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Calibration of Serologic Centrifuges - Dearborn 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 ± 1 month time span

B. Beaumont Health Biomedical: performs repairs and does some maintenance of equipment for Beaumont 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. Test Tube Sizes

1. 12 x 75 mm glass test tubes are used for all samples that are tested by hemagglutination procedures.
2. 10 x 75 mm glass test tubes may be used to prepare cell suspensions for use in gel testing on the Vision. These test tube sizes are not used to test samples by hemagglutination procedures; therefore, centrifuges are not calibrated with 10 x 75 mm test tubes.

C. 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 are used for the following processes (and are therefore calibrated for these processes):

1. Using 12 x 75 mm test tubes for immediate-spin (IS) testing
2. Using 12 x 75 mm test tubes for washing with antiglobulin (AG) testing.

D. **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.

E. **Determining the Optimal Spin Time (for IS and AG testing)**
The optimal spin time for immediate spin (IS) or antiglobulin (AG) testing is the shortest of the 4 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 AG 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 4 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 Beaumont Health Biomedical to arrange for equipment repair.
4. Notify the Lead Medical Technologist or Department Supervisor.
5. Document the equipment failure on an internal variance.

VI. REAGENTS:

A. For the calibration check of the immediate-spin process
B. Anti-B reagent antisera
C. Ortho 7% BSA
D. Group A cells, 2-5% cell suspension (negative control)
E. Group B cells, 2-5% cell suspension (positive control)
F. For the calibration check of the washing with antiglobulin testing process
G. Ortho BioClone® Anti-D
H. Rh(D) positive screening cells obtained from 3% Ortho Surgiscreen cells
I. Rh(D) negative screening cells obtained from 3% Ortho Surgiscreen cells
J. Anti-IgG reagent
K. IgG coated Coombs control cells
L. 22% Bovine Serum Albumin

VII. EQUIPMENT:

A. Vortex mixer

VIII. SUPPLIES:

A. 12 x 75 test tubes
B. Disposable pipettes
C. Normal saline
D. Parafilm

E. Plastic specimen rack

IX. QUALITY CONTROL (QC):

A. QC testing must be performed on the all reagents as described in 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 A_1 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% albumin in the labeled tube. Mix the contents of the tube thoroughly with the vortex.
3. Test the diluted Anti-A to verify that it reacts 1+ with A_1 test cells as follows:
    a. Add 1 drop of diluted Anti-A and 1 drop of A_1 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 6-8% albumin. 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 6-8% albumin. Retest the new dilution and continue to adjust until it reacts 1+ with A1 test cells.
4. Document Part A , *Preliminary Steps for Calibration of Serologic Centrifuges* of the *Centrifuge Calibration Worksheet.*

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% Bovine

serum Albumin 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.
    a. 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-B and continue to adjust until it reacts 1+ with D Positive screening cells.
    b. If the reaction strength is less than 1+, prepare a new dilution by combining 10 µL of reagent Anti-B 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 eight (8) 12 x 75 mm test tubes in pair to identify the positive and negative controls and four 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, 10 sec	1 drop	1 drop A_1 cells
Neg, 10 sec	1 drop	1 drop B cells
Pos, 15 sec	1 drop	1 drop A_1 cells
Neg, 15 sec	1 drop	1 drop B cells
Pos, 20 sec	1 drop	1 drop A_1 cells
Neg, 20 sec	1 drop	1 drop B cells
Pos, 30 sec	1 drop	1 drop A_1 cells
Neg, 30 sec	1 drop	1 drop B cells

4. Spin the 2 correspondingly labeled tubes (containing the positive and negative controls) for 10 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 15 seconds.

6. Repeat step 4 using the 2 correspondingly labeled tubes (containing the positive and negative controls) and spinning for 20 seconds.
7. Repeat step 4 using the 2 correspondingly labeled tubes (containing the positive and negative controls) and spinning for 30 seconds.
8. 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.
9. Refer to the policy Determining the Optimal Spin Time (for IS and AG testing).
10. Interpret the IS calibration check as satisfactory.

D. Calibration Check of the Antiglobulin with Washing Process

1. Label eight (8) 12 x 75 mm test tubes in pair to identify the positive and negative controls and four 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, W30 sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, W30 sec	1 drop	1 drop Rh(D) Negative screening cell
Pos, W45 sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, W45 sec	1 drop	1 drop Rh(D) Negative screening cell
Pos, W60 sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, W60 sec	1 drop	1 drop Rh(D) Negative screening cell
Pos, W90 sec	1 drop	1 drop Rh(D) Positive screening cell
Neg, W90 sec	1 drop	1 drop Rh(D) Negative screening cell

3. Incubate the tubes for 15-30 minutes at 37C.
4. Fill each of the tubes with saline.
5. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for 30 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 45 seconds. Repeat step 6 for the tubes that were centrifuged for 45 seconds.
8. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for 60 seconds. Repeat step 6 for the tubes that were centrifuged for 60 seconds.

