

Decontamination of the BD FACSAria II or BD FACSAria III System Using the Prepare for Aseptic Sort Procedure

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Application Note

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Abstract

The ability to perform cell sorts that are free from bacterial contamination is vital for studies that rely on the subsequent culture of the sorted cells in an antibiotic-free environment. The presence of antibiotics can have a deleterious effect on cultured cell physiology and can also result in the generation of low levels of antibiotic-resistant strains of bacteria in the culture medium. The ability to decontaminate a cytometer in preparation for a sort is imperative, especially when there are multiple users and for studies that require a high level of sterility. The BD FACSAria™ II and III cell sorters were specifically designed with this in mind, and both provide several features that minimize the occurrence of contamination and facilitate the cleaning and decontamination of the fluidics system. The BD FACSAria II and III systems are research use only (RUO) instruments.

In this proof of principle study, a BD FACSAria II flow cytometer was contaminated with an atypical level of bacteria to generate a “worst case” scenario that would not normally occur. These data demonstrate that the Prepare for Aseptic Sort procedure outlined in the *BD FACSAria II User's Guide* is effective at decontaminating a BD FACSAria II flow cytometer contaminated with up to 9.8×10^5 CFU/mL of a mixture of different bacteria. The BD FACSAria II system remained bacterium free for at least four days after the Prepare for Aseptic Sort procedure was performed. The fluidics for the BD FACSAria III are identical to the BD FACSAria II, making the results of this study applicable to both instruments.



Introduction

Bacterial contamination of cell products derived by sorting on a flow cytometer can compromise studies subsequently performed on the sorted cells. Many laboratories protect their sorted cell products from such contamination by using antibiotics in the medium for post-sort culture. Antibiotics can have a deleterious effect on the cells in culture and can lead to the presence of low levels of antibiotic-resistant bacterial contamination. The presence of these antibiotic-resistant bacteria might result in a depletion of essential nutrients and factors required by the cells in culture and the accumulation of bacterial metabolic waste products. These waste products might have unknown, unwanted, or toxic effects on cell metabolism or responses.

A cell sorter is often used by several operators (eg, in a flow core lab) for the analysis and sorting of a variety of samples including, but not limited to, human- or animal-derived blood products and cultured cells. These samples might be contaminated with bacteria, viruses, or other pathogens that can potentially cause contamination of subsequent samples. In addition to samples, a cause of contamination is insufficient cleaning and maintenance of the cytometer and the use of fluids that have become contaminated (such as sheath fluid, deionized [DI] water, bleach, or ethanol).

New fluidics design

The BD FACSAria II and III flow cytometers were designed for easy cleaning and decontamination. When compared to the BD FACSAria™, the BD FACSAria II and III instruments have 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 autoclavable. 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 contamination and increase the efficacy of the cleaning and decontamination procedures.

Prepare for Aseptic Sort procedure

The Prepare for Aseptic Sort wizard in BD FACSDiva™ software has been designed to lead the BD FACSAria II and III 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 or III systems will be free from all potentially infectious material, but only that this cleaning procedure can be used prior to sorting and can result in sorted cell suspensions free from bacterial contamination. When the operator initiates the procedure, the DI 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 with DI water. Finally, the system is thoroughly flushed with ethanol to remove any residue. During the subsequent Fluidics Startup procedure, the umbilical and cytometer pathways are rinsed and the sample line is backflushed with sheath fluid, preparing the instrument for setup and sample introduction.

The Prepare for Aseptic Sort 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.

Objective

The objective of this application note is to demonstrate that in this proof of principle experiment the Prepare for Aseptic Sort procedure can remove high levels of bacterial contamination from the fluidics system of a BD FACSAria II flow cytometer. The fluidics for the BD FACSAria III are identical to the BD FACSAria II, making the results of this study applicable to both instruments.

Procedures

Table 1. Materials

Material	Vendor	Part number
BD Falcon™ cell culture flask, 75 cm ²	BD	353024
<i>Pseudomonas aeruginosa</i> (Schroeter) Migula	ATCC	35422
<i>Escherichia coli</i> (Migula) Castellani and Chalmers	ATCC	25922
<i>Bacillus cereus</i> Frankland and Frankland; deposited as <i>Bacillus siamensis</i> Siribaed	ATCC	7064
<i>Staphylococcus epidermidis</i> (Winslow and Winslow) Evans	ATCC	55133
Deionized (DI) water	BD in-house system	N/A
Clorox® Bleach Ultra	VWR	37001-060
Ethanol (70% solution, denatured, sterile)	VWR	JTP004-3
PBS (sterile concentrate, OmniPur®, 10X)	VWR	EM-6506
Sheath filter	BD	331394

Instrument setup

The BD FACSAria II flow cytometer was prepared for this study by autoclaving the sheath tank and DI water containers (121°C, 15 psi, for 30 minutes). Freshly prepared sterile Dulbecco's PBS solution was used to fill the sterile sheath tank. The three auxiliary cleaning fluid containers were then filled with sterile DI water, 70% ethanol, and 10% bleach (0.5% sodium hypochlorite solution in sterile DI water), respectively. The tank and containers were primed and the fluid filters bled.

