



Biopharmaceutical Development Program

Standard Operating Procedure

Title: Rapid Endotoxin Determination Using the Charles River Portable Test System (PTS)

SOP Number: 22204

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1.0 Purpose

This procedure is a rapid point-of-use test system, which is comprised of a test cartridge along with a portable spectrophotometer. The Endosafe-PTS Endotoxin test utilizes existing FDA-licensed LAL formulations. The PTS system measures color intensity directly related to the Endotoxin concentration in a sample and provides quantitative LAL test results in approximately 15 minutes.

2.0 Scope

Process Analytics qualified personnel will perform this procedure. The PTS will be used for Endotoxin detection in WFI quality water, raw material samples, and final product samples.

3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics (PA) has the authority to define this procedure.
- 3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 PA personnel are responsible for the performance of this procedure.
- 3.4 PA is responsible for reviewing the data and documentation of this procedure.
- 3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials

- 4.1 Endosafe-Licensed PTS Endotoxin Cartridge, BDP PN 30752.
- 4.2 Portable Test System, BDP MEF 81770 or 81780.
- 4.3 Endosafe- PTS mini pipettor, BDP PN 21860, or BDP approved equivalent.
- 4.4 Epson TM-U220D Printer or any BDP controlled computer.
- 4.5 Charles River-LAL Reagent Water, BDP PN 30328, or BDP approved equivalent.

Note: LAL Reagent Water used to make product dilutions should be tested for the absence of detectable Endotoxin

- 4.6 MBP 200 Pipet Tips, BDP PN 20469, or BDP approved equivalent.
- 4.7 Endoscan software (Version V7.10A).

5.0 Charging the Battery

- 5.1 The PTS comes with its own AC power adapter, which also serves as the battery charger for the reader. The battery life is approximately 4 hours of continuous usage after a full charge. To ensure proper functioning under battery power, charge the reader after 3 hours of continuous usage or at the end of each working day.

Note: Discharged batteries will accept a full charge in approximately 6-8 hours.

- 5.2 To charge the battery, insert the power cord into any grounded outlet and then insert the DC plug into the back of the reader. The AC power cord adapter displays a green LED when the cord is securely plugged into an outlet.

6.0 Procedure

To perform the test, the user pipettes 25 µL of a sample into each of the four sample reservoirs of the cartridge. The reader draws and mixes the sample with the LAL reagent in two channels (the 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 then combined with the chromogenic substrate. After mixing, the optical density of the wells is measured and analyzed against an internally-archived, batch-specific standard curve. The PTS simultaneously performs testing in duplicate and averages the results in keeping with USP guidelines.

Note: Proper laboratory technique is essential to prevent contamination of test materials with environmental Endotoxins.

6.1 Press the menu key on the PTS keypad to turn the instrument on. The reader initiates a system self test as it heats up to 37°C. This will take approximately 5 minutes.

6.2 The reader will display "SELF TEST OK" and then "INSERT CARTRIDGE." If the reader fails the self test, do not use it, and notify the PA Supervisor.

Note: Cartridges are stored at 2-8°C. Allow the cartridge to reach room temperature while remaining in the pouch before use.

6.3 Each lot-specific cartridge contains a Certificate of Analysis. The COA will contain all the information that is required for the initial setup of an Endotoxin run; refer to Attachment 4 for an example of a lot-specific COA.

6.4 Remove the cartridge from the pouch, careful not to touch the sample reservoirs or optical cells. Insert the cartridge with the sample reservoir facing up into the slot at the front of the PTS reader. Press the cartridge firmly into the slot.

6.5 Once the cartridge has been firmly inserted into the reader, the reader will prompt the user to enter the following information.

6.5.1 Enter OID (Operator ID). The technician performing the assay will use his/her initials for the OID.

6.5.2 Enter the Lot number of cartridge.

6.5.3 Enter the calibration code. If the calibration code for the particular lot number has already been entered, the reader does not prompt for the code again. To erase all stored lot numbers and corresponding calibration codes, select menu, 2, and then 4 from the initial menu.

