STEM CELL LABORATORY (STCL)

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Stem Cell Laboratory Quality Management-Quality Control Policies for Flow Cytometry

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FLOW-GEN-020
STEM CELL LABORATORY QUALITY MANAGEMENT/ QUALITY CONTROL POLICIES FOR FLOW CYTOMETRY

1 PURPOSE

1.1 The following paragraphs outline the strategies employed by the Stem Cell Laboratory to maintain our quality management and quality control program for flow cytometry testing. Having read this procedure, the user should have a firm grasp of the measures required to maintain quality assurance within the flow cytometry section of the Stem Cell Laboratory.

2 INTRODUCTION

2.1 The Stem Cell Laboratory Quality Management Plan identifies the processes for maintaining quality throughout the processing and testing of pediatric and adult transplant products received and/or tested by this laboratory. Flow cytometry testing is considered high complexity with unique requirements for quality control of the complete testing system. This document details the specific measures to maintain quality throughout the flow cytometry testing system.

3 SCOPE AND RESPONSIBILITIES

3.1 This document describes the quality management guidelines for flow cytometry instrumentation, reagents and specimen processing. The flow cytometry staff, along with the laboratory manager and directors, are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1 ALC Absolute lymphocyte count

4.2 BD Becton Dickinson

4.3 BMT Bone marrow transplant

4.4 CAP College of American Pathologist

4.5 CD34+ cells - Refers to the progenitor cell population as determined by established fluorescent antibody staining and light scatter properties using flow cytometry.

4.6 CV Coefficient of Variation

4.7 FIFO First In First Out

4.8 IR Immune reconstitution

4.9 MSDS Material Safety Data Sheet (MSDS reference is being phased out)

4.10 N/A Not Applicable

4.11 PB Peripheral Blood

4.12 PI – Precision Index is the ratio of a lab's CV to the Group CV. PI is a measure of relative precision and should fall between 0 and +2 to meet acceptable performance.

4.13 PMT Photomultiplier Tube

4.14 Post Processing - Specimens removed from a product after some manipulation which alters it from its original state.

4.15 Process control cells - Blood cells, usually a commercially purchased stabilized cell preparation with predetermined assay values for markers of interest.

4.16 PT Proficiency test/testing

4.17 QC Quality Control

4.18 QM Quality Management

4.19 SDI - Standard Deviation Index is the number of group standard deviations by which a Lab's mean differs from the group mean. It measures relative accuracy and should fall between -2 and +2 to meet acceptable performance.

4.20 SDS Safety Data Sheet

4.21 SOP Standard Operating Procedure

4.22 STCL Stem Cell Laboratory

4.23 UCB Umbilical cord blood

4.24 WBC White Blood Count

5 MATERIAL

5.1 "Do Not Use This Lot Number" label

5.2 "This Lot is Ready for Use" label

5.3 "IN USE ON" label

6 EQUIPMENT

6.1 N/A

7 SAFETY

7.1 All newly employed Stem Cell Laboratory personnel are required to review, with the lab safety officer, the Stem Cell Laboratory Safety manual and Duke University Medical Center Safety and Infection Control manuals. Thereafter, STCL personnel will be required to complete annual safety-related training on-line via the Safety website.

7.2 MSDS/SDS for flow cytometry testing reagents are kept in a notebook in the flow cytometry section of the laboratory. The notebook is labeled *Flow Reagent MSDS/SDS Sheets*.

8 PROCEDURE

8.1 Flow cytometry test specimen management:

8.1.1 Specimens originate from both internal (in-lab cellular products) and external (peripheral blood) sources.

8.1.2 Applicable sections of *FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet* must be filled out for each specimen.

8.1.3 Any dilution of the specimen prior to sending for flow testing must be communicated verbally or written on the specimen container.

