Beaumont

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Making a Test Red Cell Suspension

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide the Blood Bank employees with stepwise instructions for preparing test cell suspensions.The Blood Bank uses several different pipettes to make various red cell suspensions that will be tested by various instruments and methodologies. This document will provide directions for making these cell suspensions.

II. ACRONYMS:

A. **DAT:** Direct Antiglobulin Test.

B. **RBC:** Red Blood Cell.

C. **LISS:** Low Ionic Strength Saline

III. ACCEPTABLE SPECIMENS:

A. Test red cell suspensions may be made from:

1. Patient samples
2. Donor RBC segments
3. Commercial reagent RBCs

IV. REAGENTS / EQUIPMENT / SUPPLIES:

A. Test tubes, 10 x 75mm or 12 x 75mm, glass or plastic

B. Segment splitters or scissors

C. Plastic disposable pipettes

D. Buffered saline

E. MTS Diluent 2™

F. MTS Diluent 2 PLUS™

G. MTS™ pipette tips

H. Electronic or manual pipette

V. INSTRUCTIONS:

A. All test tubes used when making a red cell suspension must be labeled appropriately to identify the tube's contents.

B. Red cell suspensions must be free of hemolysis and interfering proteins or immunoglobulins.

C. Too heavy or too light of a red cell suspension may cause false negative or false positive results.

D. To help prevent contamination, the MTS Diluent 2™ and MTS Diluent 2 PLUS™ bottles should not be entered with a pipette, and care must be used to avoid touching the tip of the bottle dispenser with a sample or test tube. In addition, when pipetting diluent:

1. Use the Dispense mechanism on the diluent bottle to dispense an aliquot of the diluent into a test tube.
2. Pipette/aspirate the diluent from the test tube, not from the bottle.

E. Red cell suspensions made from patient samples should be labeled with the patient's last name and will be discarded immediately after testing. Cell suspensions should be made immediately prior to testing.

F. Cell suspensions made from donor RBC segments should be labeled with the donor unit number and will be discarded immediately after testing.

G. When a reagent red cell is diluted from a 3% suspension to a 0.8% suspension, the diluted reagent cell suspension may be kept up to 24 hours (not to exceed the original expiration date of the reagent red cell) if stored at 2°C - 8°C. If the diluted reagent cell suspension is being retained following the initial testing, it must be labeled with a *Selected Cell Sticker*, which consists of:

1. Test Cell Number (from panel)
2. Panel manufacturer and lot number
3. Original expiration date
4. New expiration date and time
5. Date of preparation / technologist
6. Storage Requirements

H. If a diluted reagent red cell suspension is going to be discarded immediately after testing, it should be labeled with the panel lot number and test cell number (from the panel).

I. Expired test/panel cells should not be used unless in-date cells with the desired antigenic profile are unavailable. If the original expiration date of a test cell that must be diluted for use in gel testing has already passed, then the diluted cell shall be discarded immediately after testing (and should not be given an extended outdate of 24 hours).

J. To obtain donor RBCs, donor RBC segments may be cut with scissors or a segment splitter. Note that segment splitters have a tendency to hemolyze the cells.

VI. PROCEDURE:

A. **Preparing a 2 - 4% Red Cell Suspension for Use in Tube Testing:**

1. Obtain the specimen from which the 2 - 4% cell suspension will be made. This may be a patient sample or a donor RBC segment.
2. Label an empty test tube with the patient's last name or donor unit number, as well as indicating it

will be containing a 2 - 4% cell suspension.

3. Using a plastic disposable pipette, transfer a few drops of packed RBCs from the patient sample or donor RBC segment into the labeled test tube.
4. If any of the following conditions in a sample are present, then wash the contents of the labeled test tube 1 - 4 times with large volumes of buffered saline. Decant the buffered saline completely after each wash.
    a. Is hemolyzed.
    b. Has rouleaux forming properties.
    c. Has a strong autoantibody.
    d. Contains Wharton's Jelly.
    e. If LISS is going to be used during the RBC testing.
    f. If a donor RBC segment is to be used in an AHG tube crossmatch.
    g. Antigen typing.
5. Add enough buffered saline to the test tube to suspend the cells to 2 - 4%, comparing the suspension to the consistency and color of a 2 - 4% commercial red cell reagent.
6. Mix the 2 - 4% cell suspension well.

