



PROVINCIAL STANDARDS & GUIDELINES

Dialysate Water System Microbiology & Endotoxin Sampling

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Approved by the BCPRA Hemodialysis Committee

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IMPORTANT INFORMATION




This BCPRA guideline/resource was developed to support equitable, best practice care for patients with chronic kidney disease living in BC. The guideline/resource promotes standardized practices and is intended to assist renal programs in providing care that is reflected in quality patient outcome measurements. Based on the best information available at the time of publication, this guideline/resource relies on evidence and avoids opinion-based statements where possible; refer to www.bcrenalagency.ca for the most recent version.

For information about the use and referencing of BCPRA provincial guidelines/resources, refer to <http://bit.ly/28SFr4n>.



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1.0 SCOPE OF GUIDELINE

This guideline applies to in-centre and community dialysis units (CDUs) that provide hemodialysis (HD) and/or hemodiafiltration (HDF). It is applicable to both adult and pediatric units.

The purpose of this guideline is to support the implementation of common standards and processes for dialysis water systems (portable and facility) microbiology and endotoxin sampling within BC's HD units. It also provides standards and processes for follow-up of test results for which counts/concentrations exceed acceptable limits.

2.0 SUMMARY OF THE LITERATURE & INTERNET

Patients undergoing conventional hemodialysis three times per week are exposed to 300-600 litres of water per week, depending on their prescription (Coulliette, 2013). More than 90% of the dialysate delivered to the dialyzer is water (Layman-Amato, 2013).

Bacterial and/or endotoxin contamination of the dialysis water and/or dialysate can threaten the life and health of an HD patient.

- **Bacterial contamination:** May result in bacteremia and/or chronic inflammation. Chronic inflammation, in turn, contributes to or complicates cardiovascular disease (CVD), the leading cause of death for dialysis patients. Chronic inflammation has also been linked: poor nutritional status,

reduced response to erythropoietin therapy, decline in residual renal function and carpal tunnel syndrome (Coulliette, 2013).

- **Endotoxin contamination:** Fragments of endotoxins in the dialysate bath may pass through the dialyzer membranes and result in symptoms of septicemia or a pyrogenic reaction (Coulliette, 2013).

The source of water used in hemodialysis consists basically of drinking water, purified by various techniques, whose composition and quality depend on its origin. The quality of the water can change from season to season or even day to day (Layman-Amato, 2013). Monitoring of the quality of water used for dialysis is a vital aspect of hemodialysis treatment.

3.0 DEFINITIONS & ABBREVIATIONS

Action level: Concentration of a contaminant at which steps should be taken to interrupt the trend toward higher, unacceptable levels.

Aseptic: The complete absence of living microorganisms (sterile).

Biofilm: Coating on surfaces that consists of microcolonies of bacteria embedded in a protective extracellular matrix. The matrix, a slimy material secreted by the cells, protects the bacteria from antibiotics and chemical disinfectants.

Colony forming unit (CFU): Measure of bacterial or fungal cell numbers that

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theoretically arise from a single cell or group of cells when grown on solid media; a cell or group of cells capable of replicating to form a distinct, visible colony on a culture plate.

Dialysate (standard): Aqueous fluid containing electrolytes, usually buffer and glucose, which is intended to exchange solutes with blood during hemodialysis; also known as dialysis fluid, dialyzing fluid, or dialysis solution.

Dialysis water: Water that has been treated to meet the requirements of the CSA standard and is suitable for HD use in applications.

Disinfection: Destruction of pathogenic and other kinds of microorganisms by thermal or chemical means.

Endotoxin: Major component of the outer cell wall of gram-negative bacteria.

Endotoxin units (EU): Units assayed using the limulus amoebocyte lysate (LAL) test when testing for endotoxins.

HDF: Hemodiafiltration

Hemodialysis (HD): Form of renal replacement therapy in which waste solutes are removed primarily by diffusion from blood flowing on one side of a membrane into dialysis fluid flowing on the other side.

LAL: Limulus amoebocyte lysate

Membrane filtration: Filtration of the sample through a membrane filter with pore diameter 0.45 µm or less. It is used when the sample is to be concentrated to detect low levels of

contamination (usually less than 1 CFU/mL).

Microbial: Referring to microscopic organisms, such as bacteria, fungi, and algae.

Microbial contamination: Contamination with any form of microorganism (e.g., bacteria, yeast, fungi and algae) or with the by-products of living or dead organisms such as endotoxins, exotoxins and cyanobacterial toxins (derived from blue-green algae).

Pour plate: A technique using 15 to 20 mL of molten medium (< 45°C) added to a 1 mL of sample placed in a Petri dish. The sample and medium are carefully mixed by gentle rotation and allowed to set. If 1 mL of sample is used, the detection limit of this technique is 1 CFU/mL.