8.1.4 As part of the pre-analytical phase, all specimens for flow testing must be logged in at the staining station with the following information, if applicable:

8.1.4.1 Date/time of draw

8.1.4.2 Specimen ID

8.1.4.3 Specimen type

8.1.4.4 Test request

8.1.4.5 Reason for rejection (*if applicable*)

8.1.4.6 Notification if rejected (*if applicable*)

8.2 Specimen rejection criteria include, but are not limited to, the following::

8.2.1 Specimen improperly labeled or unlabeled

8.2.2 Specimen improperly collected and/or preserved

8.2.3 Specimen volume or cell concentration is not sufficient for requirement of test protocol

8.2.4 Specimen contains interfering substances for requested procedure

8.2.5 Specimen is not received within specified time for analysis.

8.3 If the specimen must be rejected, the tech will log the specimen in the flow cytometry specimen log-in book and note the reason for specimen rejection. A *STCL-SOP-037* ***Unacceptable Specimen Log (FRM1)*** must be completed and given to the lab manager. Once the lab manager's follow-up is complete, file the form in the designated binder found in the flow cytometry section of the laboratory.

8.4 All specimens are retained in the flow staining area at the appropriate temperature no less than 3 days and up to 1 week after completion of testing prior to disposal.

8.5 All CD34 testing includes the use of the viability dye 7AAD and the result is reported as viable CD34+ cells.

8.6 According to CAP guidelines, a statistically valid number of CD34 events must be collected at sample acquisition to ensure clinically relevant precision and accuracy. To achieve this goal, 300,000 viable CD45+ events (or 15 minute run time for samples with low cell concentration) are collected. This ensures that a minimum of 100 CD34+ events are acquired for test samples at or above clinical decision points.

8.7 Flow specimen sources with their specific requirements are listed as follows:

8.7.1 Peripheral blood pre-apheresis CD34+ cell enumeration requests:

8.7.1.1 The specimen must be drawn into an EDTA or a sodium heparin blood tube and may be held at room temperature up to 24 hours until testing can be performed.

8.7.1.2 A white blood count, via the automated hematology analyzer, must be obtained to determine if a dilution is needed and if the cell count meets the minimum of 3.0 x 10^6/ml established for this testing.

8.7.1.3 If the minimum count is not met, the apheresis nurse must be notified that the specimen will not be tested unless there are extenuating circumstances related to patient care in which the CD34+ cell concentration is required. *STCL-SOP-037* ***Unacceptable Specimen Log (FRM1)*** must be completed and given to the lab manager.

8.7.1.4 Cell concentrations ≥ 40 million cells/µl must be diluted.

8.7.1.5 BD TruCOUNT absolute counting tube integrity must be verified by comparing the WBC concentration obtained via the hematology analyzer to that obtained via TruCOUNT CD45+ cells/ µl determination. If values fall outside 15% of one another, testing must be repeated. The flow supervisor or designee must be notified if the discrepancy still exists after repeating as further troubleshooting may be required.

8.7.1.6 The result of the positive reagent control must be within specified range prior to staining test specimens.

8.7.1.7 Data analysis and result calculations must be verified by a second qualified technologist. The verification must be documented in the specified location on the Flow Cytometry Worksheet.

8.7.1.8 The CD34+ cells/µl must be 9.74 or greater to meet the lower limit of accuracy for this testing.

NOTE: Results that do not meet the lower range limit, receive a stamp in red ink stating that the CD34 content did not meet the lower limit of accuracy established for this testing.

The result must be called to the apheresis nurse and a read back of patient identifier and result is required. This notification is documented in the specified location on the Flow Cytometry Worksheet.

NOTE: Typically, patients with CD34+ cells/µl <10 will not be apheresed. This decision, however, is made at the discretion of the clinical team while considering the growth

factors being administered to the recipient, their clinical protocols, etc.

8.7.2 Peripheral blood stem cells and bone marrow CD34+ cell determinations:

8.7.2.1 0.5 ml of specimen from the collection bag is the minimum volume required.

