KCMC Biotechnology Laboratory	STANDARD OPERATING PROCEDURE	Effective Date 02-10-2006	SOP-Number FLOW004.01
		Page 1 of 17	02-10-2006

Title: Quality Control (Reagents, Controls and Equipment)

SOP References: **Supersedes:**

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

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FLOW004.01	Initial	02-10-2006

Definitions and Abbreviations

BD	Becton Dickinson Biosciences
CD	Cluster Designation
EDTA	Ethylene Diamine Tetraacetic Acid
FV	Function Verification
ISAAC	International Studies of AIDS- Associated Co-infections
KCMC	Kilimanjaro Christian Medical Centre
PM	Preventive Maintenance
QC	Quality Control
SOP	Standard Operating Procedure

1. Method Summary and Application

This section outlines quality control procedures for Reagents, Controls and Equipment. Quality control (QC) is a vital component of quality assurance. All labs (clinical and non-clinical) benefit from quality control in terms of confidence in and reproducibility of test results. QC comprises those measures that must be included during each test run to verify that the test is working properly such as ensuring correct temperature conditions, quality reagents, use of appropriate controls and ensuring proper equipment function. Thus quality control indicates whether the test run was valid and has produced acceptable results.

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. Supervision

3.1. Quality control

The quality control program is under the responsibility of the laboratory director and should be reviewed once a month.

4. Reagents

4.1. Storage

Reagents must be dated and initialed upon receipt. Lot numbers must be recorded in the reagent quality control logbook. Reagents must be stored according to the manufacturer's suggested recommendations.

4.2. Preparation

4.2.1. Labeling reagent (after preparation and/or when placed in service)

4.2.2. Note the content, quantity and concentration.

4.2.3. Note storage requirement (conditions).

4.2.4. Note date prepared and by whom.

4.2.5. Note expiry date for reagents.

4.3. Use of Reagent Kit.

4.3.1. Multiple components of a reagent kit must only be used from a kit of the same lot.

4.5. Parallel Testing

Perform parallel testing on at least two samples using new reagents received and current reagents in use. In addition, the assay control sample with known T-lymphocyte values should be processed using the new reagents and current reagents. The results should have a correlation of $\geq$ 90%. The difference of results for each of the tested samples between using new lots of reagents and old lots should not exceed 15%. Results of reagent checks must be recorded, dated and initialed.

5. Flow Cytometry Quality Controls

5.1 Instrument Controls

5.1.1. Instrument control using stabilized normal human peripheral blood lymphocytes

5.1.1.1. Stabilized whole blood normal control (BD Multi-Check, Cat# 340911 or Strek Laboratories CD-Chex Plus, Cat# 213325) and low control, should be stained for CD45+CD3+, CD3+CD4+, CD3+CD8+ using the same amount of monoclonal antibody reagent used for patients' samples. They must be run with each batch of samples on the FACSCalibur instrument and results are recorded and should fall within the range established using at least 10 runs of the control (section 5.1.1.2). If values fall outside of the range, instrument status, operator technique and product deterioration need to be evaluated. Specimens should not be run on the equipment until the problem is resolved. If for any reason the normal control cannot be run on the instrument, that instrument should not be used for patient samples for that day.

5.1.1.2. Result values for the controls from the instrument for the first 10 days of a particular lot are recorded and means and standard deviations are determined. A range for each subset is established using 2 standard deviations. These values should be used to plot a Levy Jennings chart for 1, 2 and 3 SD. The next runs for the month are compared to these ranges by plotting them on the chart. The trend and shifts for the results should be monitored and outliers are evaluated. Where there is a trend for values to fall outside the range ($\pm$2SD), instrument status, operator technique and product deterioration need to be evaluated.

5.1.2. Instrument- to- Instrument Controls Using Patient Whole Blood

5.1.2.1. Two whole blood patient specimens (1 low CD4 count and 1 normal CD4 count) should be prepared and run on the FACSCount and FACSCalibur on a monthly basis. The results obtained using the two equipments should fall within 5% difference.

