PURPOSE: Endotoxin is the LPS component of the gram negative bacterial outer membrane. More specifically it resides in the lipid portion of the LPS molecule, Lipid A. Endotoxin may cause fever and leucopenia and in more severe cases shock. In hemodialysis fluids, it can contribute to a phenomenon known as the chronic inflammatory response syndrome.

This procedure uses *Limulus* amebocyte lysate (LAL) for the detection of endotoxin in dialysate water samples. The reaction is enzymatic catalyzed by the endotoxin.

SAMPLE INFORMATION:

Type	Dialysate water samples
Volume	1 mL minimum
Storage and stability	• Specimens should be stored between 2°C and 8°C and tested within 24 hours of collection.
Rejection criteria	• Samples >24 hours old

REAGENTS and/or MEDIA:

Type	LAL Test Kit Endotoxin
Storage Requirements	• Store between 2°C and 8°C • Phone Dialysis Unit when stock is running low

SUPPLIES:

- 37°C Dry bath incubator
- ½ CC syringes
- Timer

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Positive control (red top) is provided with kit and run with each sample.
- Control results are entered into LIS with patient sample results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing Endotoxin by LAL testing	
1	Label requisitions and samples. Transfer water samples to sterile orange top container.
2	Each sample will have a red top tube (Positive Control) and a green top tube (Test). Label tubes with LIS generated labels.
3	Peel back metal covering on the tubes.
4	Measure out 0.20mL of the water using a ½ CC syringe.
5	Puncture the centre of the tube septum with the syringe - leave the syringe in the tube Do this for both the Red top and Green top tubes. **DO NOT ADD THE SAMPLE TO THE TUBE** **NOTE:** If you have more than 3 samples to test be aware that the internal pressure inside the tube draws liquid from the syringe. In cases where it will take you more than 1 min to set up, consider setting them up in two separate batches.
6	Repeat for all waters.
7	Set your timer for **30 mins** but do not start.
8	Depress the plunger on all of the syringes and discard in sharps container.
9	Invert the vials 5 times – **Start the timer.**
10	Incubate tubes in the 37° dry bath test tube incubator.
11	After 30 minutes, invert the tube once to check for a clot (+/- 1min to read results).

INTERPRETATION OF RESULTS:

IF	THEN
Red top: Clot Green top: no Clot	Sample passes – no Endotoxin detected Report: "**ENDOTOXIN: Sample <0.25 EU/mL"**
Red top: Clot Green top: Clot	Sample failed – Endotoxin detected Report: "**ENDOTOXIN: Sample >=0.25 EU/mL"**
Red top: No Clot Green top: No Clot	Sample interference – repeat. If repeated results are the same Report: "**Results inconclusive, possible sample interference. Please recollect"**
Red top: No Clot Green top: Clot	• Possible error, repeat. • If repeated results are the same – repeat using different kit lot #. • If those repeated results are the same, Report: **"Results inconclusive, possible sample interference. Please recollect"**

NOTES AND PRECAUTIONS:

Standards set forth in the *American National Standard: Hemodialysis Systems* state that dialysis water must contain a colony count of <=200 CFU/ml and LAL activity < 2 EU/ml. The CDC recommends that both be measured since there are times when colony counts can be low and endotoxin activity high, and the reverse can also be true.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.
- Instruction for Use of LAL Test Kit Endotoxin Testing. (n.d.). Chief Medical Supplies LTD.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31AUG2013	Initial Release	A.Darrach
1.1	31March2016	Reviewed – No Changes	C. Russell
2.0	18-May-2017	Reviewed and revised; Safety precautions and reagent storage requirements added; New format; New document number (Old number MIC52600)	L. Steven