8.7.2.2 Optimally, undiluted peripheral blood stem cell specimens should be held at 2-8°C and tested within 24 hours of collection. Samples from products >24 hours old may have lower viability.

8.7.2.3 Optimally, bone marrow products should be held at room temperature and sampled within 24 hours of collection, if within the STCL's control. Some products are collected at other facilities outside Duke and transferred and received in the STCL more than 24 hours after collection. This is outside of the STCL's control. Samples from products >24 hours old may have lower viability.

8.7.2.4 Specimens with a cell concentration ≥ 40 million cells/µl must be diluted.

8.7.3 Immune Reconstitution Requests

8.7.3.1 Sodium heparin (green top) or EDTA (purple top) may be used.

8.7.3.2 A minimum of 1 milliliter of specimen is needed.

8.7.3.3 Specimen must be held at room temperature. Optimally testing should be performed within 24 hours of blood draw. However, at the request of the primary physician, testing may be performed on older specimens. This must be noted on the accompanying flow worksheet.

8.7.3.4 A white blood cell count (WBC) with differential is required in order to determine if a dilution is needed and to provide quality control for the BD TruCOUNT™ tube using the absolute lymphocyte count (ALC). If the ALC is not within 20% agreement with the hematology analyzer and the hematology analyzer report does not have any atypical cell type messages, the testing may need to be repeated if the error is deemed to be due to a technical issue by the technical supervisor.

NOTE: Differences are not always related to technical error. They may be a function of unusual or low density cell populations in post- transplant peripheral blood specimens which the hematology analyzer cannot categorize properly. In some cases the hematology analyzer is unable to provide the ALC. However, if there is agreement within 10%

between TruCOUNT Tube 1 and Tube 2 ALC then it may be reasonably assumed that the results of testing are valid.

8.7.3.5 Unless specifically requested by the ordering physician, Tube 1 (CD45,CD3, CD19, CD16/CD56) of the immune reconstitution panel is all that is required on specimens with hematology analyzer ALC of <200 cells/µl.

8.7.3.6 Specimens with a cell concentration ≥ 10 million must be diluted for optimal staining.

8.7.4 Fresh umbilical cord blood

8.7.4.1 0.5 milliliters from the processed cord is required for testing.

Specimens should be stained within 4 hours of the specimen draw time recorded on the flow cytometry worksheet by the responsible party.

NOTE: The performing tech must verify the time frame between specimen draw time and specimen staining by initialing and dating in the designated location on the Flow Cytometry Worksheet. If staining occurs outside this time frame it must be noted on the Flow Cytometry Worksheet that accompanies the specimen.

8.7.4.2 Products with cell concentrations ≥ 40 million cells/µl must be diluted.

8.7.4.3 As a check on the single-platform method, agreement between the hematology analyzer WBC and the CD45+ absolute count obtained from the single platform CD34 testing should be within 25%.

NOTE: Test results with discrepant CD45+ cell concentrations must be reviewed by the flow cytometry supervisor or a qualified flow cytometry staff member to determine if repeat testing is warranted**.**

8.7.5 Thawed Products

8.7.5.1 0.5 milliliters of specimen from the post thawed product is the minimum volume required.

8.7.5.2 Specimens must be stained immediately upon receipt and they should receive first priority for acquisition on the cytometer.

8.7.6 Donor lymphocyte infusions (DLI)/Car-T protocols

8.7.6.1 Several protocols exist for these products. Lymphoctye markers used and staining requirements are included in the flow panels found in FLOW-GEN-007 or associated job aids.

8.8 Supply Receipt and Quality Control

NOTE: Receipt of supplies used for flow cytometry processes are subject to the general guidelines for supply management used in the STCL.

8.8.1 Monoclonal antibodies

8.8.1.1 Storage and Usage:

8.8.1.1.1 Monoclonal antibodies are labeled with the date received and must be stored according to manufacturer's recommendation and used within the manufacturer's recommended expiration date. If stipulated by the manufacturer, an open vial expiration date must be written on the vial reflecting the new limit for use after opening.

