KCMC Biotechnology Laboratory	STANDARD OPERATING PROCEDURE	Effective Date 02-10-2006	SOP-Number FLOW002.01
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Title: FACSComp Procedure			
SOP References:			Supersedes:

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Annual Review		
Reviewed by:	**Review Date**	**Signature**

Document History:

Version Number	Reason for Changes	Date
FLOW002.01	Initial	16-08-2006

Definitions and Abbreviations

APC	Allophycocyanin
BD	Becton Dickinson Biosciences
BSL-2	Biosafety Level -2
FITC	Fluoresceine Isothiocyanate
FL	Florescence
FSC	Forward Scatter
ISAAC	International Studies of AIDS-Associated Co-infections
KCMC	Kilimanjaro Christian Medical Centre
PE	Phycoerythrin
PerCP	Peridinin Chlorophyll Protein
PMT	Photomultiplier Tube
SIP	Sample Injection Port
SOP	Standard Operating Procedure
SSC	Side Scatter

1. Method Summary

FACSComp is an application that is used to automatically adjust the detector voltages and compensation for the flow cytometer. It involves the use of CaliBRITE beads to measure separation between bead-positive signal and noise for forward scatter (FSC) and side scatter (SSC) parameters and between bead-positive signal and autofluorescence of unlabeled beads for fluorescence 1 (FL1), fluorescence 2 (FL2), fluorescence 3 (FL3) and fluorescence 4 (FL4) parameters. It then compares the measured separation to an expected minimum value.

FACSComp is used to monitor daily performance and to set up the cytometer for immunophenotyping. It thus ensures the instrument runs consistently from day to day. In its automatic mode, FACSComp adjusts the flow cytometer to analyze human cells.

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

3.1. Standard safety precautions for working with blood should be used under BSL-2 conditions. The FACSCalibur is equipped with lasers that generate powerful light beams that can be harmful to the eyes. Direct contact with the eyes should be avoided.

3.2. Wear gloves and laboratory coats.

4. Waste Generation, Handling and Disposal

Follow all relevant KCMC Research Standard Operating Procedures in disposing waste generated by this method (See Biohazard Safety SOP[SFT.01]).

5. Material, Equipment and Reagents

5.1. CaliBRITE (3) Beads (Cat# 340-486, BD Biosciences) i.e UNLABELED, FITC, PE and PerCP

5.2. APC CaliBRITE Beads (Cat#340-487, BD Biosciences)

5.3. Disposable 5 ml 12x75-mm Falcon capped polystyrene test tubes, (Cat. # 352058, BD Biosciences)

5.4. BD FACSFlow Sheath fluid, (Cat. #342-003, BD Biosciences)

5.5. Becton Dickinson FACScomp Software

5.6. FACSCalibur (BD Biosciences)

5.7. Printer

6. FACSComp Automatic Calibration

6.1. Label one 12x75 mm tube as A (UNLABELLED) and another as tube B (MIXED). Add 1 mL of sheath fluid to tube A.

6.2. Mix each bottle of unlabeled and APC beads by gentle inversion.

6.3. Add 1 free falling drop of beads from each to the tube labeled as A (UNLABELLED) test tube, cap and mix by gently vortexing.

6.4. Add 3 mL of sheath fluid to the test tube labeled as B (MIXED).

6.5. Mix each bottle of unlabeled, FITC, PE, PerCP and APC beads and add a free falling drop of each to the tube labeled as A (MIXED) test tube, cap, and mix by gently vortexing.

6.6. Store both diluted and stock beads at 2^{o} to 8^{o}C in the dark. When stored as directed, stock CaliBRITE beads are stable until the expiration date on the label, while diluted CaliBRITE beads are stable for **8 hours** when kept in darkness at 2-8^{o}C.

