HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Calibrating a Serologic Centrifuge		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 To ensure the integrity, accuracy and reliability of serologic centrifuges of blood bank.

2. DEFINITONS:

2.1 N/A

3. POLICY:

3.1 Each centrifuge should be calibrated upon receipt, after adjustments or repairs, and periodically.
3.2 Calibration evaluates the behaviour of red cells in solutions of different viscosities, not the reactivity of different antibodies.
3.3 Periodic recalibration is performed to verify that the timing in use continues to be the optimal timing. This may be accomplished by using the current timing for a particular centrifuge and each medium and those times just above and just below the current timing.

4. PROCEDURE:

4.1 **For immediate agglutination:**
- 4.1.1 Materials:
  - 4.1.1.1 Test tubes.
  - 4.1.1.2 Worksheet for recording results.
  - 4.1.1.3 For saline-active antibodies:
    - 4.1.1.3.1 Serum/ plasma from a group A person (anti-B) diluted with 6% albumin to give 1+ macroscopic agglutination (3 mL of 22% bovine albumin + 8 mL of normal saline = 6% bovine albumin).
    - 4.1.1.3.2 Positive control: Group B red cells in a 2% to 5% saline suspension.
    - 4.1.1.3.3 Negative control: Group A red cells in a 2% to 5% saline suspension.
  - 4.1.1.4 For high-protein antibodies:
    - 4.1.1.4.1 Anti-D diluted with 22% albumin to give 1+ macroscopic agglutination.
    - 4.1.1.4.2 Positive control: D+ red cells in a 2% to 5% saline suspension.
    - 4.1.1.4.3 Negative control: D– red cells in a 2% to 5% saline suspension.
- 4.1.2 Procedure:
  - 4.1.2.1 For each set of tests (saline and high- protein antibodies), label five test tubes for positive reactions and five for negative reactions.
  - 4.1.2.2 In quantities that correspond to routine use, add diluted anti-B to each of 10 tubes for the saline test and add diluted anti-D to each of 10 tubes for the high-protein test.
  - 4.1.2.3 Add the appropriate control cell suspension to one set of tubes (one positive and one negative tube for the saline test, and one positive and one negative tube for the high-protein antibody test). Centrifuge immediately for the desired time interval (e.g. 10 seconds).
  - 4.1.2.4 Observe each tube for agglutination and record observations.

4.1.2.5 Repeat steps 2 and 3 for each time interval (e.g. 15, 20, 30, and 45 seconds). Do not allow cells and sera to incubate before centrifugation.

4.1.2.6 Select the optimal time of centrifugation, which is the shortest time required to fulfill the following criteria:

4.1.2.6.1 The supernatant fluid is clear.

4.1.2.6.2 The cell button is clearly delineated and the periphery is sharply defined, not fuzzy.

4.1.2.6.3 The cell button is easily resuspended.

4.1.2.6.4 Agglutination in the positive tubes is as strong as determined in preparing reagents.

4.1.2.6.5 There is no agglutination in the negative tubes.

4.1.2.7 Record centrifuge identification, the times selected, the date, and the identity of the person performing the calibration.

4.2 **For washing and antiglobulin testing:**

4.2.1 Principle:

4.2.1.1 Tests in which antihuman globulin (AHG) serum is added to red cells may require centrifugation conditions different from those for immediate agglutination.

4.2.1.2 Centrifugation conditions appropriate for both washing and AHG reactions can be determined in one procedure. Note that this procedure does not monitor the completeness of washing; use of IgG-coated cells to control negative AHG reactions provides this check. The following procedure addresses only the mechanics of centrifugation.

4.2.2 Materials:

4.2.2.1 AHG reagent, unmodified.

4.2.2.2 Saline, large volumes.

4.2.2.3 Test tubes.

4.2.2.4 Worksheet for recording results.

4.2.2.5 Positive control: a 2 % to 5 % saline suspension of D+ red cells incubated for 15 minutes at 37 ºC with anti-D diluted to give 1+ macroscopic agglutination after addition of AHG.

4.2.2.6 Negative control: a 2% to 5% suspension of D+ red cells incubated for 15 minutes at 37 ºC with 6% albumin. [Note: D- red cells incubated with diluted anti-D may also be used as a negative control.]

4.2.3 Procedure:

4.2.3.1 Prepare five test tubes containing 1 drop of positive cells and five tubes containing 1 drop of negative control cells.

4.2.3.2 Fill tubes with saline and centrifuge them in pairs, one positive and one negative, for different times (eg, 30, 45, 60, 90, and 120 seconds). The red cells should form a clearly delineated button, with minimal cells trailing up the side of the tube. After the saline has been decanted, the cell button should be easily resuspended in the residual fluid. The optimal time for washing is the shortest time that accomplishes these goals.

4.2.3.3 Repeat washing process on all pairs three more times, using time determined to be optimal.

4.2.3.4 Decant supernatant saline thoroughly.

4.2.3.5 Add AHG to one positive control test tube and one negative control test tube. Centrifuge immediately for the desired interval (e.g. 10 seconds).

4.2.3.6 Observe each tube for agglutination and record observations.

4.2.3.7 Repeat steps 5 and 6 for each interval (e.g. 15, 20, 30, and 45 seconds). Do not allow cells and AHG to incubate before centrifugation.

4.2.3.8 Select optimal time as in immediate agglutination procedure.

4.2.3.9 Record centrifuge identification, the times selected, the date, and the identity of the person performing the calibration.

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**
- 5.1.1 Calibrating a serologic centrifuge form

5.2 **Materials and Equipment**
- 5.2.1 Serologic centrifuge
- 5.2.2 Materials as mentioned for each procedure

6. RESPONSIBILITIES:

6.1 Blood Bank technicians/ specialists to follow the detailed procedures.
6.2 Supervisor of blood bank technicians or his deputy to follow and review calibration results.

7. APPENDICES:

7.1 Calibrating a serologic centrifuge form

8. REFERENCES:

8.1 The Unified Practical Procedure Manual For Blood Banks In The Arab Countries, 1434-2013.
8.2 The Standard Policy For Blood Banks In The Kingdom Of Saudi Arabia, 1st edition, 1435-2014.
8.3 National Standards For Clinical laboratories and Blood Banks, 1st edition, 2015.
8.4 AABB Technical manual, 18th edition, 2014.
8.5 AABB Standards for Blood Banks and Transfusion Services, 30th edition, 2016.

9. APPROVALS:

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