# STEM CELL LABORATORY (STCL)

**DOCUMENT NUMBER:** STCL-GEN-018

**DOCUMENT TITLE:**

Quality Control Systems for the STCL

**DOCUMENT NOTES:**

## Document Information

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|---|---|
| **Revision:** 12 | **Vault:** STCL-General-rel |
| **Status:** Release | **Document Type:** STCL |

## Date Information

| | |
|---|---|
| **Creation Date:** 18 Apr 2025 | **Release Date:** 08 May 2025 |
| **Effective Date:** 08 May 2025 | **Expiration Date:** |

## Control Information

| | |
|---|---|
| **Author:** MSR68 | **Owner:** WATER002 |
| **Previous Number:** STCL-GEN-018 Rev 11 | **Change Number:** STCL-CCR-564 |

# STCL-GEN-018
# QUALITY CONTROL SYSTEMS FOR THE STCL

## 1 OVERVIEW

1.1. Dr. Joanne Kurtzberg, MD, the director of the Pediatric Blood & Marrow Program and Dr. Nelson Chao, the director of the Adult Blood & Marrow Program (in collaboration with Dr. Gwynn Long, MD) are the co-directors of the Stem Cell Laboratory. They provide oversight and coordinate all aspects of harvesting, processing, cryopreservation, thawing, and testing performed on peripheral blood, umbilical cord blood, peripheral blood progenitor cells (PBPCs), and bone marrow (BM) derived mononuclear cells before, during, and after hematopoietic stem cell transplantation.

## 2 INTRODUCTION

2.1 The quality assurance program in the Stem Cell Laboratory is based on both internal and external systems designed to:

2.1.1 maintain, in proper working order, the equipment used in processing, testing, selecting, storing, and analyzing human stem cell collections derived from bone marrow, peripheral blood, peripheral blood progenitor cells, and umbilical cord,

2.1.2 evaluate the results obtained while testing samples and/or quality control material (when indicated) for cell viability, bacterial contamination, progenitor cell content, flow cytometry testing (to include the proper addition of test reagents), total nucleated cell count, etc.,

2.1.3 compare test results obtained by the Stem Cell Laboratory to other accredited laboratories who subscribe to the same proficiency testing surveys. Some of the functions outlined in this program are performed at different time intervals to include (i.e. daily, weekly, monthly, quarterly, semi-annually, and annually). Results of maintenance and quality control records are reviewed at specific time intervals, reflected on maintenance sheets, by designated staff. When corrections or improvements are required, the laboratory staff is informed during lab meetings, via e-mails, or in group training sessions, as appropriate. Execution and evaluation of the program is outlined (briefly followed by detailed methods) in the following sections.

## 3 SCOPE AND RESPONSIBILITIES

3.1 The Adult and Pediatric Blood and Marrow Transplant Program Medical Directors, Laboratory Manager, CQP, and the Stem Cell Laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

## 4 DEFINITIONS/ACRONYMS

| | | |
|---|---|---|
| 4.1 | CFU | Colony Forming Unit |
| 4.2 | CQP | Clinical Quality Program |
| 4.3 | DUMC | Duke University Medical Center |
| 4.4 | QC | Quality Control |
| 4.5 | CO2 | Carbon Dioxide |
| 4.6 | LN2 | Liquid Nitrogen |
| 4.7 | STCL | Stem Cell Laboratory |
| 4.8 | HPCA | Hematopoietic Progenitor Cell Assay |
| 4.9 | CHC | Children's Health Center |
| 4.10 | $O_2$ | Oxygen |
| 4.11 | PT | Proficiency Testing |
| 4.12 | N/A | Not Applicable |

## 5 MATERIALS

5.1 N/A

## 6 EQUIPMENT

6.1 N/A

## 7 SAFETY

7.1 N/A

## 8 PROCEDURE

8.1 INTERNAL SYSTEMS:

8.1.1 Quality control for each procedure performed (stem cell harvest, selections, depletions, cryopreservation, thawing, reinfusion, progenitor cell assays):

8.1.1.1 CFU cultures, before and/or after each procedure, ordered when appropriate serve as an internal check of the processing, selection, and cryopreservation procedures as well as a check for any of the reagents used.

8.1.1.2 Quantitation of flow cytometry markers is determined based on the product being tested as outlined in *FLOW-GEN-020 Stem Cell Laboratory Quality Management-Quality Control Policies for Flow Cytometry*.