PSLS: Patient Safety & Learning System (provincial system used to report and learn about patient safety events, near misses and hazards)

Pyrogenic: Producing heat (fever), especially in the body.

R2A: Reasoners 2A

RO: Reverse osmosis

Spread plate: A technique using a pipette to apply 0.2 to 0.5 mL of a sample to a Petri dish containing agar medium and spread over the surface of the agar. The detection limit is 5 CFU/mL when 0.2 mL of sample is used as the inoculum.

TGEA: Tryptone glucose extract agar

4.0 RECOMMENDATIONS

Recommendation #1:

Conduct dialysis water (RO) testing as per the schedule on [Table 1](#) (based on CSA-ISO).

Recommendation #2:

Utilize the standards on [Table 2](#) for acceptable count/concentrate levels (based on CSA-ISO).

Recommendation #3:

Utilize recommended laboratory methods on [Table 3](#) for analyzing samples (based on CSA-ISO).

5.0 PROCEDURE

Biomedical Technologist, Renal Dialysis Technician or Renal Nurse who is trained and has demonstrated competency in dialysis water practices may collect dialysis water samples for microbiology and endotoxin testing and perform the necessary actions should test results exceed action thresholds.

Procedure applies to regular dialysate sampling (recommendation #1, [Table 1](#)). Specific situations may require more stringent monitoring (e.g., pyrogenic reaction).

5.1 Sample Collection

1. Collect samples before, and as close as practicable to, a disinfection procedure.
2. Collect samples when the system is

operating under stable conditions (RO in DIALYSIS mode) representing normal operation for a minimum of 10 minutes - portable RO that recirculates must have clean sampling kit/tap with valve open (water flowing into sink or bucket).

3. Collect samples from:
 - The distal segment of the loop, immediately prior to where water returns to the RO; OR Immediately prior to where the water re-enters the storage tank, if one is present.
 - If there is an endotoxin filter in or before the loop, consider collecting a sample between the RO and the endotoxin filter.
 - For portable ROs, collect sample from the product water outlet.
4. Collect samples as per HA/site-specific procedure. A general procedure is provided in [Table 4](#).

5.2 Follow-up on Sample Results

1. If the microbiology count/endotoxin concentration is lower than the action threshold, resume routine testing of dialysis water the following month.

Action threshold:

- >50 CFU/mL; endotoxin: >0.125 EU/mL.
2. If the microbiology count/endotoxin concentration exceeds the action threshold, take corrective action.
 - If this is the 1st result to exceed the action threshold, retake sample as soon as possible.

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- If this is the 2nd result to exceed the action threshold, contact person responsible for dialysis water quality assurance (e.g., biomed, renal tech, nursing unit), if not already notified, to perform the next steps as per flowchart on [Table 5](#).
 - If this is the 3rd (or more) result to exceed the action threshold, it is likely that biofilm is present in the water treatment and must be removed. Contact the water treatment system vendor for guidance. Retest the system or portable RO.
3. Record corrective measures taken on the Microbiology/Endotoxin Testing of Dialysis Water log sheet.
 4. Refer to [Table 5](#) for detailed follow-up processes.

5.3 Documentation

All microbiology and endotoxin test results for dialysis water must be documented. Processes are in place within the Health Authority for designated individuals to review the results and take and document action, if required.

6.0 REFERENCES

CSA Standards

CAN/CSA-ISO 13959-11-Water for haemodialysis and related therapies (Adopted ISO 13959: 2009, 2nd edition, 2009-04-15), *Canadian Standards Association*, 2011.

CAN/CSA-ISO 26722-11—Water treatment equipment for hemodialysis applications and related therapies (Adopted ISO 26722: 2009, First edition, 2009-04-15), *Canadian Standards Association*, 2011.

CAN/CSA-Z23500-12-Guidance for the preparation and quality management of fluids for haemodialysis and related therapies, *Canadian Standards Association*, March 2012.

HA Guidelines

VIHA, Facility and Portable Water Treatment System Microbiology and Endotoxin Sampling (9.2.7), Apr 1, 2014.

Articles

Coulliette, A. and Arduino, M. (2013). *Seminars in Dialysis*, 26:4 (July-August), p.p., 427-438. <http://onlinelibrary.wiley.com.ezproxy.library.ubc.ca/doi/10.1111/sdi.12113/epdf>. Accessed Feb 24, 2016.

Layman-Amato, R, Curtis, J and Payne, G (2013). *Nephrology Nursing Journal*, 40:5 (September-October 2013), p. 383.

