**Purpose:**

To provide guidelines for resolving ABO discrepancies.

**Policy Statements:**

* ABO Groups are not resulted until the discrepancy is resolved.
* Only Universal products may be released prior to discrepancy resolution per Selection of Red Blood Cell Units
* Patient’s Blood Administrative Data file is updated with resolution results.

**General Considerations:**

An ABO discrepancy exists when the results of the red cell tests (forward group) do not agree with the results of the plasma tests (reverse group). There are several ways an ABO discrepancy may present:

* Weak, mixed field, or missing cell/forward type reactions
* Unexpected (extra) cell/forward type reaction
* Weak or missing serum/reverse type reactions
* Unexpected (extra) serum/reverse type reactions
* Negative control, when run, is not negative

**Procedure:**

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents** |
| **1** | * Determine specimen acceptability
* Request new pink top EDTA tube sample from nurse if:
	+ Gross hemolysis
	+ Fibrin clots/stands
		- ***Note:*** *Gross hemolysis can interfere with visualization and fibrin clots/strands may mistakenly be interpreted as agglutinates*
 | * + - * Sample Acceptance Evaluation
 |
| **2** | * Contact patient’s nurse or physician and obtain the following patient information:
* Transfusion history
* Transplantation history
* Diagnosis
* Date of Birth
* ***Note****: Elderly patients or infants <4-6 months of age may not reverse type correctly due to low/absent levels anti-A /anti-B in their plasma*
 | Antibody Identification Worksheet |

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents** |
| **3** | * Follow appropriate procedure below for ABO discrepancy resolution based on nature of typing results
* Record test results on appropriate worksheets
	+ Patient name and identifier
	+ Date of testing
	+ Tech performing testing
	+ Initial test results, if appropriate
 | LIS Downtime Manual Bench Testing form ABO/D Discrepancy Worksheet |
| **Procedure A: Missing/Mixed Field/Weak-Reacting Cell/Forward Group Reactions** |
| **1** | * Wash cells 3-4 times
 | * Washing by Manual Method.
 |
| **2** | * Repeat forward type with anti-A, anti-B, anti-A,B and a saline control
 |  |
| **IF** | * **Discrepancy is resolved:**
* **THEN** record interpretations and proceed with testing.
* Add blood bank comment in Sunquest (BBCS) to patient order “ABO interp req wash X 3 of pt cells”
 |  |
| **IF** | **Discrepancy is not resolved:** * **THEN** Choose a Resolution Technique based on possible causes:
 |  |
| **Possible Causes** | **Resolution Techniques** |  |
| **1** | * **Subgroup of A or B**
* Most commonly due to A subgroups
* Rarely due to B subgroups
 | * + Test for A subgroup with anti-A1 lectin.
		- If no agglutination with anti-A1 patient has an A subgroup
	+ Consider
* Enhancing reactivity with increased incubation time including an O cell control (e.g. 15-30 RT incubation)
* Absorption and elution techniques
* Enzyme treatment of cells.
	+ Refer to reference laboratory for resolution if above steps do not resolve the discrepancy
 | * Reference Lab Send Out Process
 |

|  |
| --- |
| **EXAMPLES OF DISCREPANCIES DUE TO A SUBGROUPS** (+/- denotes sometimes reacts) |
| **Sub-group** | **Reactions of Patient Cells with** | **Reactions of Patient Serum with** |
| **Anti-A** | **Anti-B** | **Anti-A,B** | **Anti-A1** | **A1** **Cells** | **A2****Cells** | **B** **Cells** | **O****Cells** |
| **A2** | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 |
| **A2 with Anti-A1** | 3 | 0 | 3 | 0 | 1-3 | 0 | 3 | 0 |
| **A3** | 2/mf | 0 | 2/mf | +/- | +/- | 0 | 3 | 0 |
| **Aend** | w+/mf | 0 | w+/mf | +/- | +/- | 0 | 3 | 0 |
| **Aint** | 2/mf | 0 | 2 | 2 | 0 | 0 | 3 | 0 |
| **Ax** | 0/w+ | 0 | 0/2 | 0 | 0 | 0 | 3 | 0 |