8.1.1.3 Pre-cooling of the control rate freezer is initiated before each freezing run. Control rate temperature tracing, for each procedure, is attached to patient processing worksheets in the laboratory patient file or filed in designated area for

freezing graphs (*filed in chronological order by date*). Selection of the appropriate freezing program (Program 1.1 for small freezing bags/vials and Program 5.1 for larger freezing bags) should be confirmed prior to starting the control rate freezing run. Freezing graphs are reviewed by designated staff and stored indefinitely.

8.1.1.4 Bacterial cultures are set up on all products processed in the Stem Cell Laboratory at the end of the processing steps. Directed Donor UCB units, preemie UCB units, and bone marrow harvested at Duke are all processed before and after processing. All products collected outside DUMC are cultured both pre- and post-processing. Any product resulting in a positive culture is communicated to the appropriate clinical staff managing that patient; the pre-inoculated culture bottles are immediately sent to the Microbiology lab with an order for stat gram stain, culture, and sensitivity. Those results are available in EPIC as soon as they are reported by the Microbiology laboratory.

8.1.2 General ongoing quality control:

8.1.2.1 New antibody reagents are titered at the discretion of the Medical Director(s) or when a new antibody product is used to replace a product currently in use. In this case, product-to-product correlations are also performed.

8.1.2.2 Lot-to-lot comparisons are performed on each new lot of monoclonal antibodies for flow cytometry; information is documented on *Reagent/Instrument Quality Control Result Sheet*.

8.1.2.3 Testing of each new lot of serum if/when used in progenitor cell cultures if the reagents have not been tested by the manufacturer.

8.1.2.4 Resistivity of de-ionized water system is monitored weekly and result recorded on the designated QC worksheet.

8.1.2.5 Backup CO2 tanks are available on-site for all incubators. Additional CO2 tanks are available in the tank room on the loading dock of the North Pavilion (Room 0111).

8.1.2.6 Some temperature-sensitive equipment is also monitored on the Building's Automation System (BAS) which provides continuous monitoring (24 hrs/day, 7 days/week). The Rees Environmental Monitoring System was implemented in the spring of 2010 for all temperature sensitive equipment and locations (*as appropriate*). Both the BAS and Rees systems have a telephone notification system which is used to communicate to the appropriate laboratory staff during and after hours. The Rees system serves as the primary

continuous monitoring system used in the Stem Cell Laboratory for the following:

8.1.2.6.1 Refrigerators - temperature

8.1.2.6.2 Freezers (-20, -80, LN2) - temperature

8.1.2.6.3 CO2 incubators – temperature and CO2%

8.1.2.6.4 Designated space – temperature and humidity

8.1.2.6.5 LN2 bulk tank @ NP – low level of LN2

8.1.2.7 New lot verification for fluids used on XS-1000i instrument.

8.1.2.8 Ongoing FACS/Facsort flow cytometry analyzer quality assurance program.

8.1.2.9 Ongoing XS-1000i hematology analyzer quality assurance program.

8.1.2.10 Ongoing laboratory safety programs (See current on-line Safety Manual).

8.2 QUALITY CONTROL AND MAINTENANCE OF EQUIPMENT:

8.2.1 Daily program:

8.2.1.1 Check internal temperature of all incubators when the laboratory is open for operation (*i.e., lab is not routinely staffed on holidays or Sundays*).

8.2.1.2 Run controls on XS-1000i, FacSort, and ABO/Rh reagents, as indicated, and confirm that results are within acceptable limits before reporting patient / product results.

8.2.1.3 Check temperature of 37 +/- 1 degree Celsius water bath before use.

8.2.1.4 Cleaning of the laminar flow hoods (*before and after each use in the processing area of the laboratory*).

8.2.1.5 Check liquid nitrogen levels of BioArchive LN2 freezers (*except on weekends and holidays*). Since the REES Environmental Monitoring System was installed, checking LN2 levels manually on LN2 freezers, other than BioArchive LN2 freezers, is no longer deemed necessary unless it is needed for troubleshooting purposes.

8.2.1.6 Check the bulk LN2 tank pressures and levels. The bulk LN2 tank located at the loading dock of the North Pavilion is now monitored by the REES system to detect low levels of LN2. The bulk LN2 tank located at 2309 B Pratt Street is monitored 2-3 times per week to ensure that Airgas National Welders is making LN2 deliveries on Tuesday and Friday nights, as scheduled. Delivery dates/times are subject to change based on Airgas schedules.

8.2.1.7 Monitor temperatures of all refrigerators, -20 degree Celsius freezers, -70 degrees Celsius freezers using the Rees Environmental Monitoring system. A call tree is initiated when alarms are triggered for STCL-related equipment.

