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	1mL minimum
Stability	24 hours
Storage Requirements	2-8°C
Criteria for rejection and follow up action	Samples >24 hours old

REAGENTS and/or MEDIA:

Type	LAL Test Kit Endotoxin
Storage Requirements	• Store at 2-8°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.

QUALITY CONTROL:

- Positive control (red top) provided with kit
- Control results are entered with patient sample results

PROCEDURE INSTRUCTIONS:

Step	Action
How to Perform Water Testing – Endotoxin by LAL	
1	Accession samples **LIS CODE: DIAST**
2	Label requisitions and samples
3	Each sample has a Red top (Positive Control) and a Green Top (test) Label Green top(test) and Red top (Positive Control)
4	Measure out 0.20mL using a ½ CC syringe
5	Peel back metal covering on the tubes
6	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** **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
7	Repeat for all waters
8	Set your timer for **30mins** but do not start it
9	Depress the plunger on all of the syringes and discard syringes in a rigid sharps container
10	Invert the vials 5 times – **Start your timer**
11	Incubate tubes in the 37° Dry Bath test tube incubator
12	When time is up gently 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 (Sample passed test)**
Red top: Clot Green top: Clot	Sample failed – Endotoxin detected Report: **ENDOTOXIN: Sample >=0.25 EU/mL (Sample failed test)**
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**

CONSIDERATIONS:

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

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Signed by: Jennifer G. Daley Bernier