8.8.1.1.2 Components of reagent kits must be used within kit lots.

8.8.1.2 New lots of antibodies are stored at appropriate temperature in a designated location separate from reagents that have been quality control tested and deemed ready for use.

8.8.1.3 After a new lot of antibody is tested, it may be placed in the designated rack for new lots which have passed QC testing. Upon being placed in use, a green label stating "IN USE ON" with the date the reagent lot is put into use, is applied to the antibody vial.

8.8.1.4 The "IN USE ON" label with date may be applied to the printed copy of lot to lot testing results as a permanent record of when a given reagent is put into use.

NOTE: This is not required for clinical reagent testing that is monitored daily with pre-assayed commercial process control cells, as those results are recorded on *FLOW-FORM-008 Flow Cytometry Process Control Record Sheet,* as a permanent record.

8.8.1.5 Manufacturer's recommended concentrations must be used unless otherwise validated.

8.8.2 All kits, boxed reagents, cellular controls, and supplies receive a "Do Not Use This Lot Number" label until tested or released for use according to established guidelines (FIFO). If quality control testing is required prior to use, the reagent receives a "This Lot Is Ready For Use" label to indicate testing is complete and the new reagent lot may be put into use when needed.

8.8.3 BD Trucount™ Tubes

8.8.3.1 Store pouches at 2-25 degrees centigrade.

8.8.3.2 Write date on pouch when opened and record the open pouch expiration at 1 month from date opened.

8.8.3.3 Keep the pouch sealed tightly to store remaining tubes.

8.8.3.4 New Trucount tube lots are validated by running process control cells with tube 1 of the immune reconstitution panel to obtain the ALC result which must fall within the manufacturer provided range.

8.8.3.5 Record the results of lot to lot testing on *FLOW-FORM-008 Flow Cytometry Process Control Record Sheet*.

8.8.4 New Lot/Shipment Testing and Process Validation

8.8.4.1 Commercially available process controls with pre-assayed values for CD34 and common lymphocyte markers are used to monitor and validate flow cytometry process and verify consistent results between reagent lots and new shipments of the same lot.

8.8.4.2 These cells are used to monitor the entire flow cytometric testing process on a daily basis, including instrument performance.

8.8.4.3 Process controls provide for validation of antibodies directed to lymphocyte subsets and CD34+ progenitor cells at two concentration levels. Both percentage and absolute values for CD34+ and most common lymphocyte subsets are supplied by the manufacturer for each lot of control cell.

NOTE: The normal level control cell, when diluted by 3, provides a second critical decision level for absolute CD4 assays. The "DO NOT USE This Lot Number" label is applied to new lots of process control cells upon receipt. New lots are tested using routine testing methods to verify the applicable manufacturer's assayed ranges prior to putting the cells into use. A minimum of 8 verification runs are required. Results of this testing must fall within the manufacturer's assay range for each marker tested. If the resulting laboratory established range does not fall *within* the assay range provided by the manufacturer, these control cells should be rejected until the cause of the failure is determined or until the lot of control cells is replaced by the manufacturer. Once a new lot is verified, the "THIS LOT IS READY FOR USE" label is applied to the box containing the new lot vials.

8.8.4.4 The results of control cell testing must fall within the manufacturer's assayed range prior to the initiation of patient sample testing. The results are recorded daily on *FLOW-FORM-008 Flow Cytometry Process Control Record Sheet* which includes reagent lot number, expiration date, and the manufacturer's assayed range for each reagent tested. When recording results from a new shipment of

current lot, write "New Shipment" in the "Lot to be tested" field adjacent to the appropriate reagent and record the result in the adjacent field under new reagent result.