8.2.1.8 Monitor temperature and humidity checks in designated spaces (STCL, STCL storage room, 2309B Pratt Street).

8.2.1.9 The slide stainer is cleaned when used by laboratory staff.

8.2.1.10 Check PMT voltages, laser current, and fluidics; perform a fluidics flush on FACSort/FACS flow analyzer when running clinical samples (i.e. weekdays).

8.2.1.11 Wipe centrifuges as directed or if/when visible spills are noted.

8.2.1.12 Check that deionized water light is green.

8.2.1.13 Check barcode printers (as used).

8.2.1.14 Perform work surface decontamination.

8.2.1.15 Monitor STCL cleaning schedule as performed by Environmental Services personnel.

8.2.1.16 Perform sterile tubing welder checks and cleaning.

8.2.1.17 Perform heat sealer checks and cleaning.

8.2.1.18 Perform scale (balance) checks using calibrated weight sets.

8.2.1.19 Replace the water (with sterile water) and clean the 37°C water bath located in the processing area of the laboratory daily (*or whenever there are cellular products to thaw*).

8.2.1.20 Perform QC on Cellometer Auto 2000 instrument, if used.

8.2.2 Weekly program:

8.2.2.1 Weekly CO2 and temperature readings in incubators.

**NOTE**: Twice/Month: Replace sterile purified water, clean incubator walls/racks, and add PolyScience® drops *(20 drops/gallon*) to the water that is replaced in the chamber.

8.2.2.2 Dusting and cleaning of microscopes as specified.

8.2.2.3 Change blood bank refrigerator temperature chart.

8.2.2.4 Check deionized water resistivity reading.

8.2.2.5 Perform eye wash maintenance

8.2.2.6 Perform fire extinguisher checks.

8.2.2.7 Perform Activate bleach testing (*if the system is used*).

8.2.2.8 Perform centrifuge cleaning (*as needed*).

8.2.3 Monthly program:

8.2.3.1 Change the water (*using filtered, deionized water from Spigot # 2*) and clean the 37°C water bath used by the FLOW and HPCA areas of the laboratory.

8.2.3.2 Check hi- and low- alarms on the blood bank refrigerator.

8.2.3.3 Perform XS-1000i hematology analyzer monthly maintenance.

8.2.3.4 Perform FACSort/FACS flow analyzer monthly maintenance.

8.2.3.5 Perform Biological Safety Cabinet monthly maintenance and cleaning.

8.2.3.6 Perform $CO_2$ incubator maintenance.

8.2.3.7 Monitor cleaning performed by external cleaning agency.

8.2.4 Quarterly program:

8.2.4.1 Preventative maintenance of LN2 freezers, ultra-low temperature freezers, -70ºC freezers, and refrigerators. *The work is performed by contractors, Barlow Scientific, or other designated company if/when appropriate.*

8.2.4.2 Preventative maintenance of LN2 and ultra-low freezers' alarm systems to ensure that the BAS is contacted should an alarm be generated and/or REES Scientific alarm is generated. *The work is performed by contractors, Barlow Scientific*.

8.2.4.3 Recalibration of incubators (*$CO_2$ %*).

8.2.4.4 Microbial testing of deionized water is performed by Pureflow, Inc to ensure the water system is not contaminated.

8.2.4.5 Print, review, sign, and file REES-generated graphs for all temperature sensitive equipment. Graphs can be generated more often but minimally on a quarterly basis.

8.2.4.6 Maintenance and cleaning of validated transport containers (coolers)

8.2.5 Semi-annual program:

8.2.5.1 Preventative maintenance of de-ionized water system.

8.2.5.2 Preventative maintenance of laminar flow hoods.

8.2.5.3 Preventative maintenance of XS-1000i hematology analyzer.

8.2.5.4 Preventative maintenance of FacSort/FACS flow cytometry analyzers.

8.2.5.5 Recertification of laminar flow hoods. Viable air particles and touch plates are tested in the 5 hoods used in the processing area tested twice per year. Reports should be reviewed by the CQP and results monitored.