Contamination of the instrument with bacteria (day 0)

A bacterial cocktail containing *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus epidermidis* was created from individual bacterial cultures in log phase growth. The sheath tank and DI water container were inoculated with a sample of the bacterial cocktail. To contaminate the entire fluidics system, the sheath filter was removed from the sheath fluid line,* and the sheath tank was directly reconnected to the BD FACSAria II instrument. The cytometer was primed to move the contaminated fluids throughout the cytometer fluidics system. A Fluidics Startup was performed, the fluid stream started, a stable drop breakoff pattern established, and a sample line backflush performed to contaminate the sample line tubing. The instrument was then shut down with the bacterial contaminants in the fluidics system.

Four days later (96 hours after inoculation) the BD FACSAria II cytometer was turned on, a fluidics startup performed, the fluid stream started, and a stable drop breakoff pattern established. One hundred milliliters of the sample stream was collected into a sterile BD Falcon cell culture flask for subsequent testing for the presence of bacteria.

* In this experiment, the sheath filter was intentionally removed to allow the bacteria to circulate from the sheath tank throughout the entire fluidics system. Under normal circumstances, the 0.2-µm sheath filter present in the sheath fluid line prevents the spreading of any bacterial contamination that might be present in the sheath tank into the fluidics system.

Decontamination of the instrument using the Prepare for Aseptic Sort procedure (day 4)

The BD FACSAria II system was decontaminated using the Prepare for Aseptic Sort procedure as outlined in the *BD FACSAria II User's Guide*. The sheath tank and DI water container were emptied and autoclaved, and the nozzle (with the o-ring in place) was soaked in a 70% ethanol solution. The Prepare for Aseptic Sort procedure was performed using the wizard within BD FACSDiva software, along with the hardware step of installing a new sheath filter. After completion of the procedure, a Fluidics Startup was performed, the nozzle insertion region of the cuvette was cleaned and dried using cotton swabs, and the cleaned nozzle (with the o-ring in place) reinserted. The fluid stream was started and a stable drop breakoff pattern established.

Sample collection

As before, 100 mL of the sample stream was collected into a sterile BD Falcon cell culture flask for subsequent testing for the presence of bacteria. The cytometer was shut down using the Fluidics Shutdown command. Samples of the sheath fluid were also collected on days 5, 6, 7, and 8.

Testing of samples for bacterial contamination levels

All samples were tested for the presence of bacterial contamination using standard QA testing procedures.

Results and Conclusions

Results are presented in Figure 1. In this study, a BD FACSAria II flow cytometer was contaminated with an atypical level of bacteria to generate a “worst case” scenario of bacterial contamination that would not normally occur.

These data demonstrate that the Prepare for Aseptic Sort procedure outlined in the *BD FACSAria II User's Guide* is an effective decontamination procedure that can be used with a BD FACSAria II flow cytometer contaminated with up to 9.8×10^5 CFU/mL of a mixture of different bacteria. In this experiment the BD FACSAria II system remained bacterium free for at least 4 days after the procedure was performed.

The use of the Prepare for Aseptic Sort procedure does not guarantee that the fluidics system of a BD FACSAria II system will be free from bacteria but demonstrates that, in principle, a bacteria-free fluid path can be achieved. Customers should validate this procedure in their own laboratory. The BD FACSAria II system is a RUO instrument. The fluidics for the BD FACSAria III are identical to the BD FACSAria II, making the results of this study applicable to both instruments.

Tips for Keeping Your Cytometer Free from Bacterial Contamination

- Routinely maintain and clean your cytometer as outlined in the *BD FACSAria II User's Guide*.
- Most bacterial contamination comes from human skin cells and hair. Wear gloves when touching the fluidics system and tie back your hair.
- If you use DI water from your facility's central system, verify that it is serviced and sanitized on a regular basis. Consider using sterile water instead.
- Do not “top up” the fluid containers and sheath tank. Instead, empty the residual fluid and start with fresh fluid each time.
- Clean and autoclave the fluid containers and sheath tank on a regular basis.

