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Work Instruction

Title:	**TS - Performing ABO Grouping & Investigating ABO Grouping Discrepancies**	**WI Number** SFOWI-0082 **Revision:** 12
Department: Immunohematology **Area:** 2425 Geary Blvd SFO Hospital Lab	***Document is in the Final Approval Process. 2 - approvals are required***	
Type of Document: Work Instruction		**Review Period -** 340 **Days**

PURPOSE

A. This protocol provides instructions for performing ABO testing and resolution of ABO discrepancy.

B. The blood group of an individual is determined by testing the red cells with Anti-A and Anti-B sera. Agglutination of the red cells signifies the presence of the relevant antigen, while no agglutination signifies its absence. Confirmation of these results is provided by testing the plasma of the blood under investigation with group A_1 and B red cells.

C. When the results of the ABO for the current specimen either by manual or automated method do not agree with the previous ABO on record or when the results of the cell and plasma testing for ABO do not agree, the discrepancy must be investigated.

D. If transfusion is necessary prior to resolution of the ABO discrepancy, group O packed red blood cells of the appropriate Rh type and group AB plasma should be given.

REAGENTS

A. Anti-A sera
B. Anti-B sera
C. A_1 cells
D. B cells
E. Isotonic saline
F. Anti-A,B, A_2 cells, and O screening cells - for investigation of discrepancy

REAGENT SAFETY PRECAUTIONS

A. The antisera reagents contain 0.1% Sodium Azide and is classified as Harmful (Xn).
B. The cellular reagents are potentially infectious.
C. Store reagents at 2 - 8°C when not in use.
D. Refer to manufacturer inserts for more information.

EQUIPMENT

A. 12 x 75 mm test tubes
B. Serologic centrifuge
C. Agglutination viewer
D. Transfer pipettes
E. Cell washer

SPECIMEN

A. 7 mL EDTA (lavender) specimen preferred for adult patients
B. Red top clotted specimen is acceptable
C. Minimum 1.5 mL EDTA peripheral blood for neonate
D. Cord blood (refer to SFOWI-0104 Cord Blood Testing)

CONTROLS

A. Daily Reagent Quality Control

PROCEDURE

A. **Red Blood Cell Testing (forward typing)**

1. Label one tube for Anti-A and one tube for Anti-B.
2. Place one drop of Anti-A and one drop of Anti-B in each appropriately labeled tubes.
3. Add one drop of a 2-5% suspension of red cells in saline (washed or unwashed) to each tube.
4. Gently mix the contents of each tube thoroughly then centrifuge both tubes at the calibrated spin time.
5. Re-suspend the cell button by gentle agitation and examine macroscopically for agglutination using the agglutination viewer.
6. Record the test results immediately.

B. **Plasma Testing (reverse typing)**

1. Label one tube for A_1 cells and one tube for B cells.
2. Place two drops of plasma or serum in each appropriately labeled A_1 and B cell tubes.
3. Add one drop of 2-5% suspension of reagent A_1 or B red cells to the appropriate tube.
4. Gently mix the contents of each tube thoroughly then centrifuge both tubes at the calibrated spin time.
5. Re-suspend the cell button by gentle agitation and examine macroscopically for agglutination and/or hemolysis using the agglutination viewer.
6. Record the test results immediately.

C. **Interpretation of Results**

1. The expected **positive** reaction is **Forward Typing: 3+ to 4+ without mixed field** and **Reverse Typing: 2+ to 4+**.

Front/Forward typing with patient red cells		Back/Reverse typing with patient plasma		Interpretation
Anti-A	**Anti-B**	**A_1 cells**	**B cells**	**ABO Group**
0	**0**	**2+ - 4+**	**2+ - 4+**	**O**
***3+ - 4+**	**0**	**0**	**2+ - 4+**	**A**
0	***3+ - 4+**	**2+ - 4+**	**0**	**B**
***3+ - 4+**	***3+ - 4+**	**0**	**0**	**AB**

KEY: ***** = with no mixed field; + = agglutination; 0 = no agglutination

2. If other than expected results occur; refer to the procedure for resolving ABO discrepancies.

D. **Investigation of ABO Discrepancy**

NOTE: When transfusion is necessary and the ABO discrepancy is unresolved,

a. perform serological crossmatch of Group O red cells with the appropriate Rh type
and
b. give AB plasma.

