**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.
* ABO discrepancies that are unable to be resolved are interpreted as No Type Determined (NTD).
* Rh discrepancies that are unable to be resolved are interpreted as Unknown

**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: All Discrepancies**

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Related Documents** |
| 1 | Repeat testing to rule out technical error. |  |
| 2 | 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 |
| 3 | Contact patient’s nurse or physician and obtain the following patient information:* Transfusion history
* Patient received out of group blood and the discrepancy is explained by a mixed population including the donor RBCs, report as the patient’s original blood type
* ***Note****: If patient received both Rh pos and Rh neg RBCs before a sample is collected with no original blood type on file, interpret the Rh as Unknown (UNKN), unless otherwise instructed by Medical Director or Manager.*
* Transplantation history
* If patient has received a Bone Marrow Transplant (BMT) or other type of Hematopoietic Stem Cell Transplantation (HSCT), and the discrepancy is explained by a mixed population of donor and recipient cells and/or antibodies – result ABO interpretation as No Type Determined (NTD).
* 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 |
| ~~4~~ | 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 |

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| **Procedure A: Missing/Mixed Field/Weak-Reacting Cell/Forward Group Reactions** |
| **Step** | **Action** | **Related Documents** |
| 1 | Wash cells 3-4 times  | Washing Patient Red Cells |
| 2 | Repeat forward type with anti-A, anti-B, anti-A,B and an ABO/Rh control |  |
| 3 | **IF** | **THEN** |  |
| Discrepancy is resolved | * Record interpretations and proceed with testing.
* Add blood bank comment in Sunquest (BBCS) and PB Comment to patient order “ABO interp req wash X 3 of pt cells”
 |
| Discrepancy is not resolved | Choose a Resolution Technique based on the possible causes listed below |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **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 according manufacturer’s instructions.
 | See Tube set up Table AIdentification of Cold Reacting Antibodies |
| Grade and Record results on ABO Discrepancy Worksheet | Reading and Grading Tube HemagglutinationABO/D Discrepancy Worksheet |
| If the discrepancy cannot be resolved, give Group O RBC. Send to Reference lab for confirmation if needed. | Reference Lab Send Out Process |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **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
		- Record initial results in SQ grid

Consider * Enhancing reactivity with increased incubation time according to manufacturer’s instruction

Send to reference laboratory for resolution if the 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 |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **Mixed Field agglutination*** + Transfusion with non-type specific donor red blood cells
	+ Transplantation with non-type specific hematopoietic stem cell donor
	+ High WBC count
	+ Chimera
	+ Fetal-maternal haemorrhage
	+ Intrauterine transfusion
 | Obtain accurate history* + - The majority of mixed field can be explained by transfusion.
		- Contact transferring facility for results of pre-transfusion testing.
		- Add a PB Comment to patient order detailing transfusion history.
 |  |
| Test EDTA from Hematology laboratory if the sample was drawn prior to transfusion. Record results on LIS Downtime Manual Bench Testing Form. | ABO/D Type by Tube MethodLIS Downtime Manual Bench Testing Form |
| Rule out mixed field due to high WBC count microscopically |  |
| Grade and Record results on ABO Discrepancy Worksheet | Reading and Grading Tube HemagglutinationABO/D Discrepancy Worksheet |
| If the discrepancy cannot be resolved, give Group O RBC. Send to Reference lab for confirmation if needed. | Reference Lab Send Out Process |

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| **Procedure B: Unexpected (Extra) Cell/Forward Typing Reactions**  |
| **Step** | **Action** | **Related Documents** |
| 1 | Wash cells 3-4 times | Washing Patient Red Cells |
| 2 | Reset forward type with anti-A, anti-B, anti-A,B and an ABO/Rh control |  |
| 3 | **IF** | **THEN** |  |
| Discrepancy is resolved | * Record post-wash interpretations and proceed with testing.
* Add blood bank comment in Sunquest (BBCS) and PB Comment to patient order “ABO interp req wash X 3 of pt cells”
 |
| Discrepancy is not resolved | Choose a Resolution Technique based on the possible causes list below. |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **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.
 | Reference Lab Send Out Process |
| **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.
* 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
 | * Perform DAT
* IF the discrepancy is not resolved,

Send to Reference Laboratory for resolution | DAT by Tube Method |
| **EXAMPLE OF ABO DISCREPANCY SEEN WITH UNEXPECTED CELL/FORWARD 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 |

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| **Procedure C: Weak or Missing Serum/Reverse Typing Reactions** |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| * **Hypogamma-globulinemia**
* Hematological malignancy. e.g. CLL
* Immunosuppressive drugs
* Immunodeficiencies
* ABO incompatible hematopoietic stem cell transplant
* **Newborn**
* **Elderly**
* **Massive Transfusion of AB or Low Titer Plasma**
 | * Use Tube Set up in Table C
* Encourage the weak antibodies to react by the following methods:
* Lengthening RT incubation time for 5-10 minutes
* Increasing plasma to cell ratio to 4 drops of plasma to 1 drop of cells.
* Document reaction on ABO discrepancy worksheet and in SQ.
* Record post resolution results in SQ grid.
* Add PB comment in SQ detailing ABO discrepancy resolution.
 | ABO/D Discrepancy Worksheet |
| **EXAMPLE OF ABO DISCREPANCY WITH WEAK OR MISSING SERUM/REVERSE REACTIONS** |
|  | **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 |

