STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-SOP-056 FRM1

DOCUMENT TITLE:

OOS - Colony Forming Unit Assay FRM1

DOCUMENT NOTES:

Document Information

Revision: 02	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** STCL

Date Information

Creation Date: 08 Mar 2023	**Release Date:** 16 Mar 2023
Effective Date: 16 Mar 2023	**Expiration Date:**

Control Information

Author: Barbara Waters-Pick (WATER002)	**Owner:** WATER002
Previous Number: STCL-SOP-056 FRM1 Rev 01	**Change Number:** STCL-CCR-541

ISBT 128 Barcode	**STCL-SOP-056 FRM1** **OOS – COLONY FORMING UNIT ASSAY** Initiated by: _____ Date: Initiated: _____	**OOS #** _____

Description of Investigation

The Duke Stem Cell Laboratory (STCL) performs a colony forming unit assay for the Carolinas Cord Blood Bank (CCBB), in accordance with *STCL-SOP-056 STEMvision™ Automated Colony Counting and Enumeration of Hematopoietic Progenitor Cells in Fresh Umbilical Cord Blood*, using a sample taken from the post-processed cord blood product prior to cryopreservation. The specification for this assay is colony "growth" or "no growth". If the initial assay reveals "no growth", *STCL-SOP-059 STEMVision Automated Colony Counting and Enumeration of Hematopoietic Progenitor Cells in Thawed Umbilical Cord Blood* is followed to document assay investigation and sample retesting.

The STCL also performs colony forming unit (CFU) assay testing on designated cellular products collected, processed, and infused for pediatric products (*both autologous and allogeneic*) and for adult products (*routinely allogeneic ONLY*). Samples that demonstrate "no growth" may be repeated, if indicated, and if/when sample is available to retest. *STCL-PROC-022 HPCA – Fresh and Thawed Clinical Products* is followed to document assay investigation and sample retesting.

Initial Test –
Product Type: □ **HPC, Cord** □ **HPC, Apheresis** □ **HPC, Marrow** □ **Other:** _____
Sample Phase □ **Fresh** □ **Thawed** □ **Other** _____
Processing: □ **Pre-Processing** □ **Post-Processing** □ **CD34 / CD56 Selection** □ **Other** _____

Method: ☐ STEMVision ☐ Manual/Microscope

Material	Supplier	Lot #	Expiration Date	N/A
Methocult H4434	Stem Cell Technologies			
□ SmartDish □ CoStar	□ Stem Cell Technologies □ Corning			
IMDM	□ Stem Cell Technologies □ VWR □ Other _____			
HetaSep	Stem Cell Technologies			
PBS	Stem Cell Technologies			

Date Plated	Plated by Study ID	Date Counted	Counted by Study ID	WBC count from Sysmex (x 10e6)
				Plating Concentration (***Check ONE***) □ 2 x 10e5 cells/ml *(1 x 10e4 cells/well)*[1] □ 4 x 10e5 cells/ml *(2 x 10e4 cells/well)*[2] □ 2.5 x 10e5 cells/ml *(1.25 x 10e4 cells/well)*[3]
Raw Counts	**BFU-E**	**CFU-GM**	**CFU-GEMM**	
Well #1				[1] Fresh Clinical STC Product (***Multiply # x 10***) **
Well #2				[2] Thawed Clinical STC Product (***Multiply # x 5***) **
Well #3 □ N/A				[3] Fresh CCBB CBU Product
Average Raw Counts				Total Average All CFU =
Per 1 x 10^5 ** (Based on Plating Concentration)				Total All CFU Per 1x10^5 =

STCL-SOP-056 FRM1
OOS – COLONY FORMING UNIT ASSAY

OOS # _____

Assay Investigation (If NO, explain in additional information section)	Yes	No	NA
Q1. Was the test procedure performed correctly?			
Q2. Were the correct sample and reagents used?			
Q3. Were products stored correctly?			
Q4. Were reagents released by QSU stored correctly, tested for quality (as applicable), and used prior to the expiration date?			
Q5. Was STEMVision instrument performance acceptable?			
Q6. Were calculations for sample and reagent preparations correct?			
Q7. Were calculations associated with use of hetasep accurate?			
Q8. Is the training status of the technician up-to-date?			
Q9. Is the assay considered valid?			
Q10. Were any events associated with the performance of this assay?			
Q10A. If Yes, Event number: _____			
Investigation Performed By:	**Date:**		

