|  |  |  |  |
| --- | --- | --- | --- |
|  | **Rh Testing and Weak D Typing**BB.Routine.1028.4 | **Dept:**  | 324311 |
| **Dept Name** | Blood Bank |
| **Effective Date:** | 3/26/01 |
| **Revised Date:** |  |
| **Name & Title**: CLIA Laboratory Medical Director | **Contact:** | Julie Simmons/ Christina Warren |
| **Signature:** | G. Pomper | **Date:** | **5/9/2019** |

**1. General Procedure Statement:**

1. **Purpose:**

The Rh system is very complex consisting of over 50 different antigens. They were first discovered when it was realized that Hemolytic Disease of the Newborn was caused by a paternal antigen inherited by the newborn. These antigens are found on red cells but not platelets. These antigens are very immunogenic with the D antigen being the most immunogenic of the system. Thus the need for routine testing of the D antigen.

A second ABORh (ABO Recheck) is required for computer crossmatches if no history is found or history is before February 2006.

**B.** **Responsible Department/Scope:**

1. Procedure owner/Implementer: Julie H. Simmons/Christina S. Warren
2. Procedure prepared by: Julie Jackson
3. Who performs procedure: Department staff/management

 **C. Definitions:**

**MR#:** Medical Record number

**IS:** Immediate Spin

**Blood Bank requisition or equivalent:** Antibody ID summary, Wake Blood Bank Order requisition

**M+:** Microscopically Positive

**ND:** Not Done

**Cord blood:** sample of blood collected from the umbilical cord when a baby is born

**Wharton’s jelly:** A gelatinous substance that provides insulation and protection within the umbilical cord that can interfere with blood bank testing.

**BCW:** Blood Centers of Wisconsin, a reference lab for genotyping patients

**RHU:** Rh (D) results Unknown

**D. Sections**:

1. Immediate Spin Rh (D) Testing
2. Weak D Testing

**E. Protocol:**

1. Refer to: *ABORh Protocol, BB.Routine.1043*
2. **Weak agglutination (Rh):** Patients who demonstrate <2+ agglutination with direct agglutination D testing must be further tested using the weak D testing method to determine RhD status.
	1. Weak D testing is not repeated when performing an ABO recheck on a weak D positive patients
3. **Weak D testing on Neo/Echo:** All *Positive* Weak D tests performed on the Neo/Echo must have a DAT performed on the sample before resulting the Weak D. The Weak D result is invalid if the DAT is positive.
4. RhD typing by direct agglutination and/or Rh weak D testing reactions must show ≥2+ reactivity to be considered positive.
	1. Rh weak D reactions showing <2+ reactivity should be examined for mixed field.
	2. Patients demonstrating reactivity <2+ with weak D testing will be interpreted as Rh unknown (RHU), will have a specimen sent for Rh DNA genotyping and will receive Rh negative products until confirmation of RhD status is received.
	3. Rh weak D testing will be performed on all the following patients:
		1. Patients demonstrating <2+ reactivity with RhD direct agglutination testing
		2. Rh negative neonates up to 4 months of age
		3. Rh negative obstetrics patients
		4. Rh negative bone marrow and organ donors
		5. Rh negative patients with Rh positive directed donor units to determine the patient’s true Rh type.
		6. Those patients previously determined to be weak D positive
		7. Rh negative patient who could potentially receive Rh positive products (due to low inventory)-Pre transfusion sample
		8. As requested by physician

**2. Procedure: I. Immediate Spin Rh (D) testing**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 or 12x75 test tubes

Transfer pipets

Blood Bank requisition or equivalent

Reagents: Anti-D antisera

Anti-IgG antisera

IgG sensitized control cells

Saline (0.85%)

Equipment: Agglutination lamp

Specimen centrifuge: Plasma Prep centrifuge or EBA 20 centrifuge

Testing centrifuge: Sero-fuge

Blood Bank Computer System

Specimen Requirements:

A properly labeled EDTA tube:

6.0mL pink top is preferred.

3.0mL or 10.0mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Refer to Specimen Labeling Requirements BB.FD.1001

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Centrifuge the specimen tube:*** 1. Preferred method: 3 minutes at 7200 RPM in the “*Plasma Prep”* centrifuge
	2. Alternative method: 7 minutes at 3150 – 3350 RPM in the “EBA 20” centrifuge
 |  |
| **2.0** | **Compare all labels and identification numbers on specimen, Blood Bank requisition or equivalent, and all work orders**2.1 Identifying information must be identical on ALL items.See *Front Desk: Specimen Labeling Requirements; BB.FD.1001* |  |
| **3.0** | **Label 10x75 test tubes with a minimum of the first 3 letters of the patient’s last name and testing to be performed.**3.1 Patient test tubes must be labeled with – ***at minimum*** – the first three letters of the patient’s last name AND the test being performed in the tube.1. For anti-D (IS or weak D) label tube “D”
2. For D control (saline control) label tube “DC”
	1. Run only when needed: A Saline control is needed for AB patients (see step 10.3) and Weak D testing (see section II. Weak D Testing)
 |  |
| **4.0** | **Place all test tubes in a test tube rack, labeling facing forward.*** 1. A maximum of 3 patient specimens may be in a test tube rack at any given time
	2. There must be at least one empty row between each patient
 |  |
| **5.0** | **Add appropriate reagent to tubes.**5.1 Add one drop of anti-D antisera to the tube labeled D* 1. Add 1 drop of saline to the tube labeled DC

