Preparation and Decontamination of the Cytoflex SRT for Fluorescence-Activated Cell Sorting (FACS) of Microbes and Submicron (Small Particle) Samples such as Virus-like Particles (VLPs), Extracellular Vesicles, and more

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Purpose: To prevent long-term contamination and disrupt potential formation of unwanted biofilms of the Cytoflex SRT cell sorter following the sorting of microbial samples and/or to clean the flow cell and system in preparation for running submicron samples. This is suitable for submicron/small particle sample types including, but not limited to microbes, virus-like particles (VLPs), extracellular vesicles, organelles, and more. Instructions are included to prepare the instrument for running samples that will require DNA-free preparation for whole genome amplification prior to genomic sequencing. Decontamination protocols for cell sorters and cytometers are instrument specific; while best practices always apply, protocols for one instrument must be thoughtfully adapted for other instruments. This is a protocol that accompanies the following article and additional necessary background can be found there: Tijerina, J.C., Martinez-Hernandez, F., Beilinson, V., Finney, O., Orphan, V.J., & Diamond, R. A. (2025). Preparation of cuvette-based sorters for sorting submicron microbial cells and viruses from environmental and biological samples. Current Protocols, 5, e70176.doi: 10.1002/cpz1.70176 . The full text is available at the publisher website, as well as on Google Scholar and Research Gate.

Protocol 1: Procedure for General Microbial Sorting (samples such as *E. coli* K-12 that range in size from 2-5 microns and 500nm diameter)

Getting started: ensure that you have all reagents, tubes, and cleaning agents available and on hand. See Tijerina et al., 2025 for additional information. You will want to do a regular fluidics startup, QC, and sort calibration to ensure that the instrument is working correctly. Inspect all parts and the wet cart, filters, and instrument carefully for any signs of contamination.

Immediately before beginning to run samples, run the following through the sample line:
Run 5 minutes 25% bleach
Run 5 minutes DI water

For regular microbial experiments, you may then proceed with the experiment as normal. Make sure that samples are diluted to an appropriate concentration that results in an event rate that is not excessively high (should be less than than 10,000 events per second). If the samples appear white or opaque, the samples are too concentrated. Bring extra buffer, pipettes and tips, and tubes to the instrument to perform serial dilutions to optimize the concentration and event rate. Then follow up after the experiment with the cleaning of the flow cell and sample lines to prevent instrument contamination.

Immediately following the experiment (when all samples have been run), run the following through the sample line:
Run 5 minutes 25% bleach
Run 5 minutes DI water

Post-experiment cleaning of the flow cell and sample line:
Stop the stream. Before completing a fluidics shutdown, perform a flow cell cleaning procedure. The flow cell cleaning procedure is an automatic cleaning procedure in the CytExpert software. Instead of the 50% Contrad solution that is used during a monthly maintenance per the instruction manual, instead use Beckman Coulter FlowClean due to its germicidal properties, and the fact that it is a milder detergent for the materials that comprise the instrument, especially if this needs to be done on a regular basis.

Alternate workflow: If you are sorting late and need to leave (remember that researcher well-being is always a consideration), perform the fluidics shutdown following running bleach and water through the sample line as indicated. Then, you may perform the flow cell cleaning procedure the following morning prior to setting the instrument up for the next sort; or first thing the next morning if no sort is scheduled. Do not wait longer than 24 hours to perform this step.

Protocol 2: Procedure for Submicron Sorting (DNA-free submicron/small particle work with samples such as ultramicrobacteria, minicells, viruses and virus-like particles, extracellular vesicles, and more, that are less than 2um but with a submicron diameter, or fully under 1 micron)

Getting started: ensure that you have all reagents, tubes, and cleaning agents available and on hand. See Tijerina et al., 2025 for additional information. Do a regular fluidics startup to ensure that the instrument is working correctly. Inspect all parts and the wet cart, filters, and instrument carefully for any signs of contamination. Set aside a specific dedicated filter for submicron work that uses DNA-free 0.1 micron filtered sheath fluid – do not use the filter that is used with other sheath fluid for regular experimentation as it will reintroduce contaminants and particulates into the specially prepared and filtered sheath for this experiment application. At the time of posting, the Cytoflex SRT does not support running without a sheath filter, although this would be most optimal if possible. Speak with your service engineer or applications specialist to discuss if this changes in the future or a bypass becomes possible.

Immediately before beginning the process, run the following through the sample line:
Run 5 minutes 25% bleach
Run 5 minutes DI water

Pre-experiment cleaning of the flow cell and sample line:
Stop the stream. Before completing a fluidics shutdown, perform a flow cell cleaning procedure. The flow cell cleaning procedure is an automatic cleaning procedure in the CytExpert software. Instead of the 50% Contrad solution that is used during a monthly maintenance per the instruction manual, instead use Beckman Coulter FlowClean due to its germicidal properties, and the fact that it is a milder detergent for the materials that comprise the instrument, especially if this needs to be done on a regular basis.

DNA-Free Workflow for submicron samples requiring amplification for sequencing:

- If you are in a Shared Resource Laboratory setting, or an individual lab that runs many cell types, be sure to either use a fresh sample line, or assign a specific sample line for the sample type.
- Make sure that the shutdown solution tank is cleaned with 25% bleach for 30 minutes and then refilled with DNA-free DI water.
- Clean the instrument surfaces, making sure that no dried sheath fluid residues, dust, or dirt are present and clean the surfaces around the instrument with 70% ethanol or CaviWipes. If possible, follow by cleaning the surfaces with bleach solution.

