to remove ET and microorganisms from dialysis water or dialysis fluid

NOTE 1: The performance of an ETRF is usually expressed as LRV.

NOTE 2: ETRFs may be configured in the cross-flow or dead-end mode. Some ETRFs also remove ET by adsorption.

2. ISO23500 7.3.9 Monitoring of endotoxin-retentive filters (Evaluation of ETRF performance)

- The performance of ETRFs can be monitored by testing the fluid that is directly exiting from the filter for bacteria and ET.
- The fundamental characteristics (retentive performance) and fouling characteristics of ETRFs should be tested.
- One suitable means of testing is to monitor the pressure drop across the filter at a given product fluid flow rate. Alternatively, product fluid flow rate can be measured at a given pressure drop.
- Such monitoring can indicate the time for replacing or cleaning ETRFs. Monitoring is also necessary to ensure that the device is being operated in accordance with the manufacturer's instructions.
- Endotoxin retentive filters operated in the cross-flow mode should also be monitored in terms of the flow rate of fluid being directed to the drain at a given pressure drop.
- Results of pressure measurements, bacterial count, and ET levels should be recorded on a log sheet.

3. ISO23500 B.2.9 Endotoxin-retentive filters (Installation of ETRFs)

- Endotoxin retentive filters should be placed in dialysis water systems at locations downstream of deionization (RO) and also in the dialysis fluid line as a final barrier.
- Endotoxin-retentive membranes are typically in either a spiral-wound configuration or a hollow-fiber configuration. Spiral-wound ETRFs are usually operated in the cross-flow mode with a fraction of the feed water being forced through the membrane and the remainder being directed along the membrane surface to drain. Hollow-fiber ETRFs are operated in the cross-flow or dead-end mode.

B. Voluntary Standard on Methods for Determining ETRF Performance by Japan Medical Devices Manufacturers Association

The Japan Medical Devices Manufacturers Association has examined the methods used to determine the performance of ETRFs used as part of dialysis systems. The Japan Medical Devices Manufacturers Association has formulated the following Voluntary Standard on the methods for determining the bacterial and ET retentive performance with the aim of purifying dialysis fluids.

B1. Methods for determining bacterial retentive performance of ETRFs

1. Scope

This Standard specifies the methods for determining the bacterial retentive performance of ETRFs using *B. diminuta*. ETRFs are used as components of dialysis bedside consoles, single-patient dialysis machines, and multipurpose dialysis systems to remove bacteria from dialysis water or dialysis fluids.

Note 1: The following standard is cited in this Standard.

- JIS K 3823 (Testing methods for determining bacterial rejection of ultrafiltration modules) (7)

2. Terms and definitions

The terms and definitions used in this Standard are as follows.

2.1. Endotoxin-retentive filters (ETRFs)

Filters used to remove bacteria and ET from dialysis water or dialysis fluids

2.2. Bacterial retentive performance

The performance is expressed as the logarithmic reduction value (LRV), which is nondimensional and defined by

$$LRV \text{ for bacteria} = \log_{10}(A/B)$$

A: Viable test bacterial count in 1000 mL test solution (CFU)

B: Viable test bacterial count in 1000 mL filtrate (CFU)

3. Test bacterium, liquid, and media

3.1. *B. diminuta* ATCC 19146 or NBRC 14213 should be used as the test bacterium.

3.2. Water: Japanese Pharmacopoeia water for ET test or filtrate water that has been subjected to ion exchange and then passed through a membrane with a pore diameter less than or equal to the membrane of the target ETRF being tested

3.3. Agar media used for measuring viable bacterial count (Note: Commercially available dry powder media can also be used for preparation)

The agar media used for measuring the viable bacterial count should be as follows.

3.3.1. Soybean casein digest agar medium: This should be prepared in accordance with the manufacturer's instructions.

3.3.2. Conventional agar medium: This should be prepared in accordance with the manufacturer's instructions.

4. System for testing bacterial retentive performance

The system for testing bacterial retentive performance should be as follows.

4.1. System configuration and components (Fig. 1)

The system should comprise a tank, a pump, an ETRF, tubes connecting these components, instrumentation devices including a pressure gauge, a thermometer, a flowmeter, and valves used for adjusting pressure (Note: A bypass line can also be set in the system to adjust the amount of water supplied to the ETRF or mix the test bacterial solution uniformly.) All components should be made of corrosion-resistant materials, such as stainless steel or plastics.