**NOTE:** Read vial label and tube label before dropping antisera or saline to confirm that they match. |  |
| **6.0** | **Prepare a 3-5% cell suspension of the patient’s red cells**6.1 Label a 10x75 or 12x75 test tube for the patient’s red cell suspension with the patient’s last name either written or using a label.6.2 Add 1-2 drops of packed patient red cells into a properly labeled tube.6.3 Add 0.9% saline to produce a red cell suspension.6.4 Mix red cell suspension.6.5 Visually compare color of suspension with that of a 3-5% commercial reagent red cell suspension.1. If it appears <3%, add patient red cells to achieve a 3-5% suspension.
2. If it appears >5%, add saline to suspension to achieve a 3-5% suspension.

**NOTE:** **Cordblood** samples must be washed a **minimum of 6 times** prior to testing to remove Wharton’s Jelly. Then prepare a 3-5% suspension of patient’s cells.1. Mixed field reactions may indicate contamination of sample with Mom’s blood. The sample must be rejected and a heel stick collected.
 |  |
| **7.0** | **Add one drop of 3-5% patient red cell suspension to each tube; D and DC.** |   |
| **8.0** | **Mix tube gently, centrifuge immediately at room temperature, 3400-3600 RPM for the immediate spin (IS) calibrated time as noted on centrifuge.** |  |
| **9.0** | **Carefully remove 2-3 tubes from centrifuge at a time, observe tubes for hemolysis.**9.1 Only one patient’s tubes may be removed from the centrifuge at a time for reading.9.2 Hemolysis may 1. Indicate a positive test result in the presence of an antigen/antibody reaction
2. Be the consequence of hemolyzed patient red cells in the 3-5% cell suspension

9.3 Make note of any hemolysis present in the absence of hemolyzed red cell suspensions  in the computer or during downtime on the patient requisition.9.4 Complete or partial hemolysis must be interpreted as a positive reaction if the original serum/plasma and/or reagent red cell suspension was free of hemolysis. |  |
| **10.0** | **Dislodge / resuspend cell button from bottom of tube gently over an approved agglutination lamp.** 10.1 Interpret agglutination strength:

|  |  |  |
| --- | --- | --- |
| **If anti-D reaction****strength is** | **Interpret as** | **Action** |
| **0** | **Negative** | No further testing is needed unless patient meets criteria for weak D testing. See chart on next page and also Refer to ABO/Rh Testing Policies. |
| Micro+ to 1+ |  | Perform weak D testing.If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field) *Go to Step 14.0* |
| 2+ to 4+ | **Positive** | Interpret as Rh Positive. If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field) No further testing required |
| Hemolysis | **Positive** | Verify patient red cell suspension is free of hemolysis |

10.2 For further instructions on grading of reaction strength, Go to: *Routine: Grading of* *Positive and Negative Reactions. BB.R.1018.* |  |
| 10.3 **Saline Control:** If the patient is AB **and** the IS D is positive an immediate spin saline control must be done to rule out polyagglutination.1. Reaction must be negative to be valid
	* 1. If reaction is positive, wash patient cells, make 3-5% suspension and repeat D and DC tubes steps 3.0-12.0
		2. If DC is still positive see Routine: Common Causes of False-Negative and False-Positive Results in ABO Testing; BB.R.1022

