STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Using R&D Systems Status Flow Process Controls

DOCUMENT NOTES:

Document required for the BLA.

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FLOW-GEN-013
USING R&D SYSTEMS STATUS FLOW PROCESS CONTROLS

1 PURPOSE

1.1 This document is to instruct the user to be able to properly prepare the R&D Systems Status Flow Process controls for use in daily quality control testing.

2 INTRODUCTION

2.1 Immunophenotyping by flow cytometry is a complex, multi-step process. Validity of imunophenotyping results depends on efficient RBC Lysis and clear separation of leukocyte subpopulations based on light scatter characteristics and reactivity with cell-specific, fluorescent monoclonal antibodies. R&D Systems Stats Flow and Status Flow^{Pro} process control cells with assayed expected values may be used to monitor quality and validity of the immunophenotyping process.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure must be used when using R&D Systems process controls cells on each day of flow cytometric testing. The medical director, senior staff, and designated laboratory staff are responsible for ensuring the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	PBS	Phosphate Buffered Saline
4.2	BSA	Bovine Serum Albumin
4.3	MSDS	Material Safety Data Sheet
4.4	BD	Becton Dickinson
4.5	QC	Quality Control
4.6	SF	Status Flow

5 MATERIALS

5.1 Monoclonal antibodies (Use according to the testing procedures used each day)

5.2 Status Flow/Status Flow^{Pro} Process Control Cells, R&D Systems

5.3 PBS/ 1% BSA (Gibco BRL)

5.4 12mmx75mm test tubes, Fisher brand or Trucount™ tubes (Becton Dickinson)

5.5 Red cell lysing agent (Use according to testing procedures used each day)

6 EQUIPMENT

6.1 Automated micropipette Calibrated 2000 microliter automated pipette and tips (Rainin)

6.2 Adjustable micropipettes 10, 20, 200, 1000 microliter and tips (Rainin)

6.3 Vortex mixer, Fisher Genie 2 (or equivalent)

7 SAFETY

7.1 Review MSDS for monoclonal antibodies used in testing.

7.1.1 Sodium azide warning

7.1.2 Nucleic acid dye warning

7.2 Review MSDS for BD Trucount tubes.

7.2.1 Cobalt chloride warning

7.2.2 Silica warning

7.3 Review MSDS for BD FACS™ Lysing Solution

7.3.1 Diethylene glycol warning

7.3.2 Formaldehyde warning

7.4 Review MSDS for BD Pharm Lyse.

7.5 Wear all appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, lab coat, gloves, goggles, etc.

8 PROCEDURE

8.1 Store the SF cell vials upright and tightly capped, at 2-8 degrees Centigrade when not in use.

8.2 Unopened vials are stable until the expiration date indicated on each vial and assay sheet.

8.2.1 Upon receipt of these individual control products, a "Daily working box" may be created with one each of the levels. The High, Low, and Normal (lymph) samples should be labeled with colored tape to distinguish them within this box.

8.3 Complete 1 Flow Cytometry Worksheet for all control cell testing using CONTROL in the Patient Information section. Fill out only the sections that apply to the Control cell testing.

8.4 Use the applicable naming convention for the data file name.

8.5 To Mix: Hold a tube horizontally between the palms of the hands. DO NOT MIX ON MECHANICAL MIXER.

8.6 Roll the tube back and forth for 20-30 seconds, mixing vigorously (do not shake).

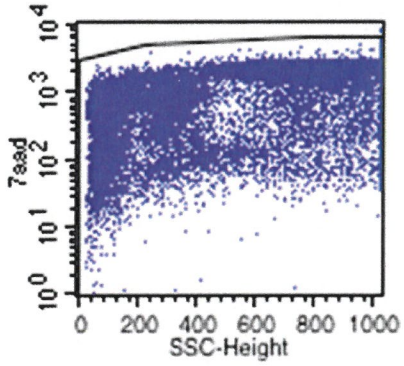
8.7 Repeat steps 2 and 3 until the cell pellet on bottom of vials are completely suspended.

8.8 Gently invert the vials 10 times immediately before sampling.

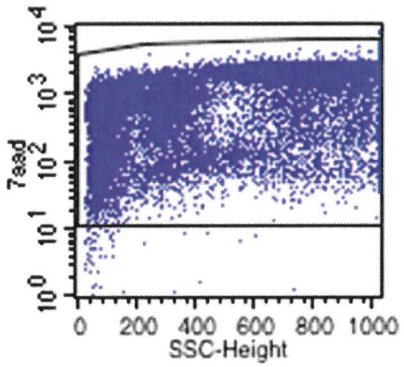
8.9 After sampling, clean residual material from the cap and tube rim with lint free tissue. Replace the cap tightly. Return tubes to refrigerator within 30 minutes of use.