The system should be easily rinsed, cleaned, and disinfected, and water should be easily drained from the tubes. The system should also prevent contamination by bacteria from the outside and keep the test bacterium inside. Figure 1 shows an example of the system configuration.

5. Preparation

5.1. Preparation of test bacterial solutions

5.1.1. Prepare a concentrated bacterial solution as described in JIS K 3823.

5.1.2. Put water (specified in 3.2.) into a predetermined tank followed by the concentrated bacterial solution, thus obtaining the test bacterial solution.

5.1.3. Prepare the test bacterial solution to have a bacterial count of 10^6 CFU/mL or higher.

6. Procedure

The test procedure is given below.

6.1. Put water (specified in 3.2.) into a tank.

6.2. Operate the pump and set the test conditions as follows.

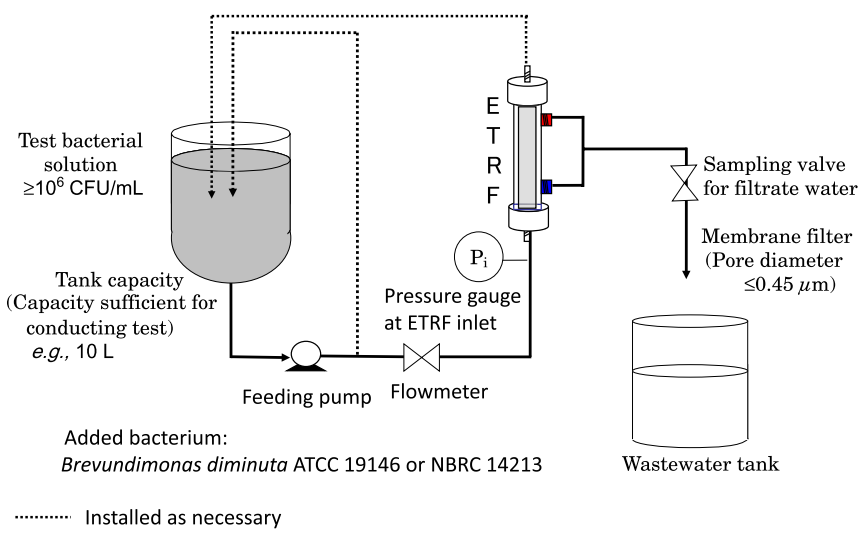

FIG. 1. Example of system configuration for measuring bacterial logarithmic reduction value (LRV)

6.2.1. The temperature at the ETRF inlet should be set between 20 and 30°C. Temperature can be measured within the tank.

6.2.2. The mean pressure difference between the inlet pressure and filtrate pressure at the outlet of the ETRF is given by the following equation and should be below the maximum allowable pressure.

$$P_{mtm} = P_i - P_f$$

P_{mtm}: mean pressure difference (kPa)
P_i: pressure at ETRF inlet (kPa)
P_f: filtrate pressure at ETRF outlet (kPa)

P_f can be considered to be 0 kPa when the end of the circuit for the filtrate is exposed to the atmosphere.

6.2.3. The filtrate flow rate should be set at 500 mL/min, which is the standard flow rate for dialysis fluid or within ± 10% of the maximum allowable flow rate for the ETRF. The flow rate can be measured at the outlet of the ETRF.

6.3. Perform a control test: Start the filtration of water in the tank. After at least 1000 mL of water has been filtered, obtain a 1000 mL sample of the filtrate and measure the bacterial count in the sample.

6.4. Add the concentrated bacterial solution to the tank and adjust the solution in the tank to obtain a bacterial count of 10^6 CFU/mL or higher.

6.5. When at least 10 min has passed after the addition of the concentrated bacterial solution, take an approximately 10 mL sample of the test bacterial solution. (Note: At least 10 min is required to mix the test bacterial solution uniformly. The solution in the tank should be circulated using a bypass line or stirred by other means. The filtrate should be sampled after the fluid in the filtrate line has been completely replaced by the filtrate.) Then, filter the test bacterial solution in the dead-end mode and sample 1000 mL of the filtrate to measure the bacterial count in the sample (Note: The filtrate should be sampled using a sterile container, without rinsing the container with the solution, to minimize the time during which the sampled filtrate is exposed to the atmosphere. Note: If the bacterial count cannot be measured immediately after sampling, store the sampled filtrate at 5 ± 1°C and start the measurement within 6 h).