**NOTE:** SCC requires that a saline control be performed on all AB patients (RH positive or negative). |
| 10.4 Determine if Weak D testing is needed: |
|  | **Patient Category** | **I.S. D****(Immediate Spin)** | **If I.S. D negative do Weak D** |  |
| Male and non-pregnant females >4 months old | **✓** | NA |
| ABO Recheck | **✓** | NA |
| Neonates < 4 months old | **✓** | **✓** |
| Pregnant Females | **✓** | **✓** |
| Any patient with I.S. D <2+ | **✓** | **✓** |
| Bone Marrow and Organ Donors | **✓** | **✓** |
| Rh negative patient with Rh positive directed donor unit | **✓** | **✓** |
| Rh negative patient who could potentially receive Rh positive products (due to low inventory)-Pre transfusion sample  | **✓** | **✓** |
| History of being Weak D positive | **✓** | **✓** |
| 10.5 For weak D testing go to **section II: Weak D Testing**  |
| **11.0** | **Document reactions in computer or during downtime on Blood Bank requisition or equivalent immediately, discard tubes only after documentation complete.**1. Confirm patient identification on test tube matches the requisition and computer screen.
2. Only one patient’s requisition should be in the work area at the time of reading, recording, and interpretation of reactions.
3. Serologic reactions must be recorded on the Blood Bank requisition or equivalent BEFORE tubes are discarded
 |  |
| **12.0** | **Document results in computer as tubes are being read or during downtime on Blood Bank requisition or equivalent.**12.1 Go to SCC>Results>Patient Test Worksheets>Build. a. Joint Worksheet may be used for TSX samples with units ordered. b. Unit Test Worksheet is used for Unit retypes. 12.2 Select appropriate Worksheet based on testing to be resulted.a. ABOCKb. CORDc. GTXd. KIDNYe. OBXf. TSXg. TSXN12.3 F12 to accept worksheet.12.4 Click Ctrl+O to select by order number. a. Scan order number. b. F12 to accept.12.5 Confirm patient identification on the requisition or specimen matches the computer  screen. 12.6 Enter reaction results obtained for Anti-D and Control (if applicable). **NOTE:** Results may also be entered in Patient > Orders > Results by clicking on each test and entering results. |  |

**2. Procedure: II. Weak D Testing**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 or 12x75 test tubes

Transfer pipets

Blood Bank requisition or equivalent

Reagents: Anti-D antisera

Anti-IgG antisera

IgG sensitized control cells

Saline (0.85%)

Equipment: Agglutination lamp

Specimen centrifuge: Plasma Prep centrifuge or EBA 20 centrifuge

Testing centrifuge: Sero-fuge

Blood Bank Computer System

Specimen Requirements:

A properly labeled EDTA tube:

6.0mL pink top is preferred.

3.0mL or 10.0mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Refer to Specimen Labeling Requirements BB.FD.1001

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Go to Patient>Orders>Modify and add Weak D to Tests if not already ordered.** 1. Select Weak D from drop down for tests.
2. F12 to accept.
 |  |
| **2.0** | **Label 10x75 test tubes with a minimum of the first 3 letters of the patient’s last name and testing to be performed.** 1. For anti-D label tube “D”
2. For D control (saline control) label tube “DC”
 |  |
| **3.0** | **Add appropriate reagent.**1. Add one drop of anti-D antisera to the tube labeled D
2. Add 1-2 drops of saline to the tube labeled DC.

**NOTE:** Read vial label and tube label before dropping antisera or saline to confirm that they match. |  |
| **4.0** | **Add one drop of prepared patient 3-5% red cell suspension (prepared in Step 6.0) to both the D and DC tubes.**4.1 Reconfirm patient specimen tube matches test tube |  |
| **5.0** | **Mix tubes gently. Incubate tubes 15-30 minutes at 36-38°C.** |  |
| **6.0** | **Remove tubes from incubator and wash 3 times with saline. Decant completely after the last wash.** |  |
| **7.0** | **Immediately add 2 drops of anti-IgG anti-human globulin to all tubes.**7.0 Vial label should be read carefully each time of use before dropping. |  |
| **8.0** | **Mix tube gently, centrifuge immediately at 3400-3600 RPM for the immediate spin (IS) calibrated time as noted on centrifuge.** |  |
| **9.0** | **Remove tubes from centrifuge carefully**9.1 Only one patient’s tubes may be removed from the centrifuge at the time of reading. |  |
| **10.0** | **Dislodge / resuspend cell button from bottom of tube gently over agglutination lamp.** 10.1 Interpret agglutination strength

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Testing** | **If the Reaction is** | **Then the Interpretation is** | **Also** | **Note** |
| **Anti-D: Weak D**  | 2+ to 4+ | Positive | If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field reaction) |
| Hemolysis present | Positive | - | - |
| Weak+ to 1+ | RHU (Rh undetermined) | If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field reaction) | Call floor/office to find out patient transfusion and pregnancy history |
| 0 and no hemolysis | Negative | - | - |
| **D Control** | W+ to 4+ | Weak D testing is invalid | If positive patient may have a positive DAT. If patient has a positive DAT Weak D testing cannot be done. |
| 0 and no hemolysis | Valid Weak D testing | - | - |

10.2 For further instructions on grading of reaction strength go to: Routine: *Grading of Positive and Negative Reactions. BB.R.1018* |  |
| **11.0** | **Document results in computer as tubes are being read or during downtime on patient BB requisition or equivalent immediately.**1. Confirm patient identification on test tube with identification on requisition.
2. Only one patient’s requisition should be in the work area at the time of reading and recording reactions.
3. Serologic reactions must be entered (or recorded) BEFORE tubes are discarded.
 |  |
| **12.0** | **Document results in computer as tubes are being read or during downtime on Blood Bank requisition or equivalent.**1. Go to SCC>Results>Patient Test Worksheets>Build.
2. Select appropriate Worksheet based on testing to be resulted.

a. ABOCK e. OBXb. CORD f. TSXc. GTX g. TSXNd. KIDNY1. F12 to accept worksheet.
2. Click Ctrl+O to select by order number.

 a. Scan order number. b. F12 to accept.1. Confirm patient identification on the requisition or specimen matches the computer screen.
2. Enter reaction results obtained for Anti-D and Control.

