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Saline Replacement Technique for Patients with Rouleaux - Blood Bank

Document type: Procedure

I. PURPOSE AND OBJECTIVES:

A. This document will provide Blood Bank staff with instructions for resolving problems resulting from rouleaux formation/interference in patient samples.

II. CLINICAL SIGNIFICANCE:

A. Difficulties may arise in forward or reverse ABO/Rh typing or crossmatching procedures on patients who have abnormalities of their serum proteins because of the tendency for rouleaux formation, which is often confused with agglutination. Rouleaux may be present in the samples of patients who have multiple myeloma, liver disease, glomerulonephritis, tuberculosis or other chronic infections, and malignancies. It is also observed in the samples of patients who have had recent intravenous infusions of macromolecular agents such as dextran and PVP.

B. Rouleaux consists of aggregates of red cells that may give a "stacked coin" appearance when observed microscopically. The aggregates may have a copper metallic luster and are refractile, like droplets of oil. Rouleaux formation results from a patient's abnormal serum protein concentration and is an in-vitro phenomenon. All tested cells may appear agglutinated at room temperature and at 37°C, including the patient's own cells. It may be difficult to detect true agglutination in a sample with rouleaux but is generally not observed in indirect antiglobulin procedures because the serum is washed away. The saline replacement technique may be used to detect directly agglutinating antibodies when rouleaux formation is present.

III. SPECIMEN COLLECTION AND HANDLING:

The preferred specimen is a 6 mL EDTA sample with affixed identifying label in accordance with Transfusion Medicine policy, Triaging And Identifying Acceptable Samples For Testing- Blood Bank.

IV. REAGENTS / SUPPLIES / EQUIPMENT:

A. Saline
B. 10 x 75 mm or 12 x 75 mm test tubes
C. Microscope
D. Centrifuge
E. Transfer pipettes

V. POLICIES:

A. All observed test results must be recorded immediately, and the final interpretation will be made upon completion of testing.
B. All observed test results shall be recorded in the Blood Bank computer or on a downtime form if applicable.
C. In order to document that rouleaux is present in a patient sample, the results must be observed with the aid of a slide and microscope. Rouleaux formation usually appears stronger the longer the slide sits under the microscope.
D. If rouleaux is observed in the forward ABO or Rh typing, then wash the cells, as described below in section VI. A *Forward Cell Typing: Using a Washed Cell Suspension*. Do not use the *Saline Replacement Technique*.
E. If rouleaux is observed in the reverse ABO typing, the *Saline Replacement Technique* described section VI.B may be used to eliminate the interference.
F. If rouleaux is suspected to interfere with an immediate-spin crossmatch, then the *Saline Replacement Technique* may be used to eliminated the interference.
G. If explained agglutination of red cells is still observed after using the either of the techniques below then:
    1. ABO or Rh results are invalid and can not be interpreted. The ABO interpretations must be blank and the Transfusion Medicine policy, *Resolution of ABO and Rh Discrepancies* must be consulted for additional action.
    2. The crossmatch must be interpreted as incompatible for immediate spin crossmatches.
    3. Consult the Lead Medical Technologist, Supervisor or Medical Director if necessary.

VI. PROCEDURE:

A. Forward Cell Typing: Using a Washed Cell Suspension

1. Proceed from ABO/Rh testing procedure (in which rouleaux interfered).
2. Wash the patient's red cells with saline a minimum of 3 times by hand or with an automated cell washer.
3. Resuspend to a 2 - 4% suspension in saline.
4. Repeat the forward ABO and Rh types.
5. Record results in Blood Bank computer in accordance with section VI.C *Resolution & Documentation of Test Results when Rouleaux Formation is Present*.

Note: If Forward typing is not resolved with routine cell washing, it may be beneficial to wash with warm saline.

B. Saline Replacement Technique

1. Proceed from the testing procedure in which rouleaux interfered (i.e. Reverse ABO typing or an immediate-spin crossmatch).
2. Repeat the test (in which rouleaux interfered) by the standard procedure.
   Note: If rouleaux was detected in the reverse of an ABO/Rh test, then both the "a" and "b" reverse test cells must be repeated using this saline replacement procedure, regardless of the patient's apparent blood type.
3. Centrifuge the tube(s) as indicated in the standard testing procedure.
4. Remove the tube(s) from centrifuge using caution to not disturb the plasma.
5. Using a clean pipette, remove and discard the plasma.
6. Replace the discarded plasma with an equal volume of saline. i.e. Assuming two (2) drops of patient plasma were used when performing the reverse typing, then two (2) drops of saline should be used for the replacement.
7. Resuspend the cell button gently and read macroscopically for agglutination.
   a. If rouleaux is not yet dispersed, repeat steps 3 - 9 (up to 3 times) until the rouleaux is dispersed.
8. Record results in Blood Bank computer in accordance with section VI.C *Resolution & Documentation of Test Results when Rouleaux Formation is Present* below.

C. Resolution & Documentation of Test Results when Rouleaux Formation is Present

1. Once rouleaux is confirmed, add the message **ROULX** ,"Rouleaux Formation Present", to the patient's demographics in the Blood Bank Computer. Refer to *Blood Bank CDM - Add/Delete/*

Edit/Display Patient Messages.

2. Save the initial test results, which indicate the presence of rouleaux, in the Blood Bank computer but do not interpret these invalid results (leave the ABO interpretation blank).
3. Repeat the testing, using one of the appropriate methods, either the *Forward Cell Typing: Using a Washed Cell Suspension* or the *Saline Replacement Technique*.
4. Document the repeated test results from Step 3, above, in the Blood Bank computer.
5. Add a comment in the Blood Bank computer to the test result indicating which method (from Step 3, above) was used.
   Refer to *Blood Bank CDM - Adding the ABORh Canned Message to Document ABORh Discrepancies*.
6. Interpret the Results.
   a. For ABO results
      i. If the rouleaux was dispersed after performing the steps in this procedure so that valid graded reactions were obtained, interpret the reactions.
      ii. If the rouleaux was not dispersed, do not interpret the invalid graded reactions (leave the ABO interpretation blank) and refer to Transfusion Medicine policy, *Resolution of ABO and Rh discrepancies*.
   b. For immediate-spin crossmatches
      i. If the rouleaux was dispersed after performing the steps in this procedure, then the crossmatch may be interpreted as compatible. Refer to Transfusion Medicine policy, Serologic Crossmatching of Red Blood Cells.
      ii. If the rouleaux was not dispersed, the crossmatch must be interpreted as incompatible.

VII. EXPECTED RESULTS:

A. Rouleaux should be dispersed if present using either of the above methods because the protein has been diluted.

B. If the apparent "agglutination" is no longer present, it may be assumed that rouleaux was the cause of the "false incompatibility".

C. True agglutinates resulting from antibody-antigen reaction should survive the manipulation (washing patient RBCs or saline replacement). If agglutination persists, it must be assumed that the serum and red cells are truly incompatible.

VIII. NOTES:

A. Rouleaux should NOT occur in the antiglobulin phase since the cells are washed free of plasma prior to that phase.

B. When the cell typing is affected by rouleaux, the serum typing will also typically be affected. However, the serum may be affected without the cell typing being affected when the cell typing is performed with a saline suspension of the test red cells.

C. When viewed microscopically, rouleaux formation usually appears stronger the longer the slide

sits under the microscope.

IX. REFERENCES:

1. AABB, *Technical Manual,* current edition.
2. AABB, *Standards for Blood Bank and Transfusion Medicine*, current edition.
3. College of American Pathologists, *Transfusion Medicine Checklist,* current edition.

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