PROVINCIAL STANDARDS & GUIDELINES

Dialysate Microbiology & Endotoxin Sampling

Updated August 2017

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.

BCRenal an agency of the Provincial Health Services Authority

BC Provincial Renal Agency
(BCPRA)
700-1380 Burrard Street
Vancouver, BC V6Z 2H3

Phone: 604-875-7340
Email: bcpra@bcpra.ca
Web: BCRenalAgency.ca

Facebook.com/BCRenalAgency
@BCRenalAgency
Youtube.com/BCRenalAgency

1.0 SCOPE OF GUIDELINE

This guideline applies to in-centre and community dialysis units (CDUs) that provide hemodialysis (HD) and/or hemodialfiltration (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 dialysate 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 to 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.

This procedure assumes that the water supplied by the hemodialysis machine used to mix the dialysate meets the Canadian Standards Association (CSA) standards. Refer to: Dialysate Water System Microbiology & Endotoxin Sampling at http://www.bcrenalagency.ca/resource-gallery/Documents/Dialysate%20Water%20System%20%20Microbiology%20and%20Endotoxin%20Sampling.pdf.

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 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 amoebocye 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

Ultrafilter: Bacteria and endotoxin retentive filter that removes bacteria and endotoxins from dialysis water and dialysis fluid.

Ultrapure dialysate: Highly purified dialysis fluid that can be used in place of conventional dialysis fluid or as feed solution for possible further processing to create fluid intended for infusion directly into the blood.

4.0 RECOMMENDATIONS

Recommendation #1:
Conduct dialysate testing as per the schedule on Table 1 (based on ISO standards with exceptions where additional sampling is recommended).

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

Recommendation #3:
Utilize recommended laboratory methods for analyzing samples (based on CSA-ISO).

Microbiology
Standard dialysate method (no filters and pre-filters, see Table 2):

- Cultured within 4 hrs if not refrigerated or within 24 hrs if refrigerated.
- Spreadplate, pour plate or membrane filtration. Other methods can be used if it can be demonstrated they provide equivalent results.
- TGEA or R2A culture media. Incubate 17 to 23°C for 7 days.

Ultrapure dialysate method (with filters, (1) HD with or without on-line priming or substitution fluid or (2) HDF):

- Membrane filtration method using .45u filter and TGEA or R2A media, 10 mL to 1,000 mL volume (1,000 mL is preferred because it is a more sensitive test), 17 - 23°C for 7 days.

Endotoxin
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.

Samples must be received by the BCCDC lab within 24 hrs of collection (BCCDC will process samples received within 48 hrs).

5.0 PROCEDURE

Biomedical Technologist, Renal Dialysis Technician or Renal Nurse who is trained and has demonstrated competency in dialysate practices may collect dialysate 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., suspected pyrogenic reaction).

5.1 Sample Collection

1. Collect samples before, and as close as practicable to, a disinfection procedure. Do not collect samples within 2-hours after a heat clean procedure.
2. Collect samples when the system is operating under stable conditions representing normal operation and there is concentrate in the dialysis machine.
3. Collect samples from the inlet to the dialyzer (pre-dialyzer). Alternatively, if the machine permits, the sample may be drawn from a sample port on the machine post-dialysate ultrafilter fluid and pre-dialyzer.
    - For membrane filtration sampling of on-line priming or substitution fluid, use as large a sample volume as practical - up to 1,000 mL (minimum 10 mL).
    - Use an aseptic technique for sampling as per manufacturer's instructions. If sampling from a port that has been cleaned with a disinfectant (usually alcohol), discard 20 mL - 100 mL of dialysate prior to taking sample.
    - While taking samples, avoid contact with the inside of the sampler, the sample container, the end of the syringe, or the clean injection site.
4. Refrigerate bacteriology and endotoxin samples or put in a cooler with icepacks and transport to the laboratory as soon as possible. Non-refrigerated samples are viable for 4 hours.
5. Collect samples as per HA/site-specific procedure. A general procedure is outlined in Table 3.

5.2 Follow-up on Sample Results

1. If the microbiology count/endotoxin concentration is lower than the action threshold, no action is required. Resume routine testing.

    Action thresholds:
    - Standard dialysate: microbiology ≥50 CFU/mL and endotoxin: ≥0.25 EU/mL.
    - Ultrapure dialysate: microbiology ≥0.05 CFU/mL and endotoxin: ≥0.03 EU/mL.

