Reduction in Endotoxin Levels After Performing the Prepare for Aseptic Sort Procedure on the BD FACSAria II Flow Cytometer

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Application Note

Contents

Abstract

The ability to sort cells in an endotoxin-controlled environment is vital for certain in vitro and in vivo studies in which the presence of endotoxin can interfere with the accurate interpretation of results. Systemic exposure of endotoxin levels as low as 1 ng/mL (10 endotoxin units (EU)/mL) can result in toxic shock and death in mammals. The maximum endotoxin level considered safe for a 20-g mouse (by intravenous injection) is 0.1 EU administered over a 1-hour period. Endotoxin (the lipopolysaccharide component of the cell wall of gram-negative bacteria) is ubiquitous since bacteria can survive in nutrient-poor environments, and is readily transferable if special precautions are not used.

Bacteria can contaminate the surfaces and fluidics system of a cytometer, especially in situations in which the machine is used by multiple users. The BD FACSAria™ II cell sorter was specifically designed to facilitate decontamination of the fluidics pathway and has several features that minimize the occurrence of bacterial contamination and facilitate the cleaning and decontamination of the fluidics system using the Prepare for Aseptic Sort (PAS) procedure. When used in this context, the word aseptic does not imply that cells sorted on the BD FACSAria II system will be free from all potentially infectious material, but only that this cleaning procedure can be used prior to sorting and can result in a sorted cell suspension free from bacterial contamination. In a previous application note, we demonstrated that the PAS procedure was effective at decontaminating a BD FACSAria II flow cytometer contaminated with up to 9.8×10^5 CFU/mL of different bacteria.[1]

In this proof of principle study to determine endotoxin levels after the PAS procedure, samples were collected from three BD FACSAria II flow cytometers and tested for the presence of endotoxin. The baseline endotoxin levels of these three machines was variable (range = 2.46–83.9 EU/mL). The PAS procedure was performed and new samples collected. The endotoxin levels in all three machines were significantly reduced (range = <0.01–0.36 EU/mL), but endotoxin levels in samples collected during a test sort were found to increase in two out of three cytometers. A low-endotoxin PAS procedure was then performed using low endotoxin or pyrogen-free reagents, additional cleaning and sterilization, replacement of all filters, and handling equipment, samples, and solutions in a biological safety cabinet whenever possible. These extra precautions resulted in an additional lowering, or maintenance of the already low endotoxin environment, to levels suitable for in vivo studies (<0.1 EU). It is important to note that the BD FACSAria II system is a research use only (RUO) instrument.

Introduction

Endotoxin is a generic term used to describe the lipopolysaccharide (LPS) components of gram-negative bacterial cell walls, which are released into the environment when a bacterial cell dies, and at low levels during bacterial cell division. Gram-negative bacteria are ubiquitous since they can survive and proliferate in nutrient-poor conditions. Endotoxin is known to be a potent inflammatory mediator and pyrogen in vivo and can result in toxic shock, tissue injury, and death. The maximum level of endotoxin that is considered safe for intravenous application is 5 EU/kg/h. For a 20-g mouse, this can be calculated to be the equivalent of no more than 0.1 EU administered over a 1-hour period.[2]

Lipopolysaccharide is known to induce monocyte and macrophage activation in vitro, resulting in the production of inflammatory mediators such as nitric oxide synthase II, cyclo-oxygenase-2, endothelin-1, tumor necrosis factor alpha, interleukin-1β, interleukin-6, and other inflammatory cytokines. Recent in vitro studies have shown that LPS acts by specifically binding to LPS-binding protein and CD14 (soluble or cell surface), that then form a complex with MD2 and toll-like receptor-4 on the cell surface, resulting in activation of the transcription factor NFκB.[3] As research on this ubiquitous family of LPS molecules and their role in many aspects of immunology progresses, especially in the fields of inflammation, innate immunity, tolerance, and allergy, it is becoming important to perform in vivo and in vitro experimentation in an endotoxin-controlled environment.

