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| METHODIST  | Page 1 of 7 | Section: UPM BBPRO | Policy #: 02.001 |
|  | BLOOD BANK PROCEDURES | Approved by: see signature block at end of document | Date: 2/4/19Review by: 2/4/21 |
|  | LABORATORY | Created: 7/13/08Supersedes: 7/15/11, 8/28/12, 1/14/14, 7/21/16, 7/27/18, 10/3/18, 2/4/19 |
|  |  | Primary Responsible Parties: Michelle EhrichSecondary Responsible Parties: June Bembenek |
|  |  | CAP Standard: NA |
| SUBJECT: | ABO/RH TUBE TYPING |

1. **Principle**

Agglutination of red cells with a given anti-serum indicates the presence of the corresponding antigen on the red cells and is a positive test result. Absence of agglutination indicates the corresponding antigen is not present. The reverse (serum) grouping procedure relies upon the appearance of the iso-agglutinins Anti-A and/or Anti-B to confirm ABO blood grouping.

1. **Clinical Significance**

ABO and Rh typings are used in many situations. Testing is performed when donating blood, in testing for transfusions of blood or blood products, in paternity testing, transplant testing and in many other procedures related to donor testing and transfusions.

1. **POLICY SCOPE**

Blood bank technologists

1. **Specimen**
2. Patient Preparation:

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques.

1. Requirements:

K2 EDTA pink or lavender top tube is preferred. Blood bank identification (BBID) label required for all UPH Methodist inpatients including ED. Outpatient drawing centers including: Outpatient lab, North lab, Methodist Diagnostic Center, and any physician office will not require a BBID label.

1. Minimum Volume:

Adult: 3.0 mL whole blood

Pediatric: 2 - K2 EDTA microtainers (each with 300-500 uL) or cord blood

1. Specimen Stability:

If stored at room temperature 15-30°C, stable for testing 24 hours.

If stored 2-8°C, stable for 72 hours.

1. Storage:

2-8°C for a minimum of 7 days after transfusion, or 10 days post crossmatch.

1. Rejection Criteria:

Hemolysis. \*\*In rare occasions where sample cannot be redrawn, a hemolyzed specimen may be used for testing only if the testing personnel can accurately interpret the reactions.

1. **Reagents**

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| **Reagent** | **Storage** | **Stability** |
| Isotonic Saline | 15-30°C  | Unopened: marked expirationOpened: 30 days |
| Monoclonal Anti-A Antisera | 2-8°C | Stable through marked expiration |
| Monoclonal Anti-B Antisera | 2-8°C | Stable through marked expiration |
| Monoclonal Anti-D Antisera | 2-8°C | Stable through marked expiration |
| A1 Reagent Red Blood Cells | 2-8°C | Stable through marked expiration |
| B Reagent Red Blood Cells | 2-8°C | Stable through marked expiration |

1. **Instrumentation/Equipment**
2. Test tube 10 x 75mm
3. Pipettes
4. Centrifuge
5. Tube Marker
6. **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. Label 6 10x75mm tubes with the patient’s initials and the following:
3. Forward group (antisera is color coded), 2 tubes: Anti-A, Anti-B.
4. Reverse group, 2 tubes: A1, B
5. Rh typing, 2 tubes: Anti-D, CT (patient autocontrol).
6. Prepare a 3-5% suspension of red blood cells to be tested in isotonic saline and label with patient’s last name, first name, and date of birth.
7. Add one drop each of reagents anti-A, anti-B, and anti-D into their correspondingly labeled tubes.
8. Add two drops of patient plasma or serum to each A1, B, and CT tube. *(Steps C and D may be performed in alternate order.)*
9. Add one drop each of reagent A1 and B cells into their correspondingly labeled tubes.
10. Add one drop of patient cell suspension to all the forward group and Rh typing tubes. *(Steps E and F may be performed in alternate order.)*

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|  | **Tube 1** | **Tube 2** | **Tube 3** | **Tube 4** | **Tube 5** | **Tube 6** |
| **Label** | Anti-A | Anti-B | A1 | B | Anti-D | CT |
| **Add First** | 1 dropanti-A | 1 dropanti-B | 2 drops patient plasma | 2 drops patient plasma | 1 dropanti-D | 2 drops patient plasma |
| **Add Second** | 1 drop patient cell suspension | 1 drop patient cell suspension | 1 drop reagentA1 cells | 1 drop reagentB cells | 1 drop patient cell suspension | 1 drop patient cell suspension |

1. Mix well and centrifuge all tubes at 3400 rpm for 15-20 seconds, or at a time and speed appropriate to the calibration of the centrifuge. Observe the reverse grouping tubes for hemolysis and then resuspend the cells by gentle agitation and examine for agglutination**. Always observe for hemolysis and agglutination.** Either of these observations indicates a positive reaction. Record reactions in LIS at time of interpretation.
2. Any patient presenting for ABO/Rh testing and transfusion of packed red blood cells for the first time must have the ABO/Rh type confirmed by a second specimen drawn at a separate time or by a second phlebotomist. If a second specimen cannot be collected, a bedside type can be used for the confirmation. Refer to UPM BBPRO 02.002: ABO Slide Typing for bedside type procedure.

