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NEPHROLOGY PROGRAM
DEPARTMENT POLICIES AND PROCEDURES

Biomed Neph - Section 03 - Water Quality Management - Neph Tech 3-02
Endotoxins Testing Procedure
No.: 01066 (TOH Standardized Policy Number)

ISSUED BY:
Nephrology Technical Practice Committee

DATE OF APPROVAL:
N/A

APPROVED BY:
Program Clinical Director / Division Head

LAST REVIEW/REVISION DATE:
2017/03

CATEGORY:
Water Quality Management

IMPLEMENTATION DATE:
2006/01

PURPOSE:

- To test for Endotoxins presence in treated water and in dialysate solution on a routine basis

FREQUENCY:

- Monthly on all in-centre water treatment systems (portable systems included)
- Monthly on all home Hemodialysis machines and water treatment systems (including non-R.O. systems)
- Quarterly on all in-centre Hemodialysis machines (quantity evenly distributed over a monthly basis)
- Microbiology testing procedure shall be completed in conjunction with microbiology monitoring

CONCENTRATION TESTING LEVELS:

- All in-centre water treatment systems = 0.125 EU/ml
- All home Hemodialysis water treatment systems (including non-R.O. systems) = 0.125 EU/ml
- All Hemodialysis machines = 0.125 EU/ml

SUPPLIES &TOOLS REQUIRED for PYROSATE TEST TUBE KITS METHOD:

- Incubator, heater block, thermometer, stop watch, pipettes, test vial holder, pyrogen free collection containers, Pyrosate test tube kits, alcohol swabs (inventory # 230660), mask and gloves

SUPPLIES & TOOLS REQURED for PTS SYSTEM METHOD:

- PTS Tester, PTS cartridge, 25uL pipettor, pipettor tips, test vial holder, pyrogen free collection containers, alcohol swabs (inventory # 230660), mask and gloves

DEFINITION(S): N/A

ALERTS: N/A

PROCEDURE: USING PYROSATE TEST TUBE KITS

- Endotoxins testing procedure shall be completed in conjunction with microbiology testing.
- Sample same locations as indicated in the Microbiology Monitoring Procedure

Setup

1. Preheat incubator to 37 degrees Celsius. Confirm that the temperature of the heating block with an external temperature probe does not exceed 37 ±1 degrees Celsius as this will cause false positive results. Record temperature on Bacteriology/Endotoxins Testing Result Sheet
2. Collect the sample in the pyrogen free container
3. Use a permanent marker to label fluid sample (SPL) and positive product control (PPC) tubes with test location or other sample identification
4. Gently tap the bottom of the pyrosate test tube on a hard surface to force all the powder in the bottom of the tube
5. Put on mask and sterile gloves
6. Remove and discard stoppers of one SPL (blue capped tube) and one PPC (red capped tube). Do not touch the tube rims while removing stoppers

Test samples

1. Open one sterile transfer pipette, without contaminating the tip or neck of the pipette. Touch only the bulb end of the pipette
2. Draw sample (from the collection container) up to the 0.5mL mark (located in near the middle of the pipette) and transfer it all to the SPL tube. Do not let the pipette touch anything as you will reuse the pipette in the following steps
3. Gently mix the contents of the SPL tube by lightly tapping the bottom of the tube several times with your finger. The contents should dissolve in 20 – 60 seconds
4. Using the same pipette as before, remove 0.25mL from the SPL tube and transfer it to the PPC tube. 0.25mL level is the ring-mark unlabeled below the 0.5mL mark on the pipette
5. DICARD THE PIPETTE AT THIS TIME
6. Gently mix the contents of the PPC tube by lightly tapping the bottom several times with your finger. It should take 10 – 20 seconds to dissolve
7. Immediately place both tubes in the incubator. Incubate @ 37 degrees C (±1 degree) for the amount of time indicated on the Certificate of Compliance included with the kit

8. If the result is positive (+) after two (2) tests, re-sample from the test point and dilute the sample by adding 0.25ml of pyrogen free sterile water to 0.25ml of the sample collected into the SPL tube and continue with the procedure to include the PPC tube. Confirm the sterility of the pyrogen free water by performing a standard Endotoxins test using the SPL and PCC tubes

Reading test results

When time is up, **immediately and carefully** remove the tubes one by one from the incubator. **Gently invert** the tubes until the absence of a solid gel-clot is confirmed or a 180 degree inversion is reached. **Do not shake the tubes when reading the test**. If a solid clot has formed the result is positive (+). If no clot has formed, example; mixture remains liquid or the clot breaks, the result is negative (-)

PPC Tube (Positive Product Control)

- This tube **SHOULD** clot
- If it does not, you have an invalid test. Repeat the test. A failure of the PPC tube to clot indicates either inhibition of the assay by the sample or improper performance of the assay

SPL Tube (Sample)

- This tube **SHOULD NOT** clot
- Absence of a clot means there is less concentration than the level you were testing for. If the SPL tube clots your sample has more concentration than what you were testing for. Disinfect equipment and re-test before next use

