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Changes from previous version:

Section	Summary of Changes	Change Control Number
ALL	1. New document	

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1. PURPOSE

This document describes the procedure for quantitatively analyzing endotoxin concentration using the Endosafe® nexgen-MCS.

2. SCOPE

This SOP encompasses the principles, use, maintenance, and troubleshooting of the Endosafe® - MCS (Multi-Cartridge System).

3. INTERNAL REFERENCES

Document ID	Title
QA001	Quality Policy

4. EXTERNAL REFERENCES

Document ID	Title
Endosafe® User's Guide	Endosafe® - Multi-Cartridge System User's Guide
LAL Endosafe® Cartridge package insert	Limulus Amebocyte Lysate Endosafe® Cartridges
ICH Q7 (API)	Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
ICH Q9	Quality Risk Management
ICH Q10	Pharmaceutical Quality System
USP 85	Bacterial Endotoxins

5. RESPONSIBILITIES

Job Function and/or Department	Responsibility
All Personnel	It is the responsibility of all employees, including temporary employees and consultants, involved in recording, or reviewing information on documents to provide clear and consistent details on record keeping and follow good documentation practices to support the quality of work.

6. DEFINITION

Term	Definition

7. PROCEDURE

The basis of the test is that endotoxin produces changes in the appearance of LAL that are measured quantitatively by kinetic chromogenic methods.

7.1. Sample receipt and disposition

7.1.1. Samples should be analyzed as soon as possible. When samples cannot be analyzed as soon as possible, samples will be stored frozen unless specific sample stability information is available. Frozen samples will be allowed to reach ambient temperature prior to analysis.

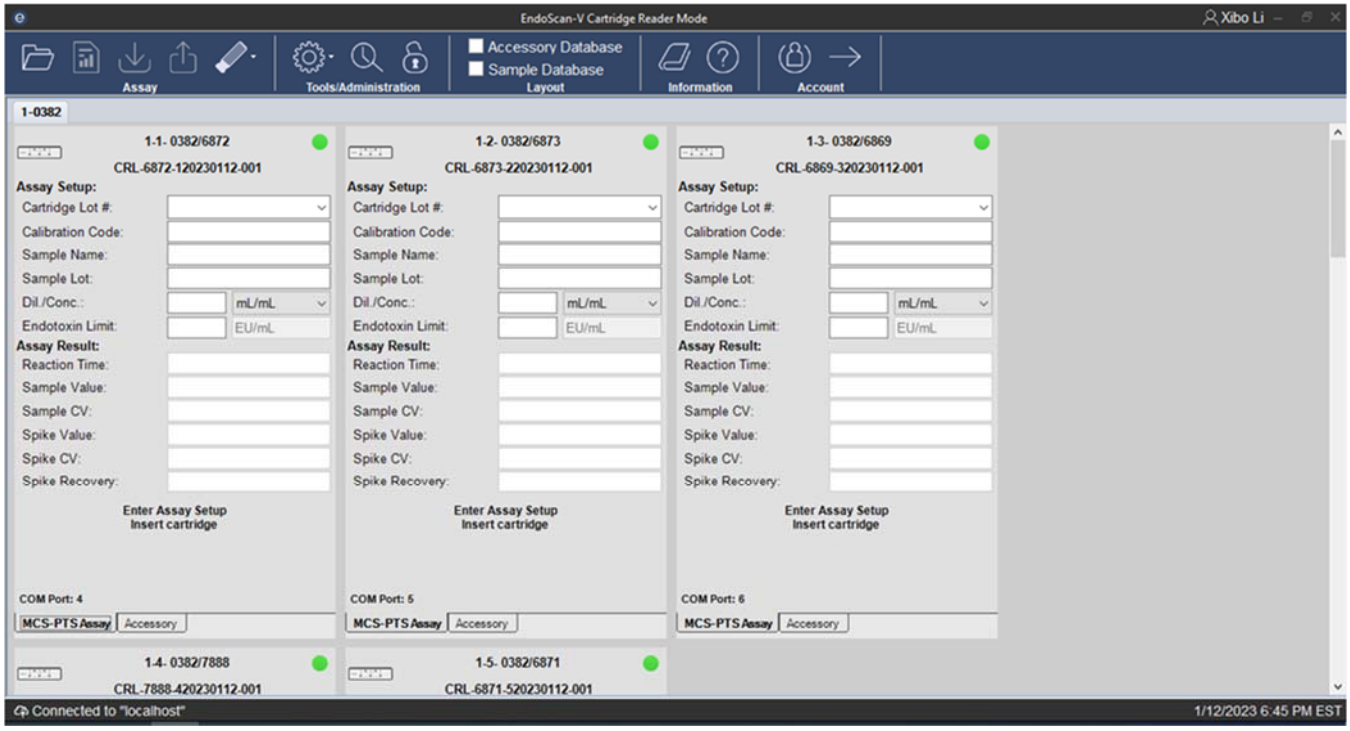
7.1.2. Following analysis samples will be stored frozen until acceptable to discard as hazardous waste.

