PROCEDURE

Title: Centrifuge Calibration

Procedure #: 2015BLOODBANK78

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

Prepared by: Anita Smith Date: 6/12/2015

Title: Laboratory Administrative Director

Accepted by: _____ Date: 6/12/15

Title: Laboratory Medical Director

Date Patient Testing Implemented: 10/1/1997

Review of procedure every two years

Reviewed by: _____ Date: _____

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Reviewed by: _____ Date: _____

Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: Blood Bank Centrifuge/Cellwasher Calibration **Department:** Blood Bank

Departmental Review: **Policy #:**

INITIATE DATE	**DATE REVIEWED/REVISED**	**PAGE 1 of 4**
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PURPOSE:
Each centrifuge should be calibrated upon receipt, after adjustments or repairs, and periodically. Calibration evaluates the behavior of red cells in solutions of different viscosities, not the reactivity of different antibodies.

PROCEDURE:

I. SALINE/IMMEDIATE SPIN PHASE

MATERIALS:
Anti-B anti sera B cells (positive control) A1 cells (negative control) saline

1. Prepare a 1:10 dilution of the anti-B sera by adding 1 drop of anti-B to 9 drops of saline.
2. For each set of tests prepare 4 12 x 75 tubes for positive reactions and 4 tubes for negative reactions. Add a drop of the diluted antisera to all tubes. Add a drop of the B cells to the 4 positive tubes and a drop of A1 cells to the negative tubes. Mix
3. Spin in pairs (1 each of positive and negative tubes) for the following times: 15, 20, 30 and 35 seconds. Observe each tube for agglutination and record observations on the logsheet.

II. HIGH PROTEIN PHASE

MATERIALS:
Anti-D sera D positive cells D negative cell Rh control sera

1. Prepare a 1:12 dilution of anti-D sera by adding 1 drop of anti-D sera to 11 drops of Rh control sera.
2. Prepare 4 positive reaction tubes and 4 negative reaction tubes.
3. Add a drop of the diluted anti-D to each positive and negative tube.
4. Add a drop of the D positive cells to the positive reaction tube and a drop of the D negative cells to the negative reaction tubes. Mix.
4. Spin in pairs (1 each of positive and negative tubes) for the following times: 30, 35, 40 and 45 seconds. Observe each tube for agglutination and record observations on the logsheet.

III. ENZYME (N-HANCE)

MATERIALS:
Gamma N-Hance D positive cells D negative cells anti-D sera

1. Prepare a 1:12 dilution of anti-D sera by adding 1 drop of anti-D sera to 11 drops of Rh control sera.
2. Prepare 4 positive reaction tubes and 4 negative reaction tubes.
3. Add a drop of the anti-D and a drop of the N-Hance to all tubes.
4. Add a drop of the D positive cells to the positive reaction tubes and a drop of the D negative cells to the negative reaction tubes. Mix.
5. Spin in pairs (1 each of positive and negative tubes) for the following times: 30, 35, 40 and 45 seconds. Observe each tube for agglutination and record observations on the logsheet.

IV. COOMBS WASH

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

MATERIALS:
Poly-specific AHG
D positive cells incubated for 15 min at 37°C with anti-D (positive control)
D negative cells incubated for 15 min at 37°C with saline (negative control)
Saline

1. Prepare 4 pairs of tubes containing 1 drop of positive and 1 drop of negative control cells.
2. Fill tubes with saline and centrifuge pairs for the following times: 20, 45, 60 and 70 seconds. The red cells should form a clearly delineated button, with no cells trailing up the side of the tube. After the saline had been decanted, the cells button should be easily re-suspended in a residual fluid. The shortest time that accomplishes this goal is the optimal time for washing.
3. Decant supernatant saline thoroughly.

4. Add AHG to each of the pairs and centrifuge for the following times: 10, 15, 20 and 25 seconds.

INTERPRETATION OF RESULTS:

The optima time of centrifugation is the shortest time required to fulfill the following criteria:

1. Agglutination in the positive tubes is as strong as determined in preparing reagents.
2. There is no agglutination or ambiguity in the negative tubes.
3. The cell button is clearly delineated and the periphery is sharply defined, not fuzzy.
4. The supernatant fluid is clear.
5. The cell button is easily re-suspended.

PROCEDURE NOTES:

Periodic recalibration is performed to verify that the timing in use continues to be the optimal timing. This may be accomplished by using the shortened version of the procedure above. For example, use the current timing for a particular centrifuge and each medium and those times just above and just below the current timing.

REFERENCES:

AABB Technical Manual, 2011, 17th edition

Highlands Regional MEDICAL CENTER

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Reviewed by	Reviewed Date	Reviewed by	Reviewed Date
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	5-29-15		

Initial Implementation Date: 10-1997

Reviewed by: Christel Portnile Date: 11-14-14
Department Supervisor

Reviewed by: Angela Lanston Date: 11/17/14
Department Adm. Director

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Department Medical Director