2. If the microbiology count/endotoxin concentration exceeds the action threshold for *standard dialysate* (i.e., machine is used for HD without on-line priming or substitution fluid), take corrective action.

    If this is the 1st result to exceed the action threshold, retake sample as soon as possible.

If this is the 2nd result to exceed the action threshold:

- Remove the machine from service.
- Disinfect the machine.
- Notify the area renal manager, biomedical &/or technical lead, infection control and nephrologist.
- Record corrective measures (i.e., disinfection).
- Retake sample.

If this is the 3rd result to exceed the action threshold:

- Complete a PSLS report.
- Troubleshoot possible reasons for the positive sample: collect and test samples from other parts of the dialysis system, evaluate microbial data for previous 3 months to look for trends, evaluate/correct sample collection technique, evaluate/correct compliance with the disinfection procedure, etc.
- DO NOT return the machine to service until a negative test result (i.e., below the action threshold) has been achieved.

3. If the microbiology count/endotoxin concentration exceeds the action threshold for *ultrapure dialysate* (i.e., machine is used for HD with on-line priming or for HDF), take corrective action.

   If this is the 1st result to exceed the action threshold:

   - Remove the machine from service.
   - Disinfect the machine.
   - Notify the area renal manager, biomedical &/or technical lead, infection control and nephrologist.
   - Record corrective measures.
   - Retake sample. Do not return the machine to service until a negative result has been achieved.

   If this is the 2nd result to exceed the action threshold:

   - Complete a PSLS report.
   - Troubleshoot possible reasons for the positive sample: collect and test samples from other parts of the dialysis system, evaluate microbial data for previous 3 months to look for trends, evaluate/correct sample collection technique, evaluate/correct compliance with the disinfection procedure, discuss troubleshooting options with service provider (e.g., change filter), etc.
   - Record corrective measures.
   - Retake sample. Do not return the machine to service until a negative result has been achieved.

Refer to:

- Table 4a: Microbiology/Endotoxin Dialysate Sampling, HD with No On-line Priming or Substitution Fluid.
- Table 4b: Microbiology/Endotoxin Dialysate Sampling, HD with On-line Priming or HDF Substitution Fluid.

5.3 Documentation

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

6.0 REFERENCES

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

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-ISO 11663-11 - Quality of dialysis fluid for hemodialysis and related therapies (Adopted ISO 11663:2009, First 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.

BCPRA Paper
BCPRA Hemodialysis Technical Group Recommendation to the BCPRA Hemodialysis Committee: Proposed Update to June 2016 Guideline on Microbiology & Endotoxin Sampling, March 1, 2017 (under separate cover)

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 Sept 10, 2015.

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

http://go.galegroup.com.ezproxy.library.ubc.ca/ps/i.do?p=HRCA&u=ubcolumbia&id=-GALEIA345458912&v=2.1&it=r&sid=-summon&userGroup=ubcolumbia&authCount=1. Accessed Sept 10, 2015.

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.

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.

