University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA, 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 02-11-16 Revision Effective Date: 06-20-2019	Number: PC-0027.02

TITLE: **Grading Reactions**

PURPOSE
To provide a standardized method among members of the Transfusion Service Laboratory (TSL) staff for grading of serological reactions

PRINCIPLE & CLINICAL SIGNIFICANCE
Strength of reactions are used to assist in the determination of testing for blood group typing, antibody screen, antibody identification, direct antigen testing, antigen typing, crossmatch and titration studies. A numerical value is assigned to the observed reactions based on the strength of agglutination or adherance in each test method.

POLICY
Reactions are read and recorded immediately in the LIS or appropriate manual testing form.

SPECIMEN REQUIREMENTS
NA

REAGENTS/SUPPLIES/EQUIPMENT

Reagents:	Supplies:	Equipment:
NA	NA	• Agglutination viewer • Microscope

QUALITY CONTROL
Quality Control is performed on day of use

Instructions

Table of Contents

Macroscopic Tube Grading

STEP	ACTION
1	Gently shake to resuspend the cell button **NOTE**: Holding the tube with the cell button up may facilitate resuspension
2	Use an aggluntination viewer to observe the way the cells are dispersed from the cell button

STEP	ACTION
3	Read the reactionas soon as the cell button is resuspended from the bottom of the tube observing the way the RBCs leave the cell button
4	Grade and record the degree/absence of agglutination according to tables in section Interpretation/Results Reporting and Results Reporting Tube Reaction Grades

Microscopic Tube Grading – not required unless specified in the SOP

STEP	Action
1	Turn on the light and set magnification to 10x on a light microscope
2	Place tube on a tube holder on the stage and rotate while viewing through the eye piece
3	Grade and record results the degree/absence of agglutination according to tables in section Interpretation/Results Reporting and Results Reporting Tube Reaction Grades

Ortho MTS™ Gel Grading

STEP	ACTION	
1	**If testing is performed via**	**Then**
	Ortho Vision	Read the reaction images and grading assigned by the Vision according to tables in section Interpretation/Results Reporting and Results Reporting Ortho Gel Reaction Grades
	Manual Ortho gel method	• Read macroscopically the front and back of each microtube for agglutination and/or hemolysis. • Grade and record the reactions according to tables in section Interpretation/Results Reporting and Results Reporting Ortho Gel Reaction Grades
2	Refer to **Appendix 1: Ortho GelTesting Trouble Shooting** for interpretation and resolution of reactions not found in Ortho Gel Grading table	

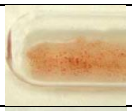
CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES:

Interpretation

Interpretation	Reaction Description
Positive Test	Agglutination, hemolysis or adherence in any phase indicates the presence of an antigen-antibody reaction
Negative Test	Absence of agglutination, hemolysis or adherence indicates the absence of a detectable antigen-antibody reaction
Invalid Test	Failure of the test system to produce agglutination following the addition of Antiglobulin Control Cells to a negative AHG tube test And/or Positve control reaction. The discrepancy must be investigated and resolved before reporting the results

Results Reporting

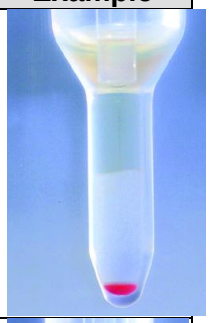
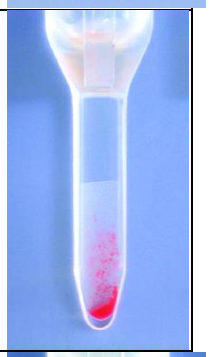
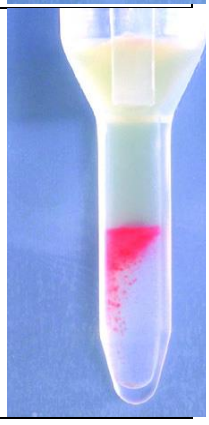
Tube Reaction Grades

