

Beaumont

Origination 6/21/2022
 Last Approved 5/25/2023
 Effective 6/24/2023
 Last Revised 5/25/2023
 Next Review 5/24/2025

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 Applicability All Beaumont Hospitals

Calibration of Serologic Centrifuges - Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide steps to be used in the calibration of the Blood Bank serological centrifuges.

II. INTRODUCTION:

- A. The centrifuge calibration is performed to determine the optimal time for centrifugation of hemagglutination procedures. Due to differences in the viscosity of suspending media, the optimal spin time may vary with different test processes. Therefore, each centrifuge should be calibrated for each test process in which centrifugation is used to enhance agglutination.
- B. Following is a summary of the centrifuge calibration. Reagents of varying viscosity are combined with red blood cells (RBCs) in test tubes. The tubes are centrifuged and/or washed as described in this document. Several observations are then made relating to the cell button, supernatant, etc. Based on these observations, the optimal spin time for each process is determined.

III. SCOPE:

This document applies to each Blood Bank centrifuge that is used to test samples by hemagglutination procedures.

IV. DEFINITIONS/ACRONYMS:

- A. **Yearly:** Within a 12 month \pm 1 month time span.
- B. **Corewell Health Biomedical:** Performs repairs and does some maintenance of equipment for Corewell Health.

- C. **Major adjustments or repairs:** Adjustments or repairs that have the potential to affect the function of the centrifuge. For example, those with the potential to affect the rotor or control panel. Does not include, for example, minor adjustments or repairs such as those to the lid spring, locking device, etc.

V. POLICIES:

- A. Centrifuges are calibrated upon receipt, after major adjustments or repairs, and yearly.
- B. Each centrifuge is calibrated according to the processes and using the tube sizes that are used during normal operations. These processes are immediate-spin testing and washing with antiglobulin testing. The centrifuges at the Blood Bank workbenches including student workstations are used for the following processes (and are therefore calibrated for these processes):
1. Dearborn/Taylor/Trenton/Wayne: 12 x 75 mm glass test tubes are used for all samples that are tested by hemagglutination procedures.
 2. Farmington Hills/Grosse Pointe/Royal Oak/Troy: 10 x 75 mm glass test tubes are used for all samples that are tested by hemagglutination procedures.
 3. Tubes used to specifically prepare cell suspensions (i.e. 13 x 100 mm) or those specifically used in gel testing are not calibrated.

C. **Calibrating a New Centrifuge**

Before a new centrifuge is placed into service, an initial calibration must be performed to determine the optimal spin time. The spin times at which to perform the initial calibration of the new centrifuge should be based on manufacturer's recommendations, previously determined optimal spin times for similar centrifuges, etc. Several spin times should be tested to determine the optimal spin time; these spin times should cover a wider range than the three spin times that are used for a calibration check.

D. **Frequency of Calibration Checks**

1. Once the optimal spin time has been determined for a new centrifuge, the centrifuge calibration is checked after major adjustments or repairs and yearly. The calibration check is performed for the following 3 spin times:

Spin Checks During Calibration* (Immediate Spin or AHG Process)	Spin Checks During Calibration* (Washing Process)
x - 5 seconds	w - 15 seconds
x seconds	w seconds
x + 5 seconds	w + 15 seconds
* x = the optimal spin time for the IS or AG process from the most recent calibration as documented on the <i>Centrifuge Calibration Sticker</i> w = the optimal spin time for the washing process from the most recent calibration as documented on the <i>Centrifuge Calibration Sticker</i>	

E. **Determining the Optimal Spin Time (for IS and AHG testing)**

The optimal spin time for immediate spin (IS) or antiglobulin (AHG) testing is the shortest of the 3 spin times required to fulfill the following criteria:

1. The supernatant fluid is clear
 2. The cell button is clearly delineated, and the periphery is sharply defined
 3. The cell button is easily resuspended
 4. Agglutination is observed in the positive control
 5. No agglutination is observed in the negative control
- Note: The optimal spin time may vary slightly from one calibration check to the next, due to the subjective nature of grading reactions, shaking off cell buttons, visualizing cell buttons.
- The calibration check of the IS or AHG process is satisfactory if the optimal spin time from the current calibration is within 5 seconds of the optimal spin time from the most recent calibration.

F. Determining the Optimal Spin Time (for the washing process)

The optimal spin time for the washing process is the shortest time of the 3 spin times required to fulfill the following criteria:

1. The cell button is clearly delineated with minimal cells trailing up the side.
2. After decanting the saline, the cell button is easily resuspended in the residual fluid.

G. Appropriate Actions if the Calibration Check is Unsatisfactory

If the calibration check is unsatisfactory, the following apply:

1. Do not use the centrifuge.
2. Tag the centrifuge with *Equipment out of Service Form*.
3. Contact Corewell Health East Biomedical department to arrange for equipment repair.
4. Notify the Lead Medical Technologist or Department Supervisor/Manager.
5. Document the equipment failure on an internal variance.

VI. REAGENTS:

A. For the calibration check of the immediate-spin process

1. Anti-A reagent antisera
2. Ortho 7% BSA
3. Group A cells, 2-5% cell suspension (positive control)
4. Group B cells, 2-5% cell suspension (negative control)
5. For the calibration check of the washing with antiglobulin testing process

B. Ortho BioClone® Anti-D

1. Rh(D) positive screening cells obtained from 3% Ortho Surgiscreen cells
2. Rh(D) negative screening cells obtained from 3% Ortho Surgiscreen cells
3. Anti-IgG reagent
4. IgG coated Coombs control cells

5. 22% Albumin

VII. EQUIPMENT:

- A. Vortex mixer
- B. Pipette capable of dispensing 10 µL

VIII. SUPPLIES:

- A. 10 x 75 mm or 12 x 75 mm test tubes
- B. Disposable pipettes
- C. Pipette Tips
- D. Normal saline
- E. Parafilm
- F. Plastic specimen rack

IX. QUALITY CONTROL (QC):

- A. QC testing must be performed on the all reagents as described in site specific Transfusion Medicine policy, *Quality Control of Blood Bank Reagents*.

X. PROCEDURE:

A. Prepare Diluted A

Diluted anti-A is required for the calibration check of the immediate-spin process. Prepare diluted anti-A (so that it reacts 1+ with A₁ test cells) as follows:

1. Label a test tube to identify the contents of the diluted anti-A.
2. Prepare the diluted anti-A by combining 10 µL of reagent anti-A antisera and 20 drops of 7% BSA in the labeled tube. Mix the contents of the tube thoroughly with the vortex. Note: Alternatively for larger quantity of testing antisera mix 9.9 mL of 7% BSA with 0.1 mL anti-D, dilution 1:100.
3. Test the diluted anti-A to verify that it reacts 1+ with A₁ test cells as follows:
 - a. If only one centrifuge is being calibrated, use a different centrifuge to confirm appropriateness of anti-A dilution.
 - b. Add 1 drop of diluted anti-A and 1 drop of A₁ test cells to a new, labeled test tube. Centrifuge the tube for x seconds (x = the optimal spin time for the IS process from the most recent calibration as documented on the *Centrifuge Calibration Sticker*). Read and grade the reaction strength.
 - i. If the reaction strength is greater than 1+, adjust the dilution by adding additional drops of 7% BSA. Retest the diluted anti-A and continue to adjust until it reacts 1+ with A₁ test cells.

- ii. If the reaction strength is less than 1+, prepare a new dilution by combining 10 µL of reagent anti-A antisera and only 15 drops of 7% BSA. Retest the new dilution and continue to adjust until it reacts 1+ with A₁ test cells.

B. Prepare Diluted D

Diluted (high protein) anti-D is required for the calibration check of the washing and antiglobulin process. Prepare diluted anti-D (so that it reacts 1+ with positive and negative screening cells) as follows:

1. Select appropriate positive and negative control cells from current lot of 3% Ortho Surgiscreen screening cells and record reagent lot# and expiration date on the *Centrifuge Calibration Worksheet*.
 - a. Positive control – positive for Rh(D) antigen
 - b. Negative control – negative for Rh(D) antigen
2. Record the lot number and expiration date of the Ortho-BioClone® anti-D, and 22% BSA on the *Centrifuge Calibration Worksheet*.
3. Label a test tube to identify the contents of the diluted anti-D. Prepare the diluted anti-D by combining 1 drop of reagent anti-D antisera and 20 drops of 22% albumin in the labeled tube. Mix the contents of the tube thoroughly with the vortex. Note: Alternatively for larger quantity of testing antisera mix 9.9 mL of 22% albumin with 0.1 mL anti-D, dilution 1:100.
 - a. If only one centrifuge is being calibrated, use a different centrifuge to confirm appropriateness of Anti-D dilution.
 - b. If the reaction strength is greater than 1+, adjust the dilution of the antisera by adding additional drops of 22% albumin. Retest the diluted anti-D and continue to adjust until it reacts 1+ with D Positive screening cells.
 - c. If the reaction strength is less than 1+, prepare a new dilution by combining 10 µL of reagent Anti-D antisera and only 15 drops of 22% albumin. Retest the new dilution and continue to adjust until it reacts 1+ with D Positive screening cells.

C. Calibration Check of the Immediate Spin Process

1. Document the centrifuge ID (Asset Tag), the date, and initials of the technologist who is calibrating the centrifuge on the *Centrifuge Calibration Worksheet*.
2. Label six (6) test tubes in pair to identify the positive and negative controls and three spin times for which the calibration check will be performed. Refer to the table below.
3. To each of the labeled tubes add the following reagents:

Label Tube	Diluted Anti-A	Test Cells
Pos, x* -5 sec	1 drop	1 drop A ₁ cells
Neg, x-5 sec	1 drop	1 drop B cells
Pos, x sec	1 drop	1 drop A ₁ cells
Neg, x sec	1 drop	1 drop B cells
Pos, x+5 sec	1 drop	1 drop A ₁ cells
Neg, x+ 5 sec	1 drop	1 drop B cells
*x = the optimal spin time for the Immediate Spin process from the most recent calibration as documented on the <i>Centrifuge Calibration Sticker</i> .		

4. Spin the 2 correspondingly labeled tubes (containing the positive and negative controls) for x-5 seconds. Read and grade the tubes for agglutination, record the graded reactions, and record the observations as YES or NO on the worksheet.
 - a. The supernatant fluid is clear (for both the positive and negative controls).
 - b. The cell button is clearly delineated with a sharply defined periphery (for both the positive and negative controls).
 - c. The cell button is easily resuspended (for both the positive and negative controls).
 - d. Agglutination is observed in the positive control.
 - e. No agglutination is observed in the negative control.
 - f. After documenting the worksheet, discard the 2 tubes.
5. Repeat step 4 using the 2 correspondingly labeled tubes (containing the positive and negative controls) and spinning for x seconds.
6. Repeat step 4 using the 2 correspondingly labeled tubes (containing the positive and negative controls) and spinning for x + 5 seconds.
7. Determine the optimal spin time for the IS process from the current calibration check by finding the time which corresponds to the column with the shortest spin time for which all observations are TRUE and record it on the worksheet. Refer to the *Determining the Optimal Spin Time (for IS and AG testing)* section of this document.
8. Interpret the IS calibration check as satisfactory. Record the interpretation on the worksheet.

D. Calibration Check of the Antiglobulin with Washing Process

1. Label six (6) test tubes in pair to identify the positive and negative controls and three spin times for which the calibration check will be performed. Refer to the table below.
2. To each of the labeled tubes, add the following reagents:

Label Tubes	Diluted Anti-D	Test Cell
Pos, w* - 15 sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, w -15 sec	1 drop	1 drop Rh(D) Negative screening cell
Pos, w sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, w sec	1 drop	1 drop Rh(D) Negative screening cell
Pos, w +15 s	1 drop	1 drop Rh(D) Positive screening cell
Neg, w +15 sec	1 drop	1 drop Rh(D) Negative screening cell
*w = the optimal spin time for the washing process from the most recent calibration as documented on the <i>Centrifuge Calibration Sticker</i> .		

3. Incubate the tubes for 15-30 minutes at 37°C.
4. Fill each of the tubes with saline.
5. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for w - 15 seconds.
6. For both tubes, record the observations as YES or NO on the worksheet.
 - a. The cell button is clearly delineated with minimal cells trailing up the side.
 - b. After decanting the saline, the cell button is easily resuspended in the residual fluid.

Retain, do not discard the tubes.

7. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for w seconds. Repeat step 6 for the tubes that were centrifuged for w seconds.
8. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for w+ 15 seconds. Repeat step 6 for the tubes that were centrifuged for w+15 seconds.
9. Determine the optimal spin time for the washing process from the current calibration check and record it on the worksheet. Refer to the policy *Determining the Optimal Spin Time (for the washing process)*.
10. Interpret the washing calibration check as satisfactory or unsatisfactory. Record the interpretation on the worksheet. Refer to the policies *Satisfactory Calibration Check* and *Unsatisfactory Calibration Check*.
11. Wash all tubes in the centrifuge 3 additional times (centrifuging for the optimal spin time for washing as determined from the most recent calibration check). Decant the saline completely after the last wash.
12. Add 2 drops of Anti-IgG reagent to the 2 correspondingly labeled tubes (containing the positive and negative control cells, labeled for optimal wash seconds). Spin the 2 tubes for x-5 seconds. Read and grade the tubes for agglutination, record the graded reactions, and record the observations as YES or NO on the worksheet.
 - a. The supernatant fluid is clear (for both the positive and negative controls).
 - b. The cell button is clearly delineated with a sharply defined periphery (for both the positive and negative controls).

- c. The cell button is easily resuspended (for both the positive and negative controls).
 - d. Agglutination is observed in the positive control.
 - e. No agglutination is observed in the negative control.
 - f. After documenting the worksheet, discard the 2 tubes.
13. Repeat step 11 using the 2 correspondingly labeled tubes (containing the positive and negative control cells) and spinning for x seconds.
 14. Repeat step 11 using the 2 correspondingly labeled tubes (containing the positive and negative control cells) and spinning for x + 5 seconds.
 15. Determine the optimal spin time for the AHG process from the current calibration check and record it on the worksheet. Refer to the policy *Determining the Optimal Spin Time (for IS and AHG testing)*.
 16. Interpret the AHG calibration check as satisfactory or unsatisfactory. Record the interpretation on the worksheet. Refer to the policies *Satisfactory Calibration Check* and *Unsatisfactory Calibration Check*.
 17. If the calibration check for the immediate-spin process and for the antiglobulin with washing process is satisfactory, remove and discard the *Centrifuge Calibration Sticker* from the previous calibration check. Document a new sticker and affix it to the side of the applicable centrifuge.
 18. Repeat the calibration check for each required centrifuge and submit the *Centrifuge Calibration Worksheets* to the Supervisor or Lead Medical Technologist for review.

XI. INTERPRETATIONS:

A. **Satisfactory Calibration Check of the IS or AHG Process**

The calibration check of the IS or AHG process is satisfactory if the optimal spin time from the current calibration is within 5 seconds of the optimal spin time from the most recent calibration. For example:

The optimal spin time from the most recent calibration (last year) was 20 seconds. The optimal spin time from the current calibration is 15 seconds (each of the criteria are fulfilled when the calibration was performed with a 15-second spin time). The calibration check is satisfactory. The Centrifuge Calibration Sticker is updated with the current date and the optimal spin time of 15 seconds.

B. **Unsatisfactory Calibration Check of the IS or AHG Process**

The calibration check of the IS or AHG process is unsatisfactory if the optimal spin time cannot be determined when performed at each of the 4 spin times, even after repeating the calibration check procedure. If the calibration check is unsatisfactory, refer to the policy *Appropriate Actions if the Calibration Check is Unsatisfactory*.

C. **Satisfactory Calibration Check of the Washing Process**

The calibration check of the washing process is satisfactory if the optimal spin time from the current calibration is within 10 seconds of the optimal spin time from the most recent calibration (last year).

D. **Unsatisfactory Calibration Check of the Washing Process**

The calibration check of the washing process is unsatisfactory if the optimal spin time from

the current calibration is not within 15 seconds of the optimal spin time from the most recent calibration. If the calibration check is unsatisfactory, refer to the policy *Appropriate Actions if the Calibration Check is Unsatisfactory*.

XII. NOTES:

- A. The calibration check of the antiglobulin with washing process does not monitor the completeness of washing; the use of IgG-coated cells provides this check as described elsewhere in the Standard Operating Procedures. The calibration check addresses only the mechanics of centrifugation.

XIII. REFERENCES:

1. AABB Technical Manual, Method 8-5, Calibrating a Serologic Centrifuge, seventeenth edition.

Attachments

[Centrifuge Calibration Worksheet](#)

[Serofuge Calibration Sticker.pdf](#)

Approval Signatures

Step Description	Approver	Date
Policy and Forms Steering Committee (if needed)	Jeremy Powers: Chief, Pathology	5/25/2023
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	Muhammad Arshad: Physician	5/19/2023
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