KCMC Biotechnology Laboratory	STANDARD OPERATING PROCEDURE	Effective Date 02-10-2006	SOP-Number FLOW006.01
		Page 1 of 19	02-10-2006

Title: Flow Cytometry External Proficiency Testing

SOP References: **Supersedes:**

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Approvals/Date:

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ISAAC Program Director

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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Guido Ferrari, M.D.
ISAAC Pathogenesis Program Co-Director

This SOP has been read and understood by:

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

Document History:

Version Number	Reason for Changes	Date
FLOW006.01	Initial	16-08-2006

Abbreviations and Definitions

ALC	Absolute Lymphocyte Count
BSL-2	Biosafety Level-2
CD	Cluster Designation
EQA	External Quality Assessment
FL	Fluorochrome
IQA	Immunology Quality Assurance
ISAAC	International Studies of AIDS-Associated Co infections
KCMC	Kilimanjaro Christian Medical Centre (Moshi)
MAb	Monoclonal Antibody
NHLS	National Health Laboratory Services
QA	Quality Assurance
QC	Quality Control
REQAS	Regional External Quality Assessment Scheme
SD	Standard Deviation
SDI	Standard Deviation Index
SOP	Standard Operating Procedure
UKNEQAS	United Kingdom National External Quality Assessment Scheme
WCC	White Cell Count
WHO	World Health Organization

1. Method Summary and Application

Proficiency testing entails participation in an established external quality assessment program. Successful performance in proficiency testing programs is considered an ultimate measure of laboratory function because it indicates that an acceptable quality assurance program is in place. The laboratory participates in the United Kingdom National External Quality Assessment Scheme (UKNEQAS) proficiency program for leucocyte immunophenotyping. It has additionally recently enrolled in Regional External Quality Assessment Scheme (REQAS).

Proficiency testing programs are offered by accrediting organizations, individual institutions or groups. The primary objectives of proficiency testing programs are: to assess the quality of laboratory performance on a nationwide basis; to provide assurance to consumers (physician and patients) that laboratory results are reliable. Secondary objectives are: to identify common errors and recommend corrective measures; to encourage good laboratory practice, using standardized procedures, appropriate definitions and high-quality reagents; to encourage the implementation of quality assurance and control measures in the participating laboratories; to stimulate information exchange and networking among laboratories at the national and/or international level.

Although some programs are not designed to regulate participating laboratories, they offer invaluable opportunities for external assessment and they promote an approach to standardization of clinical laboratory testing. UKNEQAS Immune Monitoring and REQAS are for those laboratories, which determine the lymphocyte subpopulations in immunological disorders. Both absolute and percentage values are requested from stabilized whole blood.

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. Proficiency Test Provider

3.1. United Kingdom National External Quality Assessment Service (UKNEQAS)

This program is contacted by the Clinical Pathology Accreditation (UK) Ltd. Accredited EQA Schemes of Sheffield Teaching Hospitals. Helping to ensure clinical laboratory test results are accurate, reliable and comparable wherever they are produced.

3.1.1. Address: Rutledge Mews
3 Southbourne Road
Sheffield S10 2QN

United Kingdom

Tel. # +44 (0) 114 267 3600
Fax. # +44 (0) 114 267 3601

3.1.2. Schedule of shipment:

Jan., March., May, July, Sept., Nov.

3.1.2.2. Number of samples/yr:

6 samples/year.

3.1.2.3. Type of sample:

Stabilized whole blood (patented) collected from either normal donors or using CD4 depleted blood.

3.2. Regional External Quality Assessment Scheme (REQAS)

This program is conducted by the South African National Health Laboratory Services (NHLS) in collaboration with World Health Organization (WHO).

3.2.1. Address: QC Co-ordinator
Molecular Medicine and Haematology
Flow Cytometry R & D
NHLS, Wits Medical School

Phone: +27-11-489 8541 (lab) 489 8555 (office)
Fax: +27-11-386 6296

3.2.2. Number of samples/ year
6 samples/ year

3.2.3. The sample should be kept at $2^{o}C$-$8^{o}C$ until the time of processing.

4. Safety Precautions

4.1. Standard Safety precautions for handling blood should be used under BSL-2 conditions.

4.2. Wear disposable gloves and lab coat.

4.3. All procedures should be done inside certified biosafety cabinets.

4.4. The lasers in the FACSCalibur emit laser beams which can be harmful to the eyes. Avoid contact with naked eyes when the top cover of the FACSCalibur is opened.

5. Waste Generation, Handling and Disposal

Follow all relevant Standard Operating Procedures (SOP) in disposing biohazard waste generated by this method. Follow KCMC SOP for waste storage and disposal (Biohazard Safety SOP [SFT.01]).