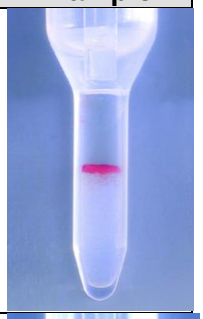
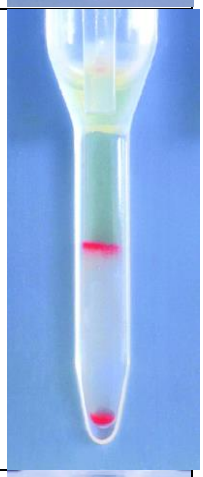
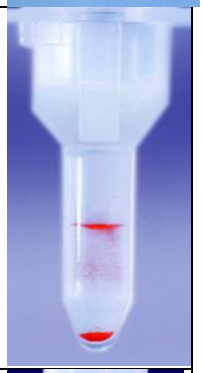
Reaction Description	Manual Grade	Sunquest Key	Example
No agglutination: Smooth cell suspension	0	0	
Very small agglutinates, turbid background	W+	W	None
Small agglutinates, turbid background	1+	1	
Many medium sized agglutinates, clear background	2+	2	
Several large agglutinates, clear background	3+	3	
One solid agglutinate, no free cells, clear background	4+	4	
Mixed field: looks like 1+ reaction on a background of unagglutinated cells	1+mf	6	None
Mixed field: looks like 2+ reaction on a background of unagglutinated cells	2+mf	7	None
Mixed field: looks like 3+ reaction on a background of unagglutinated cells	3+mf	8	None
Mixed field: looks like 4+ reaction on a background of unagglutinated cells	4+mf	9	None
Rouleaux: Microscopic examination reveals agglutinates in tight clumps or in the “stack of coin” configuration called rouleaux, which was dispersed following saline replacement	0R	R	None
Hemolysis observed (not graded in test reactions)	H	H	None
Not Done	ND	N	None
Reactivity of IgG coated control cells following a negative antiglobulin test.	✓	*+	None

*Any positive reaction may be entered, but it is not necessary to grade control cell reactivity

Ortho Gel Reaction Grades (Ortho Vision and Manual Method)

IMPORTANT: Reaction not found in this table should **NOT** be resulted – refer to **Appendix 1: Ortho GelTesting Trouble Shooting**

Reaction Description	Grade	Sunquest Key	Example
Negative Reactions: Unagglutinated red blood cells form a well-defined button in the bottom of the microtube. **NOTE**: Debris, fibrin, or other artifacts associated with serum, cord blood, or frozen samples may cause a few unagglutinated red blood cells to trap on top of the gel, but these tests should be interpreted as negative	0	0	
Red blood cell agglutinates predominantly observed in the lower half of the gel microtube. Unagglutinated red blood cells form a button in the bottom of the microtube. **NOTE:** Weaker reactions may be represented by few agglutinated red blood cells in the gel microtube in the area just above the red blood cell button near the bottom of the microtube. The button associated with these weaker reactions is often disrupted.	1+	1	
Characterized by red blood cell agglutinates dispersed vertically throughout the length of the gel microtube. A few unagglutinated red blood cells may be observed in the bottom of the microtube. **NOTE:** The horizontal position of the agglutinated cells within the microtube (i.e., their relative location front-to-back or side-to-side) should not be considered. The size of red blood cell buttons in the bottom of the microtube may vary.	2+	2	
Characterized by the majority of agglutinated red blood cells trapped in the upper half of the gel microtube. **NOTE:** A thick group of agglutinated red blood cells, or band, with some red blood cells dispersed below the predominant band in the upper half of the gel microtube. A 3+ reaction may also be characterized by an even distribution of agglutinated red blood cells in the upper portion of the gel. Occasionally, a few unsensitized red blood cells may migrate to the bottom of the microtube.	3+	3	

Reaction Description	Grade	Sunquest Key	Example
Characterized by a solid band of agglutinated red blood cells on top of the gel. **NOTE**: The size of these agglutinated red blood cells makes it difficult for red blood cells to migrate into the gel. Occasionally, a few unsensitized red blood cells may migrate to the bottom of the microtube but the middle of the gel should remain free from agglutinated red blood cells.	4+	4	
Mixed field is characterized by agglutinated red blood cells on top of the gel or dispersed throughout the microtube and accompanied by a button of unagglutinated red blood cells in the bottom of the microtube. **NOTE:** The potential exists for unexpected or mixed field results in samples from recently transfused patients, bone marrow transplant patients, and patients with blood group chimerism due to the existence of two distinct, separable populations of red blood cells in the sample. It may be difficult to distinquish between a 1+mf and 2+mf.	MF	M	
Rouleaux are aggregations of red blood cells in a characteristic “stacking” pattern. Rouleaux caused by serum or plasma with abnormally high concentrations of protein may infrequently cause difficulties in gel test interpretation. **NOTE:** Resolve by using the tube test with Saline Replacement– refer to SOP *Saline Replacement*	0R	R	
Hemolysis observed. A pink or red appearance in the upper chamber that extends to the top of the gel microtube is most likely indicative of hemolysis, either from the use of a hemolyzed sample or as a result of complement binding during incubation. **NOTE:** If the red blood cell button is of normal size and the original sample is noted to be hemolyzed, the test should be interpreted as negative (as illustrated)	Enter appropriate reaction	Enter appropriate Key	 (Example: Neg)
Not Done	ND	N	NA