6.5.4 Enter Lot number (Confirms cartridge lot number entered).

6.5.5 Enter Sample Lot number (QC number).

6.5.6 Enter Sample ID. Selecting and scrolling with the menu key under the sample ID header allows for 50 samples to be entered and stored.

- 6.5.7 Enter the dilution factor. If no dilution is required for the sample, then a 1 should be entered as the dilution factor.

Note: When the dilution factors are entered into the PTS Reader during initial setup of test information, the software will correct for the specified dilution. The Endotoxin value reported on the test report is the Endotoxin concentration in the undiluted sample.

- 6.5.8 Once all the test information is entered, the reader displays: ADD SAMPLE; PRESS ENTER.

- 6.6 Pipette 25 µL of the sample into all 4 sample reservoirs of the cartridge and press enter on the reader keypad. The sample must be delivered to each sample port without contamination or cross contamination between samples.

- 6.7 Pumps draw samples aliquots into the test channels, thereby initiating the test. Results will be obtained in approximately 15 minutes.

7.0 Test Results

- 7.1 When the test is complete, the PTS reader gives an audible notification that the assay is finished and the Endotoxin measurement and the assay acceptance criteria are displayed on the screen.

- 7.2 The reader display alternates between the following results.

- Sample EU/mL
- Sample % CV
- Spike EU/mL
- Spike % CV
- % Spike Recovery
- Remove Cartridge

- 7.3 The PTS reader is capable of storing the last 99 test results.

Note: The reader continues to display the assay results until the cartridge is removed.

- 7.4 Printing Test Results Using the Epson Printer

- 7.4.1 Connect the serial port cable to the printer and the RJ45 plug in back of the PTS reader.

- 7.4.2 Press the MENU key on the PTS reader keypad.

- 7.4.3 Select 4 for the print menu. Selecting one of the following choices on the reader keypad will send results to the printer.

- Print Last Test
- Print by Date
- Print all Tests

8.0 Pass/Fail Criteria

- 8.1 The coefficient of variation (C.V.) must be < 25% between the 2 sample replicates for the assay to be valid. The C.V. must also be < 25% between the 2 PPC replicates for the assay to be valid. If the C.V. is > 25%, then the assay is invalid.
- 8.2 Recovery of the PPC or spiked values must be within 50-200% for the test to be valid. Spike recoveries outside of this range invalidate the test. Dilute the sample and rerun the assay if possible to remove interference.

9.0 Documentation

- 9.1 Document all test results and reagent information on Form 22204-01, Attachment 1.
- 9.2 Attach PTS printout results to the QC Test Request form and forward it to the PA Supervisor for PA review.
- 9.3 Each report is signed and dated by the analysts.
- 9.4 Document all usage, maintenance, calibration, cleaning, etc. in the instrument logbook as per **SOP 21531, *Equipment/Facility Logs***.

10.0 References and Related Documents

- 10.1 **SOP 21531** *Equipment/Facility Logs*

11.0 Attachments

- 11.1 **Attachment 1** Form 22204-01, PTS Kinetic LAL Analysis
- 11.2 **Attachment 2** Image of the Endosafe PTS System
- 11.3 **Attachment 3** Image of Sample Printout
- 11.4 **Attachment 4** Certificate of Analysis (Reference Only)

Attachment 1

NCI-Frederick
Form No.: 22204-01
SOP No.: 22204
Revision 01:

PTS Kinetic LAL Analysis

Requestor:	QC Number:
Test Specifications:	Pipette ID Numbers:
Other:	Valid Assay: <input type="checkbox"/> yes <input type="checkbox"/> no