Here is a simple chart showing a valid test:

CONTROL	SAMPLE	RESULT
CLOT (+)	*CLOT (+)*	Sample contains > testing level
CLOT (+)	*NO CLOT (-)*	Sample contains < testing level
NO CLOT (-)	*CLOT (+)*	INVALID TEST
NO CLOT (-)	*NO CLOT (-)*	INVALID TEST

Fill the Bacteriology/Endotoxins Testing Result Sheet and send to the Technical Manager or his delegate. Enter information in NephroCare database and Equipment Maintenance Logsheets

PROCEDURE USING PTS SYSTEM

- Endotoxins testing procedure shall be completed in conjunction with microbiology testing
- Sample same locations as indicated in the Microbiology Monitoring Procedure

Performing routine Endotoxin test

1. **Instrument Operation**

   - Press the **MENU** key on the PTS keypad to turn instrument on (Menu 5 turns instrument off)
   - The PTS Reader initiates a "SYSTEM SELF TEST" as it heats up to 37°C – this takes approximately 5 minutes
   - The PTS Reader displays "SELF TEST OK" and then "INSERT CARTRIDGE"

2. **Insert the Cartridge**

   - Note: Allow the cartridge to come to room temperature in pouch before use
   - Remove cartridge from pouch and insert with sample reservoirs facing up into slot at front Press cartridge firmly into slot of the PTS Reader

3. **Enter Required Information**

   Once the cartridge has been firmly inserted into the PTS Reader, the PTS Reader prompts the user to enter the following information:

   - ***Enter OID*** (Operator ID)
   - ***Enter Lot #*** (Cartridge Lot #)
   - ***Enter Calibration Code*** (If the Calibration Code for the particular lot # has already been entered, the PTS Reader does not prompt for the code again
   - ***Lot #*** (Confirms cartridge lot number entered)
   - ***Enter Sample Lot #***
   - ***Enter Sample ID***
   - ***Enter Dilution Factor*** (1)

   While the above information is being entered into the PTS Reader, the cartridge is being pre-warmed

4. **Dispense the Sample**

   Once all test information is entered, the PTS Reader displays:
   "Add sample and press enter"

   - Pipette 25 µL of sample into all four (4) sample reservoirs of the inserted cartridge and press Enter on the PTS
   - Reader keypad. Pumps draw sample aliquots into the test channels, thereby initiating the test. Results will be obtained in approximately 15 minutes

Endotoxin test results

1. When the test is complete, the PTS Reader gives an audible notification that the assay is finished

2. At the conclusion of the test, the Endotoxin measurement and the assay acceptance criteria are displayed on the screen
3. The PTS Reader display alternates between the following results:
   - Sample EU/mL
   - Sample %CV
   - Spike EU/mL
   - Spike %CV
   - % Spike Recovery
   - Remove Cartridge
4. The PTS Reader continues to display the assay results until cartridge is removed

RELATED POLICIES / LEGISLATION:

1. Nephrology Policies and Procedures – Policy # 01060 - Equipment Maintenance - Biomed Neph - Section 01 - Equipment Maintenance - Neph Tech 1-07 NephroCare Work Orders

2. Nephrology Policies and Procedures – Policy # 01065 - Water Quality Management - Biomed Neph - Section 03 - Water Quality Management - Neph Tech 3-01 Microbiology Monitoring Procedure

REFERENCES:

1. CSA-ISO 13959-15 *Water for haemodilaysis and related therapies*
2. CAS-ISO 26722-16 *Water treatment equipment for haemodialysis applications and related therapies*
3. CSA-ISO 13958-15 *Concentrates for haemodialysis and related therapies*
4. CSA-ISO 11663-15 *Quality of dialysis fluid for haemodialysis and related therapies*
5. CSA Standard Z364.5-10 (R2015) *Safe Installation and Operation of Hemodialysis and Peritoneal Dialysis in a Home Setting*
6. Bacteriology/Endotoxins Testing Result Sheet (Rev. June 18, 2015)
   - See Below
7. Associates of Cape Cod, Inc. 124 Bernard Saint Jean Drive, East Falmouth, MA 02356-4445,(888) 395-2221
8. Charles River Laboratories Endosafe – Portable Test System (PTS) Kinetic Reader Installation, Operation and Performance Qualification Manual, dated 12-Jun-2012

COMMENTS / SIGNIFICANT REVISIONS: N/A

Bacteriology / Endotoxins
Testing Results Sheet

Location	Sample Name	Bacteriology Sampling Done?	Endotoxins Sample Result: EU/ml	Sample RT CV: <25%	Spike: EU/ml	Spike RT CV: <25%	Spike Recovery: >50% - <200%

Location	Sample Name	Bacteriology Sampling Done?	Endotoxins Sample Result: EU/ml	Endotoxins Sensitivity Level

Endotoxin lot number: _____ Incubator temperature: _____

Dialysis Technologist: _____

Date: _____ Campus: _____

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Revised: June 18, 2015