CALIBRATION:
NA

NOTES AND LIMITATIONS
Tube Method
- Reading agglutination reactions is somewhat subjective, technique dependent, and requires some degree of experience to recognize the fine differences in types/strengths of agglutination
- Failure to obtain a standard result may be due to incorrect technique, most commonly, shaking the tube too vigorously

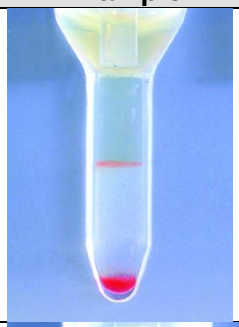
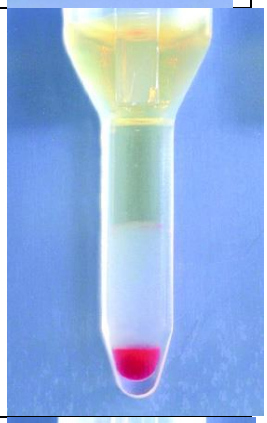
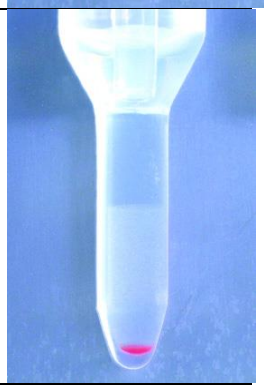
Gel Method
- It is recommended that reactions be read immediately following centrifugation.
- Interpretations may be affected by the drying out of the gel, hemolysis of the red blood cells, and slanting of the reaction patterns due to storage in a non-upright position.
- The manufacturer reports gel cards stored in the refrigerator (2–8 °C) and effectively protected from evaporation were able to be interpreted for more than 14 days from the time of testing.
- Failure to obtain a standard result may be due to incorrect testing sample preparation, poor pipetting technique, wrong testing volume pipetted, etc.

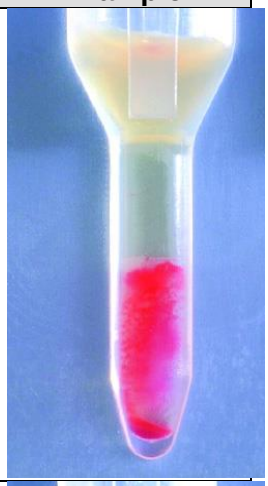
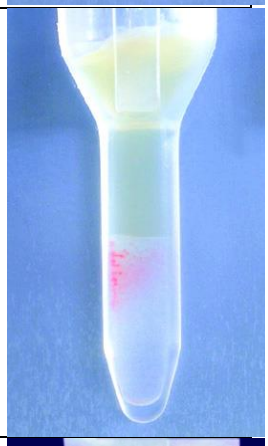
REFERENCES
- Technical Manual. Bethesda, MD; AABB, current edition.
- Standards for Blood Banks and Transfusion Services. Bethesda, MD; AABB, current edition
- Interpretation Guide ID-Micro Typing System™.Pompano Beach, FL: Micro Typing Systems, Inc. Current revision.

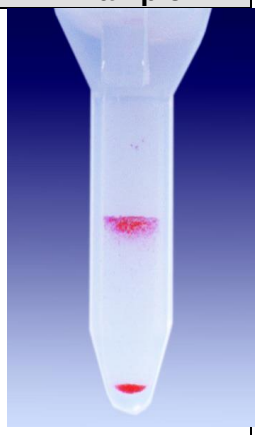
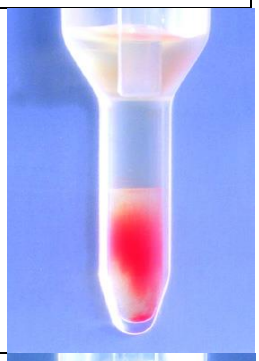
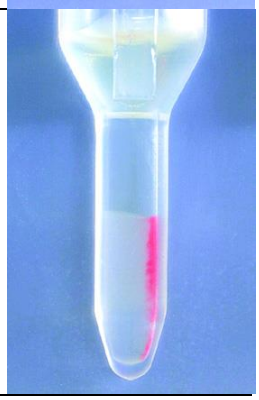
RELATED DOCUMENTS
Any SOPs that require agglutination grading of test results