6. Processing Procedure:

Upon arrival, samples should be kept at 2°–8°C until testing can be performed. Whole blood specimens are processed and analyzed using the laboratory's routine procedure for whole blood CD4 and CD8 enumeration (See SOP # FLOW003.01). Testing must be performed within 16 hours of receipt of specimen. All technicians who routinely process patient samples should process proficiency samples in rotation at each trial so that the EQA sample can be used as a competency testing tool.

7. Sample analysis

Proficiency samples should be analysed the same way as routine patient samples. When the lymphocyte population falls outside the CD45 expert gate, then manual gating should be used to adjust the gate so that it can encompass the lymphocyte population. When performing manual gating, the CD3 attractor should also be adjusted to encompass the CD3 population. Similarly, the CD4 and CD8 attractors should be adjusted accordingly depending on their current orientation respective to the cell population. To perform manual gating, select manual from the options menu of the laboratory report dot plot. Double click on the plot to be edited and adjust the appropriate gates to encompass the lymphocyte population. Click on analyze to effect the changes. Click on 'continue' to obtain the laboratory report and the physician report. Check the assay controls report to make sure that the values are within the expected ranges. If the results for the control are out of range do not report proficiency sample results. Evaluate the technique and equipment. The technician should initial the results. The results should be verified by the laboratory supervisor or his/her designee before it is reported to UKNEQAS or REQAS.

8. Tests monitored:

Absolute count determination: CD3+, CD3+CD4+ and CD3+CD8+; and percent count: CD3+/CD45+, CD3+CD4+/CD45+ and CD3+CD8+/CD45+

9. Reporting:

Report absolute values/uL and percentage values. For UKNEQAS trial samples, the results should be posted on the UKNEQAS website. www.ukneqasli.org/SampleEntry. Log on using the following information; Lab Number, Identity and Password. This information is available with the laboratory supervisor. Select IMMUNE MONITORING as the programme from the pop-up menu. Trial choices will appear. Choose the appropriate trial number (the current sample processed). The sample entry form will appear with required sample details (see appendix 3). Completely fill the form, check for the details entered to confirm the entries. For tests that do not apply such as hematology, enter "00". The laboratory supervisor or his/her designee should double check the results entered on the sample entry form before this data is saved. Click on save to secure the entries made, and click on complete to submit the form with entered results. Log out when this is completed. This information will be accessed by the UKNEQAS personnel from the website.

For the REQAS trial samples, the results should be entered onto the result entry form accompanying the sample (see appendix 4). Similarly, the completely filled result form should be double checked by the laboratory supervisor or his/her designee for verification of entries. The completely filled form should be faxed to NHLS using the fax number provided above.

10. Evaluation:

There is not pass/fail report. Laboratory results are compared to evaluation means. Standard deviation and standard deviation index are given for each set of values compared. A score is allocated for absolute and percentage values against running scores. Overall performance is also given. The results report will be posted on the website and a notification will be given through email address of the contact person (laboratory supervisor). This report will be available for six months from the day of posting. Access the report through laboratory number and password and print for filling.

To determine laboratory performance, view the overall performance table on the UKNEQAS report and the chart for REQAS report. On the UKNEQAS report identify the trimmed mean for all sites for each analyte reported as the expected mean for that sample. Also identify the trimmed standard deviation for each analyte and multiply by two to obtain 2 SD. Use this value to determine the expected range for each analyte by subtracting from the mean to obtain the lower limit, and adding to the mean to obtain the upper limit. Summarize the results as show in the example (see appendix 1). When your reported values fall within the $\pm$ 2SD range, then the performance is satisfactory. Values falling outside this range are unacceptable and a proficiency review form (appendix 2 or 5) must be completed for corrective action, signed by the supervisor and send to UKNEQAS (proficiency provider).

When a result report is received from REQAS, view the Levy Jennings Chart drawn using the values for the participating sites. Using the reported laboratory value determine where the value for the laboratory is falling on the chart. The acceptable range is $\pm$ 2 SDI. In addition, values falling out of range will be marked with a red star.

11. Corrective Action

If results are unacceptable investigate the source of the error. First evaluate the equipment by inspecting the FACSComp report for the day of sample processing. If all the parameters are acceptable then evaluate the technique of the personnel on the day of sample processing, first by checking on the results of the assay control against the expected ranges. Perform reanalysis with manual gating to check that sample analysis was correctly done. If this is within range then perform pipetting error testing using TruCount control beads for low, medium and high to check for reproducibility. If equipment calibration reports are good and assay controls are out of range check on pipettors for their accuracy as well. Perform pipette calibration. Through this approach, the source of error can be identified and corrected, and documented on the proficiency review form and IQA corrective action investigation form (appendix 2 and 5 respectively). For REQAS results there is no corrective action form provided however, an investigation should be carried out to determine the source of unacceptable results. This should be corrected and a similar form to IQA corrective investigation form filled (appendix 5).

