



STEM CELL LABORATORY (STCL)



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Specimen Dilution Protocol (JA1)

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Author: MGREESE

Owner: MGREESE

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FLOW-GEN-023 SPECIMEN DILUTION PROTOCOL

1 PURPOSE

- 1.1 The purpose of this procedure is to provide the user with definitive guidelines for diluting samples with white blood cell concentration exceeding maximum staining concentration.

2 INTRODUCTION

- 2.1 **Labeling:** Always label the tube in which the dilution is prepared with the patient's name or barcode number and the dilution factor.
- 2.2 **Reporting:** When analyzing data, enter the dilution factor in the specified box of the analysis page, to ensure proper calculation of the final result.
- 2.3 **Minimize dilution error:**
 - Prepare all dilutions from the original specimen.
 - Use the smallest dilution factor that will achieve the proper staining concentration and that will provide enough specimen volume to complete the test.
 - Conserve specimen by not using more specimen than is required to achieve a final sample volume that will afford an accurate analysis of the specimen. In the event that the dilution does not yield a valid result the first time, specimen may be available for a second attempt.

3 SCOPE AND RESPONSIBILITIES

- 3.1 Trained medical laboratory technologists are responsible for carrying out this process. The Medical Directors, supervisory flow staff, and the Laboratory Manager are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

- 4.1 PBS-Phosphate buffered saline
- 4.2 BSA-Bovine serum albumin

5 MATERIALS

- 5.1 12mm x 75mm test tubes (Falcon)
- 5.2 PBS/1% BSA (Gibco)

6 EQUIPMENT

- 6.1 1000,200,100 microliter adjustable pipettor (Rainin)
- 6.2 RT-L1000F and L200F tips (Rainin)
- 6.3 Calculator

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Stem Cell Laboratory, DUMC
Durham, NC

7 SAFETY

- 7.1 Wear all appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, goggles, lab coats, sleeve covers, disposable gowns, disposable aprons, etc.

8 PROCEDURE

NOTE: Use a pipettor that will dispense the most accurate volume. Do not prime pipette tips and use reverse pipetting for both diluent and sample addition. Keep pipettor upright when aspirating and dispensing volume and touch the tip to the inside of the dilution tube while dispensing diluent or sample

- 8.1 Aspirate diluent first (PBS – Phosphate Buffered Saline), and expel into the dilution tube. Aspirate slowly to prevent air bubbles. If bubbles are noted, expel all liquid and aspirate again. Use a new tip if unable to get rid of the bubbles.
- 8.2 Change pipette tips (always use a different tip with each different fluid being aspirated).
- 8.3 Vortex the specimen 2-3 seconds or if the specimen is in a vacutainer tube, invert a minimum of 5 times prior to aspirating the specimen amount needed for the dilution.
- 8.4 The dilution tube should be mixed thoroughly (~3 seconds on vortex mixer) prior to staining the sample.
- 8.5 Record the dilution factor on the flow worksheet or graft characterization sheet for umbilical cord blood testing.

Use the following guidelines when preparing dilutions:

DILUTION TYPE		SPECIMEN VOLUME	DILUENT VOLUME
2-FOLD	1:2	150 µl	150 µl
3-FOLD	1:3	100 µl	200 µl
4-FOLD	1:4	100 µl	300 µl
5-FOLD	1:5	100 µl	400 µl
10-FOLD	1:10	50 µl	450 µl
15 FOLD	1:15	50 µl	700 µl
20 FOLD	1:20	50 µl	950 µl

9 RELATED DOCUMENTS/FORMS

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

9.2 FLOW-FROM-010 (FRM1) Graft Characterization

10 REFERENCES

10.1 NA

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	M. Reese	<ul style="list-style-type: none"> Removed JA from title. Removed cell concentration limit requirement in section 2.3 to accommodate varying test requirement limits. Modified Scope and Responsibilities to include supervisory flow staff. Modified Safety section to include more detail.

Signature Manifest**Document Number:** FLOW-GEN-023**Revision:** 06**Title:** Specimen Dilution Protocol (JA1)

All dates and times are in Eastern Time.

FLOW-GEN-023 Specimen Dilution Protocol**Author**

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