1. **Principle**

To standardize reading, grading and recording of hemagglutination reactions. To detect missing, extra or weak unexpected reactions that requires investigation.

A standardized procedure for recording graded reactions of hemagglutination will contribute to uniformity and reproducibility of test results.

1. **Scope and Related Policies**

This procedure applies to all tube testing that requires reading, grading and/or recording of agglutination test results.

1. **Specimens – N/A**
2. **Materials**

**Equipment**: Microscope

Serological centrifuge

**Supplies:** Test tubes – 10 x 75mm

1. **Quality Control**
	1. Hemolysis and agglutination are both visible endpoints of an antigen-antibody reaction.
	2. Reading skills proficiency testing should be done with staff performing testing using tube grading.
2. **Procedure**

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| **STEP** | **ACTION** |
| * 1. Examine for Hemolysis
 | * 1. Remove the test tubes from the centrifuge without disturbing the cell button.
	2. For direct agglutination tests, examine the supernatant for hemolysis.
	3. Record hemolysis if observed.
 |
| * 1. Examine for Agglutination
 | * 1. Hold the test tube upright between the thumb and forefinger, a visual aid may be used (e.g., illuminated concave mirror or lighted white background). Do not hold tubes up to the light above the face.
	2. Rotate the tube to provide the best view of the cell button.
	3. Mix contents by holding the tube at an angle, gently tilting the tube until all the red cells are dislodged from the bottom of the tube.
	4. Stop mixing the contents as soon as all the cells are dislodged from the bottom of the tube. DO NOT OVERMIX***Note: If the tube has been correctly centrifuged, the cell button will be well formed and will dislodge easily.***
	5. Slowly and gently, tilt the tube until the contents run about one third of the way down the tube and observe the cells for agglutination. Read, grade and record results. See 7.0 – Reporting.
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| * 1. Grading Agglutination
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| ***If*** | ***Then*** |
| the test is an ABO or Rh grouping, or antibody screen or panel incubated at room temperature,  | * Read macroscopically only (unless a discrepancy is suspected).
* A cloudy background with some large aggregates may indicate a mixed population (i.e., mixed field).
* Microscopic examination may be required if mixed field agglutination or rouleaux is suspected.

Grade and record immediately each reading (i.e., at the time you make each reading and grading). See 7.0 – Reporting.* If mixed field is observed, record “mf” along with the grading results (e.g., grade 2 mf).
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| the test is an indirect antiglobulin test (IAT) antibody screen or panel or a direct antiglobulin test (DAT), | * Read microscopically if the results were negative macroscopically.
* Record results immediately (i.e., at the time you make each reading and grading). See 7.0 – Reporting.
 |
| The test is an antigen typing (phenotype) | * Follow the manufacturer’s directions for reading and result interpretation.
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| * 1. Clerical Check
 | 1. Check the labeling on each tube set and confirm accuracy.
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| * 1. Check Cells
 | * 1. Add one drop of IgG-coated cells to the tube(s) with negative reactions. Centrifuge tubes at 3400 rpm for 10-15 seconds, resuspend cells, read macroscopically and record results.

Agglutination (grade 2) must be present or the test must be repeated. |

1. **Reporting**
	1. No agglutination or hemolysis of the red cells is a negative test result.
	2. Agglutination or hemolysis of the red cells is a positive test result.
	3. Record results of agglutination as follows:

# HEMAGGLUTINATION REACTIONS - TUBE TESTS \*

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| **Record** | Description of Reaction 9.1 |
| **Macroscopic Readings\*\*** |
| 4 | One solid agglutinate background is clear. |
| 3 | Several large agglutinates; background is clear |
| 2 |  Medium sized agglutinates ; background is clear |
| 1 | Many small agglutinates ; background is turbid.  |
| **Microscopic Readings\*\*\*** |
| W  | Barely visible agglutination; turbid background |
| NEG  | No agglutination or hemolysis of red cells. Cells float freely |

\* See Procedural Notes 8.1.

\*\* Contents of each tube examined after gentle rotation and inclination using the naked eye.

\*\*\* Microscopic verification of the weaker grades of agglutination made at x60 – x100
 magnification of the contents of the tube using an inverted lens microscope.

**OTHER TYPES OF REACTIONS**

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| --- | --- |
| **Record** | **Description of Reaction 9.1** |
| mf | Mixed Field: A pattern of small compact agglutinates in a background of numerous free red cells. Usually read microscopically |
| R | Rouleaux: Red cells appear microscopically as a stack of coins  |
| H | Complete hemolysis, no intact red cells |
| PH | Partial Hemolysis with some intact red cells present |

* 1. Do not use half grade, superscript or “plus signs”

(i.e., +, ++, +++ or ++++)

* 1. Write the interpretation of the Rh typing in letters (e.g., Pos. or Neg.). Do not write the signs + or – to indicate the Rh type.
	2. Grade results of control cells on the request form, worksheet, antigram or computer screen.
1. **Procedural Notes**
	1. The clinical history of the patient (transfusion history and diagnosis) should be considered when interpreting a reaction as a mixed field.
	2. Strong cold agglutinations may give a mixed field appearance. These reactions are not truly mixed field and should be interpreted as positive.
2. **References**
	1. Roback JD Ed. Technical Manual 17th edition Bethesda MD; AABB; 2011 847.
	2. Judd WJ, Methods in immunohematology, 3rd ed. Montgomery Scientific Publications, 2008: 25-28.
3. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| January 31, 2014 | * Revised name of manual
* Revised document number from PA.006 to RT. 001
* Revised wording in section 6.2.4 to include “Do Not Over mix”
* Changed reference cited in section 7.3 from 9.2 to 9.1
* Renumbered section 8.0
* Updated list of references to include the most recent editions
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