6.7. Choose FACSComp from the Apple menu.

6.8. Enter operator name, name of Institution (KCMC), and Biotechnology Laboratory Director's name.

6.9. Click Accept.

6.10. Enter the CaliBRITE bead lot ID exactly as it appears on accompanying insert.

6.11. Check on Assay Selection as Lyse/No wash.

6.12. Using Automatic Saving Option, select Lyse/No Wash Summary Report.

6.13. Click Run after entering the information.

6.14. After the Initial photomultiplier tube (PMT) window view appear, gently vortex tube A (the UNLABELLED) bead suspension and install the test tube on the cytometer (ensure the sample flow rate switch is set to HI and the fluid control dial is set to RUN).

6.15. Click Start to begin PMT adjustment (If PMTs are not set successfully after one minute or if the event rate is less than 400 events/second, a message appears and the manual procedure must be followed). To perform manual PMT adjustment see part 7.2 of this SOP.

6.16. After the PMTs are successfully set, FACSComp automatically proceeds to Compensation adjustment (If APC beads are detected, FACSComp performs a time-delay calibration).

6.17. When the Initial Compensation view appears. Gently vortex tube B (MIXED) bead suspension and install the test tube on the cytometer.

6.18. Click Start to begin compensation adjustment. (If compensation is not set successfully after one minute or if the event rate is less than 400 events/second, a message appears and the manual procedure must be followed.) To perform manual compensation see part 7.3 of this SOP.

6.19. When the compensation is successfully set, the program automatically proceeds to the Sensitivity Test. At the completion of the sensitivity test the summary report view is displayed.

6.20. Remove tube B from the cytometer, install tube containing not more than 1 ml of distilled water on the SIP. Select STNDBY on the cytometer.

6.21. Click ‘quit’ to exit the program. Report prints automatically.

6.22. Check the FACScomp report to make sure that all the parameters have passed, including Time-Delay Calibration when running four colour (APC).

6.22. Initial and file report in FACSComp log (see Appendix 1).

7. FACSComp Manual Calibration

7.1. Reasons for Manual Calibration

7.1.1. The flow rate is too low (<400 events/second) for automatic PMT adjustment or Compensation adjustment.

7.1.2. PMT or Compensation is not set within 1 minute.

7.2. Manual PMT Adjustment

7.2.1. Click Manual during automatic PMT adjustment. The Detectors/Amps window becomes active, and the current target values appear.

7.2.2. Use the arrows in the Detectors/amps window to adjust the PMT voltages to place the beads in the desired channels. The average channel values are displayed below the plots. These values should be within $\pm$ 2 channels of the target values.

7.2.3. When satisfied with the PMT settings, click Next. The Initial Compensation view window appears.

7.3. Manual Compensation Adjustment

7.3.1. Click Manual during automatic compensation adjustment. The Compensation window becomes active, and the current target values appear.

7.3.2. Use the arrows in the Compensation window to adjust the compensation values to achieve the desired mean differences (e.g. FL1 Mean Diff: -6, Fl2 Mean Diff: -25).

7.3.3. When satisfied with the compensation settings, click Next. The Initial Sensitivity Test view window appears.

7.3.4. Continue with the Sensitivity Test. This determines the noise level versus the signal for each flourochrome. At the completion of the sensitivity test the summary report view is displayed. Remove the tube B from the SIP, install tube containing not more than 1 ml distilled water on the SIP and select STNDBY on the cytometer control.

8. References

1. BD Biosciences FACSComp Software User's Guide.

Appendix 1: FACSComp log

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

INSTRUMENT DAILY LOG

FACSCalibur
FACSComp QC Log
Calibrite Beads: Kit Lot #:________

SSC	FL1			FL2			FL3			FL4			Compensation						Operator Initials
P/F	Sep^{n}	Volt	P/F	Sep^{n}	Volt	P/F	Sep^{n}	Volt	P/F	Sep^{n}	Volt	P/F	FL1-%FL2	FL2-%FL1	FL2-%FL3	FL3-%FL2	FL3-%FL4	FL4-%FL3	

Supervisor Review: ______________ Date: __________

			Volt																					
			Sep^n																					
		FSC	P/F																					
			Amp																					
			Sep^n																					
		Laser Current																						
		Date																						