Table 1: Frequency of Dialysate Testing

MICROBIOLOGY TESTING		ENDOTOXIN TESTING	
No Filters **HD, no on-line priming or substitution fluid**	**With Filters** **(1) HD with or without on-line priming or substitution fluid or (2) HDF**	**No Filters** **HD, no on-line priming or substitution fluid**	**With Filters** **(1) HD with or without on-line priming or substitution fluid or (2) HDF**
New machine validation period: One dialysate test that meets standard. Final test results take 7 days. Machine can be used after the first 48 hrs while awaiting final test results as long as the daily interim reports indicate microbial growth is within the acceptable range and the requirements under "endotoxin testing" are also met. Thereafter: At least once per year with a monthly rotation through the fleet of machines.	New machine validation period: One test for gross contamination of pre-filters (if possible), dialysate & substitution fluid. Machine can be used for HD (no on-line/no substitution/no HDF) after the first 48 hrs while awaiting final test results as long as the daily interim reports indicate microbial growth is within the acceptable range and the requirements under "endotoxin testing" are also met. Thereafter: • No regular sampling is required. Situations requiring sampling (exception sampling) are provided below. • While awaiting final test results, machine can be used for HD (no on-line/no substitution/no HDF) after the first 48 hrs as long as the daily interim reports indicate microbial growth is within the acceptable range and the requirements under "endotoxin testing" are also met. No on-line/no substitution/no HDF until the final microbiology test results are available. • After final test results are available, follow the manufacturer's recommendations to bring the machine back into validation for on-line priming/substitution/HDF. Situations requiring sampling (exception sampling): • Manufacturer's recommended disinfection schedule has not been followed (e.g., no disinfection in >72 hrs). • After transport of a machine to a different facility by truck/train, etc. • After completion of service on the endotoxin — retentive filtration or substitution fluid components. • After an internal blood leak if the machine has the option for recirculating waste fluid into the dialysate hydraulics. • If the machine received a physical bump during dialysis treatment which may have ruptured both/all filters. Note: If there is a suspected pyrogenic reaction which is potentially machine related, pull the machine from service. Do not use for HD (+/-on-line priming or substitution fluid) or HDF. See guideline on pyrogenic reactions for sampling & other testing required (to be developed).	New machine validation period: One dialysate test that meets standard. Final test results take 24 hrs (can be longer depending on transport distance to BCCDC). Machine can be used upon receipt of a report indicating endotoxin growth is within the acceptable range and the requirements under "microbiology testing" are also met. Thereafter: Once per year with a monthly rotation through the fleet of machines.	New machine validation period: One test for gross contamination of pre-filters (if possible), dialysate & substitution fluid. Machine can be used upon receipt of a report indicating endotoxin growth is within the acceptable range and the requirements under "microbiology testing" are also met. Thereafter: • No regular sampling is required. • Situations requiring sampling (exception sampling) are provided below. • Machine can be used upon receipt of a report indicating endotoxin growth is within the acceptable range and the requirements under "microbiology testing" are also met. • After final test results are available, follow the manufacturer's recommendations to bring the machine back into validation for on-line priming/substitution/HDF. Situations requiring sampling (exception sampling): • Manufacturer's recommended disinfection schedule has not been followed (e.g., no disinfection in >72 hrs). After transport of a machine to a different facility by truck/train, etc. • After completion of service on the endotoxin — retentive filtration or substitution fluid components. • After an internal blood leak if the machine has the option for recirculating waste fluid into the dialysate hydraulics. • If the machine received a physical bump during dialysis treatment which may have ruptured both/all filters. Note: If there is a suspected pyrogenic reaction which is potentially machine related, pull the machine from service. Do not use for HD (+/-on-line priming or substitution fluid) or HDF. See guideline on pyrogenic reactions for sampling & other testing required (to be developed).

Table 2: Acceptable Microbiology & Endotoxin Concentrates

	MICROBIOLOGY				ENDOTOXINS			
	No Filters HD, no on-line priming or substitution fluid	**With Filters (1) HD with or without on-line priming or substitution fluid or (2) HDF**			**No Filters HD, no on-line priming or substitution fluid**	**With Filters (1) HD with or without on-line priming or substitution fluid or (2) HDF**		
		Pre-Filter	**Ultrapure Dialysate**	**Substitution Fluid**		**Pre-Filter**	**Ultrapure Dialysate**	**Substitution Fluid**
Viable count/ concentration	<100 CFU (colony-forming unit)/mL	<100 CFU/mL	<0.1 CFU/mL	<0.1 CFU/mL	<0.5 EU (endotoxin unit)/ mL	<0.5 EU (endotoxin unit)/mL	<0.03 EU/mL	<0.03 EU/mL
Action level	≥50 CFU/mL	≥50 CFU/mL	≥0.05 CFU/mL	≥0.05 CFU/mL	≥0.25 EU /mL	≥0.25 EU /mL	≥0.03 EU/mL	≥0.03 EU/mL

Table 3: General Procedure for Dialysate Sample Collection

STEP/DESCRIPTION		KEY POINTS/INFORMATION
1	Gather supplies.	
2	Put on clean gloves.	Protects hands from exposure to rubbing alcohol.
3	If drawing sample from a port, clean the port.	Wiping from inside to outside ensures clean to dirty technique.
4	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.
5	If drawing sample from a port, draw fluid and discard the first 20 mL - 100 mL.	Removes any residual alcohol and remaining external contaminants.
6	Collect the *microbiology* sample: • Collect a mid-stream sample; OR • Collect in a clean syringe; OR • Collect using a sterile sampling kit.	
7	Collect the *endotoxin* sample: • Collect a mid-stream sample; OR • Collect in a clean syringe; OR • Collect using a sterile sampling kit.	
8	Prepare the samples for transport.	Endotoxin samples are placed separately in a brown paper bag labeled with instructions to the laboratory to send to BC Centre for Disease Control (BCCDC).
9	Document.	

