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Title: Daily Shut Down and Monthly Cleaning Procedures for FACSCalibur			
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This SOP has been read and understood by:

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Annual Review		
Reviewed by:	Review Date	Signature

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Definitions and Abbreviations

BD	Becton Dickinson Biosciences
BSL-2	Biosafety Level - 2
HI	High flow rate control button of the fluidic control panel
ISAAC	International Studies of AIDS- Associated Co-infections
KCMC	Kilimanjaro Christian Medical Centre
LOW	Low flow rate control button on the fluidic control panel
RUN	The button on the control panel that puts the fluidic system in an active mode
SIP	Sample Injection Port
SOP	Standard Operating Procedure
STNDBY	Standby (idle) mode of the cytometer control panel

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1. Method Summary

The following procedure describes the Daily Shut Down and Monthly Cleaning Procedures for the FACSCalibur Flow Cytometer. For the purpose of preserving the reliability of the FACSCalibur, basic preventive maintenance procedures should be followed to keep the instrument in better performing condition. Proper cleaning will ensure that the instrument functions consistently.

2. Scope

This SOP applies to all staff at the KCMC Biotechnology laboratory who use the FACSCalibur. Only authorized staff may use the FACSCalibur.

3. Safety Precaution

Follow appropriate biosafety procedures. Standard safety precautions for working with blood samples should be used under BSL-2 conditions. Wear gloves and a laboratory coat. Avoid having direct eye contact with laser beams when the top cover of the cytometer is opened as this may cause damage to the eyes.

4. Reagents and Material

- 4.1. Distilled Water
- 4.2. Bleach (1:10 dilution)
- 4.3. BD FACSClean (Cat # 340345)
- 4.4. BD FACSRinse (Cat # 340346)
- 4.5. BD FACSFlow (Cat # 342003)
- 4.6. 12 x 75mm polystyrene tubes (BD Biosciences, Cat# 352058)
- 4.7. Two spare tanks; one labeled for distilled water and the other for 10% bleach, (BD Biosciences).

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<div><div>5. Procedure</div><div>Always the instrument should be cleaned before running FACSComp software for machine calibration at the start of the day’s task, and at the end of the day before switching off.</div><div><div>5.1. Daily Cleaning</div><div>To prevent the sample injection port (SIP) from clogging, a bleach solution (or FACSClean), FACS Rinse and distilled water rinse cycle should be run through the system at the end of each day before the instrument is shut down.</div><div><div>5.1.1. Install a tube containing 3 ml of a 1:10 dilution of bleach (or FACSClean) on the SIP of the FACSCalibur. Set the fluid control to ‘RUN’ on ‘HI’ flow rate. Move the tube support arm to the side and allow the vacuum to aspirate for 1 minute. Center the tube support arm (the vacuum is off) and allow the bleach to run for 5 minutes.</div><div>5.1.2. Install a tube containing 3 mL of FACS Rinse on the SIP. Move the tube support arm to the side and allow the vacuum to aspirate for 1 minute. Center the tube support arm and allow FACS Rinse to run on ‘HI’ flow rate for 5 minutes.</div><div>5.1.3. Install a tube containing 3 mL of distilled water on the SIP. Move the tube support arm to the side and allow the vacuum to aspirate for 1 minute. Center the tube support arm and allow the water to run on “HI” for 5 minutes.</div><div>5.1.4. Set fluidic control to "STNDBY" and ‘LOW’ flow rate. Place a tube containing 1ml of distilled water onto the SIP. This prevents salt deposits from forming in the injection tube.</div><div>5.1.5. Flip the vent valve toggle switch to ‘relieve pressure’ position to release pressure from the sheath tank.</div><div>5.1.6. Shut down the computer.</div><div>5.1.7. Turn off the power to the cytometer.</div><div>5.1.8. Record the time of equipment shutdown in daily log sheet (appendix 1).</div></div><div>5.2. Monthly Cleaning</div></div></div>			

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<p>Overall fluidics system cleaning is required to remove debris and contaminants from the sheath tubing, waste tubing and flow cell. This prevents clogging of the fluidic system.</p> <p>5.2.1. Turn on the power to the FACSCalibur.</p> <p>5.2.2. Remove the sheath tank</p> <p>5.2.3. Disconnect the upper tubing of the sheath filter from the Saline Filter port by squeezing the metal clip on the quick-disconnect and pulling the connector from the fitting.</p> <p>5.2.4. Fill and install a spare tank with 1 to 2 L of 1:10 dilution of bleach solution.</p> <p>5.2.5. Connect the sheath tubing (white) from the tank to the port labeled Saline Filter.</p> <p>5.2.6. Flip the vent valve toggle switch to ‘pressurize-run’ position to pressurize the bleach tank.</p> <p>5.2.7. Install a tube containing 3 mL 1:10 bleach solution on the SIP.</p> <p>5.2.8. Press the RUN fluid control button and run on HI for 20 to 30 minutes.</p> <p>5.2.9. Set fluidic panel control to “STNDBY”.</p> <p>5.2.10. Remove the tube of bleach solution from the SIP.</p> <p>5.2.11. Flip the vent valve toggle switch towards the arrow to relieve pressure from the tank.</p> <p>5.2.12. Repeat steps 5.2.4. to 5.2.9. using distilled water instead of the 1:10 dilution of bleach solution.</p> <p>5.2.13. Replace the original sheath tank.</p> <p>5.2.14. Reconnect the sheath tubing (white) to the port labeled as sheath, and the sheath filter connector into its fitting (labeled as saline filter).</p> <p>5.2.15. Place a tube containing 1 mL of distilled water on the SIP.</p> <p>5.2.16. Set fluidic control panel to “STNDBY”.</p>			

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5.2.17. Turn off the power to the FACSCalibur. The tube of distilled water should remain on the SIP to prevent salt deposits from forming in the injection tube.

5.2.18. Record date on Monthly cleaning sheet (appendix 2).

6. Reference

1. BD Biosciences Immunocytometry Systems FACSCalibur Users Guide, August 1996.

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Appendix 2: Monthly cleaning log

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FACSCalibur 1**

MONTHLY CLEANING LOG

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Reviewed by: _____ Date: _____