8.2.5.6 Preventative maintenance of centrifuges performed by Clinical Engineering Department.

8.2.5.7 Preventative maintenance of scales (balances) performed by Clinical Engineering Department.

8.2.5.8 Preventative maintenance of control-rate freezers performed by Barlow Scientific.

8.2.6 Annual program:

8.2.6.1 Preventative maintenance of microscopes.

8.2.6.2 Preventative maintenance of incubators.

8.2.6.3 Preventative maintenance (recertification) of certified weights.

8.2.6.4 Preventative maintenance (recertification) of all pipettes used for clinical purposes.

8.2.6.5 Preventative maintenance of all Sepax cell processing instruments.

8.2.6.6 Preventative maintenance of Biosafe Coolmix freezing instrument.

8.2.6.7 Preventative maintenance of Thermogenesis LN2 freezer.

8.2.6.8 Preventative maintenance of BacT/ALERT 3D system.

8.2.6.9 Preventative maintenance (recertification) of REES Scientific Temperature Monitoring system.

8.2.6.10 Preventative maintenance (recertification) of NIST-Standard thermometers.

8.2.6.11 Preventative maintenance of NIST-traceable thermometers checked against NIST-Standard thermometers.

8.2.6.12 Preventative maintenance of Cellometer Auto 2000 instrument

8.3 EXTERNAL SYSTEMS:

8.3.1 Sterility and pyrogen-free testing of reagents if not done by manufacturers (ie. BioTools, etc).

8.3.2 Routing of any/all positive cultures to the Microbiology laboratory for stat gram stain, culture, and sensitivity before and/or after processing in the STCL to identify contaminants in all clinical products.

8.3.3 All freezers, refrigerators, incubators, etc. are connected to a remote alarm system and REES Environmental Monitoring System which will

call identified personnel to respond to alarms if a problem arises with CO2 concentrations, LN2 levels, O2 sensor alarms (*if increased exposure to LN2 fumes*), temperature, humidity, or loss of power.

8.3.4 All laboratory equipment is maintained under service contracts, etc. by external vendors or Duke's Clinical Engineering Department. Quality control data is recorded and initialed by the designated employee(s) as appropriate: daily, weekly, monthly, quarterly, semi-annually, and yearly. Service reports that are provided to the laboratory are filed with other equipment records. Some service documentation is kept in the Clinical Engineering Department and is available upon request.

8.3.5 Daily and weekly quality control records are reviewed by designated laboratory staff working in that respective work area. If problems are observed with daily or weekly controls, the Laboratory Manager or designee should be consulted if problems can not be resolved in a timely manner. Any troubleshooting performed and resolution should be recorded on the troubleshooting log for future reference. All quality control records are maintained for a minimum of 10 years, unless otherwise dictated by applicable laws and/or guidelines

8.3.6 If a patient's cellular product is contaminated with bacteria or a situation arises which would compromise the quality of the product, the Laboratory Manager or designee must be notified immediately so actions can be taken appropriately while consulting with the respective program Medical Director. Options may include an increase in antibiotic coverage to the recipient, authorization to discard contaminated cells (*if applicable*), collection of additional cells from patient/donor, etc.

8.4 ORGANIZED QUALITY ASSURANCE PROGRAMS:

8.4.1 Participation in standardized QC surveys

8.4.1.1 The College of American Pathologists (CAP)

8.4.1.1.1 CAP surveys specifically applicable to the tests and procedures performed in the Stem Cell Laboratory. The CAP surveys include: hematology, differentials, flow cytometry, ABO/Rh, Stem Cell Processing, Blood Culture, and progenitor cell assays.

8.4.1.1.2 CAP specimens are incorporated into the routine, daily workload and tested by personnel who routinely test patient/client samples, using the same primary method systems as for patient/client samples. Inter-laboratory communication regarding proficiency testing samples is <u>PROHIBITED</u> until after the deadline for submission of data has passed. Referral of CAP specimens to

another laboratory for testing is also PROHIBITED.

8.4.1.1.3 CAP proficiency results are either submitted by fax or entered electronically via the CAP website. If results are entered electronically, in an effort to minimize submitting typographical errors, two technologists review, date, and initial the electronic results that were printed. Each technologist will also review the original results against the "source documentation" (*i.e. instrument printouts, etc*), to ensure that the original results were transcribed correctly. If clerical errors are discovered during this review process, corrections can be made before the CAP due date without penalty.

8.4.1.1.4 Graded proficiency challenges are evaluated against the peer group. If outliers are identified by the CAP, the laboratory conducts an investigation of the outlier, as outlined in the *Review of Proficiency Testing procedure (LTR19362)*. The Quality Management Office generates a "*Request for Investigation*" form and the laboratory has two weeks to complete and summarize the investigation.

8.4.1.1.5 Non-graded proficiency challenges that result due to a lack of consensus, etc. still require the laboratory to evaluate those ungraded results against the peer group and referees. This process will ensure that the ungraded results are represented by the majority. The Quality Management Office generates a "*Request for Investigation of Ungraded Proficiency Survey*" form and the laboratory has two weeks to complete and summarize the investigation.