Since endotoxin is ubiquitous and stable, and can be readily transferred from a contaminated to a non-contaminated surface, extreme vigilance is required to prevent the cross-contamination of products, samples, and equipment, even in the absence of viable gram-negative bacteria. Endotoxin can be present on surfaces that a sample comes in contact with, such as tubing, tubes, pipettes, and containers, due to inherent contamination. Endotoxin can be inadvertently introduced by the use of solutions or reagents used to prepare the surfaces prior to introducing the cells, such as cleaning, pre-wetting, or using contaminated reagents during instrument setup. Solutions or other auxiliary reagents used in the direct preparation of cells such as antibodies, buffers, cell culture media, and sera can also be a source of endotoxin. Endotoxin levels present in a solution, reagent, or surface can only be determined by testing. Many cell culture products and reagents that have been tested for the presence of endotoxin and are known to be low in endotoxin or are classified as non-pyrogenic are now available. The level of endotoxin of a cell preparation can only be as low as the "most contaminated" solution or surface that the cells have come in contact with.

There is a developing need for limiting or controlling levels of endotoxin present in cell suspensions generated using aseptic sorts performed on flow cytometry based sorting platforms in general, and the BD FACSAria II system in particular. This is not only important for studies of immune mechanisms highlighted previously, but also for the sorting of specific cell populations such as stem cells or specific immune cells for use in research-based, in vitro, and in vivo (pre-clinical) studies. Limiting endotoxin levels in these studies is important for developing an accurate understanding of pathways, efficacy, and toxicity. The presence of endotoxin can bias or interfere with results by inducing unwanted effects.

New fluidics design

The BD FACSAria II flow cytometer has been designed for easy cleaning and decontamination. When compared to the BD FACSAria™ flow cytometer, the BD FACSAria II instrument has a simplified sheath path with a reduced number of components, decreasing the fluid volume and the surface area available for contamination. The sheath tank is removable and can be autoclaved. Some of the tubing is now made from Teflon®, and the new fluidics system requires fewer valves. Valves are a manifold style (with less dead volume than the original style) and are very reliable. The sheath fluid path has a dedicated fluid line separate from the cleaning fluid line that is used only during cleaning and shutdown. Taken together, these new features reduce the opportunity for bacterial contamination, and therefore reduce the risk of endotoxin contamination, and increase the efficacy of the cleaning and decontamination procedures.

Prepare for Aseptic Sort procedure

The Prepare for Aseptic Sort (PAS) wizard in BD FACSDiva™ software has been designed to lead the BD FACSAria II operator through a decontamination of the fluidics system. When used in this context, the word aseptic does not imply that cells sorted on the BD FACSAria II will be free from all potentially infectious material, but only that this cleaning procedure can be used prior to sorting and may result in a sorted cell suspension free from bacterial contamination. When the operator initiates the PAS procedure, the DI (deionized) water, umbilical, and cytometer fluid pathways are rinsed, and the sample line is backflushed, followed by a soak in 10% bleach (0.5% sodium hypochlorite solution). This is followed by a rinse, backflush, and soak in DI water. Finally, the system is thoroughly flushed with 70% ethanol to remove any residue. During the subsequent Fluidics Startup procedure, the umbilical and cytometer fluidics pathways are rinsed and the sample line is backflushed with sheath fluid, preparing the instrument for setup and sample loading. Studies undertaken during the development of the PAS procedure showed that residual levels of bleach and ethanol were less than 0.2 parts per million (<0.2 ppm) after the Startup procedure was performed.

The PAS procedure can be used after a known bacterial contamination has occurred, immediately prior to an aseptic sort, or as a routine maintenance procedure to minimize or prevent the occurrence of bacterial contamination in the cytometer's fluidics system. A reduction in the risk of the occurrence of bacterial contamination will also limit the risk of elevated endotoxin levels in the cytometer's fluidics system, provided that low-endotoxin reagents are used in the PAS procedure.

Previous studies undertaken at BD Biosciences have demonstrated that the PAS procedure is effective at removing high levels of bacterial contamination from a BD FACSAria II system spiked with bacterial cultures.[1] This study demonstrated that the PAS procedure is effective in decontaminating a BD FACSAria II cytometer contaminated with up to 9×10^5 CFU/mL of a mixture of bacteria. The cytometer remained bacterium free for at least four days after the PAS was performed.