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| --- |
| **Procedure D: Unexpected (Extra) Reverse Typing Reactions** |
| **Step** | **Action** | **Related Documents** |
| 1 | Check for rouleaux (refractile stacking of red cells resembling a stack of coins) microscopically

|  |  |
| --- | --- |
| **IF Rouleaux is** | **THEN** |
| Present | Perform saline replacement.See below for possible causes. |
| Not present | Go to step 2 |

Record results on ABO/D Discrepancy Worksheet and in SQ | Saline Replacement TechniqueABO/D Discrepancy Worksheet |
| 2 | Run an Auto control and choose a resolution technique based on the possible causes listed below. | Identification of Cold Reacting AntibodiesABO/D Discrepancy WorksheetAntibody Identification Worksheet |
| 3 | **IF Autocontrol is** | **CONSIDER** |  |
| Positive | Cold Auto AntibodiesNOTE: It is possible to have a patient with an anti-A1 and a positive autocontrol. |
| Negative | Cold Allo Antibodies or An A Subgroup with an anti-A1 (If patient has applicable blood type) |
| **Possible Causes** | **Resolution Techniques** | **Related Documents** |
| **Transfusion of group O Whole Blood to non-group O patients****Transfusion of Low Titer Plasma to non-group A or O patients** | * Additional reactivity in reverse may be seen. Rule out other ABO discrepancies first.
* Check for pretransfusion sample and test on LIS Downtime Manual Bench Testing Form
* If unable to resolve discrepancy, report ABO as “NTD”
 | LIS Downtime Manual Bench Testing Form |
| **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
 | * Verified in step # 1 above.
 | Saline Replacement Technique |
| **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
 | * Set up tubes using table D.
* 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** cold antibody identified, perform ABO reverse typing using antigen negative cells (antigen typed donor cells may be used)
* Record initial reverse discrepancy in SQ grid (This will generate an override)
* **IF** there is no definite pattern and the discrepancy is not resolved, send to Reference Lab for resolution.
 | Identification of Cold Reacting AntibodiesReference Lab Send Out Process |
| **Unexpected Cold Auto Antibodies** | * Rule out the possibility of cold allo antibodies.
* Utilize the Pre-Warm technique to resolve extra reactivity caused by cold auto antibodies.
* Record results on ABO/D Discrepancy Worksheet
* Record initial reverse discrepancy in SQ grid (This will generate an override)
 | Judd’s Methods in Immuno-hematologyABO/D Discrepancy Worksheet |
| **Presence of Anti-A1 in A Subgroups** | * Set up tubes using table E.
* Test patient cells with anti-A1.
	+ If no agglutination with anti-A1, patient has an A subgroup.
* Test reverse, substituting A1 cells for A2 cells.
* Test the patient’s plasma against 3 random A donor units.

|  |  |  |
| --- | --- | --- |
| Units | Then | Interpretation |
| All Positive  | Testing complete | Patient has anti-A1 |
| Any Negative Reaction | Antigen type negative units with anti-A1 lectin | If units Neg Patient has anti-A1 |
| If units Pos Patient does not have anti-A1 |

* Record results on ABO/D Discrepancy Worksheet
* Record initial A1 reverse discrepancy in SQ grid (This will generate an override) and interpret per table below.
* Enter anti-A1 in ABI
* Add PB comment indicating patient is a subgroup of A that has made anti-A1.
* Give O RBCs to Asub patients who have made an Anti-A1
 | Antigen Typing of Red CellsPackage Insert for Anti-A1 LectinABO/D Discrepancy WorksheetAntigen Typing Worksheet |
| **EXAMPLE OF PATIENT WITH AN A SUBGROUP AND AN ANTI-A1 ANTIBODY** |
|  | **Reactions of Patient Cells with** | **Reactions of Patient Serum with** |
|  | **Anti-A** | **Anti-B** | **Anti-A1 Lectin** | **A1 Cells** | **A2 Cells** | **B Cells** | **Random A Donor Cells** |
| **A subgroup with an Anti-A1** | 3+ | 0 | 0 | W+/1+ | 0 | 3+ | W+/1+ |

**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. A,B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A,B reagent |
| **4.** **ABO/Rh Control** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of ABO/Rh Control |

**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. A,B** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A,B reagent |
| **4. ABO/Rh Control** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of ABO/Rh control |

**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. BC** | **1** drop of B reagent cells | **2-4** drops of pt's plasma |
| **3. O cell** | **1** drop of O reagent screening cells | **2-4** drops of pt's plasma |
| **4. Auto Control** | **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. BC** | **1** drop of B reagent cells | **2-4** drops of pt's plasma |
| **3. S1** | **1** drop of S1 reagent screening cells | **2-4** drops of pt's plasma |
| **4. S2** | **1** drop of S2 reagent screening cells | **2-4** drops of pt's plasma |
| **5. S3** | **1** drop of S3 reagent screening cells | **2-4** drops of pt's plasma |
| **6. Auto Control** | **1** drop of 3-5% cell suspension of pt's cells | **2-4** drops of pt's plasma |

**Table E: Tube Set Up for Subgroup Workup**

|  |  |  |
| --- | --- | --- |
| **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 Lectin** | **1** drop 3-5% cell suspension of pt's cells | **1** drop of Anti-A1 (Lectin) |
| **4. A1C** | **1** drop of A1 reagent cells | **2-4** drops of pt's plasma |
| **5. A2C** | **1** drop of A2 reagent cells | **2-4** drops of pt's plasma |
| **6. BC** | **1** drop of B reagent cells | **2-4** drops of pt's plasma |
| **7. Random A Donor Units x3** | **1** drop 3-5% cell suspension each of individual donor cells | **2-4** drops of pt's plasma |

**References:**

Current manufacturer’s package insert

Technical Manual, Current Edition. AABB Press

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