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents** |
| **Procedure A: Missing/Mixed Field/Weak-Reacting Cell/Forward Group Reactions continued** |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **2** | * **Weakened A or B Antigens** due to **disease state**: e.g.
	+ Leukemia
	+ Hodgkin’s disease

Note: Mixed Field is typically seen in these patients due to transplant or transfusion support therapy. An accurate history is vital to the resolution. | * + Encourage the weak antigens to react by:
* Lengthening incubation time (e.g. 15-30 min RT incubation)
* Reducing temperature (e.g.15-30 min incubation at 4C)
* Include an O cell control
 | * + See Tube set up Table A
 |
| * + Using Tube Set up in Table A, Test all tubes using Cold Panel Technique
 | * + Antibody Identification using Cold Method
 |
| * + Grade and Record results on ABO Discrepancy Worksheet
 | * + Reading and Grading Reactions
 |
| * + If the discrepancy cannot be resolved, Give Group O. Send to Reference lab for confirmation
 |  |
| **EXAMPLES OF ABO DISCREPANCY SEEN WITH LEUKEMIAS OR LYMPHOMAS** |
| **Patient Group** | **Reactions of Patient Cells with** | **Reaction of Patient serum with** |
| **Anti-A** | **Anti-B** | **Anti-A,B** | **A1 Cells** | **B Cells** |
| **A** | 1mf | 0 | 2mf | 0 | 3 |
| **B** | 0 | W+/1 | 1 | 4 | 0 |

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents** |
| **Procedure A: Missing/Weak-Reacting Cell/Forward Group Reactions continued** |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **Mixed Field Reactions due to Dual RBC populations** |
| **3** | * **Mixed Field agglutination**
	+ Transfusion with non-type specific donor red blood cells
	+ Transplantation with non-type specific hematopoietic stem cell donor
	+ Chimera
	+ Fetal-maternal haemorrhage
	+ Intrauterine transfusion
 | * Obtain accurate history
	+ - The majority of mixed fields can be explained by transfusion.
		- Contact transferring facility for results of pre-transfusion testing
 |  |
| * + Test EDTA from Hematology laboratory if the sample was drawn prior to transfusion
 | * + ABO D Type by Tube Method
 |
| * + Grade and Record results on ABO Discrepancy Worksheet
 | * + Reading and Grading Reactions
 |
| * + If the discrepancy cannot be resolved, Give Group O. Send to Reference lab for confirmation
 |  |
| **Procedure B: Unexpected (Extra) Cell/Forward Typing Reactions**  |
| **1** | * Wash cells 3-4 times
 |  |
| **2** | * Reset forward type with anti-A, anti-B, anti-A,B and a saline control
 |  |
| **IF** | * **Discrepancy is resolved**
* **THEN** record interpretations and proceed with testing. Add BBCS to patient order “ABO interp req wash X 3 of pt cells”
 |  |
| **Procedure B: Unexpected (Extra) Cell/Forward Typing Reactions (continued)** |
| **IF** | * **Discrepancy is not resolved:**
* **THEN** Choose Resolution Technique:
 |  |
| **Procedure B: Unexpected (Extra) Cell/Forward Typing Reactions (continued)** |
|  | **Possible Causes** | **Resolution Techniques** |  |
|  | **Acquired B phenomena*** Intestinal obstruction (e.g. carcinoma of the colon or rectum) – bacterial overgrowth and deacetylation of the A antigen
 | * Check Diagnosis
* Expected results using Tube Set up Table B
* Strong agglutination with Anti-A
* Strong agglutination with Anti-A1

if patient is A1 subgroup.* Weaker agglutination with Anti-B
* Patient serum agglutinates with B cells but not A cells
* Send to Reference Laboratory for confirmation.
 |  |
|  | **B(A) Phenotype*** Red cells of some group B individuals may be agglutinated by Anti-A reagent that contains a particular murine monoclonal antibody.
 | * Test with different manufacturers of Anti-A or Anti-B if available.
* Test against plasma of 3 A units and 3 B units.
* IF the discrepancy is not resolved, send to Reference Laboratory for resolution.
 |  |
|  | **Positive DAT*** Red cells coated with immunoglobulin may demonstrate a positive control in AB forward testing
 | * Wash 3-4 times with normal saline
* Retest
* IF the discrepancy is not resolved,