In this study, we investigated the levels of endotoxin present in three BD FACSAria II cytometers used routinely in the laboratories located at BD Biosciences, San Jose. Baseline samples were collected (Phase 0 – Baseline), then a standard PAS procedure was performed and samples collected a second time (Phase 1 – Standard PAS). A second enhanced PAS procedure was then performed, introducing extra cleaning and sterilization steps, and the replacement of as many fluids as possible with low endotoxin, sterile reagents. A third set of samples was then collected (Phase 2 – Low endotoxin PAS). All samples were tested for the presence of endotoxin using a kinetic Limulus Amoebocyte Lysate (LAL) assay.

Objective

The objective of this application note is to demonstrate that, in addition to being an effective way to eliminate bacterial contamination, the PAS procedure is also effective at reducing endotoxin levels present in the fluidics system of a BD FACSAria II flow cytometer.

Methods

A summary of the study workflow is presented in Figure 1.

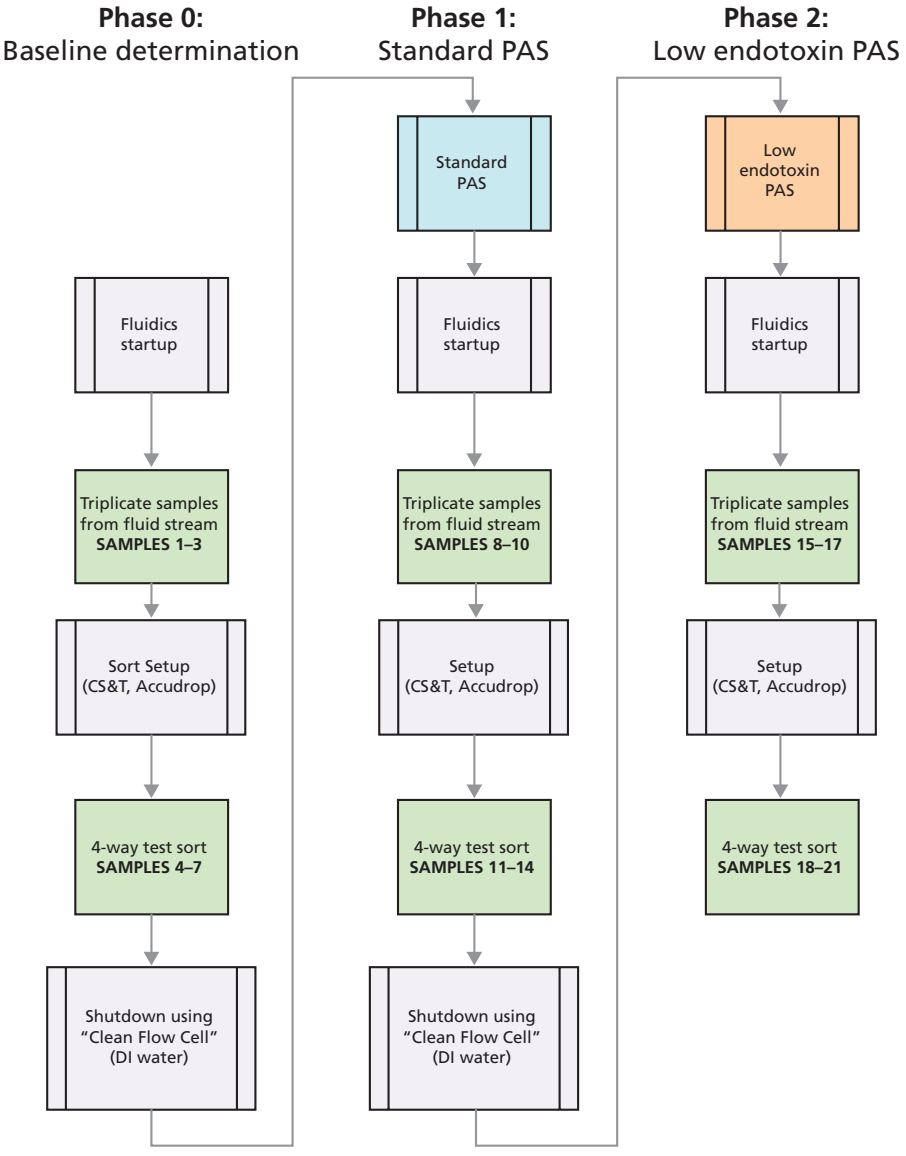

Figure 1. Study Flow Chart

Table 1. Materials

Item	Vendor	Product Number	Phase 0 (Baseline)	Phase 1 (PAS)	Phase 2 (Low endotoxin PAS)
12 x 75-mm tubes (Non-sterile)	BD	352008	X		
12 x 75-mm tubes (Sterile, non-pyrogenic)	BD	352063		X	X
BD FACS™ Accudrop beads	BD	345249	X	X	X
CONTRAD® 70 (Decon Labs Inc.)	BD	99-30109-00			X
BD™ CS&T beads	BD	642412	X	X	X
BD FACSClean™ solution	BD	340345	X		
Sheath filter	BD	331394		X	X
Wet cart filters	BD	643867			X
In-house DI water supply	N/A	N/A	X		
Test tubes: sterile, non-pyrogenic (15 mL)	Pacific BioLabs	10.9151	X	X	X
Clorox® Bleach Ultra	VWR	37001-060	X		
DECON-AHOL® 70% WFI (Veltek Associates, Inc.) (Sterile, USP low endotoxin product)	VWR	14003-278			X
DPBS w/o Ca & Mg, 10X, Sterile IVD product (Mediatech)	VWR	MT-20-031-CV		X	X
Ethanol, denatured 63–63.5%	VWR	BDH1164-4LP	X		
Gamma Wipes™ (Berkshire Corp.)	VWR	21913-812			X
HYPO-CHLOR® (Veltek Associates, Inc.) (Sterile, USP product)	VWR	14003-332			X
HyPure™ WFI quality water (Hyclone®) (Sterile, USP product)	VWR	82013-254		X	X
PBS, Sterile Concentrate, OmniPure 10X	VWR	EM-6506	X		
STER-AHOL® 70% WFI (Veltek Associates, Inc.) (Sterile, USP low endotoxin product)	VWR	14003-354			X

Abbreviations: CS&T – Cytometer Setup and Tracking; DI water – deionized water; DPBS – Dulbeccos's phopsphate buffered saline; IVD – In vitro diagnostic; PBS – Phosphate buffered saline; USP – United States Pharmacopeia; WFI – water for injection.