6.6. Measure the viable bacterial count in the sampled filtrate.

In the measurement of viable bacterial count, the membrane filtration (MF) method using a filter with a pore diameter smaller than 0.45 μm is adopted. The used MF itself should then be incubated on a soybean casein digest agar medium or conventional agar medium at 30 ± 2°C for 48 h.

7. Calculation

The bacterial retentive performance of ETRFs should be calculated using the following equation.

$$LRV \text{ for bacteria} = \log_{10}(A/B)$$

A: Viable test bacterial count in 1000 mL test solution (CFU)

B: Viable test bacterial count in 1000 mL filtrate (CFU)

(Note: LRV for bacteria depends on the test conditions, particularly the bacterial count in the test solution.)

Remark: When the bacterial count is 0 CFU, it is possible to make $LRV \geq \log_{10}(A/B)$ by assuming $B = 1$.

8. Test items to be reported

The following items should be reported.

8.1. Endotoxin retentive filters used in the test

(a) Shape and type
(b) Product number
(c) Manufacturer's name
(d) Manufacturer's serial number

8.2. Test conditions

(a) Pressure on filter inlet side
(b) Filtrate pressure on filter outlet side
(c) Temperature of test bacterial solution
(d) Flow rate

8.3. Bacterial count

Bacterial counts obtained in the control test, test bacterial solution, and filtrate specified in 6.

8.4. Bacterial retentive performance

LRV for bacteria

B2. Methods for determining ET retentive performance of ETRFs

1. Scope

This Standard specifies the methods for determining the ET retentive performance of ETRFs using test solutions that contain ET originating from *E. coli*. ETRFs are used as part of dialysis bedside consoles, single-patient dialysis machines, and multipurpose dialysis systems to remove ET from dialysis water or dialysis fluids.

Note: The following standard is cited in this Standard.

- JIS K 3824 (Testing methods for determining endotoxin rejection of ultrafiltration modules) (6)

2. Terms and definitions

The terms and definitions used in this Standard are as follows.

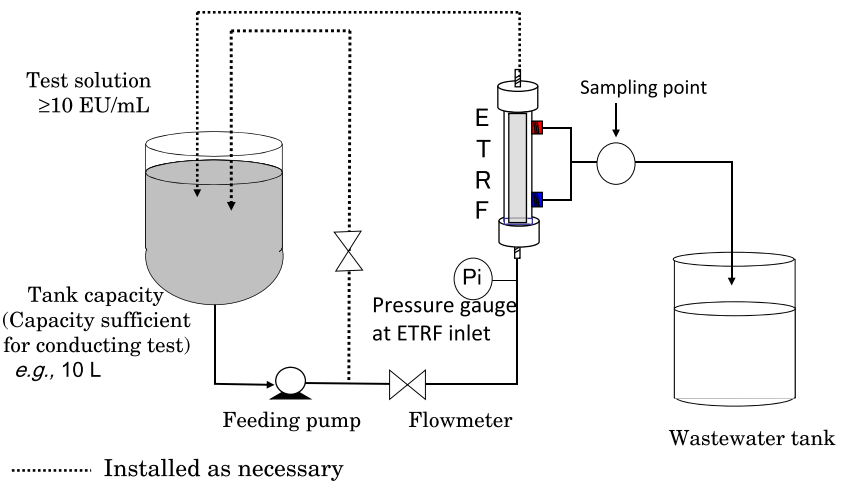

FIG. 2. Example of system configuration for measuring endotoxin logarithmic reduction value (LRV)

2.1. Endotoxin retentive filters (ETRFs)

Filters used to remove bacteria and ET from dialysis water or dialysis fluids

2.2. ET retentive performance

The performance is expressed as the logarithmic reduction value (LRV), which is nondimensional and defined by

$$LRV \text{ for ET} = \log_{10}(F/G)$$

F: ET level in test solution (EU/mL)

G: ET level in filtrate (EU/mL)

2.3. Concentrated ET solution

Solution with an ET level of at least 10^2 EU/mL obtained using Japanese Pharmacopoeia Reference Standard ET or commercially available ET certificated using the Reference Standard ET

2.4. Test ET solution

A solution with an ET level of at least 10 EU/mL that is obtained by diluting concentrated ET solution with water

3. Test ET and liquid

3.1. ET: Japanese Pharmacopoeia Reference Standard ET or commercially available ET certificated using the Reference Standard ET

3.2. Water: Japanese Pharmacopoeia water for ET test or filtrate water that has been subjected to ion exchange then passed through a membrane with a pore diameter less than or equal to the membrane of the target ETRF

4. System for testing ET retentive performance

The system for testing ET retentive performance should be as follows.

