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|  | | **Grading of Positive and Negative Reactions**  BB.R.1018.3 | **Dept:** | 324311 |
| **Dept Name** | Blood Bank |
| **Effective Date:** | <7/2009 |
| **Revised Date:** | Title 21 |
| **Name & Title**: CLIA Laboratory Medical Director | | | **Contact:** | Julie Simmons/  Christina Warren |
| **Signature:** | Refer to Title 21 | | **Date:** | **Title 21** |

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1. **General Procedure Statement:**
2. **Purpose:** To provide guidelines for grading of reactions for methods currently in use in Blood Bank.
3. **Responsible Department/Scope:**
   * 1. Procedure owner/Implementer: Julie H. Simmons/Christina S. Warren
     2. Procedure prepared by: Julie Simmons
     3. Who performs procedure: Department staff/management
4. **Definitions:**

MF: Mixed Field. Agglutinated cells and free cells when viewed microscopically

M: Microscopic. Agglutination is visible under microscope but not macroscopically

1. **Sections:**
2. Grading Tube Testing Methods (LIS, PEG, Enzyme, Saline)
3. Microscopic Reactions In Tube Testing Methods
4. Gel Method – Grading Manually and on Vision Max
5. **Protocol: Grading of Positive and Negative Reactions:**

**I. Grading Tube Testing Methods (LISS, PEG, Enzyme, Saline)**

1. Agglutination and Hemolysis
   1. Interpretation:
   2. Agglutination or hemolysis of red cells is a positive test result. No agglutination or hemolysis is a negative test result. Refer to chart in Step 8 for grading and codes for recording for agglutination and hemolysis.
2. Read no more than two to three tubes at one time.
3. Centrifuge tubes for the calibrated time. Remove tubes from the centrifuge. DO NOT MIX, SHAKE OR TILT.
4. Immediately after centrifugation and before mixing or tilting tubes, observe and inspect carefully for hemolysis.
   1. Complete or partial hemolysis must be interpreted as a positive reaction if the original serum/plasma was free of hemolysis.
5. Gently resuspend.
6. Read tube for agglutination beginning with button side up with agglutination viewer or microscope with tube holder.
   1. Gently roll the tube but only until all cells are dislodged from the bottom.
   2. Use an agglutination viewer to observe the way red cells leave the red cell button.
   3. Gently tilt back and forth until an even suspension is obtained.

Caution: Always observe the tube as agglutination is dislodged from button.

* 1. Stop when the button is resuspended.

1. Observe the resuspended cells and use the Grading Chart for Agglutination and Hemolysis.
   1. Negative reactions appear as a smooth homogeneous opaque suspension.
   2. All positives must be observed and the character of the agglutination noted, for example, mixed field.
   3. Mixed-field and questionable reactions must be observed using a microscope with a tube holder.
2. Record all results immediately after tubes are read in computer and/or on form used before discarding tubes.

**Grading Chart for Agglutination and Hemolysis**

| **Reaction Strength** | **Description** | **Example** |
| --- | --- | --- |
| **4+** | One solid aggregate, clear background.  No free cells. | http://webmedia.unmc.edu/alliedhealth/CLS/CLS422%2009/Grading%20reactions%2009/05%20Grading%20reactions_slide0003_image004.jpg |
| **3+** | A few large aggregate, clear background |  |
| **2+** | Medium-sized aggregates, clear background |  |
| **1+** | Small aggregates, turbid reddish background |  |
| **wk+** | Weakly positive. Tiny aggregates, turbid  reddish background,  Barely visible macroscopically |  |
| **m+** | Macroscopically negative. Microscopically  positive with 6-8 aggregates per field |  |
| **mf** | Mixed-field agglutination noted either  macroscopically as well-defined aggregates  in a suspension of opaque red cells or  microscopically as a few well-defined  aggregates with many free cells. |  |
| **-** | Negative. No agglutination or hemolysis.  Smooth homogenous suspension. |  |
| **R** | Rouleaux: Red cells appear to stack "like coins"  on each other. |  |
| **?** | Doubtful or questionable microscopically.  Lab Use only |  |
| **NT** | Not Tested |  |

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| **Hemolysis** | **Description** | **Example** |
| **h** | This is a positive reaction. Serum overlaying the centrifuged cell button is slightly hemolyzed with small agglutinates and some free cells in the background. |  |
| **H** | This is a positive reaction. Serum overlying the centrifuged cell button is moderately hemolyzed with many agglutinates (small to medium) or no free cells. |  |

**II. Microscopic Reactions in Tube Testing Methods**

1. A microscopic reading MUST be used:
   1. If indicated in manufacturer's direction.
   2. All transfusion reaction workups - repeat antibody screening, crossmatches, direct antiglobulin tests.
   3. Direct antiglobulin tests performed manually.
   4. HTLA titrations for endpoints.
   5. Fetal bleed screening test
2. Microscopic positive reactions must be approached carefully.
   1. Microscopically cells do not appear to fall smoothly as a negative reaction.
   2. Small clumps of cells may appear in a field of free cells or many clumps of cells may appear in every field.
3. ABO Microscopic Reactions:
   1. Rule: Reactions with anti-A, anti-B (anti-A,B) less than 2+ positive (2+) must always be investigated further.
   2. Examine microscopically for mixed field.
   3. If mixed field, check transfusion history for out of group packed cells or other products (e.g. platelets).
   4. Patient must not be reported group A, B or AB if there is less than 2+ positive (2+) agglutination with anti-A, anti-B typing sera.
   5. The cell typing (anti-A, anti-B and when tested anti-A,B) should not be routinely read microscopically on patients except when investigating out of group transfusions, allogeneic bone marrow transplants and neonates.
4. Rh Microscopic Reactions:
   1. Reactions with anti-D less than 2+ positive (2+) must always be investigated further.
   2. Examine weak reactions for mixed field.
   3. Examine reactions less than 2+ on OB procedure patients microscopically for mixed field.
      1. Perform weak D testing
5. Direct Antiglobulin Test Microscopic Reactions:
   1. Observe all positive reactions for mixed field regardless of strength of reactivity.
   2. Macroscopically negative reactions should be checked microscopically for agglutination.
6. Antibody Screens Microscopic Reactions:
   1. Antibody screens are not routinely read microscopically. If read and microscopically positive specimen must be further investigated before reporting results.
   2. Microscopic to weak reactions without seeing stronger serological reactions at another phase may be an indication of a cold allo or auto antibody requiring cold testing.
   3. Perform prewarm technique if applicable or autoabsorption techniques.
   4. Perform saline replacement techniques when rouleaux is suspected.
   5. Antibody detection techniques using enzyme treated cells must **NOT** be read microscopically.
   6. PEG testing at 37C must not be read for agglutination, only for hemolysis.
7. Crossmatch Microscopic Reactions:
   1. When interpreting routine crossmatch tests, it is **NOT** necessary to read microscopically. If the cell button is rough or suspicious, proceed to examine tube microscopically.

**III. Gel Method - Grading Manually and on Vision Max**

1. Grading Gel Test Reactions
   1. Read macroscopically the front and back of each microtube for agglutination and/or hemolysis by holding up to light or against a white background.
   2. The grading system for agglutination is based upon the position of the agglutinated red blood cells within the microtube.
   3. Caution must be taken in interpreting a reaction as mixed field. Additional patient history and testing will be necessary for resolution.
   4. Debris, fibrin or other artifacts may cause a few unagglutinated red cells to trap on top of the gel, but should be interpreted as negative.

**Grading Chart of Gel Cards**

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| **Reaction Strength** | **Description** | **Example** |
| **4+** | A well-defined band of agglutinated red blood cells in the top part gel column. A few agglutinated cells may be visible below the band. | See the source image |
| **3+** | Medium-sized clumps of agglutinated cells in the upper half of the gel column. |
| **2+** | Small or medium-sized clumps of agglutinated cells throughout the gel column. A few unagglutinated cells may be visible at the bottom of the gel column. |
| **1+** | Some small-sized clumps of agglutinated cells most frequently in the lower half of the gel column. A small pellet may also be observed at the bottom of the gel column. |
| **W+** | Barely visible small-sized clumps of agglutinated cells in the lower part of the gel column and a pellet of unagglutinated cells at the bottom. |
| **mf** | A band of red blood cells at the top part of the gel or dispersed through the gel column, and a pellet in the bottom as a negative result. |
| **-** | Well defined pellet of non-agglutinated red blood cells at the bottom of the gel column and no visible agglutinated cells in the rest of the gel column. |
| **H** | Hemolysis in the microtube with very few or no red blood cells in the gel column. Report if hemolysis is present in the microtube but not in the sample. |

1. **Review/Revised/Implemented:**

All procedures must be reviewed as stated in the Document Control protocol.

All new procedures and procedures that have major revisions must be signed by the CLIA director.

All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director or designee.

1. **Related Procedures: NA**
2. **References:**

AABB Technical Manual, revised periodically

AABB Standards for Blood Banks and Transfusion Services, revised periodically

Ortho Interpretation Guide

1. **Attachments**: NA
2. **Revised/Reviewed Dates and Signatures:**

See Archived Document Change Control