Additional Information:

STCL-SOP-056 FRM1
OOS – COLONY FORMING UNIT ASSAY

OOS # _____

Sample Retesting				
Retest □ **Not Tested** (*NO Sample Available*) □ **Thawed DMSO vial** □ **Thawed Segment** □ **Other** _____ **Method:** ☐ STEMVision ☐ Manual/Microscope				
Material	**Supplier**	**Lot #**	**Expiration Date**	**N/A**
Methocult H4434	Stem Cell Technologies			
□ SmartDish □ CoStar	□ Stem Cell Technologies □ Corning			
IMDM	□ Stem Cell Technologies □ VWR □ Other _____			
ADLEFLUOR® Buffer	Stem Cell Technologies			
ErythroClear RBC Depletion Reagent Kit	Stem Cell Technologies			

Date Plated	Plated by Study ID	Date Counted	Counted by Study ID	WBC count from Sysmex (x 10e6)
				Plating Concentration (***Check ONE***) □ 4 x 10e5 cells/ml *(2.0 x 10e4 cells/well)*[2] □ 10 x 10e5 cells/ml *(5.0 x 10e4 cells/well)*[3]
Raw Counts	**BFU-E**	**CFU-GM**	**CFU-GEMM**	
Well #1				
Well #2				[2] Thawed Clinical STC Product (***Multiply # x 5***) **
Well #3 □ N/A				[3] Thawed CCBB CBU Product
Average Raw Counts				Total Average All CFU =
** Per $1x10^5$ (Based on Plating Concentration)				Total All CFU Per $1x10^5$ =

Is Result within Specification?

☐ Yes	The retest sample demonstrates GROWTH
☐ No ***SELECT ONE in next column***	□ CCBB specimens: if NO GROWTH report to CCBB supervisor and exclude unit per CCBB-LAB-005. □ Clinical STCL specimens: Repeat assay if additional sample is available. Repeated Again? □ Yes □ No

STCL-SOP-056 FRM1
OOS – COLONY FORMING UNIT ASSAY

OOS # _____

Related Events	Yes	No
Q11. Were there any events associated with collection or manufacturing of this product that may impact SQIPP of the product? Q11A. **If Yes enter Event Number**: _____		
Q12. Were there any events associated with the equipment used for the assay (*bracketed in a way that would include this product*)? Q12A. **If Yes enter Event Number**: _____		
Q13. Were there any events associated with the supplies used for the assay (*bracketed in a way that would include this product)*? Q13A. **If Yes enter Event Number**: _____		
Q14. Were there any events associated with the control lot used for the assay (*bracketed in a way that would include this product)*? Q14A. **If Yes enter Event Number**: _____		

Evaluation

Is Root cause of OOS identifiable? □ Yes □ No

If yes, please check all contributing factors that apply:

□ Equipment Issue/STEMVision malfunction
□ Invalid Initial Test Result
□ Processing (*i.e. technical issue when plating sample/issue during processing product*)
□ Supply issue (*i.e. expired, contaminated*)
□ Test Sample unacceptable (*i.e. volume, leaking, cell concentration*)
□ Training/SOP not followed
□ Unable to determine
□ Other, explain below.

Risk to other product quality identified? □ Yes □ No
If Yes enter Event Number: _____

STCL-SOP-056 FRM1
OOS – COLONY FORMING UNIT ASSAY

OOS # _____

CAPA	Yes	No
Q15. Were any Corrective or Preventive actions performed as a result of this OOS? Q15A. **If Yes enter CAPA REPORT Number**: _____		

Attachment(s) *(include SV CFU Assay Reports, etc)*

Signature Manifest

Document Number: STCL-SOP-056 FRM1 **Revision:** 02
Title: OOS - Colony Forming Unit Assay FRM1
Effective Date: 16 Mar 2023

All dates and times are in Eastern Time.

STCL-SOP-056 FRM1 OOS - Colony Forming Unit Assay FRM1

Author

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		09 Mar 2023, 05:01:24 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		09 Mar 2023, 05:01:55 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		09 Mar 2023, 05:16:31 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Isabel Storch De Gracia (IMS19)		16 Mar 2023, 07:48:41 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		16 Mar 2023, 02:18:01 PM	Approved