1. **Definition of ABO Discrepancy:**
   a. Current ABO Grouping does not match historical result
   b. Reaction strength is weaker than expected: Forward < 3+; Reverse < 2+
   c. Mixed field reaction
   d. Forward and Reverse typings do not match

2. **Determine where the Typing (Forward/Reverse) Problem exists:**
   a. Identify the atypical reactions from what is expected in healthy adults or infants. **NOTE: Refer to section for Interpretation of Results.**
   b. **Forward Typing** - Atypical Reactions
      i. Weaker than 3+
      ii. Mixed field
   c. **Reverse Typing** – Atypical Reactions
      i. Weaker than 2+
   d. **Forward and Reverse together** – Atypical Reactions
      i. Forward and Reverse Typings do not match due to Weak/Missing reaction or Extra reaction e.g. Forward typed as group A but Reverse typed as group O.
   e. **If atypical reactions cannot be clearly determined, investigate both Forward and Reverse Typings simultaneously.**

3. **Common ABO Discrepancy Patterns**

Anti -				Cells								Possible Source of Discrepancy
A	B	A_1	H	A_1	A_2	B	I	II	III	AC	I Neg	
+	0			+*		+*				+*		*Stack of coins (Rouleaux)
+	0	0		+	0	+				0		A subgroup with anti-A_1
+	0			+		+	+	0	+	0		Cold alloantibody
+	0			+		+	+	+	+	+	0	Auto anti-I
0	0		0	4+		4+	4+	4+	4+	0	4+	O_h (Bombay phenotype) with anti-H
+	+			0		+	0	0	0	0		Acquire B Phenotype
0	0			0		0				0		Group O Infant or old age
+	0			0		0				0		Group A Infant or old age
0	+			0		0				0		Group B Infant or old age
+	+	0		+	0	0	0	0	0	0		A subgroup B with anti-A_1
+	0			+	+	+	0	0	0	0/+		Anti-IA
0	+			+	0	+	0	0	0	0/+		Anti-IB
+	0	+		0/W+*	+	+	+	+	+	0/W+*	0/W+*	Group A_1 with anti-IH (*can sometimes be weakly reactive)

4. **If the current blood type does not match the historical blood type, perform one or more of the following steps:**

   a. Check identification of the specimen.

   b. Request a new specimen if suspect contamination or misidentification.

   c. Repeat testing to rule out technical error. Refer to section for ‘Sources of Technical Error’.

   d. Repeat testing with 3X washed patient cells or reagent cells.

   e. Check patient's clinical history and diagnosis for bone marrow/progenitor cells transplant or massive blood transfusion of a blood group different from their own.

   f. If the discrepancy cannot be explained, request a new specimen.

      i. If the second specimen agrees with the first, change the historical ABO to the current ABO interpretation.

      ii. If the second specimen differs from the first but matches the historical, the discrepancy is resolved.

      iii. Retrieve the historical sample if it is still available and repeat the ABORh testing for investigation purposes. Perform the necessary corrective action.

      iv. If the historical result was verified by another facility, notify the facility to perform the investigation.

5. **Possible Causes and Solutions for ABO Typing Discrepancies**

Category	Causes	Solution
Mixed-field red cell reactivity	Recent red cells transfusion ABO subgroup A_3, B_3 Bone Marrow or Hematopoietic Stem Cell Transplant ABO mismatched transplants Fetomaternal hemorrhage Twin or dispermic (tetragametic) chimerism	1. Mixed field agglutination is encountered when there is two distinct, separate populations of red blood cells. 2. Resolve by patient's transfusion history, diagnosis and transplant history. 3. Transfusion History: Obtain transfusion dates, the number of red cell units transfused, patient's historical and donor cell's blood types. 4. BMT/HSCT History: Obtain transplant date(s), auto versus allo transplant, patient's historical and donor's blood types. 5. If there is no clinical explanation for the mixed field, test the patient's red cells with anti-A_1 lectin to determine if the patient is a subgroup of A.

Category	Causes	Solution
Plasma weak /missing reactivity	Age related < 4-6 months old, elderly ABO subgroup weak A or B subgroup Hypogammaglobulinemia immunocompromised Bone Marrow or Hematopoietic Stem Cell Transplant ABO mismatched transplants	1. Increase plasma to reagent red cell ratio i.e. 3-4 drops plasma to 1 drop reagent cell. 2. If the reaction is still weak or negative, incubate the plasma with A_1 cell, B cell, ABSC and autocontrol (AC) for 15-30 minutes at room temperature. 3. If the reaction is still weak or negative, incubate at 4°C for 15-30 minutes. 4. The autocontrol and ABSC (group O red cells) are included for room temperature and 4°C testings as controls for reactivity of common cold autoagglutinins. 5. Further investigation is needed if either or both the AC and ABSC is/are reactive. 6. Including group O cord cell for room temperature and 4°C testing may give valuable information when trying to rule out/in anti-I/i. 7. Check patient's age and diagnosis.

