



STEM CELL LABORATORY (STCL)



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JA3 - Calibration of Fixed Speed Centrifuges

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CALIBRATION OF FIXED SPEED CENTRIFUGES

1. PURPOSE

- 1.1. Each centrifuge will be calibrated upon receipt and after adjustments or repairs. Calibration evaluates the behavior of red cells in solutions of different viscosities, not the reactivity of different antibodies. Optimal centrifugation time is determined by varying the time for each suspending medium used and the antiglobulin technique to detect the strongest agglutination of a weak antibody when reacted with positive cells. No reaction/agglutination or ambiguity in the negative cells.

2. INTRODUCTION

- 2.1. Functional calibration results will be posted on the centrifuge and stored in the QC Notebook. Mechanical calibration includes quarterly speed and timer checks for accuracy and is under service contract.

3. SCOPE

- 3.1. Medical Technologists processing cellular products will perform this testing. The Medical Directors and Laboratory Manager are responsible for ensuring that the requirements of this procedure are successfully met.

4. DEFINITIONS/ACROYMNS

- 4.1. mls milliliters

5. MATERIALS

- 5.1. Saline phase
 - 5.1.1. Commercial anti-A diluted 1:100 in saline
 - 5.1.2. Positive control – A cells
 - 5.1.3. Negative control – B or O cells

** ALL CELL SUSPENSIONS ARE 2-5% IN SALINE or CELL PACK**

(NOTE: To prepare 2-5 % cell suspension, add ~ 5 drops of cells to 1 ml of saline or cell pack)

6. EQUIPMENT

- 6.1. Clay Adams Serofuge or comparable centrifuge

7. SAFETY

- 7.1. Wear all appropriate personal protective equipment when handling potentially hazardous blood and body fluid to include, but not limited to, gloves, lab coat, goggles, etc.

8. PROCEDURE

Record all results on the Centrifuge Functional Calibration Worksheet. (Attachment I)

8.1. SALINE

- 8.1.1. Label six tubes 1-6 pos and six more 1-6 neg.
- 8.1.2. Add one drop diluted anti-A to all tubes

- 8.1.3. Add one drop A cells to all positive tubes
- 8.1.4. Add one drop B or O cells to all negative tubes
- 8.1.5. In pairs, one pos and one neg, centrifuge the tubes for the different times as indicated on the worksheet. Observe each tube for agglutination and record observation appropriately.

8.2. 37°C ENHANCEMENT (if ordered)

- 8.2.1. Label tubes as indicated for the saline phase
- 8.2.2. Add 2 drops of diluted anti-D to each tube
- 8.2.3. Add one drop D positive cells to positive tubes
- 8.2.4. Add one drop D negative cells to all negative tubes
- 8.2.5. Add two drops of enhancement media to all tubes and mix.
- 8.2.6. Incubate cell suspensions at 37 degrees Celsius for 15 minutes.
- 8.2.7. In pairs (one positive tube and one negative tube) centrifuge for the different times as indicated on the worksheet. Observe each tube for agglutination and record observations appropriately.

8.3. ANTIGLOBULIN (if ordered)

- 8.3.1. Label tubes 1-4 pos and 1-4 neg
- 8.3.2. Add one drop diluted (1:100) anti-D to all tubes
- 8.3.3. Add one drop D positive cells to positive tubes
- 8.3.4. Add one drop D negative, Du negative cells to negative tubes
- 8.3.5. Mix all tubes
- 8.3.6. Incubate at 37C for 15-20 minutes. NO ENHANCEMENT!
- 8.3.7. Wash 4 times and completely decant.
- 8.3.8. In pairs, one pos and one neg, add two drops of antiglobulin serum; mix and centrifuge for indicated times and record observations appropriately.

8.4. MANUAL WASH

- 8.4.1. Label tubes 1-3
- 8.4.2. Add one drop of a 5% suspension of O cells to each tube
- 8.4.3. Fill with saline and centrifuge each tube as indicated
- 8.4.4. Record all observations

8.5. INTERPRETATION

- 8.5.1. The optimum time of centrifugation is the LEAST time required to fulfill the following criteria:
 - 8.5.1.1. The supernatant fluid is clear.
 - 8.5.1.2. The red cell button is clearly delineated and the periphery is sharply defined, not fuzzy.
 - 8.5.1.3. The red cell button is easily resuspended.