7.2. Operation of the Endosafe® - MCS (Multi-Cartridge System)

7.2.1. Power on the Endosafe® MCS reader and log into the associated PC with EndoScan-V software. User names and passwords are case sensitive. If the Endosafe® MCS reader is not on prior to logging into the software a warning is displayed that no cartridge instrument are connected, click OK. Once the instrument is powered on, select Cartridge icon, then Reconnect.

7.2.2. Allow the Endosafe® reader warm to 37°C, each of the 5 slots will beep when the appropriate temperature is reached, the red dot will turn into green.

7.2.3. Once the PC and MCS reader are successfully connected and warmed, the following screen will appear:



7.2.4. Enter the Cartridge Lot number and Calibration Code specific for the lot of cartridges being used into the respective fields. The lot number can be found on the box, cartridge pouch, and/or the Certificate of Analysis. The calibration code can be found on the Certificate of Analysis which can be accessed on the manufacturer's website. If the calibration code for the particular lot # has already been entered, the software automatically inputs this information until the lot numbers and calibration codes are erased by the user.

7.2.4.1. Each new lot of cartridges must be qualified prior to use.

7.2.4.2. Initial qualification testing requires one cartridge with LAL Reagent Water as the sample.

7.2.4.3. The evaluation must demonstrate no detectable endotoxin and acceptable spike recovery.

7.2.5. Enter the sample name and sample lot.

7.2.6. Enter the concentration or dilution of the sample in the Conc/Dil field. Select the appropriate unit of measure for the sample from the drop-down list. When the sample has an unknown concentration and is analyzed without a dilution, 1:1 is entered in the Conc/Dil field and ml/ml is selected from the unit's drop-down list.

7.2.7. LAL Endosafe® Cartridges are stored at 2-25°C. Allow cartridges to come to room temperature before opening the pouch and testing. Touch only the sides and handle of the cartridge.

7.2.8. Add 25µL of sample into each of the 4 wells of the test cartridge.

7.2.9. Insert the cartridge firmly into the cartridge bay at the front of the reader with the sample reservoirs facing up. Up to five cartridges may be tested simultaneously.

7.2.10. Select the Send Setup button to send information to the MCS Reader.

7.2.11. Once all test information is entered the software displays "Add sample and click start."

7.2.12. Select Start. While in progress, the screen will display messages showing the current status.

7.2.13. Repeat steps 7.2.4 through 7.2.12 for each cartridge to be tested.

7.2.14. Upon completion of the assay, a message will be displayed "Assay complete" with results of the assay. Refer to section 7.3 for validation of results.

7.2.15. Completed cartridges are disposed of as hazardous waste.

7.2.16. To begin a new assay click on the "New Assay" button and repeat beginning with step 7.2.4.

7.2.17. Results are automatically saved electronically in a file labelled with the date of analysis. A printed report

may also be generated as needed.

7.2.18. When analysis is complete, log off the system and shut off the MCS reader.

7.3. Result Validation

7.3.1. Acceptance criteria for a valid assay consists of a positive product control recovery (Spike Recovery) value of 50-200%, and a coefficient of variation (%CV) of less than 25% on reaction times for both sample and Spike Recovery channels.

7.3.2. When the results do not adhere the acceptance criteria, a dilution will be made to eliminate interfering factors such as pH, ionic strength, and high background endotoxin. Refer to section 7.4for dilution instructions.

7.4. Sample Dilution

7.4.1. Samples exhibiting inhibition (interference) are diluted with only LAL Reagent Water.

7.4.2. Samples will not be diluted beyond the MVD (Maximum Valid Dilution)

MVD is calculated as:

$$MVD = \frac{Exdotoxin\ Limit \times Sample\ Concentration}{Lambda}$$

Where Lambda= sensitivity (lowest point on the archived curve) of test cartridge

7.5. Maintenance

7.5.1. While the instrument is turned off, the exterior of the Endosafe® reader may be wiped with a cloth dampened with water or a mild detergent.

7.5.2. Use a clean dry cloth to dry all wet surfaces.

7.6. Troubleshooting

7.6.1. If the cartridge reader becomes disconnected, from the main menu select Cartridge icon, then Reconnect.

7.6.2. Listed in the table below are various Error or Fault messages and a description of the corresponding cause of the error or fault.

a. One or more long audible tones will precede each error message.
b. Any fault listed here will prevent the reader from running a test,
c. Unless it is a hardware problem, the only way to clear an error message is to turn off the reader and turn it back on.

Message	Description
A2D SETUP	Photo A/D setup did not complete
TEMP TOO LOW	Cartridge temperature is too low
TEMP TOO HI	Cartridge temperature is too high
MOTOR NOT HOME	Cannot run pump motor
CLOCK FAULT	Cannot read Real Time Clock
Computing Error	Divide by 0 or range error
UNKNOWN	Any unspecified system fault
One or more test LEDs out of spec: Abort Test CANCEL or ENTER	One or more of the LEDs does not have sufficient intensity to run the test properly
Sample Not Seen flashes during test	Sample was not detected in one or more of the optical cells

7.6.3. If any of these or other problems persists, contact Charles River Laboratories Technical Service.