Instruments

This study was performed on three independent BD FACSAria II flow cytometers used routinely in the laboratories located at BD Biosciences, San Jose, CA.

Sample collection

The BD FACSAria II cell sorter was turned on, a Fluidics Startup performed, a tube of sheath fluid loaded as a mock sample, and three samples (approximately 4.5 mL) of the fluid stream (sample and sheath fluid that normally pass to waste) were collected into 15-mL test tubes (sterile, non-pyrogenic). A performance check was performed (CS&T) and the cytometer was set up for a sort using automatic drop delay as instructed in the *BD FACSAria II User's Guide*.[4] Four collection tubes (12 x 75-mm tubes) were installed into the collection device and a Test Sort (4 tube) performed, collecting approximately 4.5 mL of the side streams into each tube. These samples were aseptically transferred into 15-mL test tubes (sterile, non-pyrogenic) for storage and transported to Pacific BioLabs for endotoxin testing. The cytometer was shut down using the Clean Flow Cell command using DI water.[5,6]

Phase 0 – Baseline determination

Samples were collected as outlined previously, prior to any cleaning, to determine the baseline level of endotoxin present in the fluidics system of each flow cytometer.

Phase 1 – Standard Prepare for Aseptic Sort procedure

After collection of baseline samples, each cytometer was prepared for an aseptic sort as outlined in the *BD FACSAria II User's Guide*[4] and using the software wizard in BD FACSDiva™ software. Samples were collected as outlined to determine the level of endotoxin present in the fluidics system of each flow cytometer immediately after a standard PAS procedure.

Phase 2 – Low endotoxin Prepare for Aseptic Sort procedure

After the completion of Phase 1, each cytometer was prepared for an aseptic sort using the standard PAS procedure with the following modifications:

- Handling of components and samples was performed wearing gloves to prevent the transmission of bacteria and/or endotoxin to the cytometer or sample. Gloves were changed frequently.
- Solutions and cloths used for the cleaning and refilling of tanks were sterile and/or low in residual endotoxin whenever possible as determined by the manufacturer and documented by a Certificate of Analysis (C of A). The product description and endotoxin levels of individual reagents are shown in Table 2.