Reagent Information

Reagent	Mfg./Lot #	Expires
PTS Cartridge		
LAL Reagent Water		
Other		

Sample Preparation

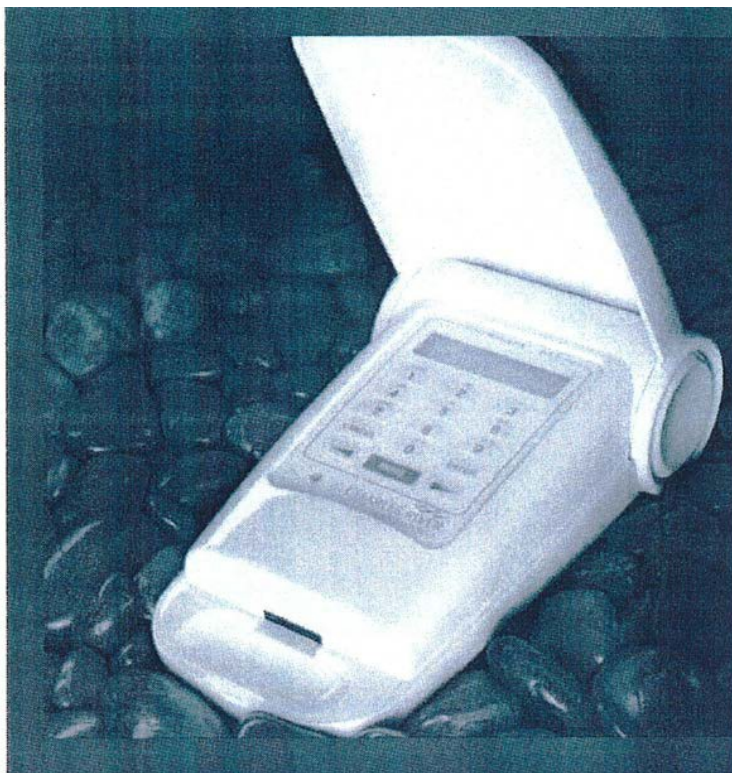
Sample	Dilution Tested	Endotoxin Value	Spike Recovery

Attach PTS printout

Analyst/Date: _____

Reviewed By/Date: _____

Attachment 2
Image of the Endosafe PTS System



Attachment 3
Image of Sample Printout

```
***** ENDOSAFE Test Record *****
V7.09A 02/14/05
DateTime: ..... 03/03/05 @ 13:12:35
Device: ..... 0019
OperatorID: ..... BCP
Cartridge: ..... Endotoxin
Temperature: .. Start: 37.0C End: 37.0C
Method: ..... KX-122
Cartridge Lot#: ..... 4317154
Range: ..... 1-0.01
Range Time: ..... Sec: 163-848
Onset Time (sec): ... >848 296 >848 290
Slope: -0.358 ..... Intercept: +2.213
Dilution: ..... 1
Sample Lot: ..... B42030
Sample ID: ..... LRW
Sample Value: ..... <0.010 EU/ml
Sample CV: ..... 0.0%
Spike Value: ..... 0.184 EU/ml
Spike CV: ..... 1.5%
Spike Recovery: ..... 102%
```


Attachment 4
Certificate of Analysis (Reference Only)

Endosafe® - PTS Cartridges	
Certificate of Analysis	
Reorder Code: [REDACTED]	Expiration Date: 12/2007
Cartridge Lot #: [REDACTED]	RSE/CSE Ratio: 3 EU/ng
Calibration Code: [REDACTED]	Archived Standard Curve Linearity: -0.999
Archived Standard Curve Range: 5-0.05 EU/mL	Archived Standard Curve Linearity: -0.999
Archived Standard Curve Mean Reaction Times:	5.0 EU/mL 102 seconds
	0.5 EU/mL 284 seconds
	0.05 EU/mL 686 seconds
Archived Spike Concentration: 0.56 EU/mL	
Negative Control: Non-reactive/Pass	
This lot of PTS Cartridges has been tested and meets Quality Control testing requirements for an archived curve, negative controls and positive product control results.	
Store cartridges at 2-25°C. Allow the unopened foil pouch to reach room temperature prior to opening. Cartridges should be used immediately once the foil pouch seal has been opened. Cartridges are for single-test use only.	
CAUTION: DO NOT FREEZE THE CARTRIDGES	
Qualified Analyst: [REDACTED]	Date: [REDACTED]
Reviewed By: [REDACTED]	Date: [REDACTED]
Charles River Laboratories 1023 Wappoo Road Charleston, SC 29407	