8.8.4.5 If the test result is not within range, then an investigation into the root cause for the out of range value must be undertaken prior to using the reagent for patient test specimens. The rules for determining the root cause of the out of range value are as follows, assuming instrument related issues have been ruled out:

8.8.4.5.1 Rule 1-If upon review of analysis of the data file, it is determined that the error is related to a gating error, that when corrected brings the result within range, patient testing may go forward.

8.8.4.5.2 Rule 2-If rule 1 is not the problem and the acquisition setup is determined to be correct, then testing must be repeated to rule out staining error. The results of this repeat testing must be documented on the reagent quality control result sheet.

8.8.4.5.3 Rule 3-If upon re-staining and re-analysis of the control, the result is still out of range, then a new reagent vial or new lot of the reagent, if the current lot is suspect, must be tested. If it is determined that the reagent is not performing properly, then that reagent must be removed from use and an event report must be filed.

8.8.4.5.4 Rule 4-If there is error across the board for all markers, the control cell or other factors which influence the complete process may be suspect and therefore resolution may be achieved by substituting an unopened vial of control cell or identifying another common factor (i.e. sheath fluid or lysing agent) which might require troubleshooting.

8.8.4.6 If the issue is not resolved; patient testing for this marker must be put on hold until the cause of failure is determined.

8.8.4.7 The process control cell test results are monitored and verified daily using the control cell manufacturer's web based data entry software. This software allows participating labs to track daily results for each flow cytometer used in the lab.

8.8.4.8 The final report is printed and reviewed by the flow cytometry supervisor or other qualified staff member. If the PI and SDI do not meet the manufacturer's recommended criteria, an investigation to determine the root cause must be undertaken. The reviewer's initials and date should be recorded on the first page of the report. Reports are then filed in the flow cytometry section and kept for a minimum of 2 years.

8.8.4.9 Any required action, based upon unacceptable results of control cell testing failures, must be documented according to STCL QA policy.

8.8.4.10 Lot-to-lot testing or new shipments of same lot for un-assayed antibodies (e.g., immune reconstitution studies) are tested via direct comparison during patient testing. The current reagent lot should be substituted with the new reagent in a duplicate tube and acquired at the end of the panel run. The acceptability criteria between reagent lots is a CV $\leq$20% for the reported values obtained from the analysis.

8.8.4.11 The rules for follow-up when results don't meet this specification are the same as those followed in 8.7.3.5.1-4 above (with the exception being no commercial control cells are used).

8.8.4.12 A printed label indicating the antibody being tested, lot #, expiration date, and pass/fail designation is applied to the printed result page. A copy of the current reagent result is stapled to the new lot test result and these results are then stored in the designated binder for a minimum of two years. Once the lot passes testing, move all vials onto the designated "Ready For Use" rack in the storage refrigerator.

8.8.4.13 When the lot is placed in use the green "In Use On" label with date the reagent is placed into use, should be applied to all vials of that lot and to the results page of the lot to lot test.

8.8.4.14 At approximately one month intervals the immune reconstitution testing is performed on a peripheral blood sample from a healthy adult donor to assess the ongoing performance of the entire analytic process for this testing. Results of this testing are compared to the laboratory established range (within 2SD of the mean).

8.8.4.15 Commercially available red cell lysing reagents must be used according to manufacturer's recommendations. Any departure from recommendations must be shown to yield comparable results through comparison testing. Performance characteristics of the lysing agents are tested

using daily process control cells. A new lot is verified using process control cells prior to acceptance. If a new working solution must be prepared during the course of the day due to high testing volume, perform a QC check prior to use with test specimens by lysing 50 µl of peripheral blood while observing the test to see if the lysed appearance (clear red) occurs within 15 minutes. Working solutions of the lysing agent are labeled with the lot number, date diluted, date of expiration, and tech initial.

NOTE: All of the results from testing listed in this section are reviewed daily by the flow supervisor or other qualified flow cytometry personnel.