Send to Reference Laboratory for resolution |  |
|  |
| **EXAMPLE OF ABO DISCREPANCY SEEN WITH UNEXPECTED CELL REACTIONS** |
| **Patient Group** | **Reactions of Patient's Cells with** | **Reactions of Patient's serum with** |
| **Anti-A** | **Anti-B** | **Anti-A,B** | **Anti-A1****Lectin** | **A1** **Cells** | **A2 Cells** | **B Cells** | **O** **Cells** |
| **Acquired B** | 3 | 1/2 | 4 | 3 | 0 | 0 | 4 | 0 |
| **B (A)** | 1/2 | 4 | 4 | 0 | 4 | 3 | 0 | 0 |

|  |
| --- |
| **Procedure C: Weak or Missing Serum/Reverse Typing Reactions** |
|  | **Possible Causes** | **Resolution Techniques** | **Related Documents** |
|  | * **Hypogamma-globulinemia**
* Heme malig. e.g. CLL
* Immunosuppressive drugs
* Immunodeficiencies
* ABO incompatible hematopoietic stem cell transplant
* **Newborn**
* **Elderly**
 | * Encourage the weak antibodies to react by:
* Lengthening incubation time
* Reducing temperature (e.g.15-30 min incubation at 4C)
* Increasing plasma to cell ratio.
* Use Tube Set up Table C
* Incubate all tubes at RT for 5-10 minutes.
* Spin, read, and record.
* **If** discrepancy is not resolved, incubate tubes using reduced temperature.
 |  |
| **EXAMPLE OF ABO DISCREPANCY WITH WEAK OR MISSING ANTIBODIES** |
|  | **Reactions of Patient Cells with** | **Reactions of Patient Serum with** |
|  | **Anti-A** | **Anti-B** | **Anti-A,B** | **A1 Cells** | **B Cells** | **O Cells****(S1,2,3)** | **Auto****Control** |
| **Elderly patient -** **Group B** | 0 | 3 | 3 | 0 | 0 | 0 | 0 |
| **Unexpected (Extra) Reverse Typing Reactions** |  |
|  | **Possible Causes** | **Resolution Techniques** |  |
| **1** | * **Rouleaux formation** **secondary to Hyper-gammaglobulinemia**
* Multiple Myeloma
* Waldenstrom’s macroglobulinemia
* Some Hodgkin lymphomas
* Wharton’s jelly in cord blood samples
* Use of plasma expanders such as dextran
 | * Perform Saline Replacement Technique.
* Confirm rouleaux microscopically
* If the discrepancy is not Rouleaux, consider unexpected alloantibodies, and proceed with step 2.
 | * Saline Replacement Technique
 |
|  | **Action** | **Related Documents** |
| **Unexpected (Extra) Reverse Typing Reactions (continued)** |
|  | **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **2** | * **Unexpected Cold Alloantibodies** in patient plasma reacting with corresponding antigen on reagent red cells. Most common examples are:
* Anti-M
* Anti-P1
* Anti-N
* Lewis Antibodies
 | * Prepare tubes using Table D. Test using Cold Technique
* Add additional panel cells as necessary in order to have one cell negative for each of the following antigens:
* M
* N
* P
* Lewis.
* Observe for definitive pattern.
* **IF** there is no definite pattern and the discrepancy is not resolved, send to Reference Lab for resolution.
 | * Identification of Cold Reacting Antibodies
 |
| **3** | * **Presence of Anti-A1 in A Subgroups**

 | * Type patient cells for A 1  using anti-A1 lectin and A1 cells for pos control and A2 cells for neg control
* **If patient types negative for A1**confirm by testing patient plasma with segments of three A units.
* Interpret per table below.
 | Antigen Typing of Red CellsPackage Insert for Anti-A1 Lectin |

|  |  |  |
| --- | --- | --- |
| **If Patient Cells with** | **And Patient Plasma with** | **Action** |
| **Anti-A1 Lectin**  | **A1 cells** | **A2 cells** | **Random A donor cells** |  |
| **POS** | **POS/NEG** | **POS/NEG** | **POS/NEG** | **Perform identification for Cold Reacting Antibodies** |
| **NEG** | **POS** | **NEG** | **Majority are POS** | **Anti-A1****Issue group O RBCs** |

