STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: FLOW-GEN-044

DOCUMENT TITLE:

Performance Verification of Un-assayed Antibodies

DOCUMENT NOTES:

Document Information

Revision: 03	**Vault:** FLOW-General-rel
Status: Release	**Document Type:** FLOW SOPs

Date Information

Creation Date: 28 Jun 2019	**Release Date:** 05 Aug 2019
Effective Date: 05 Aug 2019	**Expiration Date:**

Control Information

Author: MGREESE	**Owner:** MGREESE
Previous Number: FLOW-GEN-044 Rev 02	**Change Number:** STCL-CCR-462

FLOW-GEN-044
PERFORMANCE VERIFICATION OF UN-ASSAYED ANTIBODIES

1 PURPOSE

1.1 The purpose of this procedure is to describe how antibodies that currently have no commercially available pre-assayed positive control are tested to ensure proper performance in defined assays.

2 INTRODUCTION

2.1 One aspect of quality control in flow cytometry assay is monitoring reagent performance based on predetermined criteria. Many commonly used lymphocyte subset markers are easily monitored by the use of commercially available stabilized process control cells with established range of expected values. However, when an antibody is less commonly used, it becomes more challenging to find an appropriate source for positive control material. One good source material is peripheral blood from normal heathy donors, particularly when using antibodies to antigens that are found on the surface of lymphocytes, monocytes, or granulocytes consistently.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure is to be used to monitor proper performance of antibodies which have been optimized for use in a defined assay and for which there is no commercially available process control with pre-assayed ranges. It is the responsibility of the lab director, lab manager, flow cytometry supervisor, flow cytometry staff to ensure that this procedure is followed.

4 DEFINITIONS/ACRONYMS

4.1 BD™-Becton Dickinson

4.2 SCE-Stem Cell Enumeration

4.3 CD-Cluster Designation

4.4 PBS-Phosphate buffered saline

4.5 BSA-Bovine Serum Albumin

5 MATERIALS

5.1 Antibody reagents to be tested

5.2 Peripheral blood collected in sodium heparin or EDTA anticoagulant vacutainer

5.3 FACS Lysing Solution (at 1X concentration with deionized and filtered water), BD Biosciences

5.4 PBS with 1% BSA (wash reagent), Gibco

6 EQUIPMENT

6.1 Flow cytometer

6.2 Vortex mixer

6.3 Timer

6.4 12 x 75 mm BD Falcon™ polystyrene tubes (or equivalent)

6.5 Calibrated 10, 200, 1000 µl micropipette, (Rainin or equivalent)

6.6 Micropipette tips for appropriate volumes.

6.7 2 ml automated pipette and tips (Rainin or equivalent)

6.8 Centrifuge, Beckman Coulter Alegra 6KR

7 SAFETY

7.1 Review MSDS for reagents used in testing.

7.2 Sodium azide warning

7.3 BD FACS Lysing solution

7.4 Use universal precautions and wear appropriate personal protective equipment (PPE) when working with biohazardous materials.

8 PROCEDURE

8.1 Identify antibody or antibody cocktail to be tested.

8.2 Once per month or as needed obtain peripheral blood test samples from normal healthy donor.

8.3 Follow the assay procedure for staining, acquisition, and analysis as you would a patient test sample.

8.4 Use the file name NORMAL mmddyy.

NOTE: In order to assess antibodies that are used to set fluorescent threshold in the lyse/no wash method, it is necessary to wash the excess reagent from the tube in order to use a forward scatter threshold and gate on the appropriate population via light scatter properties free of excess antibody and debris.

8.5 Add 1 ml of PBS wash reagent to the appropriate tubes (i.e. tubes 3,5,6,7 of immune recon panel) and to the compensation optimization tubes used to optimize the LNW settings.

8.6 Centrifuge these tubes at 300g (~1200 rpm) for 5 minutes.

8.7 Remove tubes and decant the supernatant.

8.8 Re-suspend the pellet in 300 µl of PBS wash reagent.

8.9 It will be necessary to perform the compensation optimization using the Calib File (Lyse/Wash) settings prior to re-acquiring test tubes that are washed.