12. References:

1. College of American Pathologists-1992 Survey Manual

2. Centers for Disease Control Model Performance Evaluation Program LymphocyteImmunophenotyping, Summary results for 1991 Shipment.

3. Fast Systems, Inc. Annual Report on Flow Cytometry Quality Control Nov.1, 1990-Oct. 1991.

4. Clinical pathology accreditation (UK) Ltd. Accredited EQA schemes website.

Appendices

Appendix 1: PROFICIENCY EVALUATION FORM

Section: Kilimanjaro Christian Medical Center (FACSCalibur)
Proficiency Provider: UKNEQAS
Survey: 0604

Date of Evaluation: July 17, 2006

ANALYTE	SPECIMEN NUMBER	RESULT	MEAN	ACCEPTABLE RANGE
1. CD3+ Lymphocyte	178	771	771	569-973
2. CD3+/CD4+ Lymphocyte	178	178	177	115-239
3. CD3+/CD8+ Lymphocyte	178	503	473	315-631
4. CD3 +%	178	64	64.19	59.87-69.51
5. CD3+/CD4+%	178	15	14.63	11.51-17.75
6. CD3+/CD8+%	178	42	39.48	31.64-47.32
7. CD3+ Lymphocyte	179	848	769	569-969
8. CD3+/CD4+ Lymphocyte	179	193	177	121-233
9. CD3+/CD8+ Lymphocyte	179	553	475	321-629
10. CD3 +%	179	64	64.14	58.70-69.58
11. CD3+/CD4+%	179	15	14.53	11.37-17.69
12. CD3+/CD8+%	179	42	39.68	32.16-47.20

Laboratory Manager/supervisor: Date:

Appendix 2: PROFICIENCY REVIEW FORM

AACTG, CHAVI, HPTN, NIH-ISAAC

Section: KILIMANJARO CHRISTIAN MEDICAL CENTRE Proficiency Provider: UKNEQAS
Survey: _____

Today's Date: _____ Date of Evaluation: _____
Score: _____ Unacceptable / Total

ANALYTE	SPECIMEN NUMBER	YOUR RESULT	MEAN	ACCEPTABLE RANGE	REPEAT ANALYSIS (give result when applicable	QC FOR DAY OF ANALYSIS? [1]	QC NOTES?[2]
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							

[1]To be noted: In-Control / Out of Control
[2]To be noted: Trend noted / Shift noted / Bias noted

UNACCEPTABLE RESULT ANALYSIS

ANALYTE (S)	REASON	ACTION TAKEN

Code for Reason:

MP – Methodological Problem **SMP** – Survey Material Problem
TP – Technical Problem
NE* – No Explanation after Investigation(Random Error) **CE** – Clerical Error
XX – Other (include explanation)

**NE may be used only after all other possibilities have been excluded.*

Technologist Date PI or Lab Manager Date

Appendix 3: UK NEQAS Sample Entry Form

Appendix 4: REQAS Sample Entry Form

The South African NHLS/WHO/ QASI Collaborative CD4 Regional External Quality Assessment Scheme (REQAS)

Sample Condition: SATISFACTORY/ Unsatisfactory

Reason:_____

Return Date: 12h00 Tuesday **28 November 2006**

Participant name and participant code number must be written in

Trial Number: Trial 21			
Participant Name:		**Code number:**	
Received: (DD/MM/YY)		Date of Analysis: (DD/MM/YY)	

1. *The emphasis of these trials is CD4 EQAS, and participants who report only a CD4 absolute count, and CD4 percentage of lymphocytes (e.g. with PLG CD4), are not compromised on this analysis by non submission of CD3 or CD8 results*

2. ***Please note:*** *The nature of QA material is such that it cannot reliably generate an ALC on a haematology analyser. It is therefore imperative that your laboratory generates a flow differential (either 45 or LS based), and use the percentage lymphocytes obtained from this differential multiplied by the WCC from a haematology analyser to generate your ALC (absolute lymphocyte count).*
   For DP – lymphocyte users only.

Please tick all the relevant blocks that are applicable to your laboratory / analysis method.