B. **Preparing a Diluted 0.8% Reagent Red Cell Suspension from a 3% Reagent Red Cell Suspension:** These 0.8% cell suspensions are used in the MTS™ gel system. This procedure will yield sufficient volume for 1 - 3 test cells. If more than 1 - 3 test cells are required, refer to the note below.

1. Dispense 100 µL of a 3% reagent red cell into a 12 x 75 mm test tube, using either a manual pipette or an electronic pipette.
2. Add a few drops of MTS Diluent 2™ into the 12 x 75 mm test tube.
3. Centrifuge the 12 x 75 mm test tube for 60 seconds to pack the RBCs.
4. Invert the 12 x 75 mm test tube to completely remove the supernatant.
5. Dispense 200 µL of MTS Diluent 2™ into the 12 x 75 mm test tube of packed RBCs.
6. Mix the 12 x 75 mm test tube to resuspend the 0.8% red cell suspension.

    Note: This will yield sufficient volume for 1 - 3 test cells. This volume may be increased based on testing requirements, but the 1:2 ratio of 3% reagent red cells: MTS Diluent 2™ should remain constant. In some cases, it may be necessary to prepare sufficient volume for a large number (more than 30) of test cells; i.e., when diluting 3% screening red cells to 0.8% for use by an entire shift. If this is necessary, then follow the steps of the above procedure with the following modification: use 500 µL (0.5 mL) of 3% reagent red cell and 1.5 mL of MTS Diluent 2™. This 1:3 ratio of 3% reagent red cells: MTS Diluent 2™ is recommended by Ortho Clinical Diagnostics when preparing sufficient volume for 30 or more tests, as opposed to the 1:2 ratio used when preparing smaller volumes.

C. **Using a Sartorius or BioHit Electronic Pipette to Prepare a 0.8% Red Cell Suspension for use in the MTS™ Gel System as an Auto Control, DAT, Antigen Typing, or Donor Cell/Crossmatch:**

1. Label a 12 x 75 mm test tube with the patient's last name or donor unit number.
2. Dispense 1 mL of MTS Diluent 2™ into the test tube.

3. Obtain the patient sample or donor RBC segment from which the 0.8% red cell suspension will be made.
4. Packed RBCs will be aspirated in step 5, so centrifuge the patient sample or donor RBC segment, if necessary, so that packed RBCs will be available.
    a. Packed RBCs may be obtained from a segment by cutting the segment at the end containing packed cells and placing the MTS™ pipette tip into the segment opening, or using a segment splitter and emptying the segment RBCs into a separate 12 x 75 mm test tube.
5. Continue with the steps of Program #6 of the Sartorius or BioHit pipette to prepare the 0.8% red cell suspension. During mixing, the 12 x 75 mm test tube's contents will mix 3 times as the MTS™ pipette tip is kept in the contents of the test tube. Program #6 goes as follows:
    a. Fill the MTS™ pipette tip with 100 µL of the MTS Diluent 2™ (from 12 x 75 mm the test tube).
    b. Aspirate 15 µL of air into the MTS™ pipette tip.
    c. Fill the tip with 10 µL packed RBCs and wipe the outside of the tip.
    d. Purge all contents from the tip into the 12 x 75 mm test tube.
    e. Mix the contents of the 12 x 75 mm test tube containing the 0.8% red cell suspension.
    f. Eject the mixed contents into the 12 x 75 mm test tube.
6. If the 0.8% red cell suspension does not appear adequately mixed, it may be necessary to mix the contents with a plastic disposable pipette.

D. **Using a Manual Pipette to Prepare a 0.8% Red Cell Suspension for use in the MTS™ Gel System as an Auto Control, DAT, Antigen Typing, or Donor Cell / Crossmatch:**
1. Label a 12 x 75 mm test tube with the patient's last name or donor unit number.
2. Dispense 1 mL of MTS Diluent 2™ into the 12 x 75 mm test tube.
3. Pipette 10 µL of the packed donor or patient RBCs into the 12 x 75 mm test tube.
4. Mix well to resuspend the packed RBCs into a 0.8% red cell suspension.