**NOTE:** Results may also be entered in Patient > Orders > Results by clicking on each test and entering results. |  |
| **13.0** | **Check all negative test results with IgG sensitized control cells**1. Add one drop of IgG sensitized control cells to all negative tubes.
2. Mix tube gently, centrifuge immediately at room temperature for the immediate spin (IS) calibrated time.
3. Remove tubes from centrifuge carefully
	1. Only one patient’s tubes may be removed from the centrifuge at a time for reading.
4. Dislodge / resuspend cell button from bottom of tubes gently over an approved agglutination lamp
5. Check cell results must be ≥2+ to consider the test valid.
6. Negative check cell results or those reacting <2+ indicate technical error and require the test be repeated
7. Document check cell results in computer or during downtime on BB requisition or equivalent. Discard tubes.
8. Confirm patient identification on test tube matches requisition
9. Serologic reactions must be recorded on the BB requisition or in computer BEFORE tubes are discarded
 |  |
| **14.0** | **Interpret weak D test results in computer and F12 to accept. Refer to chart below:** |  |
|  | **Tube Testing Interpretation** |  |
|  | **Weak D** | **D Cont** | **Check Cells** | **Weak D Interp and final Rh interp** |  |
| **Wk D** | **D Cont** |
| **2+ – 4+** | **0** | **ND** | **2-4+** | **Positive+** |
| **0** | **0** | **2-4+** | **2-4+** | **Negative** |
| **M+ – 1+** | **0** | **ND** | **2-4+** | **RHU\*+** |
| **0 – 4+** | **M+ – 4+** | **ND** | **ND** | **RHU\*+****Perform DAT** |
| **0** | **0** | **0–1+** | **0–1+** | **Invalid- Repeat** **Refer to step 13.4** |
| **Instrument Testing- Neo/Echo** |
| **Weak D** | **Control** | **DAT** | **Weak D Interp and final Rh Interp** |
| **2+ – 4+** | **0** |  | **Perform DAT** |
| **0** | **0** |  | **Negative** |
| **Wk+ – 1+** | **0** |  | **Perform DAT** |
|  | **0 – 4+** | **M+ – 4+** |  | **Perform DAT** |  |
|  | **Wk+ - 4+** | **0** | **POS** | **RHU\*+** |  |
|  | **2+ – 4+** | **0** | **NEG** | **Positive+** |  |
|  | **Wk+ – 1+** | **0** | **NEG** | **RHU\*+** |  |
|  | **0 – 4+** | **M+ – 4+** | **POS** | **RHU+** |  |
| \*If patient has a history of being Rh positive at another facility and the patient was transfused enough Rh negative blood to dilute the cells to a weak to 1+ reaction, interp as RHU until the patient starts testing 2+ to 4+. Let Management know, they will change historical type to Rh positive at that time.\*If the patient is pregnant a weak reacting Weak D may indicate a fetal maternal bleed. Determine if patient has been transfused. Consult management, a quantitative fetal hemoglobin test may be indicated.**+NOTE:** Any obstetrical patient with a positive Weak D test must be sent to BCW for a Weak D/Partial D analysis. (Order BCW tests: Weak RhD analysis and Partial RhD analysis; complete Reference Lab tracking log.)**NOTE-Echo only:** When tested on the Echo and the result is *Positive* on the Echo the Weak D test will *not* show in Results > Tests Verify > Patient Test Verify. The results will cross (but not the interpretation) into Patient > Orders > Results. The Interpretation will need to be manually selected, either POS or RHU. See chart above. |

 **3. Review/Revised/implemented:**

All procedures must be reviewed according to the Document Change Protocol.

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

All reviewed procedures with minor revisions can be signed by the designated section Medical Director

1. **Related Procedures:**

ABO Testing Manual Method BB.R.1001

Grading of Positive and Negative Reactions; BB.Routine.1018

Front Desk; Specimen Labeling Requirements; BB.FD.1001

**5. References**:

Reagent package inserts, updated periodically

AABB Technical Manual, updated periodically

Modern Blood Banking and Transfusion Practice, Harmening; updated periodically.

Merriam-Webster Dictionary

**6. Attachments**:

NA

**7. Revised/Reviewed Dates and Signatures:**

See Archived Document Change Control