Participants: Please fill in your monoclonal antibodies below

Antibody panels – EXAMPLE ONLY				
	Identify the antibody (CD) and fluorochrome for each tube of the panel (only use the relevant column e.g. if using a CD3/4/8, then only fill in MAb-FL1, MAb – FL2 and MAb-FL3 in row for tube 1			
Panel	MAb - FL1	MAb – FL2	MAb – FL3	MAb – FL4
Tube 1	CD8 FITC	CD4 RDI	CD3 PC5	
Tube 2				
Antibody panels				
	Identify the antibody (CD) and fluorochrome for each tube of the panel			
Panel	MAb - FL1	MAb – FL2	MAb – FL3	MAb – FL4
Tube 1				
Tube 2				

Please submit the BCXL raw data with results

IMPORTANT: ALL PLG USERS

Results:

Predicate method = normal method used in your laboratory.
PLG method: If PLG method is predicate then write results in PLG row.
If both methods are is use, then write all results in the relevant rows.

	Absolute Counts		
Sample	CD4 T cells (Cells /µL)	CD8 T cells (Cells /µL)	CD3+ Total (Cells /µL)
Predicate QC 17			
Predicate QC			
PLG QC 17			
PLG QC			

Sample	Lymphocyte Percentages		
	CD4T cells	CD8 T cells (if applicable)	CD3+ Total (if applicable)
Predicate QC 17			
Predicate QC			
PLG QC 17			
PLG QC			

Flow Cytometer Used:		
	BDS FACScan	
	BDS FACSCalibur	
	BDS FACSCount	
	BC FC 500	
	BC Elite	
	BC XL	
	Partec	
	Ortho Cytoron	
	Other:	

Basic Methodology		
	Single Platform	
	Dual Platform	

Single Platform				
Dedicated	Yes	No	BDS FACSCount	
			Other:	
Bead product			BDS Trucount	
			BC FlowCount	
			Other:	

Pipette Method	**Manual**	
	Use same pipette for blood and beads with normal pipetting	
	Manual Reverse pipetting (positive displacment pipetting)	
	Automated	
	AutoPrep reverse pipetting	
	BDS (SP-1)	
	BC	
Are your pipettes regularly calibrated?		
If so, how often:		

Dual Platform		
Haematology Analysis:		
Haematology Analyser Used		
White Cell Count		x 10^9/l
	Counting reference	
	WCC	
	Absolute Lymphocyte Count (ALC). (Refer to cover page no.2)	

Flow Cytometer Differential:		
% Granulocytes		
% Lymphocytes		
% Monocytes		
Flow cytometry gating strategy		
	Lymphocyte gating FS/ SS CD3/4/8	
	CD45 bright (Lymphs) abbreviated version of guideline CD3/4/8/45	
	CD45 bright (Lymphs) 3 or 4 colour panel (1998/ 2002 CDC/ NIH guidelines)	
	CD14/ CD45 2 colour panel (6 tube 1992 CDC/ NIH guideline)	
	PLG CD4 Total CD45	

Red Cell lysis		
	Lysed – No wash	
	Separated cells (eg FICOLL)	
	Lysed and washed	
	No wash - No lyse	

To be filled in by new participants

Maintenance:	
Are your instruments regularly maintained?	
If so, how often:	
Are your instruments regularly serviced?	
If so, how often:	
Routine laboratory specimens:	
How many CD4 specimens does your laboratory generate monthly? (approx)	
At the time of joining REQAS	
At the current date	

Title: Flow Cytometry External Proficiency Testing

SOP References: **Supersedes:**

Comments:	

Suggestions:	

Date:	**Signed:**
	Name:

Thank you for your participation.
Professor D.K Glencross and Mrs H. Aggett.
CD4 SA REQAS

Appendix 5: IQA Corrective Action Investigation Form

Date:	Site:
Proficiency Provider & Panel:	
Analyte:	**Reported Result:**
Acceptable Result/Range:	**Previous survey problems:**

Laboratory Investigation: *(Attach supporting documentation. Add additional lines as needed for comments.)*

1. Survey report examined for discrepancies, clerical errors and appropriate codes?
Comments and action taken:

2. Survey material receiving temperature, handling, reconstitution, storage and analysis investigated?
Comments and action taken:

3. Method history reviewed (quality control, maintenance, reagents, etc.)?
Comments and action taken:

4. Manufacturer consulted?
Comments and action taken:

5. Survey samples reassayed?
Comments and action taken:

6. Calibration data reviewed? Recalibrate if needed.
Comments and action taken:

7. Linearity data reviewed? Perform a linearity study if needed.
Comments and action taken:

8. Personnel competency reviewed? Conduct staff education or retraining if applicable.
Comments and action taken:

9. Assess patient results for adverse impact? Review impacted patient results, amend results and notify physicians, if applicable.

Comments and action taken:

Deficiency Classification:

- ☐ Methodological
- ☐ Technical
- ☐ Clerical
- ☐ Survey evaluation problem
- ☐ Other (explain) _____

Prepared by:

_____ _____

Name/Title Signature

Note: Please complete the report and submit it to IQA.