Category	Causes	Solution
Plasma extra reactivity	Plasma antibody to reagent constituent Transfusion of large volume of non-type specific plasma components Bone Marrow or Hematopoietic Stem Cell Transplant ABO mismatched transplants Infusion of intravenous immune globulin IVIg can contain ABO isoagglutinins	1. Washed reagent red cells three times. 2. Repeat plasma testing with washed reagent red cells. 3. Read macroscopically and microscopically. 4. If the discrepancy is not resolved, check patient's transfusion history, transplant history, medication history and diagnosis. 5. Transfusion History: Obtain transfusion dates, the number of plasma and/or platelets transfused, patient's historical and donor cell's blood types. 6. BMT/HSCT History: Obtain transplant date(s), auto versus allo transplant, patient's historical and donor's blood types. 7. Medication History: Inquire if and when IVIg was infused. Note: IVIg is frequently given to patient diagnosed with Idiopathic Thrombocytopenic Purpura (ITP).

Category	Causes	Solution
Plasma extra reactivity	ABO subgroup subgroup of A with anti-A_1	**Unexpected Reaction with A1 cells** 1. Reactivity of the A_1 reagent cells when anti-A is strongly reactive with the patient's red cells suggests the presence of anti-A_1 in the plasma of an individual who is a subgroup of A (most commonly A_2 or A_2B). 2. Test the patient's cells with anti-A_1 lectin to differentiate group A_1 from subgroup of A. 3. If there is no reactivity between patient's cells and anti-A_1 lectin, test the plasma against 2-3 A_1 cells, and 2-3 A_2 cells. Only if the antibody agglutinates all A_1 red cells and none of the A_2, can it be called anti-A_1.
	Cold alloantibody anti-P_1, anti-M, anti-Le^a	4. If the patient is A_1, then the extra reactivity may be due to an unexpected alloantibody that reacts at room temperature. **Unexpected alloantibodies that react at room temperature** 1. Identify the room temperature alloantibody by performing an IS or RT ABSC with autocontrol, and phenotype the reagent A_1 and B cells to determine which, if either, carries the corresponding antigen. 2. Repeat back typing with new lots of A_1 and B cells if available or obtain A_1 and/or B red cells that lack the antigen if available and use them for back typing. If expected reactions are obtained, then the discrepancy is resolved.
	IgM alloantibody reacting at RT anti-c, anti-K	3. Alternatively, warm the plasma and reagent A_1 and B red cells to 37°C <u>separately</u> for 5-10 minutes before testing. Mix and centrifuge immediately. If expected reactions are obtained, then the discrepancy is resolved. 4. If discrepancy is not resolved, prepare new tubes and warm the plasma and reagent A_1 and B red cells to 37° C <u>separately</u> for 5-10 minutes. Mix and incubate at 37°C for 1 hour and observe for agglutination without centrifugation ('settled' reading). If the thermal amplitude of the alloantibody is below the temperature at which anti-A and anti-B react, this may resolve the discrepancy. **NOTE:** This procedure is **NOT** to be used for Antibody Screen and Crossmatch. 5. If the antibody detection test is negative, test the plasma against several examples of A_1 and B red cells. The plasma may contain an antibody directed against

		an antigen of low incidence, which will be absent from most randomly selected A_1 and B red cells.