8.9 Nonspecific Binding Controls

8.9.1 Isotype controls or irrelevant markers of the same class as the positive antibody may be used to establish the degree of nonspecific binding taking place between the antibody and cell. These methods are not perfect, however. It is important to review the results of each stained specimen to determine if nonspecific staining is influencing the result so that corrections can be made if needed.

8.10 Use of CD45 antibody to Determine Leukocyte Gate and Progenitor Cells

8.10.1 CD45 is a pan leukocyte marker found on the surface of normal white blood cells. CD45+ fluorescent antibody staining is a useful aid in separating lymphoid from myeloid populations when combined with side scatter characteristics. It is also helpful in gating out erythroid cell contamination and it can be used in identifying progenitor cells, as they stain characteristically dim with the CD45 antibody.

8.11 Flow Cytometer Instrument Quality Control

8.11.1 Calibration, Troubleshooting, and Maintenance

8.11.1.1 BD FACSCalibur Instruments

8.11.1.2 BD FACScomp software and BD Calibrite beads are used each day of operation to determine instrument performance and reliability using performance criteria associated with each BD Calibrite bead lot. The results are documented in a log book kept for each instrument. A troubleshooting log is available for documenting problems, corrective action, and service calls.

8.11.1.3 At approximately 30 day intervals, results of FACScomp testing are reviewed using BD Levy Jennings software to track instrument performance and detect trends which could indicate a need for instrument service. The mean values will be impacted most commonly by instrument preventative maintenance adjustments, a major part failure and replacement (i.e. laser), or following a bead lot change.

These events must be documented or noted in the troubleshooting log.

8.11.1.4 FACScomp must be performed after instrument service to demonstrate acceptable performance.

8.11.1.5 Any instrument repairs must be documented in the proper location on maintenance log sheets or in the instrument troubleshooting log so that unusual values or repeat values captured in the Levy Jennings charts can be correlated.

8.11.1.6 Examples of expected daily variation in PMT voltage values are provided by the bead manufacturer in the product insert.

8.11.1.7 Monthly cleaning of the fluidics system is performed to maintain instrument performance.

8.11.1.8 If an instrument does not meet the established performance standards, the instrument must be removed from service and BD instrument support contacted to set up a service call.

8.11.2 BD FACSCanto II Instrument

8.11.2.1 Daily, weekly, and monthly maintenance is documented on the FACSCanto II maintenance log kept at the cytometer.

8.11.2.2 BD 7-Color Setup Beads are run each day that testing will be performed with the FACSCanto automated instrument setup when preparing the instrument for the Stem Cell Application.

8.11.2.3 Results of all instrument quality control testing must pass acceptability standards prior to use for clinical testing each day.

8.11.2.4 If minor troubleshooting does not resolve bead setup failures, BD technical support should be contacted and the instrument must be removed from service until the issue is resolved and the instrument passes the quality control testing.

8.12 Optimization

8.12.1 Because plastic particles have different optical properties from blood cells, the scatter and fluorescence properties of biological specimens must be previewed prior to acquiring data on test specimens. Amplifier gain settings, SSC PMT settings, or Threshold settings may require adjustment to keep cell populations on scale. Guidelines are posted on the side of the cytometer when manual setup for these parameters is required.

8.12.2 Instrument settings are linked to the particular staining method and reagents used. Specific testing procedures must be referenced to determine which instrument settings to apply and whether further optimization is required.

8.13 Instrument Cross-check

8.13.1 Process controls provide for monitoring of consistent instrument performance as results of testing must fall into a specified range provided by the control cell manufacturer. The process controls are analyzed on each flow cytometer 2-3 times each week of testing unless an instrument is out of service.

8.13.2 Flow cytometer instruments that are used interchangeably for a particular test request are cross checked monthly during the verification of new process control cells. Each flow technologists prepares at least one set of tests for high and low level CD34, lymphocyte subset, and T-cell subset using STCL SOPs for this testing. A total of 8-10 runs are compared. The results of all runs must fall within the manufacturer's assayed range for the tested marker and should meet laboratory specification for agreement between instruments (i.e. within specified CV or % agreement). Results of testing are reviewed by the flow cytometry supervisor and are kept in lab for 2 years.

