Flow Cytometry Research Group 2016-2017 Study

# Endotoxin Contamination of Cell Sorters: Evaluating Cleaning and Testing Procedures

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## Abstract

Cell sorting plays an important role in many *in vitro* and *in vivo* studies. Sorted cells are often placed back into culture for expansion, used for *in vitro* experiments or adoptively transferred into animals. Thus, anything that the cells come into contact with while passing through the cell sorter has the potential to affect their function, including cytokine production or proliferation. This is particularly true for endotoxin, a lipopolysaccharide derived from gram-negative bacteria, which can elicit a variety of direct and indirect cellular responses, depending on cell type. Every flow cytometry shared resource lab has its own routine cleaning procedures for sorters. However, endotoxin is not commonly considered and is not usually included in testing as a contaminant in the cell sorter fluidics. To investigate the prevalence of endotoxin contamination samples were collected from sorters in various cell sorting facilities across the USA. In addition, a hydrogen peroxide cleaning procedure was tested by a subset of the facilities to determine its effectiveness in eliminating endotoxin contamination. The results will be presented here.

## Background

Endotoxin aka lipopolysaccharide (LPS) is a large molecule consisting of a lipid and O-linked polysaccharide. It is found in the outer membrane of gram negative bacteria and is typically released upon the death of the bacteria. It is known to elicit an immune response even at very low levels (0.02ng/ml)[1]. Macrophages, dendritic cells and B cells are particularly sensitive to endotoxin. LPS activates these cells by binding to a receptor complex made up of TLR4, CD14 and MD-2. If these cell types are passed through a sorter that is contaminated with endotoxin they could become inadvertently activated, thus affecting the results of downstream assays.

Last year the FCRG began a study to look at "how clean is your sorter really?". The idea behind the study was that anything (i.e. RNAses, endotoxins, bacteria and fungus) that a cell comes in contact with during the sorting process can affect the results of downstream assays. In last year's screen of FCRG members' sorters a number of them had endotoxin contamination. For this year, we chose to expand the testing to sorters in other facilities across the USA. In addition, to remove endotoxin from contaminated sorters a $H_2O_2$ protocol based on two publications[2,3] was tried. $H_2O_2$ was chosen because it oxidizes the endotoxin thereby inactivating it.

## Participants Profile

47 sorters from 17 Flow Cytometry Core Facilities in USA
Bacteria/Yeast run in 27 instruments
Instrument Types:

| | |
|---|---|
| Astrios – 6 | Jazz - 1 |
| FACS Aria – 29 | MoFlo - 2 |
| Fusion – 1 | S3 – 5 |
| Influx – 3 | |

## Protocol for Collecting and Testing Samples for Endotoxin Contamination

1. Sorters were started up as normally done following standard startup protocol for the lab
2. A sterile 10 ml pipet was used to collect samples from the sheath tank connected to the instrument
3. After turning on the sorter and fluidics following normal procedures, 10 ml of sheath fluid was collected in a single 15 ml tube of sheath fluid by placing a sterile 15 ml conical tube under the fluid stream exiting the nozzle
4. Samples were shipped on wet ice or with freezer packs to the testing lab
5. Endotoxin levels in the samples were determined using the Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo Fisher catalog #88282)
   Detection range: 0.01 – 0.1 ng/ml LPS

**Figure 1. Sixty-two Percent of the 0-5 Year Old Sorters had Detectable Endotoxin Levels**

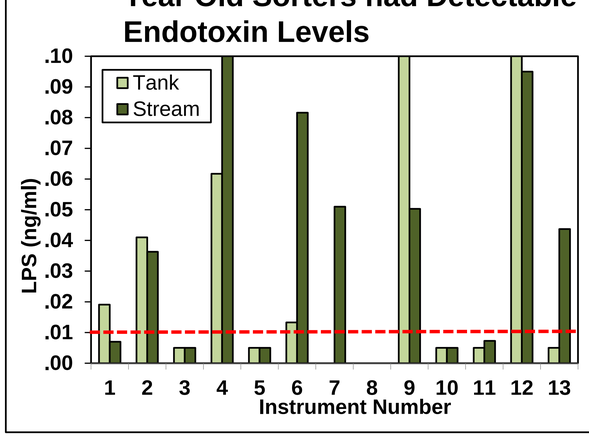



**Figure 2. Seventy-eight Percent of the 6-10 Year Old Sorters had Detectable Endotoxin Levels**

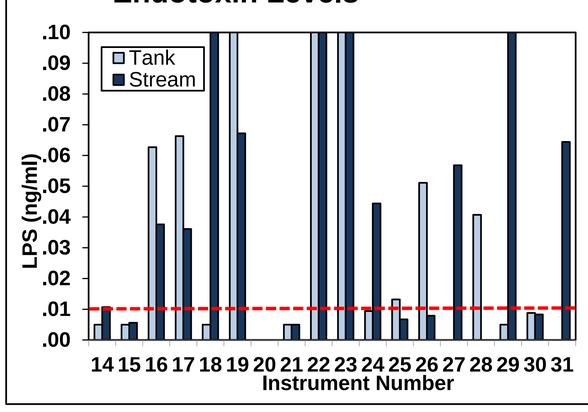
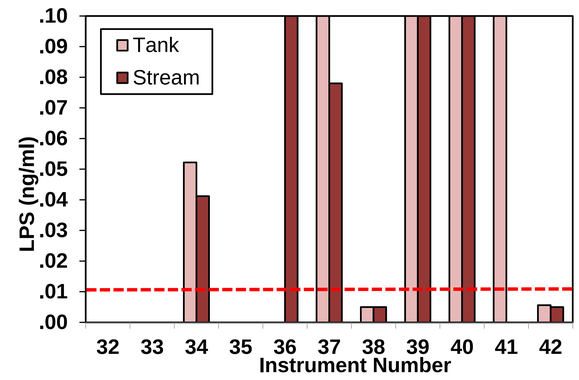



**Figure 3. Fifty-Five Percent of the > 10 Years Old Sorters had Detectable Endotoxin Levels**




**NOTE:** The age of 5 sorters were not reported. Of those five, 3 had endotoxin levels of > 0.1 ng/ml and 2 had 0.068 and 0.076 ng/ml in the samples collected from the stream of the instrument.

## Parameters That Did Not Correlate with Endotoxin Results

1. Date of last PM (preventative maintenance)
2. Source and type of sheath (homemade vs company)
3. Recent contamination
4. Cleaning methods including bleach, water, ethanol, detergent and sporacidin
5. Modifications to the fluidics system of the instrument
6. Whether or not bacteria or yeast have been run through the instrument

## Protocol for Removing Endotoxin from a Sorter

1. Remove sort nozzle (on ARIAs only) and bypass sheath filter
2. Fill sheath tank halfway with 1% $H_2O_2$ (30% $H_2O_2$ stock diluted with sterile $H_2O$)
3. Perform a fluidics startup
4. Load tube containing 1% $H_2O_2$ on sample loader
5. Perform a "Clean Flow Cell" procedure 2-3 times
6. Turn on stream
7. Run tube of 1% $H_2O_2$ at highest flow rate for 2h
8. Empty sheath tank, fill with 1% $H_2O_2$ and let sit for 2h then rinse thoroughly with sterile water and fill with sheath
9. Perform "Fluidics Startup" – repeat 3 times
10. Load tube containing sterile sheath on sample loader
11. Perform "Clean Flow Cell" procedure 5 times
12. Turn on stream
13. Run tube of sterile sheath at highest flow rate for 3 h
14. Perform an ethanol fluidics clean
15. Replace $H_2O_2$/$H_2O$ saturated sheath filter with a brand new filter

## Results of $H_2O_2$ Cleaning

**Table 1. Cleaning #1 – Endotoxin Levels in the Stream**

| | Pre-clean | Day 3 Post clean | Day 65 Post Clean |
|---|---|---|---|
| **Sorter A** | 0.054 ng/ml | BDL* | 0.089 ng/ml |
| **Sorter B** | 0.089 ng/ml | BDL* | 0.087 ng/ml |
| **Sorter C** | 0.082 ng/ml | 0.091ng/ml | 0.052 ng/ml |

*Below Detection Limit (0.01ng/ml)

**Table 2. Cleaning #2 – Endotoxin Levels in the Stream**

| Stream | Pre-clean | Day 10 Post clean |
|---|---|---|
| **Sorter A** | Not Done | 0.070 ng/ml |
| **Sorter B** | Not Done | 0.082 ng/ml |
| **Sorter C** | Not Done | 0.072 ng/ml |

**Table 3. Cleaning #3 – Endotoxin Levels in the Sheath Tank & Stream of Sorter D**

| | Pre-clean | Day 1 Post clean | Day 6 Post Clean | Day 10 Post Clean |
|---|---|---|---|---|
| **Tank** | >0.100 ng/ml | BDL* | BDL* | >0.100 ng/ml |
| **Stream** | >0.100 ng/ml | 0.100 ng/ml | Not done | Not done |

*Below Detection Limit (0.01ng/ml)

## Conclusions

1. Many sorters in core facilities have some level of endotoxin contamination.
2. There does not appear to be any specific pattern or explanation on why some instruments are contaminated.
3. Cleaning with $H_2O_2$ does not always remove endotoxin but when it does clean the instrument does not stay clean long.

## Acknowledgements

We would like to thank all the individuals who provided samples for our study.

## References

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3. Lin S.M., Svoboda K.K.H., Giletto A., Seibert J. and Puttaiah R. 2011. Effects of hydrogen peroxide on dental unit biofilms and treatment water contamination. European Journal of Dentistry. 5:47-59.