8.10 Optimize the FSC threshold level to eliminate excess debris (usually around 100-150).

8.11 Save these settings as Calib file opt. and leave them in place for acquiring washed samples.

8.12 Continue acquisition run order using the same data file name and starting with next file increment number.

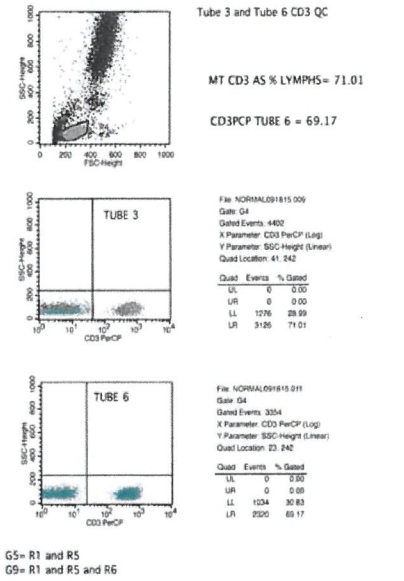
8.13 Align the curser in the parameter description window to correspond with the tube that is being re-acquired.

8.14 The table below includes the analysis templates currently used for this quality control testing.

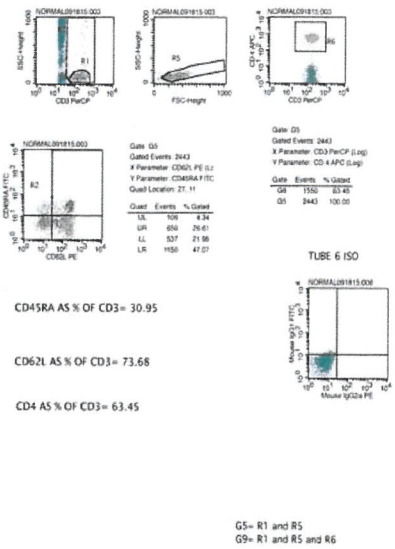
Analysis templates		
Template	File Increment	Results
Clinical IR QC T-3&6 CD3	9 (washed 3)	CD3% MT* as % Lymphs
	11 (washed 6)	CD3% PCP as % Lymphs
Clinical IR QC T-3 CD45RA-CD62L	3 and 6 (iso)	CD62L% MT as CD3
		CD45RA% MT as CD3 RTE**
IR QC T4 CD25andCD62L	4 and 6 (iso)	CD25% FITC as %CD4+ T Cells
		CD62L% PE as %CD4
		CD4% APC as %CD3
IR QC T-5 CD57 CD28	5 and 6 (iso)	CD57% FITC as %CD8 bright
		CD28% FITC as %CD8 +
IR QC T-5 CD8 &HLA-DR APC	10 (washed 5)	CD8% PCPcy5.5 as % Lypmhs
		HLA-DR% APC as % monocytes
IR QC T-7 LIN & HLA-DR PCP	12 (washed 7)	LIN-1% FITC as % monocytes
		HLA-DR% PCP as % monocytes
* MT = multitest		
**RTE = Recent Thymic Event		

8.15 Analysis examples for Immune Reconstitution panel:

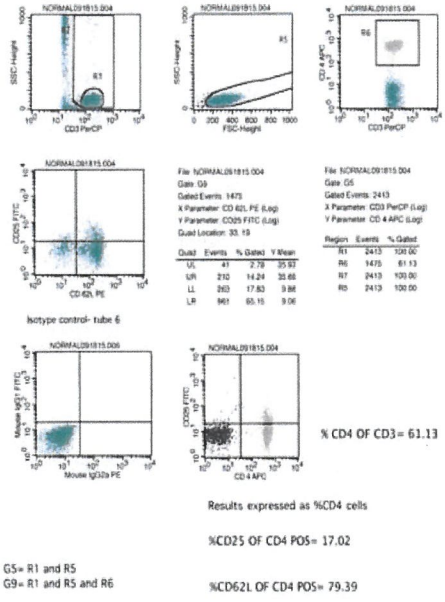
Clinical IR QC T-3&6 CD3 (wash)



