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| /Volumes/dsm/UPH/Creative Services/Graphic Design/Logos/UnityPoint Health/UnityPoint Health/png/1 UP Health 2c H.png  METHODIST | | | Page 1 of 4 | Section: UPM BBPRO | Policy #: 02.017 |
|  | BLOOD BANK PROCEDURES | | Approved by: see signature block at end of document | | Date: 2/12/19  Review by: 2/12/21 |
|  | LABORATORY | | Policy Created: 4/5/99  Supersedes: 3/1/12, 3/5/14, 7/21/16, 6/26/18, 2/4/19 | | |
|  | |  | Primary Responsible Parties: Michelle Ehrich  Secondary Responsible Parties: June Bembenek | | |
|  | |  | CAP Standard: NA | | |
| SUBJECT: | | WEAK D ANTIGEN TESTING | | | |

1. **Principle**

There are over 84 known weak D antigen types. Some of these types, classified as partial D, allow for the production of anti-D following exposure to the complete D antigen. Anti-D produced by partial D patients has resulted in fatal hemolytic disease. Current monoclonal reagents licensed by the FDA have been designed to be nonreactive with partial D patients at immediate spin. Treatment of all patients negative for the D antigen at immediate spin as D negative reduces the risk of production of anti-D.

However, weak D antigen testing should be performed on newborns who have a negative reaction at immediate spin with anti-D during ABO/Rh typing. Weak D testing of infants born to D negative mothers ensures proper administration of RhIG prophylaxis to all women at risk of sensitization.

Additionally, rosetting tests used to detect a feto-maternal hemorrhage may be falsely positive if maternal cells are weak D positive. All positive rosetting tests should be verified to be negative for the weak D antigen prior to any confirmatory testing.

The presence of a weak D antigen type can best be detected by incubating the red cell suspension with anti-D. The antiglobulin technique is the most sensitive method for detecting the weak D antigen.

1. **Specimen**
   1. Patient Preparation:  
      No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques.
   2. Requirements:   
      K2 EDTA pink or lavender top tube is preferred.
   3. Minimum Volume:

Adult: 3.0 mL whole blood  
Pediatric: 2 - K2 EDTA microtainers (each with 300-500 uL) or cord blood specimen

* 1. Specimen Stability:   
     If stored at room temperature 15-30°C, stable for testing for 24 hours.  
     If stored 2-8°C, stable for testing for 72 hours.
  2. Storage:  
     2-8°C for a minimum of 7 days after transfusion, or 10 days post crossmatch.
  3. Rejection Criteria:  
     Hemolysis: \*\*In rare occasions where sample cannot be redrawn, hemolyzed specimens may be used for testing as long as the testing personnel can accurately interpret the reactions.

1. **Policy Scope**

Blood Bank Technologists

1. **Reagents**

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| **Reagent** | **Storage** | **Stability** |
| Isotonic Saline | 15-30°C | Unopened: Marked expiration Opened: 30 days |
| Monoclonal Anti-D Antisera | 2-8°C | Stable through marked expiration |
| Anti-IgG Anti-Human Globulin | 2-8°C | Stable through marked expiration |
| Coombs Check Cells | 2-8°C | Stable through marked expiration |

1. **Instrumentation/Equipment**
2. Tubes 10x75mm
3. Pipettes
4. Cell Washer (optional)
5. Centrifuge
6. Incubator
7. **Quality Control**

To be performed once per lot number per day of testing. Refer to UPM BBQA 03.003: Reagent Quality Control for complete procedure.

1. **Procedure**
2. Using the tubes from the ABO/Rh typing procedure, (BB PRO 02.001 ABO/Rh Tube Typing), incubate both the anti-D and the patient autocontrol tubes at 37°C for 15-30 minutes
3. After incubation, wash both tubes three times with normal saline.
4. Add 2 drops of anti-IgG to each tube.
5. Mix well and centrifuge for 15-20 seconds at 3400 rpm, or at a time and speed appropriate to the calibration of the centrifuge.
6. Resuspend the cells by gentle agitation and examine for macroscopic agglutination. Negative results may be viewed using an agglutination reader.
7. Record reactions.
8. Add one drop of coombs check cells to each negative result, and centrifuge for 15-20 seconds at 3400 rpm, or at a time and speed appropriate to the calibration of the centrifuge.
9. Gently dislodge the cell button and observe for macroscopic agglutination. Non-reactive check cells indicate that testing is invalid and must be repeated.
10. **Interpretation**

Refer to UPM BBPRO 02.001: ABO/RH Tube Typing for reaction strength grading and interpretation

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| **Anti-D** | **Patient Control** | **Rh interpretation** |
| NEG | NEG | Rh Negative |
| POS | NEG | Rh Positive |
| POS | POS | Treat as Rh negative  unless discrepancy is resolved\* |

\* Refer to UPM BBPRO 02.032: Resolving ABO Discrepancies

1. **Procedural Notes/Problem-Solving Tips**
   1. Weak D positive reactions on women who just delivered/miscarried may be due to a fetal bleed. Refer to UPM BBPRO 02.020: Feto-maternal Hemorrhage Rapid Screen for further information.
   2. In 1998 a consensus of larger transfusion services decided that weak D testing was no longer necessary on selected patients as only 0.2-0.3% of the population is truly weak D positive. It was determined that the reason to ascertain the weak D status on all individuals was to conserve on the use of Rh negative blood.
   3. The blood bank will continue to perform weak D types on:
2. All newborns – **For weak D positive newborns, add PB comment: Weak D positive, in the LIS**
3. All positive feto-maternal hemorrhage rapid screens
4. **References**
5. AABB Technical Manual, 15th Edition. 2005. Pages 165, 322-324.
6. AABB Technical Manual, 18th Edition. 2014. Pages 323-328.

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| ***POLICY CREATION :*** |  |
| ***Author: Mary McCallister*** | ***August 29, 2004*** |
| ***Medical Director: Douglas McGrady, MD*** | ***September 17, 2004*** |

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| **REVISION HISTORY** | | | |
| **Rev** | **Description of Change** | **Author** | **Effective Date** |
| 1 | Minor formatting changes, added Document ID to header. Updated for HBB | S.Schaffer | 12/6/11 |
| 2 | Made changes to add BIO-RAD reagents, system name updated, formatting changes. | K. Turpin/ T. Lanan | 2/17/14 |
| 3 | Added negative reactions to daily controls, removed microscopic reading, included postpartum Rhig in Du, removed “MMCI” Added the word The before Blood Bank | V. Strow  K. Turpin | 2/10/16  7/6/16 |
| 4 | Removed requirements to test all women <60 and for all RhIG administration. Rewrote principle to comply with new standards. Removed addition of extra antisera to comply with manufacturer’s instructions. Referred QC and discrepancy resolution to the appropriate procedures. Replaced dated “Du” terminology with “weak D.” Added reference. Minor formatting | M. Ehrich | 01/31/19 |
| 5 | Added enter PB comment “Weak D positive” for newborns. | J. Bembenek | 2/11/19 |

**REVIEWED BY**

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| **Lead** | **Date** | **Coordinator/**  **Manager** | **Date** | Medical Director | **Date** |
|  |  | schafer | 3/1/12 |  | 3/1/12 |
|  |  |  | 3/5/14 |  | 3/5/14 |
| V. Strow | 2/10/16 |  | 7/6/16 |  | 7/21/16 |
| L. Miller | 7/27/18 |  | 7/30/18 |  | 8/14/18 |
| Michelle Ehrich | 1/31/19 |  | 1/31/19 |  | 2/4/19 |
|  |  |  | 2/11/19 |  | 2/12/19 |