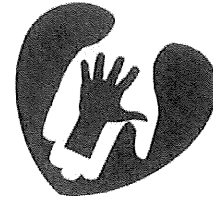


STEM CELL LABORATORY (STCL)



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DOCUMENT TITLE:

Medium Lot to Lot Testing JA2

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STCL-SOP-052 JA2 MEDIUM LOT-TO-LOT TESTING

1 PURPOSE

- 1.1 Methycellulose based medium (MethoCult) and Iscove's Modified Dulbecco's media (IMDM) are the standard gelling agent and preparation media used in Colony Forming Cell assays (CFC) in the Stem Cell Laboratory (STCL). When a new lot number arrives, the lab must verify the quality and evaluate the performance of these reagents before they are put into use.

2 INTRODUCTION

- 2.1 Each batch of MethoCult and IMDM is sterility tested by Stem Cell Technologies. The quality control performance of MethoCult is also tested in CFC assays using human Bone Marrow (BM), Cord Blood (CB), HPC-Apheresis (HPC-A) also known as peripheral stem cells (PSC), or Peripheral Blood (PB) samples by the manufacturer. The STCL performs internal quality control on new lots of media to ensure reproducibility of the colonies enumerated. New lot numbers of media are compared to the current lot number (that is in use) by plating the same specimen using each lot. The plates are read after the 14-16 day incubation period and the colony counts compared against one another.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Medical Director, Laboratory Manager, QA Manager, and applicable STCL staff are responsible for ensuring the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

- | | | |
|------|-------|-------------------------------------|
| 4.1 | CFC | Colony Forming Cell |
| 4.2 | STCL | Stem Cell Laboratory |
| 4.3 | BM | Bone Marrow |
| 4.4 | CB | Cord Blood |
| 4.5 | HPC-A | Hematopoietic Progenitor Cell Assay |
| 4.6 | PSC | Peripheral Stem Cells |
| 4.7 | PB | Peripheral Blood |
| 4.8 | HPCA | Hematopoietic Progenitor Cell Assay |
| 4.9 | QA | Quality Assurance |
| 4.10 | HPC | Hematopoietic Progenitor Cell |
| 4.11 | IMDM | Iscove's Modified Dulbecco's Media |
| 4.12 | SOP | Standard Operating Procedure |
| 4.13 | CO2 | carbon dioxide |

5 MATERIALS

5.1 Reagents:

- | | | |
|-------|-----------------------|-----------------------------------|
| 5.1.1 | MethoCult 4434 Medium | Stem Cell Technologies/ Cat# 4434 |
| 5.1.2 | IMDM with 2% FBS | Stem Cell Technologies/ Cat# 7700 |
| 5.1.3 | Sterile water 1L | Sigma/ Cat# W3500-1L |

5.2 Supplies:

- | | | |
|--------|--|--|
| 5.2.1 | 3 ml sterile syringes with luer lock tip | BD Biosciences/ Cat# 309657 |
| 5.2.2 | Blunt-end needle, 16G | Stem Cell Technologies/ Cat# 28110 |
| 5.2.3 | 24-well Costar cell culture plates | Corning Inc./ Cat# 3524 |
| 5.2.4 | Sample ID barcodes | Computype/ Cat# 1018184 |
| 5.2.5 | Permanent marker | Sharpie/ Cat# 30001 |
| 5.2.6 | Sterile 12 x 75 polystyrene tubes | Port City Diagnostics/ Cat# T2063STR |
| 5.2.7 | Sterile 15 ml conical tubes | Port City Diagnostics/ Cat# 1100SRGFT |
| 5.2.8 | Sterile 200µl pipette tips | Port City Diagnostics/Cat# 7509-96RS |
| 5.2.9 | Sterile serological pipettes | Port City Diagnostics/ 1ml Cat# SER-010-S01
2ml Cat#SER-0020-S01
5ml Cat#SER-0050-S01
10ml Cat#SER-0100-S01 |
| 5.2.10 | Do Not Use label | |
| 5.2.11 | This Lot is Ready for Use label | |

6 EQUIPMENT

- | | | |
|-----|------------------------------------|---|
| 6.1 | Barcode scanner | Zebra/ Model ZM400 |
| 6.2 | Inverted Microscope | Olympus IMT-2 |
| 6.3 | Thermo Scientific CO2 Incubators | HERAcell 150 SN# 225658 & SN# 225659
Isotemp Plus SN# Z01J464990ZJ |
| 6.4 | Vortex Mixer | VWR/ Mini Vortexer MV1 |
| 6.5 | DIFFCOUNT electronic cell counter | Modulus Data Systems/ SN# 319806 |
| 6.6 | Stamping Clock | Lathem 1000E/ SN# 1E014032 |
| 6.7 | 3 channel traceable timer | Fisher Scientific/ SN# 111878606 /
SN# 111878753 |
| 6.8 | Class II Biological Safety Cabinet | NUAIRE 425-600/ SN# 123044050508
Baker SG400/ SN# SL29877V |
| 6.9 | Micropipettes | Rainin/ 20µl : SN# M11689G
20µl: SN# I0985053K
200µl: SN# I0984537K
200µl: SN# M10263E |