- Sheath tank preparation:
    - Autoclave then chemically decontaminate two sheath tanks
    - One tank should be filled with DNA-free sheath fluid for use during the sort. The other should be used for the DNA-free aseptic sort procedure.
    - Perform the automated aseptic sort cleaning protocol in the system using 25% bleach (1.5L), 70% ethanol prepared with DNA-free DI water (2L due to the weight of the ethanol solution being insufficient for the tank sensor at 1.5L), and DNA-free DI (1.5L).
        - The required DNA-free DI water, sheath fluid protocol and accompanying tank protocols and more are contained in Tijerina et al, 2025
        - Tank cleaning protocols (autoclave and chemical decontamination) can be found for BD instruments and adapted to the SRT tanks in Tijerina et al, 2025. The key difference is that the SRT tank will not require disassembly or removal of any probes or fittings since the sheath is monitored by an internal scale on the wet cart that monitors the sheath fluid level by weight.

Proceed with the experiment as normal. Make sure that samples are diluted to an appropriate concentration that results in an event rate that is not excessively high. If the samples appear white or opaque, the samples are too concentrated. Bring extra buffer, pipettes and tips, and tubes to the instrument to perform the dilutions to achieve an event rate of 1000 events per second or less. Performing voltration for the gain settings and adjusting the threshholds is necessary. Although Beckman Coulter has calculations available indicating certain ratios for thresholds, these are simply guidelines and not always applicable: you will need to verify your own values qualitatively and use additional technologies to verify sort results such as microscopy, cultivation, or sequencing. This is due to the stochastic nature of submicron small particle detection. Follow up after the experiment with the cleaning of the flow cell and sample lines to prevent instrument contamination.

Immediately following the experiment (when all samples have been run), run the following through the sample line:
Run 5 minutes 25% bleach
Run 5 minutes DI water

Post-experiment cleaning of the flow cell and sample line:

Stop the stream. Before completing a fluidics shutdown, perform a flow cell cleaning procedure. The flow cell cleaning procedure is an automatic cleaning procedure in the CytExpert software. Instead of the 50% Contrad solution that is used during a monthly maintenance per the instruction manual, instead use Beckman Coulter FlowClean due to its germicidal properties, and the fact that it is a milder detergent for the materials that make up the instrument, especially if this needs to be done on a regular basis.

Alternate workflow: If you are sorting late and need to leave (remember that researcher well-being is always a consideration), perform the fluidics shutdown following running bleach and water through the sample line as indicated. Then, you may perform the flow cell cleaning procedure the following morning prior to setting the instrument up for the next sort; or first thing the next morning if no sort is scheduled. Do not wait longer than 24 hours to perform this step.

General Notes for Both Protocols:

- Beckman Coulter Flow Clean is less effective for bacterial samples at shorter contact times because it is meant for decontamination following running more traditional samples in flow cytometry. However, the 30-minute contact time in the flow cell cleaning procedure is what makes it effective enough to prevent contamination long-term. Additionally, it is mild enough to use more frequently than once a month if bacterial samples are run on a regular basis; using it will not damage parts and will not void the service contract since it is a Beckman Coulter product recommended for use on the instrument. For many common pathogens on regular surfaces, 10% bleach with 10 minutes of contact time is considered sufficient, although given that chlorine resistance is an emerging concern, this is a bare minimum and is not sufficient to remove endotoxin contamination. 25% is best in the context of decontaminating a cell sorter for submicron work and microbial work given that the surfaces of the sorter are not open, the possibility of endotoxin contamination (for which a sodium hypochlorite concentration of 2% is necessary to remove; this concentration is not achieved with 10% bleach), and because it is very important to disrupt the formation of biofilms as quickly and effectively as possible. This protects the instrument from repairs such as line changes that are not typically covered under service contract when contamination is the issue, and protects other client experiments in a shared resource laboratory. It is important to rinse bleach from stainless steel surfaces to prevent rust formation. It is also important to rinse the bleach from the lines using DI to prevent residues from forming. Additionally, sequencing of small samples such as extracellular vesicles, virus-like particles, exosomes, organelles, and more benefits from strict decontamination and complete removal of amplifiable free-DNA to provide more reliable sequencing results, most especially when whole genome amplification is necessary.

- BD FACSClean has a sodium hypochlorite concentration of 1%. This is not sufficient for removal of endotoxin and is less optimal to use. 25% bleach prepared according to Tijerina et al 2025 results in a sodium hypochlorite concentration of 2% which is optimal

for microbial work and is sufficient for endotoxin removal. However, if it is not possible to prepare 25% bleach, FACSClean is an acceptable alternative due to its stability.

- Also note that there are instances in which you may encounter a contaminant that is resistant to heat, bleach (chlorine resistance), and other common decontaminating agents. If this occurs, more complex measures will need to be taken such as changing out lines and even identifying the microorganism through genomic sequencing to apply an appropriate agent for decontamination. This is an emerging issue in clinical settings especially, but it is not sufficiently discussed as a possible confound in research laboratory settings.