5.1.2.2. Twenty whole blood patient samples in EDTA anticoagulant will be stained and analyzed in the KCMC Hospital Research Laboratory and the KCMC Biotechnology, Immunology Laboratory for inter-laboratory comparison to determine if the procedure for preparing and processing is optimal between the two laboratories. This will be done TWICE a year (6 month intervals). Each Laboratory will use its own routine procedure for whole blood immunophenotyping. The lymphocyte subsets monitored will be $CD45^{+}CD3^{+}$, $CD3^{+}CD4^{+}$ and $CD3^{+}CD8^{+}$. The results will be evaluated by the Laboratory Director. Laboratory results for each laboratory should meet the Allowable Experimental Error (difference from the mean as a percent of allowable error) of each subset.

5.1.3. Instrument Performance, Reproducibility Control Using Healthy Donor Whole Blood

A whole blood specimen from a healthy control donor must be prepared and analyzed daily. The sample should be used to inspect the distribution of blood cells as expected for a normal sample on the dot blot. Values for CD45+CD3+, CD3+CD4+ and CD3+CD8+ should be recorded.

5.1.4. Operators Reproducibility Control Using a Healthy Donor Whole Blood

A whole blood specimen from a healthy control donor must be prepared and analyzed by each technologist on the FACSCount and FACSCalibur. This will be done once a month. Values for CD45+CD3+ Abs, CD3+CD4+ Abs and CD3+CD8+ Abs must be recorded. Agreement between runs should be within 5%. Pipetting errors should also be checked using TruCOUNT Control beads (Cat# 340335). Low, Medium, and High control bead suspensions are added to normal blood prepared with the appropriate reagents using TruCOUNT Tubes. This should be done at least every month.

6. Equipment

Laboratory instruction manuals should be available to answer questions regarding the proper use and maintenance requirements, and should be read by all personnel using the equipment. Establishing a function verification and preventive maintenance program will assure that equipment is reliable and properly maintained. The equipment listed below

should be monitored and record for quality control procedure, function check, preventive maintenance and repairs should be documented and filed in separate logbooks.

6.1. Flow Cytometer (immunology laboratory)

BD FACSCalibur Serial # E4375

6.1.1. Function Verification (FV). Before use, the instrument must pass the BD FACSComp calibration. Laser output must be checked daily. Laser output must be between 14.4-15.2 mW.

6.1.2. Quality Control (see separate SOP for Biotechnology Laboratory)

6.1.3. Operating Conditions: Temperature should not exceed 53^{o}C.

6.1.4. Preventive Maintenance (PM): Daily and monthly maintenance procedures are performed on each Instrument. Preventive maintenance check should be scheduled with Becton Dickinson service once every 6 months. Minor trouble shooting procedures should be available to all personnel using the instrument.

6.2. Refrigerators

Type: GR 151

Type: Electrolux

6.2.1. FV

Temperature is monitored on a daily basis. Average temperature must be maintained between 2^{o}-8^{o}C. Temperatures are monitored using a manual thermometer (SOP number EQP.002).

6.2.2. QC

Using a manual thermometer, temperatures are recorded daily. Thermometers should be calibrated at least once a year (SOP number EQP.001).

6.2.3. PM

Cleaned inside once every 6 months.

6.3. Laminar Flow Hoods

Laminar Flow Hood Serial #84101052103

6.3.1. FV

Inspected for proper operation daily.

6.3.2. PM

Preventive maintenance check is scheduled with Laboratory Safety Services once every 6 months. Clean after each use (SOP number SAF.002).

6.4. Dispensers/pipettes

6.4.1. FV

Inspect for proper operation and for breakages.

6.4.2. QC

Calibrate every 6 months (SOP number, EQP.004)

6.4.3. PM

Clean after each use.

8. Documentation of Corrective Measures

Remedial action to be taken when values for controls do not fall within range limit. This must be documented in the "out of range control book". Equipment malfunction and reagent problems must also be documented in the same book.

9. Quality Control Performance and Documentation

Quality control procedures for flow cytometry will be performed, documented and filed in Immunology Laboratory.

10. Reference

1. College of American Pathologist, Guidlines for Flow Cytometry, 1989.

2. National Committee for Clinical Laboratory Standards; Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. NCCLS, H 42-P., 1989.