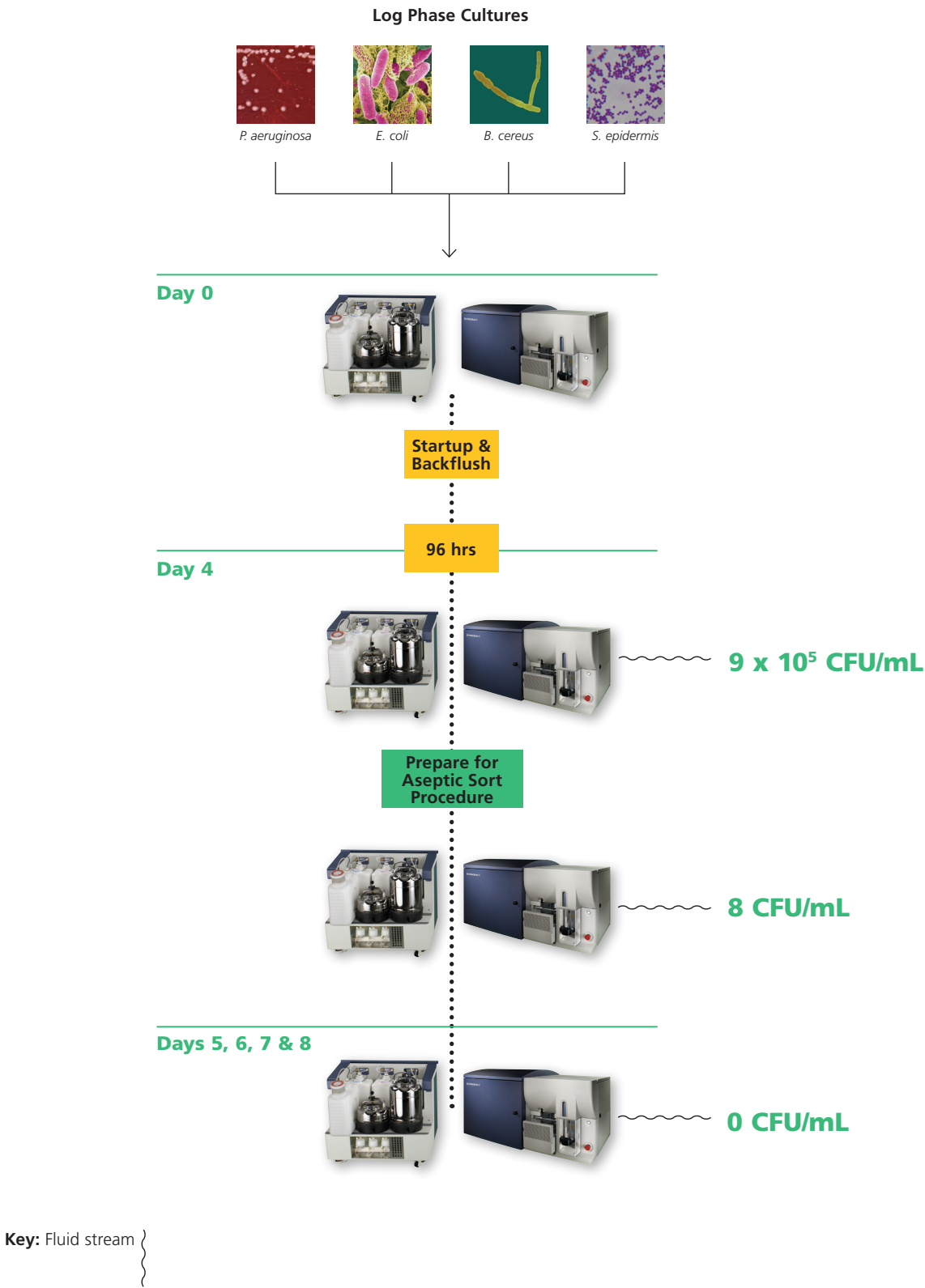
Tips for Performing a Contamination-Free Aseptic Sort

- Use aseptic technique and sterile reagents (including antibodies) to prepare cells prior to sorting.
- Clean the nozzle, clean the flow cell and deflection plates, and perform the Prepare for Aseptic Sort procedure immediately prior to using the cytometer for sorting.
- Clean and autoclave the fluid tanks and containers and refill them with sterile reagents using aseptic technique.
- Handle BD™ Cytometer Setup and Tracking beads, BD™ CompBeads, and BD FACS Accudrop beads using aseptic technique. Consider using fresh (unopened) bottles for an aseptic sort.
- Collect sorted cells into sterile tubes and handle the cells using aseptic technique.

References

BD FACSAria II User's Guide, Part Number 643245, Rev A, 2007

Figure 1. The Prepare for Aseptic Sort procedure decontaminates the BD FACSAria II system



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