4.1. System configuration and components (Fig. 2)

The system should comprise a tank, a pump, an ETRF, tubes connecting these components, instrumentation devices including a pressure gauge, a thermometer, a flowmeter, and valves used for adjusting pressure. (Note: A bypass line can also be set in the system to adjust the amount of water supplied to the ETRF or mix the test ET solution uniformly.) All components should be made of corrosion-resistant materials, such as stainless steel or plastics.

The system should be easily rinsed, cleaned, and disinfected, and water should be easily drained from the tubes. The system should also prevent contamination by bacteria from the outside and keep the test solution inside. Figure 2 shows an example of the system configuration.

5. Preparation

5.1. Preparation of ET solution

The ET solution used in the test should be prepared as follows.

5.1.1. Use Japanese Pharmacopoeia Reference Standard ET or commercially available ET certificated using the Reference Standard ET.

5.1.2. Prepare the test solution to have an ET level of at least 10 EU/mL.

5.1.3. Sample an aliquot of the test solution to measure ET level by an optical measurement method (turbidimetric or colorimetric test) specified in 4.01 of the Japan Pharmacopoeia general test methods, ET Test Method.

6. Procedure

The test procedure is given below.

6.1. Put water (specified in 3.2) into a tank.

6.2. Operate the pump and set the test conditions as follows.

6.2.1. The temperature at the ETRF inlet should be set between 20 and 30°C. Temperature can be measured within the tank.

6.2.2. The mean pressure difference between the inlet pressure and filtrate pressure at the outlet of the ETRF is given by the following equation and should be below the maximum allowable pressure.

$$P_{mtm} = P_i - P_f$$

P_{mtm}: mean pressure difference (kPa)
P_i: pressure at ETRF inlet (kPa)
P_f: filtrate pressure at ETRF outlet (kPa)

P_f can be considered to be 0 kPa when the end of the circuit for the filtrate is exposed to the atmosphere.

6.2.3. The filtrate flow rate should be set at 500 mL/min, which is the standard flow rate for dialysis fluid, or within ± 10% of the maximum allowable flow rate for the ETRF. The flow rate can be measured at the outlet of the ETRF.

6.3. Perform a control test: Start the filtration of water in the tank. After at least 1000 mL of water has been filtered, obtain a filtrate sample of approximately 10 mL (Note: A container suitable for sampling is one that can be dry-heat sterilized at 250°C for at least 30 min or a commercially available ET-free container) and measure the ET level in the sample.

6.4. After the control test, add the concentrated ET solution to the water in the tank, thus obtaining the test ET solution.

6.5. When at least 10 min has passed after the addition of the concentrated ET solution (Note: At least 10 min is required to mix the test ET solution uniformly. The solution in the tank should be circulated using a bypass line or stirred by other means. The filtrate should be sampled after the fluid in the filtrate line has been completely replaced by the filtrate) sample approximately 10 mL of the test ET solution. Then filter the test ET solution in the dead-end mode and sample approximately 10 mL of the filtrate.

The samples obtained as described in 6.3 and 6.5 should be immediately subjected to an optical measurement method (turbidimetric or colorimetric test) as specified in 4.01 of the Japan Pharmacopoeia general test method, ET Test Method. (Note: If the measurement cannot be performed immediately after sampling, store the samples below 5°C and start the measurement within 6 h).

7. Calculation

The ET retentive performance of ETRFs should be calculated using the following equation.

$$LRV \text{ for ET} = \log_{10}(F/G)$$

F: ET level in test solution (EU/mL)
G: ET level in filtrate (EU/mL)

(Note: *LRV* for ET depends on the test conditions, particularly the ET level in the test solution).

Remark: When G is lower than the detection limit of the *Limulus* reagent, it is possible to make $LRV \geq \log_{10}(F/G)$ by assuming G to be equal to the detection limit.

8. Test items to be reported

The following items should be reported.

8.1. Endotoxin retentive filters used in the test

(a) Shape and type
(b) Product number
(c) Manufacturer's name
(d) Manufacturer's serial number

8.2. Test conditions

(a) Pressure on filter inlet side
(b) Filtrate pressure on filter outlet side
(c) Temperature of test ET solution
(d) Flow rate

8.3. ET level

ET levels obtained in the control test, test ET solution, and filtrate specified in 6.

8.4. ET retentive performance

LRV for ET

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