TITLE:

TEST RESULT DISCREPENCIES

PURPOSE

To be considered valid, the results of red cell grouping and serum grouping should agree. This method describes a general approach to the initial investigation of an ABO grouping discrepancy caused by either missing reactions or unexpected positive reactions. A detailed discussion on ABO grouping is found in Chapter 12 of the AABB *Technical Manual*, 18th edition.

DEFINITIONS

N/A

POSSIBLE CAUSES

~~ABO/Rh or other test result discrepancies can be caused by a variety of factors, from clerical/tech errors to patient characteristics. Common causes for specimen problems are:~~

1. A patient who has received red cell transfusions or marrow transplant may have cells circulating of more than one ABO/Rh type. Serum antibodies may not agree with red cell antigens.
2. Abnormal concentrations of serum proteins, such as Wharton's jelly in cord blood samples may cause nonspecific aggregation.
3. Potent cold-reactive autoagglutinins may cause red cells to spontaneously agglutinate in the presence of diluent, independent of the specificity of the reagent antibody.
4. Small fibrin clots may be mistaken for agglutinates if plasma or incompletely clotted serum is used. Spinning the sample for the suggested five minutes usually will remove fibrin from the serum/plasma.
5. Abnormal concentrations of proteins can cause nonspecific red cell aggregation or rouleaux, that is difficult to distinguish from true agglutination. Rouleaux formation is easily recognized on microscopic examination if the red cells assume what has been described as a "stack of coins" formation.
6. Antibodies other than anti-A and anti-B in a test sample can agglutinate reagent A_1 or B red cells if they carry the corresponding antigen. For example, if the patient has a room temperature reactive anti-M, it may cause an unexpected reaction with M-positive A_1 and/or B reagent red cells.
7. Patients who are immunodeficient due to disease or therapy may have such depressed immunoglobulin levels that there is little or no ABO agglutinin activity. Samples from elderly patients whose antibody levels have declined with age, or from patients whose antibodies have been greatly diluted by plasma exchange procedures may also have weak agglutinins.
8. Negative or weak results are seen in serum test from infants under 4-6 months of age.

POLICY

1. Any testing discrepancy encountered during testing must be resolved. This includes, and is not limited to:
   a. Front Type/Back Type discrepancies on the current specimen.
   b. Result discrepancies between current and historical test results on file.
2. If any discrepancy is unresolved by using the following steps, the sample must be sent to United Blood Services Immunohematology Reference Lab (IRL) for resolution as soon as possible.~~(1-800-288-2199 EXT. 7133).~~
3. If transfusion is necessary prior to resolution of the discrepancy, transfuse with type O, Rh compatible red blood cells, and AB fresh frozen plasma.

4. When a discrepancy is encountered, the discrepant results must be recorded, but interpretation of the ABO/Rh group or other test results must be delayed until the discrepancy is resolved.
5. If the specimen is from a donor unit, the unit must not be released for transfusion until the discrepancy is resolved. Contact United Blood Services, for further instruction on handling of the unit if the discrepancy can not be resolved.
6. **CLERICAL/TESTING ERRORS:** To eliminate error as the cause of the discrepancy:
   a. Repeat current testing. See common causes of False-Negative and False-Positive results in ABO/Rh testing listed below.
      1) False-Negative Results
         a) Reagent or test serum not added to a tube
         b) Hemolysis not identified as a positive reaction
         c) Inappropriate ratio of serum (or reagent) to red cells
         d) Tubes incubated at temperatures above 20-24°C
         e) Incorrect interpretation or recording of test results
      2) False-Positive Results
         a) Over centrifugation of tubes
         b) Use of contaminated reagents, red cells, or saline
         c) Use of dirty glassware
         d) Tubes not centrifuged sufficiently
         e) Incorrect interpretation or recording of test results
   b. Check patient identity.
      1) Patient must be wearing a Blood Bank ID (BB ID) band with MRN and BB ID number that matches the information on the blood specimen.
      2) Check medical record number and date of birth for patient in question.
      3) If there is any unresolved discrepancy or doubt about the identity of the patient, the patient must be redrawn, and the sample must be retested.
   c. If possible, check previous worksheets for actual serological reactions. Determine whether previous reactions match current findings and previous and current interpretations.
   d. If a discrepancy still exists and all current testing and patient identification is shown to be correct, enter a Blood Bank Comment describing the discrepancy and its resolution.
   e. An "~~*Situation* Investigation~~ *Error Variance Report*" must be initiated when a discrepancy is found to be due to a clerical/technologist error.
7. **RESULT DISCREPANCIES:**
   a. Absence of expected antigens
      1) Red cells of most A or B people are strongly agglutinated (3-4+) by the corresponding reagent antibody.
      2) A or B antigens may be weakly expressed on cells from individuals who have inherited variant alleles, or with disease-related antigen depression.
      3) To enhance the detection of weakly expressed antigens:
         a) Incubate washed red cells with anti-A, anti-B, and anti-A,B for 30 minutes at room temperature or 4°C to enhance antibody attachment.
         b) Perform the "Weak D" test, if the expected antigen is the D antigen (See procedure "*ABO and Rh Testing*").
         c) To help remove cold-reactive IgM autoagglutinins, incubate the cell suspension at 37°C for 5 minutes and then wash the cells several times with saline warmed to 37°C prior to testing.
      4) Obtain a new blood specimen from the donor unit or patient and retest the sample.
      5) Obtain the patient's history of diagnosis, transfusion, marrow transplantation and medications. One of these factors may be the cause of the discrepancy.