1000µl: SN# R73215A
1000µl: SN#H0962198K

7 SAFETY

- 7.1 All procedures for cell processing and set-up of cell culture assays should be performed using sterile technique under a biohazard safety cabinet certified for Level II handling of biological materials, and universal handling precautions. When handling a biological hazardous substance, such as umbilical cord blood, appropriate personal protective equipment (PPE), must be worn as the primary barrier of protection. PPE may include, but is not limited to face protection, lab coats and gloves. Appropriate PPE should be donned before handling potentially hazardous biological materials and removed immediately and replaced if gross contamination of the equipment occurs.

8 PROCEDURE

- 8.1 Record the NEW lot number information on the *HPC Reagent Verification Log*. If it is a new lot number, place a **“DO NOT USE”** red sticker on each bottle and store the media at -20°C, as per manufacturer’s recommendations.
- 8.2 When ready to perform the lot-to-lot verification, thaw a bottle of media from the new lot number according to manufacturer’s directions (See MethoCult Technical Manual).
- 8.3 Aliquot thawed media into 15 ml sterile conical tubes and label each tube with name of reagent, lot number, aliquot date, technologist’s initials, and expiration date.
- 8.4 Prepare a 24 well culture plate.
- 8.5 Prepare two syringes and two tubes of the MethoCult and/or IMDM, one with the current lot and one with the new lot number.
- 8.6 Select a sample (CB, PSC, BM, or PB) and plate according to the SOP; using the same plate, plate one sample using the current lot number(s) of media and the other sample using the NEW lot number(s) of media. Label each sample on the plate accordingly.
- 8.7 Place the plate in a 37°C humidified 5% CO₂ incubator for 14-16 days.
- 8.8 Enumerate the colonies for each sample and record the results on the Progenitor Assay Form. Place a copy of that form in the *HPC Reagent Verification Log*.
- 8.9 Since there are factors that could contribute to the variability of the results obtained from this assay (i.e., equipment and/or culture conditions, integrity of the sample being used, etc.), the total colony count, between lots, should not exceed 20%.
- 8.10 If the criterion is **NOT** met, repeat the lot-to-lot comparison using a new sample; document this failure and subsequent corrective action on the *HPC Reagent Verification Log*.
- 8.11 If the criterion **IS** met, results of this comparison will be recorded on the *Media Lot to Lot QA Release Log* and reviewed by the QA or lab manager to approve/release the lot for use. Record the **“IN USE”** date on the *Reagent Verification Log*. Replace the **“DO NOT USE”** red labels with **“THIS LOT READY FOR USE”** green labels.

9 RELATED DOCUMENTS/FORMS

- 9.1 STCL-SOP-052 HPCA – Cord Blood Bank Products

- 9.2 STCL-SOP-052 (JA1) – HPC Processing of Fresh UCB Samples – Flow Chart
- 9.3 STCL-SOP-052 (FRM1) Progenitor Cell Assay Form (Cord Blood Products)
- 9.4 STCL-PROC-022 (FRM 1) Hematopoietic Progenitor Cell Assay Sheet
- 9.5 STCL-PROC-022 (FRM 2) Media Lot-to-Lot QA Release Log
- 9.6 STCL-PROC-022 (FRM 3) Reagent Verification Log
- 9.7 STCL-PROC-022 JA1 Tech Manual HPCA - MethoCult

10 REFERENCES

- 10.1 Human Colony-Forming Assays Using MethoCult, Stem Cell Technologies, Version 4.0.0, April 2012
- 10.2 Atlas of Human Hematopoietic Colonies, Stem Cell Technologies, Version 1.1.1, October 2013.
- 10.3 Atlas of Hematopoietic Colonies from Cord Blood, Stem Cell Technologies, Version 2.0.0, March 2010

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	Barbara Waters-Pick	<ul style="list-style-type: none"> • Added “Do NOT Use” labels to Supply Section 5.2.10 • Added “This Lot is Ready for Use” labels to Supply Section 5.2.11 • Added Section 11 Revision History to track modifications to this job aide

Signature Manifest**Document Number:** STCL-SOP-052 JA2**Revision:** 03**Title:** Medium Lot to Lot Testing JA2

All dates and times are in Eastern Time.

STCL-SOP-052 JA2 Medium Lot to Lot Testing JA2**Author**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		28 Feb 2017, 02:52:32 PM	Approved

Manager

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		28 Feb 2017, 02:52:44 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		01 Mar 2017, 09:27:50 AM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
John Carpenter (JPC27)		02 Mar 2017, 02:06:08 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		09 Mar 2017, 08:26:10 PM	Approved