Status **Pending** PolicyStat ID **16784810**

Beaumont

Origination	12/15/2021
Last Approved	N/A
Effective	5 Days After Approval
Last Revised	9/25/2024
Next Review	2 years after approval

Document Contact	Kelly Sartor: Mgr, Division Laboratory
Area	Laboratory-Blood Bank
Applicability	All Beaumont Hospitals

Reading, Grading, and Recording Test Reactions - Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide the Blood Bank staff with instructions for reading, grading, and recording Blood Bank test reactions.

II. POLICIES:

A. All Blood Bank personnel should be consistent in grading reactions and interpreting test results.

B. Test tube reactivity must be assessed when the red cells have been completely resuspended from the button.

C. The lighted viewing mirror must be used as an optical aid in reading test tube results.

D. Microscopic observation is not routinely recommended but may be useful in distinguishing patterns of agglutination.

E. Observed test results must be recorded immediately and concurrently upon completion of a critical activity and the final interpretation will be made upon completion of testing.

F. Observed test results shall be recorded in the Blood Bank computer system (BBIS) or on a downtime form.

III. CLINICAL SIGNIFICANCE:

A. **Rouleaux**

1. For patients whose samples display rouleaux formation, refer to Transfusion Medicine policy, Saline Replacement Technique for Patients with Rouleaux - Blood Bank.

B. **Mixed-Field Reactions**

1. In most cases, recent transfusion histories should be obtained on patients whose samples display mixed field reactivity.
2. Refer to the following Transfusion Medicine policies:
    a. Obtaining Patient Histories - Blood Bank
    b. Resolution of ABO/Rh Discrepancies - Blood Bank
3. Dual population mixed-field reactions observed in the gel system and tube method should be recorded in the Blood Bank computer as mixed field (2MF).

C. **Hemolysis**

1. Hemolysis observed in testing is considered a positive reaction when the sample used for the testing was not itself hemolyzed.
2. Tests performed with a hemolyzed sample may create difficulties in evaluating test results and antibody-induced hemolysis may be masked. Refer to Transfusion Medicine Policy, Triaging and Identifying Acceptable Samples For Testing
3. It may be difficult to distinguish a sample that is hemolyzed due to collection technique from a sample involving a hemolytic transfusion reaction. Therefore, if a hemolyzed sample was collected as part of a transfusion reaction evaluation, a 2nd post sample should be collected, to help determine whether the hemolysis is in vivo or due to collection technique. Refer to Transfusion Medicine policy, Transfusion Reaction Investigation and Workup.

IV. DEFINITIONS:

A. **Rouleaux:** An in-vitro phenomenon whereby aggregates of red cells may give a “stacked coin” appearance when observed microscopically. The aggregates may have a copper metallic luster and are refractile, like droplets of oil.

B. **Hemolysis:** Pink or red supernatant fluid in the test system that occurs when the red cell membrane ruptures.

C. **Mixed field (MF):** A sample that contains 2 distinct populations of red cells, usually as a result of recent RBC transfusions of a dissimilar ABO or Rh type as the patient. A mixed-field reaction results when one population is agglutinated in testing, while the other population is not.

D. **Reading:** Observing the hemolysis or agglutination that constitutes the visible endpoint of a red cell antigen - antibody interaction.

E. **Grading:** Determining the strength of agglutination (or adherence as seen in solid-phase methods), or the degree of hemolysis.

F. **Recording:** Capturing the graded reaction in writing or electronic media

G. **Critical Activity:** A procedure conducted by a qualified laboratory employee that yields test results. For manual tube testing, a critical activity may consist of up to 3 test tubes at a time.

H. **BBIS:** Blood Bank Information System

V. REAGENT / EQUIPMENT / SUPPLIES

See applicable *Standard Operating Procedures* for reagents, equipment and supplies needed for each particular function.

VI. SPECIMEN COLLECTION AND HANDLING:

See applicable *Standard Operating Procedures* for the specimen required for each particular test.

VII. PROCEDURE:

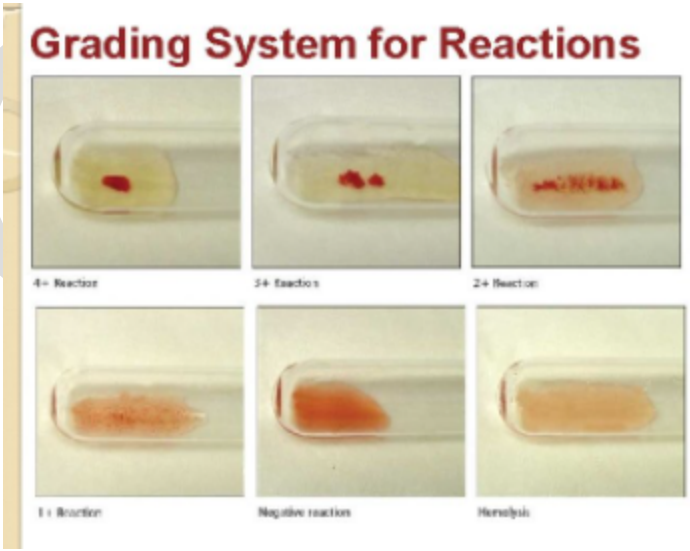
A. **Reading and Grading Test Tube Reactions**