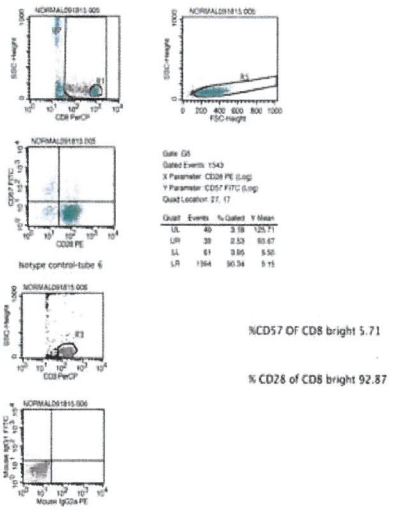
Clinical IR QC T-3 CD45RA-CD62L



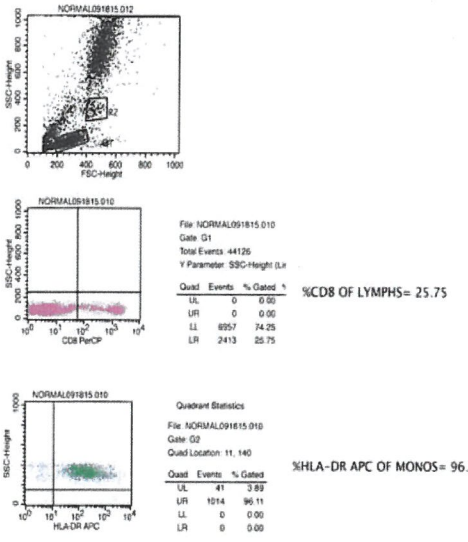
Clinical IR QC T4 CD25 and CD62L



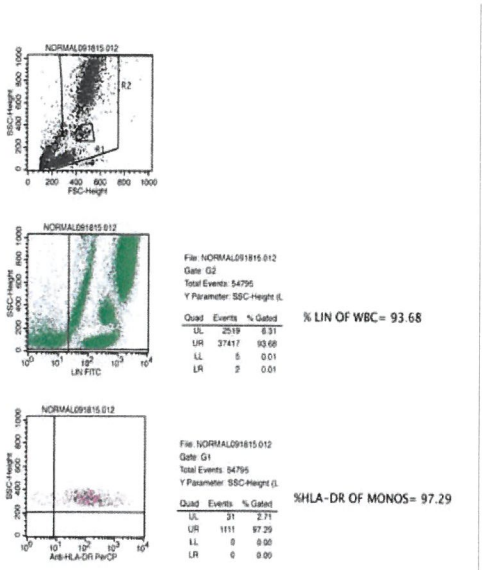
Clinical IR QC T5 CD57 CD28



Clinical IR QC T5 CD8 & HLA-Dr (wash)



Clinical IR QC T7 Lin & HLA-DR (wash)



8.16 Record the results on FLOW-GEN-044 FRM 1, Flow Cytometry Reagent Quality control Result Sheet, and compare the results of testing to the defined acceptance range to verify performance.

8.17 The acceptable ranges are established by obtaining the mean and standard deviation from testing performed on healthy volunteers monthly over at least a two year time frame. The results of testing are entered into an EXCEL spreadsheet for the purpose of statistical analysis. No fewer than 20 values from 5 or more healthy donors are used to establish the mean value for each marker of interest and the range for each marker is then determined by calculating ± 2 standard deviations from the mean. Due to the variable nature of the immune system and the changing donor pool over time, the range will be reviewed periodically to determine if adjustments are warranted. Future revisions of acceptable ranges will be performed as described in this procedure and documented in the MasterControl system via Change Control as updates to FLOW-GEN-044 FRM1.

8.18 Follow the rules for troubleshooting unacceptable results outlined in FLOW-GEN-020, Stem Cell Laboratory Quality Management-Quality Control Policies for Flow Cytometry.

8.19 Ensure that these results receive a second review by a qualified staff member to detect transcription errors, then file and keep the results available for at least 2 years.

9 RELATED DOCUMENTS/FORMS

9.1 FLOW-FORM-008, Flow Cytometry Reagent Quality control Result Sheet

9.2 FLOW-GEN-020, Stem Cell Laboratory Quality Management-Quality Control Policies for Flow Cytometry

9.3 FLOW-GEN-044 FRM1,UN-ASSAYED ANTIBODIES RECORD SHEET

10 REFERENCES

10.1 Clinical Laboratory Improvements Amendments of 1988 (102 Stat. 2903, Public Law 100-578)

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	M. Reese	Added explanation for acceptable range determination. Explained how/why acceptable ranges will be reviewed. Added FRM 1 to the related documents.

Signature Manifest

Document Number: FLOW-GEN-044 **Revision:** 03

Title: Performance Verification of Un-assayed Antibodies

All dates and times are in Eastern Time.

FLOW-GEN-044 Performance Verification of Un-assayed Antibodies

Author

Name/Signature	Title	Date	Meaning/Reason
Melissa Reese (MGREESE)		10 Jul 2019, 10:41:55 AM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		10 Jul 2019, 01:15:40 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		10 Jul 2019, 06:27:02 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
(RB232) for Bing Shen (BS76)			
Isabel Storch (IMS19)			
Lisa Eddinger (LE42)			
Taylor Orr (TSO4)			
Richard Bryant (RB232)		11 Jul 2019, 01:52:59 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		25 Jul 2019, 05:33:49 PM	Approved