E. **Using a Sartorius or BioHit Electronic Pipette to Prepare a 4% ± 1% Red Cell Suspension for use in the MTS™ Gel System for ABO/Rh Testing or Antigen Typing:**
1. Label a 12 x 75 mm test tube with the patient's last name or donor unit number.
2. Label a 2nd 12 x 75 mm test tube stating it will contain MTS Diluent 2 PLUS™.
3. Dispense at least 200 µL (0.2 mL) of MTS Diluent 2 PLUS™ into the 12 x 75 mm test tube labeled for MTS Diluent 2 PLUS™.
4. Packed RBCs will be aspirated in step 5, so centrifuge the patient sample or donor RBC segment, if necessary, so that packed RBCs will be available.
    a. Packed RBCs may be obtained from a segment by cutting the segment at the end containing packed cells and placing the MTS™ pipette tip into the segment opening, or using a segment splitter and emptying the segment RBCs into a separate 12 x 75 mm test tube.
5. Continue with the steps of Program #7 of the Sartorius or BioHit pipette to prepare the 4% ± 1% red cell suspension. During mixing, the test tube's contents will mix 3 times as the MTS™ pipette tip is kept at the bottom of the 12 x 75 mm test tube. Program #7 goes as follows:
    a. Fill the MTS™ pipette tip with 200 µL of the MTS Diluent 2 PLUS™ (from the 12 x 75 mm test

tube containing the MTS Diluent 2 PLUS™).

b. Aspirate air into the MTS™ pipette tip.

c. Fill the MTS™ pipette tip with 10 µL packed RBCs and wipe the outside of the tip.

d. Eject the contents of the MTS™ pipette tip into the 12 x 75 mm test tube labeled with the patient's last name or donor unit number.

e. Mix the contents of the 12 x 75 mm test tube containing the 4% ± 1% red cell suspension.

f. Eject the mixed contents of the MTS™ pipette tip into the 12 x 75 mm test tube labeled with the patient's last name or donor unit number.

6. If the 4% ± 1% red cell suspension does not appear adequately mixed, it may be necessary to mix the contents with a plastic disposable pipette.

F. **Using a Manual Pipette to Prepare a 4% ± 1% Red Cell Suspension for use in the MTS™ Gel System for ABO/Rh Testing or Antigen Typing:**

1. Label a 12 x 75 mm test tube with the patient's last name or donor unit number.
2. Dispense 0.5 mL of MTS Diluent 2 PLUS™ into the 12 x 75 mm test tube.
3. Add 25 µL of packed RBCs.
4. Mix gently to resuspend. The final red blood cell suspension should be 4% ±1%.

VII. REFERENCES:

A. Cohn, C.S., Delaney, M, Johnson, S.T., Katz, L.M. (2020) *Technical Manual*.(19th ed.). AABB.

B. Package insert: A/B/D Monoclonal and Reverse Grouping Card™,

C. 2005-05-02 Version 4.0, Pompano Beach, FL: Micro Typing Systems, Inc.

D. *Micro Typing Systems TM Procedures: Rh Phenotyping Using Individual Gel Cards (Monoclonal Anti-D, Anti-c, Anti-E, and Anti-e) and Rh Phenotype Using Monoclonal Rh Phenotype Gel Cards*

E. *ID-MTS Quick Reference Card: Preparing Patient and Donor Cell Suspensions*

Attachments

Selected Cell Sticker (02/17/2021)

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	7/6/2021
	Vaishali Pansare: Chief, Pathology	6/25/2021
	Ryan Johnson: OUWB Clinical Faculty	6/25/2021
	Ann Marie Blenc: System Med Dir, Hematopath	6/24/2021
	Muhammad Arshad: Chief, Pathology	6/23/2021

Step Description	Approver	Date
	John Pui: Chief, Pathology	6/23/2021
Policy and Forms Steering Committe (if needed)	Billie Ketelsen: Mgr Laboratory	6/23/2021
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	6/22/2021
	Craig Fletcher: System Med Dir, Blood Bank	6/22/2021
	Anji Miri: Supv, Laboratory	6/10/2021
	Karrie Torgerson: Supv, Laboratory	5/18/2021
	Kelly Sartor: Supv, Laboratory	5/18/2021
	Michael Rasmussen: Supv, Laboratory	5/17/2021
	Teresa Lovins: Supv, Laboratory	5/12/2021
	Billie Ketelsen: Mgr Laboratory	5/11/2021
	Billie Ketelsen: Mgr Laboratory	5/11/2021

Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne