PROCEDURE
Corewell Health East - Making a Test Red Cell Suspension - All Beaumont Hospitals

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Grosse Pointe Hospital, Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital, Corewell Health Farmington Hills Hospital, Corewell Health Taylor Hospital, Corewell Health Trenton Hospital, Corewell Health Wayne Hospital, Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Blood Bank

1. Principle
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.

2. Responsibility
Personnel who have completed the competency requirements will perform this testing.

3. Definitions
A. DAT: Direct Antiglobulin Test
B. RBC: Red Blood Cell
C. LISS: Low Ionic Strength Saline
D. Wharton's Jelly: A gelatinous substance on cord blood cells that can interfere with laboratory testing if not adequately removed

4. Specimen
A. Test red cell suspensions may be made from:
   1. Patient Samples
   2. Donor RBC segments
   3. Commercial reagent red blood cells

5. Reagent/Equipment Needed
A. Test tubes, 10 x 75 mm or 12 x 75 mm
B. Segment splitters or scissors
C. Transfer pipettes
D. Buffered saline
E. MTS™ Diluent 2
F. MTS™ Diluent 2 Plus

G. MTS™ pipette tips
H. Pipette tips
I. Electronic or manual pipette

6. Policies

A. Red cell suspensions must be free of hemolysis and interfering proteins or immunoglobulins.
B. Red cell suspensions should be made immediately prior to testing.
C. All test tubes used when making a red cell suspension must be labeled appropriately to identify the tube's contents.
    1. Red cell suspensions made from patient samples or donor segments should be labeled with the patient's last name or the donor unit number, and discarded immediately after testing.
D. When a reagent red cell is diluted from a 3% suspension to a 0.8% suspension, the diluted reagent suspension may be kept up to 24 hours if stored at 2°C-8°C (not to exceed the original expiration date of the reagent red cell).
    1. If the diluted 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.
    2. If the diluted suspension is retained following the initial testing, it should be labeled with a *Selected Cell Sticker* (see attachment), consisting of:
        a. Test Cell Number (from panel)
        b. Panel manufacturer and lot number
        c. Original expiration date
        d. New expiration date and time
        e. Date of preparation and Technologist initials
        f. Storage Requirement
E. Expired test/panel cells should not be used unless in-date cells with the desired antigenic profile are unavailable.
    1. If using a diluted cell suspension of expired reagents, then the diluted cell should be discarded immediately after testing and cannot be given an extended outdate of 24 hours.
F. To obtain donor RBCs, donor segments may be cut with scissors or a segment splitter.
G. 0.8% cell suspensions are used in the MTS™ gel system.
H. To help prevent contamination, the MTS™ Diluent 2 and MTS™ Diluent 2 Plus bottles should not be entered with a pipette and the tip of the bottle dispenser should not touch the sample or test tube. 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 the diluent from the test tube as opposed to the diluent bottle.

7. Procedure

A. Preparing a 2-4% Red Cell Suspension for Tube Testing from Packed RBCs

1. Obtain the specimen from which the 2-4% cell suspension will be made.
    a. 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 the tube contains a 2-4% suspension
3. Using a transfer pipette, transfer a few drops of packed RBCs from the patient sample or donor segment into the labeled test tube.
4. If any of the following conditions are met, wash the contents of the labeled test tube 1 to 4 times with buffered saline, decanting the saline completely after each wash:
    a. Hemolysis is present
    b. The sample exhibits Rouleaux formation
    c. A strong autoantibody is present
    d. The sample contains Wharton's Jelly
    e. LISS will be used during testing
    f. A donor RBC segment will be used in AHG tube crossmatching
    g. Antigen typing will be performed

5. After the final wash (if completed), add enough buffered saline to the test tube to suspend the cells to 2-4%, comparing the consistency and color to a 2-4% commercial red cell reagent.
6. Gently mix using a transfer pipette.

B. Preparing a 0.8% Reagent Red Cell Suspension from a 3% Reagent Red Cell Suspension

1. To produce enough volume for 1-3 uses:
   a. Dispense 100 µL of a 3% reagent red cell into an empty test tube, using either a manual or electronic pipette.
   b. Add a few drops of MTS™ Diluent 2 into the test tube.
   c. Centrifuge the test tube for 60 seconds to pack the RBCs.
   d. Completely remove the supernatant.
   e. Dispense 200 µL of MTS™ Diluent 2 into the test tube of packed RBCs.
   f. Gently mix using a transfer pipette.
2. To produce greater quantities (more than 30 uses):
   a. Use 500 µL (0.5 mL) of 3% reagent red cell and 1.5 mL of MTS™ Diluent 2.
3. Note: The volume produced may be increased based on testing requirements but the 1:2 ratio of 3% reagents cells to MTS™ Diluent 2 should remain constant up to 30 uses. When preparing sufficient volume for 30 or more tests, Ortho Clinical Diagnostics recommends a 1:3 ratio of 3% reagents cells to MTS™ Diluent 2.

C. Using an Electronic Pipette to Prepare a 0.8% Red Cell Suspension from Packed RBCs

1. For use in the MTS™ gel system (e.g. for Auto Control, DAT, Antigen Typing, or Donor Cell/Crossmatch)

   a. Label an empty test tube with the patient's last name or donor unit number.
   b. Dispense 1 mL MTS™ Diluent 2 into the test tube.
   c. Obtain the patient sample or donor RBC segment from which the 0.8% red cell suspension will be made.
   d. If necessary, centrifuge the patient sample or donor RBCs to obtain packed red cells.
   e. Use Program #6 of the electronic pipette to prepare the 0.8% red cell suspension. The Program #6 steps are as follows:
      1) Fill the MTS™ pipette tip with 100 µL of the MTS™ Diluent 2 from the test tube.
      2) Aspirate 15 µL of air into the MTS™ pipette tip.
      3) Fill the pipette tip with 10 µL of packed RBCs.
      4) Purge all contents from the pipette into the labeled test tube.
      5) Mix the contents of the test tube, keeping the pipette tip submerged in the tube's contents. The program mixes 3 times.
      6) Eject all the contents of the pipette into the test tube.
      7) If the 0.8% red cell suspension does not appear adequately mixed, it may be necessary to mix the contents with a transfer pipette.

D. Using a Manual Pipette to Prepare a 0.8% Red Cell Suspension from Packed RBCs

1. For use in the MTS™ gel system (e.g. for Auto Control, DAT, Antigen Typing, or Donor Cell/Crossmatch)

   a. Label an empty test tube with the patient's last name or donor unit number.
   b. Dispense 1 mL MTS™ Diluent 2 into the test tube.
   c. Obtain the patient sample or donor RBC segment from which the 0.8% red cell suspension will be made.
      1) If necessary, centrifuge the patient sample or donor RBCs to obtain packed red cells.
   d. Pipette 10 µL of the packed donor or patient cells into the labeled test tube.
   e. Gently mix using a transfer pipette to create a 0.8% red cell suspension.

E. Using an Electronic Pipette to Prepare a 4% ± 1% Red Cell Suspension from Packed RBCs

1. For use in the MTS™ gel system (e.g. for ABO/Rh Testing or Rh Antigen Typing)

   a. Label an empty test tube with the patient's last name or donor unit number.
   b. Label a second empty test tube stating it will contain MTS™ Diluent 2 Plus.
   c. Dispense at least 200 µL (0.2 mL) of MTS™ Diluent 2 Plus into the second tube.
   d. Obtain the patient sample or donor RBC segment from which the 4% red cell suspension will be made.

1) If necessary, centrifuge the patient sample of donor RBCs to obtain packed red cells.

e. Use Program #7 of the electronic pipette to prepare the 4% red cell suspension. The Program # steps are as follows:
   1) Fill the MTS™ pipette tip with 200 µL of the MTS™ Diluent 2 Plus from the second test tube.
   2) Aspirate 15 µL of air into the MTS™ pipette tip.
   3) Fill the pipette tip with 10 µL of packed RBCs.
   4) Purge all contents from the pipette into the test tube labeled with the patient's last name or donor unit number.
   5) Mix the contents of the test tube, keeping the pipette tip submerged in the tube's contents. The program mixes 3 times.
   6) Eject all the contents of the pipette into the test tube labeled with the patient's last name or donor unit number.
   7) If the 4% red cell suspension does not appear adequately mixed, it may be necessary to mix the contents with a transfer pipette.

F. Using a Manual Pipette to Prepare a 4% ± 1% Red Cell Suspension from Packed RBCs

1. For use in the MTS™ gel system (e.g. for ABO/Rh Testing or Antigen Typing)

a. Label an empty test tube with the patient's last name or donor unit number.
b. Dispense 0.5 mL of MTS™ Diluent 2 Plus into the test tube.
c. Obtain the patient sample or donor RBC segment from which the 4% red cell suspension will be made.
   1) If necessary, centrifuge the patient sample of donor RBCs to obtain packed red cells.
d. Add 25 µL of packed RBCs to the tube.
e. Gently mix using a transfer pipette to create a 4% ± 1% red cell suspension.

8. Limitations

A. Too heavy or too light of a red cell suspension may cause false negative or false positive results.
B. Using a segment splitter to obtain donor RBCs has a greater chance of hemolyzing the cells.

9. Revisions

Corewell Health reserves the right to alter, amend, modify, or eliminate this document at any time without prior written notice.

10. Resources

A. ID-MTS™ Quick Reference Card: Preparing Patient and Donor Cell Suspensions

11. References

A. Cohn, C. S., Delaney, M., Johnson, S. T., Katz, L. M., & Schwartz, J. (2023). *Technical manual*. AABB.

12. Procedure Development and Approval

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13. Keywords

Not Set