Table 4a: Microbiology & Endotoxin Dialysate Sampling, HD with No On-line Priming or Substitution Fluid

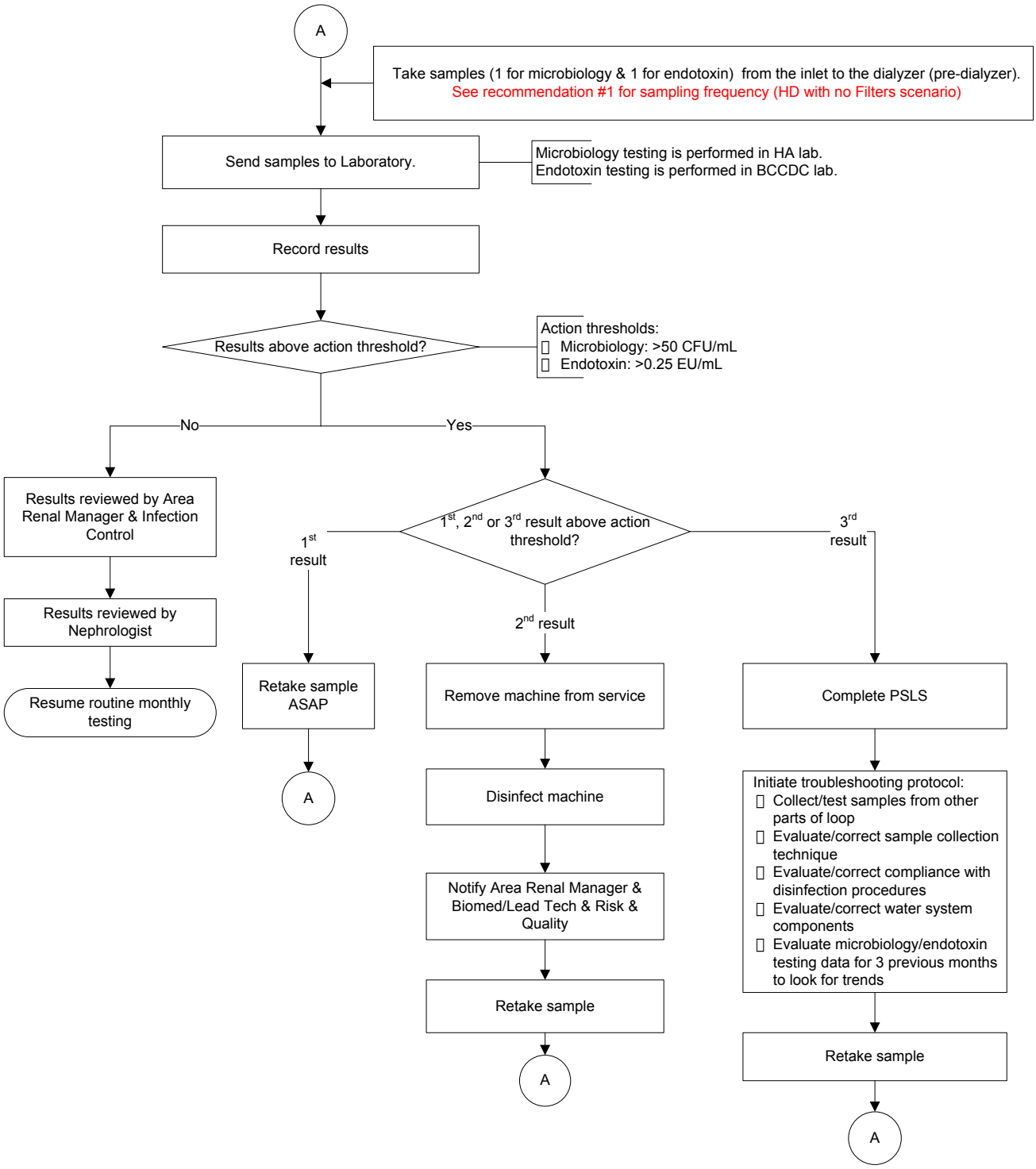

Table 4b: Microbiology & Endotoxin Dialysate Sampling Process for HD with On-line Priming or HDF