8.14 Proficiency Testing and Staff Competency Assessment

8.14.1 New flow cytometry staff must spend adequate time training in order to gain the skills needed to successfully perform testing in this area. The training and training documentation follow the guidelines established in Stem Cell Laboratory procedure *STCL-TRN-001 Training in the Stem Cell Laboratory*.

8.14.2 Monthly, each STCL flow cytometry trained staff member is required to perform a minimum of one time, the process control run including BD Trucount absolute count tubes for the lymphocyte markers and SCE CD34 testing. The results of this testing must fall within the control cell manufacturers assayed range for each marker tested and must meet the quality measures of the R&D System's QA monitoring software. If an out of range value is obtained from this testing, the staff member will be asked to repeat the test. If results are still outside of the range the staff member will be removed from task and must undergo retraining until competency is re-established.

8.14.3 The Stem Cell Laboratory participates in all relevant flow cytometry CAP proficiency testing (PT) surveys. Other peer group testing or alternate performance assessment may be utilized as necessary when CAP does not provide a PT survey for a particular analyte tested in the STCL. Proficiency test specimens are integrated into the daily workload by trained staff using routine staining and analysis methods. CAP proficiency test results are not shared with any other laboratory, nor are the specimens referred to any other laboratory. All testing is performed by a Stem Cell Laboratory staff members who have been trained to perform the testing. The CAP results are reviewed upon receipt and any failures are thoroughly investigated to determine if there is an impact on patient testing that requires corrective action.

8.14.4 The PT specimens are retained until the testing results are received and reviewed.

8.14.5 Annually, procedural competency for flow cytometry staff is assessed through procedure review and direct observation of the staff by the flow cytometry supervisor during performance of designated procedures reflected on the staff member's individual training plan. Technical competency is assessed via the monthly testing using process control cells described in 8.14.2.

8.15 Data Archiving

8.15.1 Per procedure *FLOW-GEN-021 Archiving Flow Cytometry Data* all flow cytometry data files and any PDF files are stored on a shared network drive which is backed up nightly.

8.15.2 Hard copy results of data file analysis are kept in patient folder no less than 10 years.

8.16 Result calculation by user designed spreadsheet or flow cytometry software

8.16.1 Results from flow testing that involve user defined calculations within a software program (i.e. EXCEL or CellQuest Pro) should be verified for accuracy minimally once every 2 years using a manual calculation method. Results of this check are kept in the flow cytometry section of the Stem Cell Laboratory.

9 RELATED DOCUMENTS / FORMS

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

9.2 STCL-SOP-037 Unacceptable Specimen Log (FRM1)

9.3 FLOW-FORM-008 Flow Cytometry Process Control Record Sheet

9.4 STCL-TRN-001 Training in the Stem Cell Laboratory

9.5 FLOW-GEN-021 Archiving Flow Cytometry Data

9.6 STCL-EQUIP-008 Quality Control Systems for the STCL

9.7 STCL-QA-006 Stem Cell Laboratory Quality Management Plan

9.8 STCL-SOP-037 FRM1 Unacceptable Specimen Log

9.9 STCL-SOP-038 Confirmation of Specimen Identification Form

9.10 STCL-SOP-043 Receipt of Products in the Stem Cell Laboratory

10 REFERENCES

10.1 Becton Dickinson CaliBRITE bead product insert

10.2 Becton Dickinson FACSCalibur users' manual

10.3 R&D Systems Status Flow Flow Cytometry Control Product insert

10.4 BD FACSCanto II Instructions For Use, Part no.644450 Rev. A

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
16	Melissa Reese	• Corrections made to formatting of the related document titles in Sec. 9. • Corrected related document formatting in Sec. 8 when referenced.

Signature Manifest

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