9. Spin the 2 correspondingly labeled tubes (containing the positive and negative control cells) for 90 seconds. Repeat step 6 for the tubes that were centrifuged for 90 seconds.
10. 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)*
11. 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.*
12. 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.
13. 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 10 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.
14. Repeat step 11 using the 2 correspondingly labeled tubes (containing the positive and negative control cells) and spinning for 15 seconds.
15. Repeat step 11 using the 2 correspondingly labeled tubes (containing the positive and negative control cells) and spinning for 20 seconds.
16. Repeat step 11 using the 2 same corresponding labeled tubes (containing the positive and negative control cells) and spinning for 30 seconds.
17. Determine the optimal spin time for the AG process from the current calibration check and record it on the worksheet.
    Refer to the policy Determining the Optimal Spin Time (for immediate-spin and antiglobulin testing).
18. Interpret the AG calibration check as satisfactory or unsatisfactory. Record the interpretation on the worksheet. Refer to the policies Satisfactory Calibration Check and Unsatisfactory Calibration Check.
19. 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.
20. 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 AG Process**
The calibration check of the IS or AG 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. American Association of Blood Banks Technical Manual, Method 8-5, Calibrating a Serologic Centrifuge, seventeenth edition.

Attachments

Centrifuge Calibration Worksheet

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	6/7/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	6/6/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	6/6/2022
	Kimberly Geck: Dir, Lab Operations B	6/4/2022
	Kelly Sartor: Supv, Laboratory	6/3/2022
	Kelly Sartor: Supv, Laboratory	6/3/2022

Beaumont HEALTH

Beaumont Laboratory, Dearborn

Centrifuge Calibration Worksheet

Centrifuge ID (Asset Tag #): _____ **Date:** _____ **Technologist** _____

Reagent	Manufacturer	Lot Number	Exp. Date	Reagent	Manufacturer	Lot Number	Exp. Date
Anti-A	Ortho Diagnostics			O- Screening cell # _____	Ortho Diagnostics		
A_1 cells	Ortho Diagnostics			Albumin 6-8%	Ortho Diagnostics		
B Cells	Ortho Diagnostics			Albumin 22%	Ortho Diagnostics		
Anti-D Serum	Ortho Diagnostics			Anti-IgG	Ortho Diagnostics		
O+ Screening cell # _____	Ortho Diagnostics			Coombs Control Cells	Ortho Diagnostics		

Immediate-Spin					Washing					Antiglobulin				
Criteria	10s	15s	20s	30s	Criteria	30s	45s	60s	90s	Criteria	10s	15s	20s	30s
Supernatant fluid is clear (for both the positive and negative controls)					Cell button is clearly delineated with minimal cells trailing up the side (for both the positive and negative controls)					Supernatant fluid is clear (for both the positive and negative controls)				
Cell button is clearly delineated, and periphery is sharply defined (for both the positive and negative controls)										Cell button is clearly delineated, and periphery is sharply defined (for both the positive and negative controls)				
Cell button is easily resuspended (for both the positive and negative controls)					After decanting saline, the cell button is easily resuspended in the residual fluid (for both the positive and negative controls)					Cell button is easily resuspended (for both the positive and negative controls)				
Agglutination is observed in positive control	RXN:	RXN:	RXN:	RXN:						Agglutination is observed in positive control	RXN:	RXN:	RXN:	RXN:
No agglutination is observed in negative control	RXN:	RXN:	RXN:	RXN:						No agglutination is observed in negative control	RXN:	RXN:	RXN:	RXN:
Optimal Spin Time determined from current calibration for Immediate-Spin (IS): _____ seconds					Optimal Spin Time determined from current calibration for Washing: _____ seconds					Optimal Spin Time determined from current calibration for AHG Testing: _____ seconds				
☐ IS Calibration Check Satisfactory ☐ IS Calibration Check Unsatisfactory					☐ Washing Calibration Check Satisfactory ☐ Washing Calibration Check Unsatisfactory					☐ AHG Calibration Check Satisfactory ☐ AHG Calibration Check Unsatisfactory				

QC Reviewed by: _____ Date: _____