6) Wash the test and reagent red cells several times to remove any serum or chemical constituents that may be causing unexpected positive reactions.

7) Review the antibody screen test results to determine if there are interfering effects from cold-reactive allo- or autoantibodies. It may be necessary to perform an immediate spin and/or 4°C antibody screen with an autocontrol.

b. Unexpected Serum Reactions

1) Check the age and diagnosis of the patient. Immunodeficient, newborn or elderly persons may have decreased or absent levels of antibodies.

2) To enhance weakly reacting anti-A and anti-B antibodies:

a) Incubate the patient's serum/plasma with known A_1 and B cells for up to one hour at room temperature or 4°C.
b) If incubation is performed at 4°C, run parallel testing of patient serum/plasma against autologous red cells AND type O cells to rule out interference by broad reacting agglutinins. To obtain a type O cell, select any cell from any set of ~~3~~ **2-5%** antibody screen or panel cells.

3) If an anti-A_1 is suspected, send to the IRL for further testing.

4) Strongly reactive cold autoagglutinins can agglutinate red cells of adults, including autologous cells and reagent red cells, at room temperature.

a) Agglutination caused by cold-reactive autoagglutinins is usually weaker than that caused by anti-A and anti-B.

b) Perform the following steps to help eliminate reactivity of cold autoagglutinins.

1. Warm the serum and reagent red cells to 37°C before mixing and testing.
2. Read serum tests at 37°C and convert them to the antiglobulin phase, if necessary.
3. Weakly reactive examples of IgM anti-A or anti-B may not be detected by this method because 37°C is above the optimal temperature of reactivity for these antibodies.

5) Sera from patients that have received plasma expanders, or with abnormal concentrations of serum proteins or altered serum protein ratios, can aggregate reagent red cells and mimic agglutination.

a) Some can cause aggregation described as "rouleaux".
b) Saline replacement may be used to decrease reactivity of this type. (See the "*Saline Replacement*" procedure).

6) Obtain a new blood specimen from the donor unit or patient and retest the sample.

7) Obtain the patient's history of diagnosis, transfusion, marrow transplantation and medications. One of these factors may be the cause of the discrepancy.

8) Wash the test and reagent red cells several times to remove any serum or chemical constituents that may be causing unexpected positive reactions.

9) Review the antibody screen test results to determine if there are interfering effects from cold-reactive allo- or autoantibodies.

RELATED DOCUMENTS N/A

REFERENCES

American Association of Blood Banks, 18th Ed. (2014). *Technical Manual*. Bethesda, Maryland: AABB.
American Association of Blood Banks, 30th Ed. (2015). *Standards for Blood Banks and Transfusion Services*. Bethesda, Maryland: AABB.