Table 2. Endotoxin levels present in the products used to perform the low endotoxin PAS procedure

Item	Product Name*	Product Description	Endotoxin level (from manufacturer's C of A)
Sheath	DPBS w/o Ca & Mg, 10X, (Mediatech) (Diluted 1:10 in WFI – see below)	Sterile IVD	<0.005 EU/mL
Ethanol	STER-AHOL 70% WFI	Sterile, USP, low endotoxin	<0.03 EU/mL
Bleach	HYPO-CHLOR (Diluted 1:10 in WFI – see below)	Sterile, USP	Not tested
DI water	HyPure WFI quality water (Hyclone)	Sterile, USP	<0.01 EU/mL
12 x 75-mm tubes	12 x 75-mm tubes	Sterile, non-pyrogenic	≤0.5 EU/mL
Isopropanol	DECON-AHOL 70% WFI	Sterile, USP low endotoxin	<0.03 EU/mL
Sterile cleaning cloths	Gamma Wipe	Sterile	Not tested
Collection and storage tubes for samples	Test tubes (15 mL)	Sterile, non-pyrogenic	<20 EU/tube

* Refer to Table 1 (Materials) for product catalog numbers and suppliers

- The three fluid containers, container probes, lids, sheath tank, and collection device were soaked for 2 hours in a 5% (v/v) solution of Contrad 70 diluted in water for injection (WFI), then rinsed in WFI. The fluid containers and the sheath tank were autoclaved (130°C, 30 min, 15 psi).
- The cleaned fluid container probes, lids, and the collection device were stored overnight wrapped in sterile cloths (Gamma Wipes). Immediately prior to use, the items were transferred to a biological safety cabinet (BSC) and the fluid probes and collection device were decontaminated by soaking in a 0.5% (v/v) solution of HYPO-CHLOR (diluted from 5% in WFI) for 20 minutes. Items were thoroughly rinsed in WFI followed by a final rinse in STER-AHOL WFI (70% solution of sterile, low endotoxin, USP, ethanol in WFI). The sheath tank probe and fluid container lids were cleaned using STER-AHOL WFI and sterile Gamma Wipes.

- Autoclaved containers were reassembled (probes and lids), and filled with sterile, low endotoxin reagents, using aseptic technique in a BSC. Reagents are listed in Table 1.
- The wet cart fluid filters were replaced and a Perform Prime Tank after Refill procedure performed as outlined in the *BD FACSAria II User's Guide*.[4] This procedure was repeated as necessary until all filters were purged with the fresh (low endotoxin) fluids.
- The accessible components of the cytometer (inside the sort chamber, deflection plates, and sample loading port) were cleaned using a sterile Gamma Wipe dampened with a 0.5% solution of HYPO-CHLOR (diluted from 5% in WFI) and rinsed using a Gamma Wipe dampened with WFI.
- Cytometer setup was performed using new (unopened) vials of CS&T and BD FACS™ Accudrop beads prepared in sterile DPBS in sterile 12 x 75-mm tubes in a BSC using aseptic technique. Sterile DPBS in a sterile 12 x 75-mm tube was used as "mock sample" and a Test Sort (4 tube) performed, collecting sample streams into sterile 12 x 75-mm tubes loaded into the decontaminated collection device in a BSC and attached to the cytometer immediately prior to use.

The differences between the standard and low endotoxin PAS procedure are summarized in Table 3.

Table 3. Comparison of flow cytometer status durring different phases of the study

Item	Phase 0 (Baseline)	Phase 1 (Standard PAS)	Phase 2 (Low endotoxin PAS)
Sheath	As found	Replaced –Low endotoxin and sterile IVD reagent	Replaced – Low endotoxin and sterile IVD reagent
Ethanol	As found	As found	Replaced – Low endotoxin and sterile USP reagent
Bleach	As found	As found	Replaced – Sterile USP reagent
DI water	As found	Replaced – Low endotoxin and sterile reagent	Replaced – Low endotoxin and sterile USP reagent
CS&T beads	General lab stock	General lab stock	New (unopened) vial
BD FACS Accudrop beads	General lab stock	General lab stock	New (unopened) vial
12 x 75-mm tubes	General lab stock	Sterile, non-pyrogenic	Sterile, non-pyrogenic
Sheath tank	As found	Autoclaved	Cleaned and autoclaved
Sheath filter	As found	Replaced	Replaced
Fluid containers (DI water, bleach, and ethanol)	As found	As found	Cleaned and autoclaved
DI water probe	As found	Decontaminated in bleach	Decontaminated in sterile bleach
Bleach and ethanol probes	As found	As found	Decontaminated
Wet cart filters	As found	As found	Replaced
Collection device	As found	As found	Cleaned and decontaminated
Accessible cytometer components	As found	As found	Cleaned

Determination of endotoxin levels

Samples were tested for the presence of endotoxin by Pacific BioLabs using a Kinetic-QCL® Kinetic Chromogenic LAL Assay (Lonza).