**Table A: Tube Set Up for Weak or Missing Forward Typing Reactions**

|  |  |  |
| --- | --- | --- |
| **Tube Label** | **Cells** | **Antisera/plasma** |
| **1. A** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A reagent |
| **2. B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-B reagent |
| **3. A1** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A1 (Lectin) |
| **4. A,B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A,B reagent |
| **5. Auto** | **1** drop of 3-5% cell suspension of pt's cells | **2-4** drops of pt's plasma |
| **6. A1C** | **1** drop of A1 reagent cells | **2-4** drops of pt's plasma |
| **7. A2C** | **1** drop of A2 reagent cells | **2-4** drops of pt's plasma |
| **8. O Cell Control** | **Matching tubes set up for patient cell testing substituting a group O cell for patient cells** |

**Table B:** **Tube Set up for Unexpected (Extra) Forward Typing Reactions**

|  |  |  |
| --- | --- | --- |
| **Tube Label** | **Cells** | **Antisera/plasma** |
| **1. A** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A reagent |
| **2. B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-B reagent |
| **3. A1** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A1 (Lectin) |
| **4. A,B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A,B reagent |
| **5. Auto** | **1** drop of 3-5% cell suspension of pt's cells | **2-4** drops of pt's plasma |
| **6. A1C** | **1** drop of A1 reagent cells | **2-4** drops of pt's plasma |
| **7. A2C** | **1** drop of A2 reagent cells | **2-4** drops of pt's plasma |
| **8. O Cell Control** | **Matching tubes set up for patient cell testing substituting a group O cell for patient cells** |

**Table C: Tube Set Up for Weak or Missing Reverse Group Reactions**

|  |  |  |
| --- | --- | --- |
| **Tube Label** | **Cells** | **Antisera/plasma** |
| **1. A1C** | **1** drop of A1 reagent cells | **2-4** drops of pt's plasma |
| **2. A2C** | **1** drop of A2 reagent cells | **2-4** drops of pt's plasma |
| **3. BC** | **1** drop of B reagent cells | **2-4** drops of pt's plasma |
| **4. S1** | **1** drop of S1 reagent screening cells | **2-4** drops of pt's plasma |
| **7. Auto** | **1** drop of 3-5% cell suspension of pt's cells | **2-4** drops of pt's plasma |

**Table D:** **Tube Set Up for Unexpected (Extra) Reverse Group Reactions**

|  |  |  |
| --- | --- | --- |
| **Tube Label** | **Cells** | **Antisera/plasma** |
| **1. A1C** | **1** drop of A1 reagent cells | **2-4** drops of pt's plasma |
| **2. A2C** | **1** drop of A2 reagent cells | **2-4** drops of pt's plasma |
| **3. BC** | **1** drop of B reagent cells | **2-4** drops of pt's plasma |
| **4. S1** | **1** drop of S1 reagent screening cells | **2-4** drops of pt's plasma |
| **5. S2** | **1** drop of S2 reagent screening cells | **2-4** drops of pt's plasma |
| **6. S3** | **1** drop of S3 reagent screening cells | **2-4** drops of pt's plasma |
| **7. Auto** | **1** drop of 3-5% cell suspension of pt's cells | **2-4** drops of pt's plasma |
| **8. ɪ neg cell** (if available) | **1** drop of 3-5% suspensionof cord cell or I neg cell if available | **2-4** drops of pt’s plasma |

**References:**

Current manufacturer’s package insert

Roback J (ed). Technical Manual, Current Edition. AABB Press, Bethesda, MD

Judd WJ, Johnson ST, Storry JR (eds). Judd’s Methods in Immunohematology. Current Edition. AABB Press Bethesda, MD