**All first-time patients must have two blood types performed before blood is transfused.**

1. Refer to UPM BBPRO 02.032: Resolving ABO Discrepancies for help in resolving ABO, Rh, forward, and reverse discrepancies.

1. I**NTERPRETATION**

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| 4+  | Agglutination, one solid aggregate |
| 3+  | Agglutination, several large aggregates, clear background |
| 2+  | Agglutination, medium-sized aggregates, clear background |
| 1+  | Agglutination, small aggregates, turbid background |
| +w | Agglutination, tiny or microscopic\* aggregates |
| 0, = | No agglutination or test results negative |

\* Negative results may be examined with an agglutination viewer; however, microscopic examination is not recommended and should only be used to confirm the presence of rouleaux.

Determine the ABO/Rh typing based on the presence or absence of agglutination as follows:

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| **Anti-A** | **Anti-B** | **A1 Cells** | **B Cells** | **Group Interpretation** |
| NEG | NEG | POS | POS | O |
| POS | NEG | NEG | POS | A |
| NEG | POS | POS | NEG | B |
| POS | POS | NEG | NEG | AB |
|  |  |  |  |  |
| **Anti-D** | **PT Control** | **Interpretation** |
| POS | NEG | D POSITIVE |
| NEG | NEG | D NEGATIVE\*\* |
| POS | POS | Invalid result\*\*\* |

**\*\*** Weak D testing is required for all newborns. Refer to UPM BBPRO 02.017: Weak D Antigen Testing and UPM BBPRO 02.015: Cord Blood Testing.

\*\*\* Refer to UPM BBPRO 02.032: Resolving ABO Discrepancies.

1. **Reporting Results**

 Enter the immediate spin reactions and interpretation into the LIS.

**If there is an ABO discrepancy between forward and back typing:**

Refer to UPM BBPRO 02.032: Resolving ABO Discrepancies and resolve the discrepancy

1. If resolution is attained, record process and results in LIS
2. If resolution is unable to be attained, document process and ensure follow-up
3. **Procedural Notes/Problem-Solving Tips**

ABO/Rh typing discrepancies may occur in the forward group, reverse group, or both. Any reaction results that do not correlate with the chart provided should be treated as a discrepancy.

Refer to UPM BBPRO 02.032: Resolving ABO Discrepancies for all discrepancy resolution notes and tips.

1. **References**
2. AABB Technical Manual, 16th Edition. 2008, Pg. 877-898.
3. Gamma Biologicals, Inc. Blood grouping reagent insert.
4. Ortho-Clinical Diagnostics, Inc. Blood grouping reagent insert.
5. ARC Reference Laboratory
6. Bio-Rad Medical Diagnostics GmbH, Blood grouping reagent insert.

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| ***POLICY CREATION :*** |  |
| ***Author: Mary McCallister***  | ***June 11, 2003*** |
| ***Medical Director: Juan G. Gonzales, MD***  | ***June 13, 2008*** |
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| **REVISION HISTORY**  |
| **Rev** | **Description of Change** | **Author** | **Effective Date** |
| 0 | Initial release. | M. McCallister | 7/11/03 |
| 1 | Added to number 6 ABO Discrepancy. Manually add blood attributes if no ABO history. | Kathy Turpin | 08/27/12 |
| 2 | Changed storage temperatures. Added Bio-Rad as reference. | Kathy Turpin | 07/01/13 |
| 3 | Added suspension made with isotonic saline. Removed suspension made with patient’s serum/plasma. | Kathy Turpin | 09/26/13 |
| 4 | Under reporting Results changed how results are entered into the computer if patient is a Du negative or Du positive | Kathy Turpin | 03/11/14 |
| 5 | Removed all references to specific LIS – Du, ABORh Discrepancy, ABORh. Added Negative Controls for ABORh testing. | Vincent Strow | 02/10/16 |
| 6 | Removed requirement for second phlebotomist for second specimen. Maintained stipulation that second specimen required before transfusion. | Vincent Strow | 3/31/16 |
| 7 | Added that patient’s initials should be on each of the test tubes. The red cell suspension tube must have the patient’s last name, first name and date of birth. | Leah Miller | 7/27/18 |
| 8 | Added policy references, BBID label requirement, minor formatting. | Leah Miller | 10/16/18 |
| 9 | Removed weak D testing requirement except for newborns. Clarified microscopic evaluation. Moved QC and discrepancy resolution tips to the appropriate procedures. Minor formatting | Michelle Ehrich | 01/31/19 |

**REVIEWED BY**

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| --- | --- | --- | --- | --- | --- |
| **Lead** | **Date** | **Coordinator/Manager** | **Date** | Medical Director | **Date** |
| K. Maher | 7/15/11 |  | 7/14/11 |  | 7/15/11 |
|  |  |  | 8/27/12 |  | 8/28/12 |
| D. Allen | 1/14/14 |  | 9/26/13 |  | 1/14/14 |
| V. Strow | 3/31/16 |  | 7/19/16 |  | 7/21/16 |
| L. Miller | 10/16/18 |  | 10/18/18 |  | 10/30/18 |
| Michelle Ehrich | 1/31/19 |  | 1/31/19 |  | 2/4/19 |
|  |  |  |  |  |  |