Results

Endotoxin levels measured in samples collected from the three BD FACSAria II flow cytometers at different phases of the study are summarized in Table 4 and presented in Figure 2. A concentration of 0.1 EU/mL was used as a cutoff target value and is indicated by a red line in Figure 2. This concentration had been previously calculated to be a safe level for intravenous administration to a 20-g mouse over a 1-hour period. Refer to the flow chart (Figure 1) for details of when the samples were taken.

Table 4. Endotoxin levels in samples from three BD FACSAria II flow cytometers

Instrument	Average Endotoxin Levels (EU/mL)					
	Phase 0 (Baseline)		Phase 1 (Standard PAS)		Phase 2 (Low Endotoxin PAS)	
	Startup[a]	Test Sort[b]	Startup	Test Sort	Startup	Test Sort
A	8.643 ±0.430	6.860 ±0.623	0.057 ±0.002	0.017 ±0.001	<0.05	<0.05
B	83.940 ±2.225	67.215 ±11.780	0.358 ±0.173	0.934 ±0.234	0.577 ±0.142	0.391 ±0.031
C	2.525 ±0.071	2.456 ±0.656	<0.01	1.674 ±0.339	<0.01	<0.01

[a]Average of triplicate samples ±1 standard deviation [b]Average of quadruplicate samples ±1 standard deviation

These data show that:

- The baseline endotoxin levels of samples collected from three independent BD FACSAria II flow cytometers were variable, ranging from high levels in samples collected from cytometer B (83.94 EU/mL) to relatively lower levels in samples from cytometers A and C (8.64 and 2.53 EU/mL, respectively).
- The standard PAS procedure is highly effective at reducing endotoxin levels to low levels (<0.01–1.67 EU/mL).
  - The efficacy of this reduction is influenced by the baseline endotoxin level initially present in Phase 0. That is, samples from cytometer B had the highest endotoxin levels in Phase 0 and Phase 1. Samples from cytometer C had the lowest levels in both phases.
  - Samples collected from two of the three instruments achieved the target endotoxin level of <0.1 EU/mL during startup immediately after the standard PAS procedure.
  - In two out of three instruments (B and C) the endotoxin levels rose to levels >0.1 EU/mL after instrument setup when samples were collected as a part of a Test Sort.
  - Instrument A showed a further reduction in endotoxin levels after a test sort to 0.02 EU/mL.

- The low endotoxin PAS procedure demonstrated an additional benefit by further reducing or maintaining already low endotoxin levels.
  - Samples from cytometer A maintained the already extremely low endotoxin levels measured in Phase 1 (<0.05 EU/mL).
  - Samples from cytometer B demonstrated a reduction in endotoxin levels from 0.93 ±0.23 EU/mL during the Test Sort in Phase 1 down to 0.58 ±0.14 EU/mL immediately after the low endotoxin PAS procedure and an additional reduction down to 0.39 ±0.03 EU/mL during the Phase 2 Test Sort.
  - Cytometer C achieved the extremely low level of endotoxin found during Phase 1 startup (<0.01 EU/mL) and maintained these levels during the Phase 2 Test Sort (<0.01 EU/mL).