- 8.5.2. Agglutination in the positive tubes is as strong as determined in preparing reagents.
- 8.5.3. There is no agglutination or ambiguity in the negative tubes.
- 8.5.4. The optimum time of centrifugation for a manual wash is the LEAST time required to fulfill these criteria:
 - 8.5.4.1. The supernatant fluid is clear.
 - 8.5.4.2. The red cells should form a clearly delineated button, with no cells trailing up the side of the tube.
 - 8.5.4.3. After the saline has been decanted, the cell button should be easily resuspended in the residual fluid.
 - 8.5.4.4. The volume of cells should remain the same after decanting.
- 8.5.5. Determine optimum times for each phase and complete worksheet. Post results on centrifuge using the label provided (see example). Retain the CENTRIFUGE FUNCTIONAL CALIBRATION WORKSHEET in the QC Notebook.

9. RELATED DOCUMENTS/FORMS

- 9.1. See Label attached
- 9.2. See Centrifuge Functional Calibration Worksheet attached.

10. REFERENCES

- 10.1. Principle and Practice of Quality Control in the Blood Bank, ed. Taswell and Saeed, American Association of Blood Banks, Washington, DC 1980.
- 10.2. Technical Manual, Vengelen-Tyler, V. ed. American Association of Blood Banks, 13th edition, Bethesda, MD, 1999, 742-744.

EXAMPLE OF LABEL

<p style="text-align: center;">Stem Cell Laboratory , Duke University Medical Center</p> <p>Saline Spin Time _____ seconds</p> <p>Date Calibrated: _____</p> <p>Next Calibration Due: _____</p> <p>Initials of Tech Performing Calibration: _____</p> <p>Centrifuge Serial # _____</p>
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CENTRIFUGE FUNCTIONAL CALIBRATION WORKSHEET

STEM CELL LABORATORY

Centrifuge Number: _____ Date: _____ Tech: _____

Interpretative Criteria	CELLS	10"	15"	20"	30"	45"	60"	SALINE *	37 Enhancement *	Antiglobulin *
1. Supernatant clear?	POS									
	NEG									
2. Cell button delineated?	POS									
	NEG									
3. Resuspended easily?	POS									
	NEG									
4. Strength of reaction.	POS									
	NEG									
*Enter information IF APPLICABLE. If NOT Applicable, enter N/A										

* Enter information if applicable; if NOT applicable, enter N/A.

REVIEWED BY: _____

DATE REVIEWED: _____

Signature Manifest**Document Number:** STCL-SOP-049 JA3**Revision:** 01**Title:** JA3 - Calibration of Fixed Speed Centrifuges

All dates and times are in Eastern Time.

STCL-SOP-049 JA3 Calib Fixed Sp Cen**Author Approval**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		31 May 2011, 06:11:45 PM	Approved

Manager Approval

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		31 May 2011, 06:12:08 PM	Approved

Medical Director Approval

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		31 May 2011, 09:41:59 PM	Approved

QA Approval

Name/Signature	Title	Date	Meaning/Reason
Linda Sledge (SLEDG006)	Quality Manager	02 Jun 2011, 09:03:01 AM	Approved

Notification

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		02 Jun 2011, 09:03:01 AM	Email Sent
Linda Sledge (SLEDG006)	Quality Manager	02 Jun 2011, 09:03:01 AM	Email Sent
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Barbara Waters-Pick (WATE02)		02 Jun 2011, 09:03:01 AM	Email Sent

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Name/Signature	Title	Date	Meaning/Reason
Janet Celko (CELKOJ)		09 May 2012, 12:34:43 PM	Reviewed
Barbara Waters-Pick (WATE02)		21 May 2012, 03:38:09 PM	Reviewed
Joanne Kurtzberg (KURTZ001)		22 May 2012, 07:08:55 PM	Reviewed
Linda Sledge (SLEDG006)		23 May 2012, 08:05:15 AM	Reviewed

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Barbara Waters-Pick (WATE02)		07 May 2013, 12:47:14 PM	Reviewed
Sharon Hartis (SH259)		07 May 2013, 02:03:05 PM	Reviewed
Betsy Jordan (BJ42)		07 May 2013, 02:25:02 PM	Reviewed
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Barbara Waters-Pick (WATE02)		12 Mar 2015, 09:41:22 AM	Reviewed
Joanne Kurtzberg (KURTZ001)		19 Mar 2015, 09:45:12 AM	Reviewed
John Carpenter (JPC27)		19 Mar 2015, 11:28:35 AM	Reviewed
Sharon Hartis (SH259)		19 Mar 2015, 12:14:56 PM	Reviewed
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