8.4.1.1.6 The Stem Cell Laboratory follows the DUHS Clinical Laboratories, DUH Department of Pathology, and Departmental Laboratories Quality Management / Procedures for "*Proficiency Testing Program (LTR19362)*" and "*Review of Proficiency Testing (LTR19366)*.

- The Quality Management (QM) Office receives a consultant's copy of each

laboratory CAP Proficiency Testing results.

- Investigations are required by the Stem Cell Laboratory for any outlier result or ungraded challenge. Consultant copies of the PT Evaluations and investigation or ungraded response forms are provided to the Physician Quality Management Representative of Clinical Laboratories.
- The Physician Quality Representatives review the CAP results and investigation forms and assign each outlier a rating according to type and severity. Ungraded responses and investigations are also reviewed by these Physician Quality Representatives.
- The Quality Management (QM) Office compiles the data and tracks the results of these investigations to find trends and generate reports. The QM Office maintains files of all PT evaluations and outlier investigations by laboratory.
- IF two consecutive PT Challenges are found to have outliers, the STCL senior staff, in consultation with the medical director, will evaluate the data and devise a "plan of action" to resolve the problem.

8.4.1.2 Stem Cell Technologies distributes proficiency surveys each year that are specifically for progenitor cell assays but those surveys may include additional test parameters as that program evolves (ie. flow cytometry, etc).

8.4.2 Inter-Laboratory Quality Control (ILQC) Programs at Duke:

8.4.2.1 Hematology ILQC (2 levels) samples were once distributed for testing. Distribution of those samples was sometimes sporadic, based on sample availability; due to variations in the quality of those specimens, this exchange program was discontinued in November 2019 by Dr. Chad McCall.

8.4.2.2 In April 2021, the STCL started participating in the Hematology ILQC quarterly slide exchange program. Employees will perform manual differential and cell morphology on the slide provided; a result will be submitted for comparison against other participating laboratories.

8.4.3 External Comparative QC Programs

8.4.3.1 Hematology quality control specimen results are submitted electronically at designated time intervals to the Insight QC

Program. The STCL's quality control results are evaluated and compared to those results submitted by other laboratories in other facilities that use same control material.

8.4.4 Internal Quality Control Assessment for the BacT/ALERT 3D

8.4.4.1 Perform culture of a sterile solution (*if applicable*) or load empty culture bottles (*as negative controls*) and a culture of a known positive isolate. These serve as + and – controls for the BacT/ALERT 3D instrument since there are no ILQC exchange programs for the screening of + and – specimens. Controls are not required on this instrument as long as the instrument is being used according to the manufacturer's recommendations. Stem Cell Processing (SCP) CAP survey also reports culture results on the specimens distributed for these challenges. In 2012, Blood Culture Survey (BCS) was added to the list of CAP Surveys tested in the Stem Cell Laboratory.

## 9 RELATED DOCUMENTS/FORMS

9.1 STCL-FORM-030 CAP Survey Review Form

9.2 STCL-FORM-031 SCT Proficiency Survey Review Form

9.3 Request for Investigation of Out of Limit Proficiency Survey

9.4 Request for Investigation of Ungraded Proficiency Survey

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

## 10 REFERENCES

10.1 NCCLS. Clinical Laboratory Technical Procedure Manuals - Current Edition; Approved Guideline. (GP2-A4, 2002).

10.2 College of American Pathologists Inspection Checklists 2021 or current version

## 11 REVISION HISTORY

| Revision No. | Author | Description of Change(s) |
|---|---|---|
| 12 | M. Ritt | • Removed reference to Quality Systems Unit (QSU) throughout the document and replaced with Clinical Quality Program (CQP) |

## Signature Manifest

**Document Number:** STCL-GEN-018 **Revision:** 12
**Title:** Quality Control Systems for the STCL
**Effective Date:** 08 May 2025

All dates and times are in Eastern Time.

### STCL-GEN-018 Quality Control Systems for the STCL

### Author

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Melissa Ritt (MSR68) | GMP, Quality Assurance Associate I | 21 Apr 2025, 12:11:20 PM | Approved |

### Management

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Barbara Waters-Pick (WATER002) | | 22 Apr 2025, 01:20:41 PM | Approved |

### Medical Director

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Beth Shaz (BHS16) | | 22 Apr 2025, 01:21:51 PM | Approved |

### Quality

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Bing Shen (BS76) | Associate Director, Quality Assurance | 23 Apr 2025, 01:33:23 PM | Approved |

### Document Release

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Amy McKoy (ACM93) | Document Control Specialist | 24 Apr 2025, 09:59:06 AM | Approved |