APPENDIX

Appendix 1: Ortho GelTesting Trouble Shooting

Reaction Description	Discussion	Example
Reactions associated with sample quality issues	A few unagglutinated red blood cells may be observed on the top of the gel with some negative reactions. These reactions may be associated with the following sample quality issues: • Samples with clots, particulates, or other artifacts • Freshly collected serum samples • Samples previously frozen **NOTE**: Hard spin the testing cells and retest.	
Too many red blood cells in a negative reaction	Too many red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube. If the red blood cell button is large enough, weak reactions may be missed due to masking of red blood cell agglutinates in the bottom portion of the microtube. **NOTE**: Repeat the test with properly prepared red blood cell suspension and correct quantities of red blood cells.	
Too few red blood cells in a negative reaction	Too few red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube. If too few red blood cells are added to the gel card, there may not be enough red blood cells to make a valid test interpretation. **NOTE**: Repeat the test with properly prepared red blood cell suspension and correct quantities of red blood cells.	

Reaction Description	Discussion	Example
Too many red blood cells in a positive reaction	Too many red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, poor mixing of red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube. Variations in red blood cell concentration may affect the sensitivity of the test. It is possible when red blood cell suspensions are too concentrated that weaker reactions may be observed due to the increase in the antigen-antibody ratio. **NOTE**: Repeat the test with properly prepared red blood cell suspension, well mixed, and correct quantities of red blood cells.	
Too few red blood cells in a positive reaction	Too few red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, poor mixing of red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube. Variations in red blood cell concentration may affect the sensitivity of the test. When red blood cells are too low in concentration, they become difficult to see. **NOTE**: Repeat the test with properly prepared red blood cell suspension, well mixed, and correct quantities of red blood cells.	
Haze or Pink Color in Gel	Occasionally, gel tests may appear hazy or pink with no evidence of red blood cell agglutination. These reactions differ from typical positive or negative results. Hazy or pink color in gel tests may be associated with rouleaux which has been induced by high levels of abnormal proteins in the patient's serum or plasma. Information regarding the patient's diagnosis and total serum protein may help confirm this explanation. **NOTE**: If rouleaux is suspected as interfering with the reverse blood group test, switch totube test with saline replacement to resolve the discrepancy.	

Reaction Description	Discussion	Example
Direct Agglutinating Antibody	Samples containing strong, direct agglutinating antibodies may give the appearance of a mixed-field reaction in Anti-IgG gel antibody detection or identification tests. The most common direct agglutinins are cold enhanced IgM antibodies. It may be helpful to perform a room temperature or 37°C test procedure using the MTS™ Buffered Gel Card (Pipette 50 µL of the 0.8% reagent red cells first, followed by 25µL of plasma into microtube, then incubate at desired time and temperature (room temperature or 37± 2°C), and centrifuge). Reactivity only at room temperature or reactivity that is stronger at room temperature would be consistent with a strong IgM cold agglutinin in the test sample. These reactions are not truly mixed-field if the reagent red blood cell sample used for testing is from a single donor source.	
Improper Centrifugation (centrifugation interrupted)	If the centrifugation cycle is interrupted, unagglutinated red blood cells may be observed in the gel. These red blood cells will appear dark pink and hazy. **NOTE:** In these situations, tests should be repeated. Do **NOT** recentrifuge these gel cards under any circumstances. The recentrifugation of gel cards may dissipate weak reactions producing false negative results.	
Improper Centrifugation (cards not properly seated)	If cards are not properly seated in the card holders and not allowed to spin at a 90 degree angle during centrifugation, a line of red blood cells may stream down one side forming a “J” appearance. These tests should be repeated.	

UWMC SOP Approval:

UWMC CLIA Medical Director	Mark H. Wener, MD	Date	
Transfusion Service Manager	Nina Sen	Date	
Compliance Analyst	Christine Clark	Date	
Transfusion Service Medical Director	Monica Pagano, MD	Date	

UWMC Biennial Review:

	Date	
	Date	

06/20/2019 – Added grading reactions for the Ortho Gel Method and removed grading reactions for the Tango Optimo.