Appendices

Appendix 1: Daily cleaning log

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

DAILY CLEANING LOG

Date	Name of Personnel	Comments	Sign

Reviewed by: _____ Date: _____

Appendix 2: Monthly cleaning log

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

MONTHLY CLEANING LOG

Date	Name of Personnel	Comments	Date Due for Next Cleaning	Sign

Reviewed by: _____ Date: _____

Appendix 3: Reagent log

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

REAGENT LOG

INSTRUMENT:_____ MANUFACTURER:_____

Item Description	Date Opened	Lot Number	Date Stopped	Expiry Date	Manufacturer	Tech. Initials

Reviewed by: _____ Date: _____

Appendix 4: FACSComp log

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

INSTRUMENT DAILY LOG

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

FL1			FL2			FL3			FL4			Compensation						Operator Initials
Sep"	Volt	P/F	Sep"	Volt	P/F	Sep"	Volt	P/F	Sep"	Volt	P/F	FL1-%FL2	FL2-%FL1	FL2-%FL3	FL3-%FL2	FL3-%FL4	FL4-%FL3	

		SSC	P/F																		
			Volt																		
			Sep°																		
		FSC	P/F																		
			Amp																		
			Sep°																		
		Laser Current																			
		Date																			

Appendix 5: Temperature Recording Sheet

KCMC BIOTECHNOLOGY LABORATORY, QUALITY CONTROL

Equipment Manufacturer: _____ Model/Serial #: _____

Location: _____ Month: _____

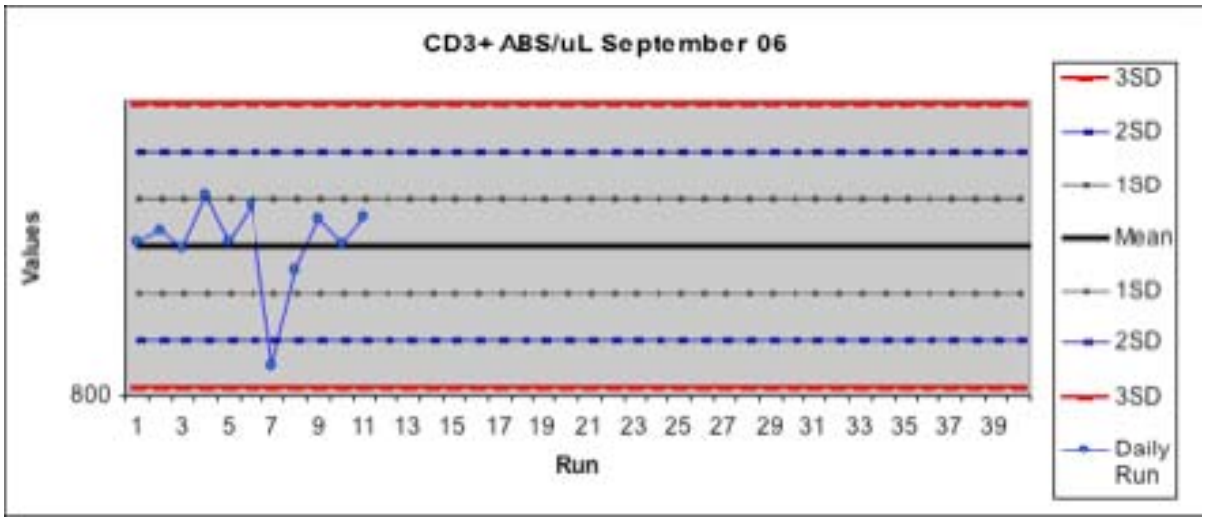
Acceptable Range: _____ ^{o}C To _____ ^{o}C Year: _____

DATE	1ST READING		2ND READING	
	6:00 a.m – 9:00 a.m	Initials of Reader	6:00 p.m – 8.00 p.m	Initials of Readrer

Reviewing Supervisor _____ Date: _____

Appendix 6: Quality Control Data- Levy Jennings Chart

Month	Manufacturer Ranges	Assay Performance for the Period
Year	Mean ± 2SD	% CV
Instrument	Units	Mean
Analyte		SD
QC Product		
Level	Expected Performance Manufacturer	
Lot#	% CV	
Expiry		



Reviewed by Supervisor: Date:

Appendix 7: Corrective Action Log

FACSCALIBUR FLOW CYTOMETER CORRECTIVE ACTION LOG
(ACTG, CHAVI, ISAAC- Moshi Lab, Tanzania)

Date	Initials	Problem and Action Taken