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Accessed Feb 24, 2016.

7.0 SPONSORS

This provincial guideline was developed to support improvements in the quality of hemodialysis care delivered to patients with chronic kidney disease in BC. Based on the best information available at the time it was published, the guideline relies on evidence and avoids opinion-based statements where possible. When used in conjunction with pertinent clinical data, it is a tool health authorities and health professionals can use to develop local guidelines.

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Developed by a working group of multidisciplinary care providers from across BC, the guideline was approved by the BCPRA Hemodialysis Committee and the BCPRA Medical Advisory Committee. It has been adopted by BCPRA as a provincial guideline.

This guideline is based on scientific evidence available at the time of the effective date; refer to www.bcrenalagency.ca for most recent version.

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Table 1: Frequency of Water (RO) Testing

MICROBIOLOGY		ENDOTOXINS	
HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid	HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid
<p>New RO validation period: 2 consecutive weeks (1 test per week) of tests that meet the standard. Final test results take 7 days. RO can be used during this period as long as the daily interim report indicates microbial growth is within the acceptable range.</p> <p>Monthly thereafter.</p>	<p>New RO validation period: 3 consecutive weeks (1 test per week) of tests that meet standard. Run as HD until completed.</p> <p>Exception: If the membranes were disinfected after installation AND the loop can be heat disinfected at least weekly AND the loop has a valid endotoxin filter THEN on-line priming or HDF can commence after 1 microbiology test (results received after 7 days) as long as the microbial growth is within the acceptable range.</p> <p>Note: Endotoxin standard must also be met.</p> <p>Monthly thereafter.</p>	<p>New RO validation period: 2 consecutive weeks (1 test per week) of tests that meet standard. Final test results take 24 hrs. The RO can be used during this period assuming the test indicates the endotoxin result is within the acceptable range.</p> <p>Monthly thereafter.</p>	<p>New RO validation period: 3 consecutive weeks (1 test per week) of tests that meet standard. Run as HD until completed.</p> <p>Exception: If the membranes were disinfected after installation AND the loop can be heat disinfected at least weekly AND the loop has a valid endotoxin filter THEN on-line priming or HDF can commence after 1 endotoxin test (results received after 24 hrs) as long as the endotoxin result is within the acceptable range.</p> <p>Note: Microbiology standard must also be met.</p> <p>Monthly thereafter.</p>

Table 2: Acceptable Microbiology & Endotoxin Concentrates

	MICROBIOLOGY		ENDOTOXINS	
	HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid	HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid
Viable count/ concentration	<100 CFU/mL		>0.25 EU/mL	
Action level	>50 CFU/mL		>0.125 EU/mL	

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Table 3: Acceptable Methods of Laboratory Microbiology & Endotoxin Testing

MICROBIOLOGY		ENDOTOXINS	
HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid	HD, no on-line priming or substitution fluid	HD with on-line priming or HDF substitution fluid
<p>Cultured within 4 hrs if not refrigerated or within 24 hrs if refrigerated.</p> <p>Spreadplate, pour plate or membrane filtration. Other methods can be used if it can be demonstrated they provide equivalent results.</p> <p>TGEA or R2A culture media. Incubate 17 to 23° C. for 7 days.</p>		<p>BCCDC is contracted to perform Endotoxin testing for all BC sites. Charles River cartridge system is used with an RDL of .01 EU/ml as the standard for all testing. This system is a validated LAL test for endotoxin.</p> <p>Samples must be received by the BCCDC lab within 24 hrs of collection (BCCDC will process samples received within 48 hrs).</p>	

Table 4: General Procedure for RO Water Sample Collection

STEP/DESCRIPTION		KEY POINTS/INFORMATION
1	Gather supplies.	
2	Put on clean gloves.	Protects hands from exposure to rubbing alcohol.
3	Disinfect sampling kit/tap.	Sampling Kit must be cleaned before taking a sample.
4	Clean the port being sampled.	Wiping from the inside to the outside ensures clean to dirty technique.
5	Put on protective gear for an aseptic procedure (wash hands & put on clean gloves & other protective gear as required by the unit).	Washing hands prevents potential spread of microorganisms. Protective gear prevents contamination of the sample.
6	Flush the port being sampled.	This will remove any residual alcohol and any remaining external contaminants.
7	Collect a mid-stream microbiology sample.	Sample container lid should not be placed on any surface unless sterile.
8	Collect a mid-stream endotoxin sample.	Sample container lid should not be placed on any surface unless sterile.
9	Prepare the samples for transport.	Labs will not process sample containers that have moisture on the outside.
10	Document.	

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Table 5: Microbiology & Endotoxin Dialysis Water System Sampling Flowsheet