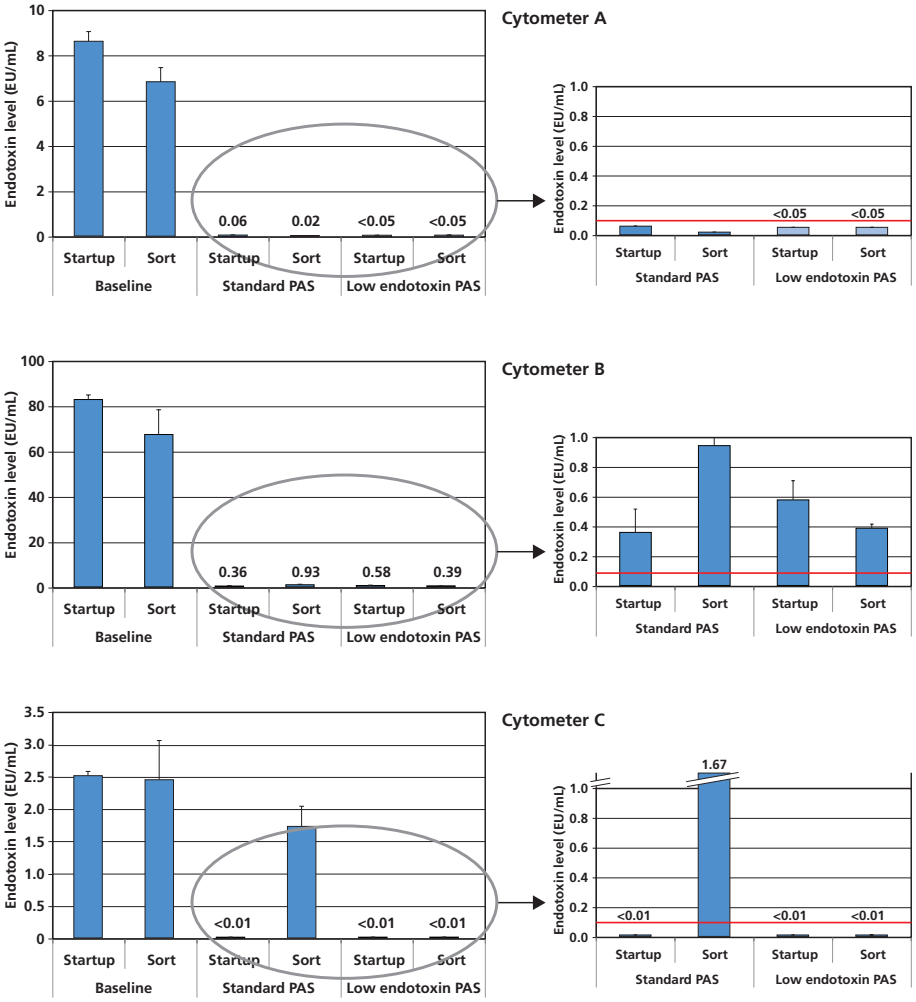

Figure 2. The large graphs in each set are scaled to the data presented since each cytometer had different baseline endotoxin levels. The small graphs in each set are all on the same scale (1 EU/mL) so that data can be easily compared. The red line on each small graph is set at 0.1 EU/mL, the calculated maximum level of endotoxin considered safe for intravenous administration to a 20-g mouse over a 1-hour period.

Discussion

The baseline levels of endotoxin found in different flow cytometers is extremely variable and is probably related to how often the cytometer is used and maintained. Prior to commencing this study, cytometers A and C had been used and maintained on a regular basis compared to cytometer B, which had not. Leaving the cytometer turned off with liquid present in the fluidics system can result in the proliferation and/or death of any bacteria present in the liquid, leading to the deposition of endotoxin inside tubing, valves, and filters.

The data from cytometer B in particular demonstrates that the standard PAS procedure is effective at reducing extremely high levels of endotoxin. The extensive cleaning, filter replacement, autoclaving, and the use of low endotoxin reagents during Phase 2 further reduced endotoxin levels, but the target level of 0.1 EU/mL was not achieved. Additional components that could be cleaned or replaced to help reduce the endotoxin levels even further could include, but are not limited to, the sample lines tubing and nozzle. See the *BD FACSAria II User's Guide* for details on how to clean or replace these components.[4]

The standard PAS procedure (Phase 1) was found to be efficient at reducing endotoxin levels to low concentrations in samples collected from cytometers A and C. An investigation of the reagents routinely used with these cytometers showed that high-quality reagents were used on a regular basis. Type 1 (ultrapure, sterile, low pyrogen) water was routinely used to prepare sheath fluid from sterile 10X PBS, refill the DI water tank, and dilute household bleach for the bleach tank. Target levels <0.1 EU/mL were achieved during Phase 1, and increasing the quality of the reagents and procedure used in Phase 2 maintained low endotoxin levels.

