1. **Purpose of protocol**

 To describe Endotoxin Testing using the Charles River PTS instrument.

1. **Introduction**

 Endotoxins are major component of the outer cell wall of gram-negative bacteria. They are lipopolysaccharides which consist of a polysaccharide chain covalently bound to lipid A.

Endotoxins can acutely activate both humoral and cellular host defences, leading to a syndrome characterized by fever, shaking, chills, hypotension, multiple organ failure and even death if allowed to enter into circulation in a sufficient dose. Long term exposure to low levels of endotoxins is suspected to contribute to complications such as cardiac disease, further diminishing patient quality of life, increasing physician treatment and medical costs. Testing for Endotoxins as a function of product release criteria can ensure patients are not exposed to harmful toxins.

1. **Surveillance Methods**

 Endotoxins can be detected with Limulus Amoebocyte Lysate and is measured in Endotoxin Units per millilitre, EU/mL. The Charles River Endosafe PTS instrument and cartridges use the Kinetic Chromogenic method for Endotoxin detection. The PTS is a spectrophotometer with heater, pump and on board software for data analysis.

1. **Surveillance Requirements**

 Three samples from each batch should be tested prior to product release, it is recommended samples from beginning, middle and end of production be taken for testing. Specific product requirements can be found in individual quality control standard operating procedures.

1. **Surveillance Parameters**

The standards required are based on dosage over time and the weight of recipient. Specific product requirements found in individual quality control standard operating procedures.

**APPENDIX 1 ENDOSAFE PTS METER OPERATING INSTRUCTIONS**

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| **Biohazard Protection*** Wear gloves while collecting, handling and testing the sample and test reagents.
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| **Specimen Requirements*** Prepare sample as per individual Endotoxin Test method.
* Test at room temperature as cold samples can cause condensation within the instrument and interfere with optical readings.
* It is recommended to assay samples as soon as possible, storage can result in false negative results due to interference from storage container or agents contained in sample.

**Running a Sample*** Ensure cartridges are at room temperature for at least 15 minutes prior to use.
* Press the MENU key to turn the reader on. The reader begins a System Self-Test and heats up to 37°C in approx. 5 mins.
* The display will change from Self-Test to INSERT CARTRIDGE.
* Remove cartridge from the pouch by tearing the foil. Touching the edges of the cartridge so not to contaminate the optical wells and insert the cartridge firmly in to the reader with the sample reservoirs facing upwards.
* Enter your Employee ID at the ENTER ID prompt, press enter button.
* Enter the Cartridge Lot number at the ENTER LOT # prompt.
* Carefully enter the Calibration Code found on Certificate of Analysis and check that the numbers match exactly.
* Enter required information at Sample Lot # prompt.
* Enter required information at Sample ID prompt.
* Enter the dilution factor if one is used. (NB: # 1 means that there is no dilution) .The result the PTS reports takes into account the dilution factor.
* The meter then displays ADD SAMPLE, PRESS ENTER.
* Pipette 25µL of sample in to each of the four wells. (NB: Do not touch the pipette tip onto any surface other than the sample and cartridge well. The prevalence of Endotoxins means that contamination will likely occur).
* Press Enter.
* At the end of the assay the meter will alternate displaying the results and the prompt to remove the cartridge.
* If required, copy the result from the display on to the report.
* Power off the meter by pressing Menu then System Off.

**Exporting data*** Ensure that the PTS Logger software is loaded on to the desktop of the PC.
* Connect the cable from the PC serial port to the Endosafe analyser.

1) Open the software on the computer:-* Select Connect on the PTS logger software and then enter Port Number 1 at the prompt.
* Click on Connect.
* Select Log then Open/Close to open the log.

2) On the Endosafe PTS analyser:-* Press the Menu key until you reach 4 Print Menu. (Shortcut = Menu/4)
* Select from the options 1. Print Last Test. 2 Print by date, 3. Print all Tests. This will automatically download the results to desktop in the form of a notepad file (.log).

3) In the software on the computer* Select Log then View and the results will be displayed in notepad format.
* Select Log Open/Close to close.
* Open notepad file to obtain result and check validity of test. See page 2 of Endosafe PTS assay guide for results interpretation.

**Storage of Reagents*** Do not use past the expiry date.
* Cartridges must be stored at room temperature i.e. between 2-25 °C.
* Cartridges must be bought to room temperature for at least 15 minutes prior to use.
* Use the cartridge within 2 hours of opening the foil pack.

**Procedural Care*** Touching the sample reservoirs can contaminate the assay.
* Touching the optical cells leaving fingerprints or smudges can cause problems with the transmittance of light through the optical cells.
* Use only endotoxin free and non-interfering accessories e.g. water, dilution tubes, pipettes and tips.
* An incorrect dilution factor will mean the analyser will give the wrong results.
* Splashing the sample out of the wells (due to poor pipetting techniques) will give inaccurate volumes and incorrect results particularly in the spiked channels.
* Bubbles cause false onset times because of the disturbance in light transmittance.
* Bubbles can interfere with the mixing of the sample with the reagents so that it is not homogeneous.
* In the event of bubbles or splashing out, pipette the sample out of the well and repeat using a new pipette tip.

**Maintenance*** The meter must be returned to the local distributor (SC Bio Limited) for calibration and maintenance annually.

**Principle of the Procedure**The reader draws and mixes the sample with the LAL reagent in two channels (sample channels) and with the LAL reagent and Positive Product Control (PPC) in the other two channels (the spike channels). The sample is incubated and mixed with Lysate and a chromogenic substrate. After mixing, the optical density of the wells is measured and analysed against an internally-archived, batch specific standard curve. The PTS simultaneously performs testing in duplicate and averages the results in keeping with USP/EU guidelines.**Required Reading:*** Charles River Laboratories Endosafe PTS Users Guide Version 7.
* PTS Loger Instructions.
* Endosafe PTS Assay Guide.
* Endosafe Quick Start Quide.
* Cartridge Package Insert.
* Endosafe PTS Power Point.
* Endosafe Video.
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**Locations of Hard Copies:**

Standard Operating Procedures Manual

**References:**

Charles River Laboratories Endosafe PTS Users Guide Version 7.

PTS Loger Instructions.

Endosafe Assay Guide.