8.9.1 **NOTE: Acquisition and analysis of these process controls should be carried out using the same staining methods, acquisition settings, and analysis templates being used for routine testing with the following exceptions:**

Since these cells are fixed, all or most of the cells are non viable and will be positive for 7aad dye. To avoid waste of 7aad, it is used only in the isotype control tube of the lymphocyte panel (see analysis examples below) and it is left out of the control cell testing for the BD SCE assay. Example A shows correct placement of the viable cell region using CellQuest Pro analysis software.



Example B shows the correct placement of the region to QC 7AAD staining.

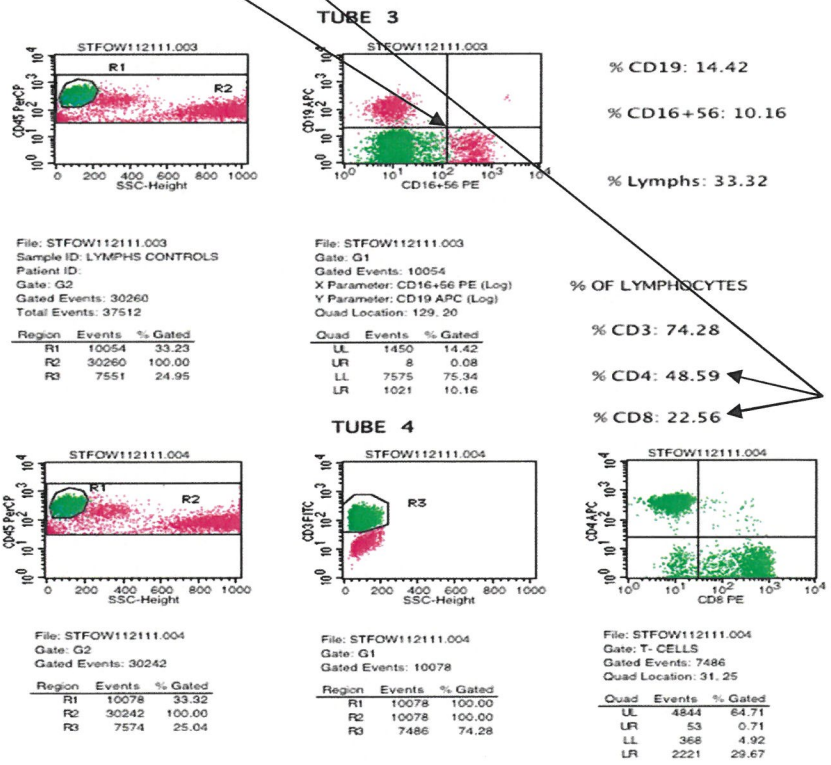


8.9.1.1 Since the cell concentration is lower than is found in routine product testing and this can impact the amount of time it requires to complete testing, the number of cells acquired is reduced to a practical level such as 50-60,000 events for rare event (CD34) testing and 5000 for lymphocyte subset testing.

8.9.1.2 The lymphocyte subset assay values and expected ranges provided by the manufacturer are expressed as % of lymphocytes. The CellQuest Pro analysis template in routine use is set up to express T-cell subset results as % of CD3 a modification was required in order to express these values as % of lymphocytes. Using the math functions in Cellquest Pro, the event values for CD4 (UL(4844)+UR(53) quadrants) and CD8 (LL(2221)+UR quadrants(53)) are divided by the lymphocyte event count (10079) to obtain the required values for this testing.

8.9.1.3 Additionally, on the dot plot illustrating the CD19+ and CD16+56 marker results, the quad stats must be placed so that the dim CD16+CD56 staining is removed. The bright population of CD16+CD56 + cells represent the CD3 negative CD16+CD56 positive NK population that is represented by the manufacturer's assayed values for these markers.

See the CellQuest Pro analysis below to illustrate exceptions 8.9.1.2 and 3.



8.10 Indication of deterioration:

8.10.1 Product should be reddish and slightly cloudy. Discoloration of the supernatant may indicate deterioration or contamination. Do not use the product if deterioration is suspected.

8.11 Storage and Stability:

8.11.1 Store the vials upright and tightly capped, at 2-8 degrees C when not in use.

8.11.2 Unopened vials are stable until the expiration date indicated on each vial and assay sheet.

8.11.3 Opened vials are stable for 9 thermal cycles which consist of one removal from storage for testing. Therefore each vial should be dated upon opening and marked each time it is removed for use.

9 RELATED DOCUMENTS/FORMS

9.1 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet

10 REFERENCES

10.1 R&D Systems Status Flow/Status Flow^{Pro} product insert.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	M. Reese	1. Changed SOP title 2. Corrected SOP titles in section 9

Signature Manifest

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FLOW-GEN-013 Using R&D Systems Status Flow Process Controls

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