The increase in endotoxin levels detected in samples collected from cytometers B and C during the Test Sort at the end of Phase 1 (from 0.36 to 0.93 and from <0.01 to 1.67 EU/mL, respectively) showed that it is possible to contaminate sort samples even if the cytometer has been previously shown to be low in endotoxin. One of the improvements implemented during the Phase 2 procedure was the cleaning and decontamination of the sort tube holder, loading and unloading the tubes in a BSC, and taking extra precautions with glove changes and sample handling, all of which can contribute to the minimizing of any cross-contamination that could occur.

Conclusions

The baseline levels of endotoxin found in different flow cytometers are extremely variable. Yet even when baseline levels are high, the PAS procedure is very effective at reducing endotoxin concentration to low levels. Even when low levels have been achieved, re-contamination of the instrument can occur. By the introduction of extra precautions and diligence, samples with an endotoxin value of <0.1 EU/mL, a level calculated to be safe for administration to a 20-g mouse, might be achieved.

The use of the PAS procedure does not guarantee that the fluidics system of a BD FACSAria II flow cytometer will be free from bacteria or endotoxin, but demonstrates that a bacteria-free, low endotoxin fluid path might be achieved. Customers should validate this procedure in their own laboratories. The BD FACSAria II cytometer is an RUO instrument and we make no claims of completely eliminating bacteria or endotoxin.

Tips to limit endotoxin contamination of the cytometer

- Endotoxin is a component of the cells walls of gram-negative bacteria. Reducing bacterial contamination will also reduce endotoxin levels.
- Most bacterial contamination comes from human skin cells and hair. Always wear gloves and tie back your hair when touching the fluidics cart and any component of the cytometer.
- Routinely maintain and clean your cytometer as outlined in the *BD FACSAria II User's Guide* using high-quality reagents (sterile, low endotoxin). Implement additional cleaning as necessary.
- Check the quality of your reagents. Ask manufacturers for a Certificate of Analysis.
- While 10% bleach (0.5% hypochlorite) and BD FACSClean solution can be used interchangeably, 10% bleach can lose its effectiveness due to oxidation and reactivity to impurities present in the water used for dilution. BD FACSClean solution is a quality-controlled product and is known to be stable over an extended period of time.
- If you use DI water from your facility's central system, check that it is serviced and sanitized and is of sufficient quality for your needs.
- Do not "top off" the fluid containers and sheath tank. Empty the residual fluid instead and start with fresh fluid each time.
- Clean and autoclave the fluid containers and sheath tank on a regular basis.
- You might want to periodically test samples for endotoxin levels to determine baseline levels.

Tips to limit endotoxin contamination during an aseptic sort

- Use aseptic technique and sterile, low endotoxin reagents, including antibodies, to prepare your cells prior to sorting.
- Use low pyrogen plasticware and low endotoxin ancillary reagents when handling cells.
- Check the quality of reagents, plasticware, and ancillary reagents. Ask manufacturers for Certificates of Analysis.
- Clean the nozzle, install a new sample line filter (if being used), clean the flow cell and deflection plates, and perform a PAS procedure immediately prior to using the cytometer for aseptic sorting using high quality (sterile, low endotoxin) reagents.
- Clean and autoclave fluid tanks and containers and refill with sterile, low endotoxin reagents using aseptic technique.
- Handle CS&T beads, BD™ CompBeads, and Accudrop beads using aseptic technique. Consider using fresh (unopened) bottles for an aseptic sort.
- Decontaminate the tube holder, handle tubes in a BSC, collect sorted cells into sterile, pyrogen-free tubes, and handle the cells using aseptic technique.

Many BD antibodies are manufactured in a low endotoxin environment and formulated in the absence of sodium azide. Search for "NA/LE" (No azide/low endotoxin) at **bdbiosciences.com** for a complete list.

BD Falcon™ products are certified non-pyrogenic based on the US FDA 21 CFR Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies, USPXXVI, Sec 85, Bacterial Endotoxin Test.

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Many BD medical products are also non-pyrogenic.

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Note: A new BD FACSAria User's Guide will shortly be available incorporating references 4, 5 and 6: *BD FACSAria II Users's Guide, Part Number 644832, Rev B, 2009.*

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