1. **Principle**

To standardize reading, grading and recording of gel reactions.

A standardized procedure for reporting gel reactions will contribute to uniformity and reproducibility of test results.

1. **Scope and Related Policies**

This procedure applies to all gel tests that require the reading, grading and/or recording of agglutination results.

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

**Equipment:** N/A

**Supplies:** Micro Typing Systems (MTS™) Anti-IgG cards that were used for the testing.

1. **Quality Control**
   1. Reading skills proficiency testing should be done with staff performing testing using MTS™ columns.
   2. The MTS™ interpretation guide will give color illustrations to assist technologists with determining reaction grades. Additional technical assistance is available by telephone on a help line. See the MTS™ Interpretation Guide.
2. **Procedure**

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| * 1. After centrifugation, remove the card(s) from the centrifuge and observe each card for the following signs of improper centrifugation: | * + 1. Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifugation cycle. |
| * + 1. A line of red cells streaming down one side and forming a “J” appearance is caused by improperly seated card in the card holders. |
| * + 1. If the card(s) show a sign of improper centrifugation, repeat the test. Do not re-centrifuge the card(s). See Procedural Notes 8.7.3. |
| * 1. Observe both the front and back of each microtube in the gel card. | |
| * 1. Read macroscopically. | |
| * 1. Grade the reactions. | * + 1. Refer to the MTS™ Interpretation guide for diagrams or pictures showing range of reactions. |
| * + 1. Record reactions as described below on the appropriate form. See also Procedural Notes 8.8.  |  |  | | --- | --- | | **Grade** | **Description of Reaction\*** | | Neg | Unagglutinated red blood cells form a well-defined button  at the bottom of the microtube. See Procedural Notes 8.1 if a few unagglutinated cells are trapped at the top or sides of the gel. | | 1 | Agglutinates predominantly observed in the lower half of the microtube. Unagglutinated red cells form a pellet in the bottom of the microtube. | | 2 | Agglutinates dispersed throughout the length of the gel column. A few free cells may be observed in the bottom of the microtube. See Procedural Notes 8.2. | | 3 | Majority of agglutinates trapped in the upper half of the microtube. See Procedural Notes 8.3. | | 4 | A solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel, but remain near the predominant band. See Procedural Notes 8.4. | | H | Hemolysis with few or no red cells in the gel. Report if hemolysis is present in the microtube but not in the specimen. See Procedural Notes 8.5. | | mf | A band of red cell agglutinates at the top of the gel or dispersed throughout the gel,accompanied by unagglutinated cells in the bottom of the microtube. See Procedural Notes 8.6. | | NT or ND | Not tested or not done | |
| * + 1. Do not use half grade, superscript or “plus signs”   (i.e., +, ++, +++, or ++++). |
| * + 1. See Procedural Notes 8.7 if the reaction in the microtube is not described in the table above. |
| * 1. Interpret the grading(s). | * + 1. No agglutination or hemolysis of the red cells is a negative test result. |
| * + 1. Agglutination or hemolysis of the red cells is a positive test result. |

1. **Reporting – N/A**
2. **Procedural Notes**
   1. Debris, fibrin or other artifacts associated with plasma or frozen specimens may cause a few unagglutinated cells to be trapped on the top or sides of the gel. These tests should be interpreted as negative. Plasma specimens previously frozen should be centrifuged prior to use.
   2. When interpreting grade 2 reactions, consider the upper and lower position of agglutinated red cells in the gel. Size of the red cell agglutinates in the bottom of the microtube may vary.
   3. A grade 3 reaction appears as a thick group of agglutinates, or band, with some red cells dispersed below the predominant band in the upper half of the gel column.

A grade 3 reaction may also be characterized by an even distribution of agglutinates in the upper portion of the gel. Occasionally, a few unsensitized cells may migrate to the bottom of the microtube.

* 1. Strong grade 4 agglutinations form a band of agglutinates and become trapped on or near the top of the gel. Occasionally a few unsensitized red cells may migrate to the bottom of the tube but the middle of the gel should remain free from agglutination.
  2. Red cells present in the gel and hemolysis in the liquid portion is usually due to a hemolyzed specimen. In this case, hemolysis should not be reported as a positive test result.

If hemolysis occurs during centrifugation, the liquid portion above the gel will appear pink or red but there will be few or no cells in the gel.

* 1. Consider the following when interpreting a reaction as mixed field.
     1. The clinical history of the patient.
     2. Strong cold agglutinations may give a mixed field appearance. These reactions are not truly mixed field and should be interpreted as positive.
  2. Consider the following troubleshooting tips when reactions in gel microtubes are difficult to grade.
     1. Rouleaux is a property of test plasma resulting in a characteristic pattern of red cell aggregation. It can occur if sufficient quantities of abnormal proteins are present in the test sample and may infrequently cause difficulties in gel test interpretation. Rouleaux must be confirmed using tube hemagglutination methods and saline replacement performed when necessary.
     2. Too few or too many cells in the microtube may cause false positive or false negative reactions. This may be due to one or both of the following errors:
* Improperly prepared cell suspension
* Adding the incorrect quantities of cells to the upper chamber.

In this case repeat the test(s) ensuring correct   
 quantities using new cell suspensions.

* + 1. Insufficient centrifugation and/or centrifugation when the card(s) were not properly seated in the centrifuge holders may cause one or more of the following reactions:
* A line of cells streaming down one side of the microtube
* The red cell agglutinate shifted from the bottom of the microtube
* Unagglutinated cells observed throughout the gel (appearing pink or hazy) in all microtubes on the card.

In this case repeat the test(s) ensuring correct   
 placement of the cards and centrifugation time.

* 1. The colour plate on page 6 of the MTS Interpretation Guide9.2 may assist in the interpretation of agglutination reactions.

1. **References**
   1. ID-Micro Typing System. Question and Answer Guide. Ortho Diagnostics. January 1996.
   2. ID-Micro Typing System. Interpretation Guide. Ortho Diagnostics. June 2010
   3. ID-Micro Typing System. Procedure manual. Ortho Diagnostics. January 1996.
   4. ID-Micro Typing System. Implementation guide. Ortho Diagnostics. May 2010
2. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| April 30, 2014 | * Revised name of manual * Changed document number from PA.007 to GM.001 * Revised and renumbered section 6.0 * Revised wording in section 8.2 to include “size of the red cell agglutinates in the bottom of the microtube may vary.” * Revised wording in section 8.8 to specify “on page 6 of the MTS Interpretation Guide 9.2” * Updated list of references to include the most recent editions |