1. Proceed from the applicable procedure after the centrifugation step of the test system (e.g. DAT, antibody screen).
2. Observe the supernate for hemolysis.
3. Disrupt the red cell button by gently shaking and/or tilting the tube while observing the button using a lighted viewing mirror.
4. While using the lighted viewing mirror, continue to gently shake and tilt the tube until the red cell button is completely resuspended.
5. Observe cell dispersion with lighted viewing mirror and grade reactions as described below.
6. Immediately and concurrently record graded test tube results in the BBIS or on the appropriate downtime form.

Test Tube System Graded Reactions

Graded Reaction	BBIS Reaction Code	Appearance of Test Tube System	Notes
4+	4	One solid agglutinate in a clear background	
3+	3	Several large agglutinates in a clear background	
2+	2	Medium-size agglutinates in a clear background	
1+	1	Small agglutinates in a turbid	

Graded Reaction	BBIS Reaction Code	Appearance of Test Tube System	Notes
		background	
w+	W+	Barely visible agglutination in a turbid background	
0	0	No agglutination or hemolysis	
MF	2MF	Mixed field: a mixture of agglutinated and unagglutinated RBCs	
RU	R	Rouleaux: aggregates of red cells that can look like "stacked coins"	
H	H	Rupture of red cell causes the plasma to be pink or red in color	
m+	M	Reactions that are observed only microscopically	Not routinely indicated



B. **Reading and Grading Microtube Reactions**

1. Proceed from the applicable procedure after centrifugation of the gel microtube test system
2. Read the front and back of each gel card macroscopically for hemolysis and agglutination.
    a. If either side is reactive, the entire reaction is considered positive
3. Grade the microtube reactions as described below.
4. Immediately and concurrently record graded test tube results in the LIS or on the appropriate downtime form.

Gel Microtube System Graded Reactions

Graded Reaction	BBIS Reaction Code	Appearance of Gel Test System	Notes
4+	4	A solid band of agglutinated red cells are observed on the top of the gel.	A few unagglutinated red cells may filter into the gel near this band
3+	3	The majority of the red cell agglutinates are trapped in the upper half of the microtube.	
2+	2	Red cell agglutinates are dispersed throughout the length of the microtube.	A few unagglutinated red cells may be observed on the bottom of the microtube.
1+	1	Red cell agglutinates are observed predominantly in the lower half of the microtube with an unagglutinated red cell button on the bottom of the microtube.	The red cell button at the bottom of the microtube may be disrupted.
w+	W+	A small number of red cell agglutinates are observed just above the red cell button near the bottom of the microtube.	
0	0	Unagglutinated red cells form a well-defined button at the bottom of the tube and hemolysis is absent.	
MF	2MF	mixed field is characterized by agglutinated red cells on top of the gel or dispersed throughout the microtube with an unagglutinated red cell button on the bottom of the microtube.	Not all mixed cell situations have a sufficient minor population to be detected. Mixed-field reactions typically occur only in testing with patient's cells, not in testing with the patient's plasma.
H	H	Hemolysis will color the liquid portion above the gel a pink or red color depending on the degree of hemolysis.	

VIII. LIMITATIONS:

A. **ID-Micro Typing System™**

1. While the manufacturer does not specify a weakly positive reaction, reactions that are consistent with the appearance of w+ as described above, shall be graded as weakly positive.
2. Debris, fibrin, or other artifacts associated with plasma, cord blood, or frozen samples may cause a few unagglutinated red cells to be "trapped" on top of the gel.
    a. These tests may be interpreted as negative.
    b. Alternatively, the sample may be re-centrifuged after rimming it with wooden sticks and retested.

IX. REFERENCES:

A. AABB Technical Manual, current edition

B. Ortho Diagnostics Systems Ins., *ID-Micro Typing System™ Interpretation Guide,* Pub. No 6902201, 06-04-2010

C. AABB *Standards for Blood Banks and Transfusion Services*, current edition

Approval Signatures

Step Description	Approver	Date

Kristen DiCicco: Mgr, Laboratory	Pending
Fatima Bazzi: Supv, Laboratory	Pending
Hilary Morey: Medical Technologist Lead	Pending
Katherine Persinger: Mgr, Laboratory	Pending
Ashley Beesley: Mgr, Laboratory	Pending
Suzanne Chahine: Medical Technologist Lead	9/26/2024
Karrie Torgerson: Medical Technologist Lead [KS]	9/25/2024
Teresa Lovins: Supv, Laboratory [KS]	9/25/2024
Kelly Sartor: Mgr, Division Laboratory	9/25/2024
Kelly Sartor: Mgr, Division Laboratory	9/25/2024

Applicability

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