

KCMC Biotechnology Laboratory	STANDARD OPERATING PROCEDURE	Effective Date 02-10-2006	SOP-Number FLOW001.01
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Title: Start Up Procedure 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
ISAAC	International Studies of AIDS - Associated Co infections
KCMC	Kilimanjaro Christian Medical Centre (Moshi)
SOP	Standard Operating Procedure

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1. Method Summary

The following document describes the basic start up procedure and operation of the BD FACSCalibur for use. The FACSCalibur system is a modular bench top flow cytometer. It consists of a sensor module, a computer module and various software packages. The procedure can be applied for the purpose of CD4/CD8 lymphocyte enumeration using whole blood specimens.

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 Precautions

Follow appropriate biosafety procedures. Standard Safety Precautions for working with blood samples should be used under BSL-2 conditions. Wear disposable gloves and lab coat. The lasers in the flow cytometer emit strong light beams. Avoid looking directly at the lasers when the flow cytometer top cover is open to avoid damage to the eyes.

4. Reagents and Materials

- 4.1. BD FACSflow (BD Biosciences, Cat #342003); alternative phosphate buffered saline (PBS) solution.
- 4.2. Distilled Water
- 4.3. Bleach
- 4.4. 12 x 75mm polystyrene tubes (BD Biosciences)

5. Procedure

- 5.1. Ensure the electrical mains connection is switched on.
- 5.2. Turn the FACSCalibur on by pressing the power switch located in the lower right corner of the instrument.
- 5.3. Turn on the computer

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- 5.4. Slide the fluidics drawer open and check the sheath and waste reservoirs.
- 5.5. To fill sheath reservoir, ensure the switch for vent valve is to “vent-tank change” position to relieve of air pressure. Remove the sheath tank metal bracket cover by gently pressing down and sliding it towards the rear of the instrument, and lift it off. Unscrew and remove the fluid detection probe and fill container $\frac{3}{4}$ full with FACSFlow.
- 5.6. Return sheath tank to its position in the fluidics draw. Replace the fluidic detection probe onto the sheath tank and finger-tighten the cap. Replace the sheath bracket onto the alignment pins and secure by sliding the cover towards the front of the instrument.
- 5.7. Disconnect and empty waste reservoir. Before reconnecting, add an amount of bleach (400ml) to the container that will bring the final concentration of commercial bleach to 10% if the container is filled.
- 5.8. Reconnect waste reservoir and switch vent valve to “pressure-run” position. Check that the sheath tank is properly pressurized. It should not be able to move around under the bracket.
- 5.9. Check the sheath filter to be sure no air bubbles are trapped inside. If air bubbles are present, gently tap sheath filter several times to dislodge the bubbles and force them to the top. Push the roller in the pinchcock forward to allow the pressurized sheath fluid to force the air bubbles into the waste reservoir. Return the pinchcock to the closed position. To remove stubborn bubbles, squeeze the metal clip and pull the sheath filter from the lower quick-disconnect port. Lift the filter up and firmly tap the filter body to dislodge the bubbles. Reconnect the filter to its lower quick-disconnect port. Push the roller in the pinchcock forward to allow the pressurized sheath filter to force air bubbles into the waste reservoir. Return the pinchcock to the closed position.
- 5.10. Ensure the tube containing distilled water is on the sample injection port (SIP).
- 5.11. Set the fluidic mode selector to "PRIME" allowing the flow cell assembly to completely drain and fill. The “STNDBY” button is orange after completion.
- 5.12. Repeat 5.11. three times
- 5.13. Record date, time and initials of operator on daily maintenance sheet.
- 5.14. Set the fluidics mode selector to "STNDBY".

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5.15. Proceed with daily Calibration.

6. References

1. BD Biosciences Immunocytometry System FACSCalibur user's guide, August, 1996.

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Appendix 2: Monthly cleaning log

**IMMUNOLOGY LABORATORY, KCMC BIOTECHNOLOGY LABORATORY, P.O
BOX 2222, MOSHI, TANZANIA
FACSCalibur 1**

MONTHLY CLEANING LOG

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Appendix 3: Reagent log

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FACSCalibur 1**

REAGENT LOG

INSTRUMENT: _____ **MANUFACTURER:** _____

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Reviewed by: _____ Date: _____

Supersedes: