**Purpose**

This procedure provides instructions for how to perform a weak D test, which can be used to help resolve Rh typing discrepancies and to determine whether RHIG is required for a postpartum patient.

**Policy Statement**

* Weak D positive individuals are considered Rh Positive as donors.
* Weak D positive individuals are considered Rh Negative as recipients.
* Candidacy for administration of Rh Immune Globulin will be determined by clinical care team.
* Weak D positive men ≥ 1 and females ≥ 50 with no current or historical Anti-D can be transfused with Rh Positive products without prior medical director approval.

**Limitations**

* Samples with a positive DAT, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution.
* Insufficient or inappropriate washing can lead to false negative or false positive reactions.
* Some conditions that may cause false positive results are:
  + Contamination of sample or reagents
  + Autoantibodies
  + Improper storage or preparation of red blood cells
  + Antibodies to antibiotics or other reagents
  + Cold antibodies
* Additional limitations may be found in the manufacturer’s package insert.

**Procedure:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Step** | | **Action** | | | | | **Related Documents** |
| **Perform Weak D Test** | | | | | | |
| 1 | Perform anti-D and ABO/Rh control by tube, if not already done.   * Add reagents to clean labeled tubes: * Place **1** drop of anti-D reagent into a tube. * Place **1** drop of ABO/Rh control into a second tube * Prepare a 3% to 5% patient red cell suspension, if not already available * Add 1 drop of the 3% to 5% cell suspension into each tube. | | | | | Labeling Tubes for Manual Bench Testing  Preparation of 3-5% Red Cell Suspension  ABO/D Discrepancy Worksheet |
| **Step** | | **Action** | | | | | **Related Documents** |
| 2 | | Mix and incubate tubes for 15 to 30 minutes at 37°C. | | | | |  |
| 3 | Wash the tubes four times with saline. | | | | | Washing Patient Red Cells |
| ~~4~~ | Add **2** drops of anti-IgG antiglobulin reagent. | | | | |  |
| 5 | Mix the tubes immediately. Centrifuge for the posted time in a calibrated serologic centrifuge. | | | | |  |
| 6 | Immediately after centrifugation:   * Resuspend the cell button and observe for agglutination. * Read macroscopically and record results per established procedure. | | | | | Reading and Grading Tube Hemagglutination  SQ Blood Order Processing Test Result Guide |
| 7 | Validate all negative antiglobulin results by adding Check cells:   * Add **1** drop of IgG-coated control cells to each tube with a negative result. * Centrifuge for the posted time in a calibrated serologic centrifuge. * Resuspend the cells. * Read macroscopically for agglutination and record the results.   ***Valid control results****: Agglutination of at least grade 2 must be present or the test results are invalid and the test must be repeated*. | | | | |
| 8 | Analyze the reactions of the IgG-coated RBCs as follows: | | | | |  |
| **If agglutination is…** | | | **Then…** | |  |
| Present | | | Test is complete. | |  |
| Absent | | | Test is invalid:   * Repeat Steps 1-7. * Consider cell washer problem or inactive AHG. | |  |
| **Validate Completed Test Results** | | | | | | |
| 9 | Testing is unresolved/invalid if:   * Positive anti-D tube with no control tube performed * Positive control * Positive DAT with anti-IgG * Mixed field reaction   Note: If sending out for additional troubleshooting, result as Inconclusive (;ICLR) | | | Additional troubleshooting could be considered:   * Additional lot numbers of reagents * Other manufacturers * Eluate * Antibody Investigation * Transfusion history * Send out to reference lab | | Eluate Testing Guidelines  Guidelines for Antibody Identification  Reference Lab Send-Out Process |
| **Results Entry** | | | | | | |
| 10 | Enter initial tube ABO/Rh results in SQ reaction grid. Do not interpret result and click Accept.   * Add Weak D test * Enter weak D testing results and interpretation.   *Note: Override maybe required if final Rh(D) interpretation does not match initial ABO/Rh test grid.* | | | | | Sunquest: Blood Order Processing Test Result Guide  ABO/D Discrepancy Worksheet |
| **Step** | **Action** | | | | | **Related Documents** |
| **Interpreting weak D test results** | | | | | | |
| 11 | Interpret the **valid** IAT results as follows: | | | | | SQ Blood Order Processing Test Result Guide  Selection of Red Blood Cell Units  Discrepant Result Resolution Process  Prenatal Clinic Sample Process  ABO Discrepancy Resolution Process |
| **If the anti-D IAT result shows** | **And the control IAT result shows** | **Interpret the initial Rh result** | | **Interpret the Weak D result as** |
| Agglutination ≥ W+ | No agglutination | Positive | | D positive.  Update BAD file:  WKDP Enter comment “Give Rh Neg”, if patient is female <50 yrs of age or male <1 yr of age. |
| No agglutination | No agglutination or not required by manufacturer | Negative | | D negative  Update BAD file:  WKDN |
| Mixed Field agglutination | No agglutination | Do Not interpret until further investigation and resolution is completed | | Do Not interpret until further investigation and resolution is completed |

**References:**

Standards for Blood Banks and Transfusion Services, Current Edition. American Association of Blood Banks, AABB Press, Bethesda MD.

AABB Technical Manual, Current Edition

Current version of reagent manufacturer’s package insert instructions