Category	Causes	Solution
Plasma extra reactivity	Cold autoantibody anti-I, anti-i	**Strongly reactive cold autoagglutinins** 1. Anti-I, anti-IH, anti-IA, and anti-IB can agglutinate red cells of adults, including autologous cells and reagent red cells, at room temperature. 2. Perform a cold ABSC at 15 minutes RT and 15 minutes 4°C, include autocontrol, A_1 cells, A_2 cells, B cells and O cord cells (if available) to confirm the presence of cold autoagglutinins. 3. **NOTE:** If the autocontrol is non-reactive, the antibody is not likely to be a cold autoantibody. 4. Anti-IH is seen in A_1 individual usually with a negative autocontrol and reacts preferentially with A_2 and adult O cells. 5. Warm the plasma and reagent A_1 and B red cells to 37 °C <u>separately</u> for 5-10 minutes before testing. Mix and centrifuge immediately. If expected reactions are obtained, then the discrepancy is resolved. 4. If discrepancy is not resolved, prepare new tubes and warm the plasma and reagent A_1 and B red cells to 37° C <u>separately</u> for 5-10 minutes. Mix and incubate at 37°C for 1 hour and observe for agglutination without centrifugation ('settled' reading). If the thermal amplitude of the alloantibody is below the temperature at which anti-A and anti-B react, this may resolve the discrepancy. **NOTE:** This procedure is **NOT** to be used for Antibody Screen and Crossmatch. 6. Reference Lab: Cold autoadsorption will remove cold autoagglutinins from the plasma. Then the adsorbed plasma can be tested against A_1 and B reagent red cells. **Sera from patients with abnormal concentrations of plasma proteins** 1. Rouleaux can be easily recognized microscopically as a "stack of coins". 2. Respin the plasma-cell mixture, remove the plasma with a pipette and replace it with an equal volume of saline. 3. Centrifuge the plasma-saline mixture, then resuspend the button. Rouleaux will disperse but true agglutination will remain.
	Excess plasma proteins causing rouleaux infusion of high molecular weight plasma expander Multiple Myeloma	

Category	Causes	Solution
Extra red cell reactivity (with or without mixed field)	Autoagglutinins heavy coating of red cells by potent autoagglutinins	**Initial Steps** 1. Repeat testing after washing the patient's red cells three times with saline. 2. If suspect cold reactive autoantibody, repeat testing after washing three times with 37^0C warmed saline. 3. If the discrepancy is not resolved, check patient's transfusion history, transplant history and diagnosis. 4. Transfusion History: Obtain transfusion dates, patient's historical and donor cells blood types. May need further investigation to make sure that patient was not initially mistyped and given incompatible blood. 5. BMT/HSCT History: Obtain transplant date(s), auto versus allo transplant, patient's historical and donor's blood types. 6. Diagnosis: Associated with Gastrointestinal (GI) disease or infection – suspect Acquired B antigen. 7. If patient's red cells are strongly reactive with anti-B and weakly reactive with anti-A (<2+), suspect B(A) Phenotype.
	Unwashed red cells abnormal concentration of plasma proteins or infused macromolecular solution	
	Unwashed red cells antibody in patient's plasma to reagent constituent	
	Out-of-group transfusion	
	Bone Marrow or Hematopoietic Stem Cell Transplant ABO mismatched transplants	**Acquired B Phenotype** 1. Acquired B antigen is usually associated with GI conditions that allow colonic bacteria to enter the circulation, but Acquired B antigens have also been found on the red cells of apparently normal blood donors. 2. Patient's red cells react strongly with anti-A and weakly with anti-B (2+ or less) while the plasma reacts strongly with B cells but not with A_1 cells and the patient's own cells. 3. Detection of the Acquired B phenotype is also influenced by reagent pH and specific monoclonal anti-B reagents (ES-4 clone). Check manufacturer's insert. 4. Repeat testing with a different monoclonal anti-B reagent or with acidified (pH 6.0) human anti-B should resolve the discrepancy.
	Acquired B antigen	
	B(A) Phenomenon	**B(A) Phenomenon** 1. Patient's red cells react strongly with anti-B and weakly with anti-A, while the plasma reacts strongly with both A_1 and A_2 red cells, but not with B cells. 2. Except for newborns and immunocompromised patients, plasma testing should distinguish this phenomenon from the AB phenotype in which a subgroup of A is accompanied by anti-A_1. 3. Repeat testing with a polyclonal anti-A or a different

Extra red cell reactivity (with or without mixed field)	A(B) Phenomenon	monoclonal anti-A reagent (one that lacks the MHO4 clone) should resolve the discrepancy. **A(B) Phenomenon** 1. This phenomenon is not well documented and there is no recommended solution at this time. 2. The A(B) phenotype has been described with monoclonal anti-B. 3. The A(B) phenotype was associated with elevated H antigen and plasma H-transferase activity. 4. It is hypothesized that the increased H precursor on these cells may permit the synthesis of some B antigen by the A glycosyltransferase.

Category	Causes	Solution
Red cell weak/ missing reactivity (with or without mixed field)	ABO Subgroup or inherent variant alleles weakened antigen expression	1. Wash patient's red cells three times with saline. 2. Incubate the washed red cells with anti-A, anti-B, and anti-A,B, including autocontrol (AC) for 15-30 minutes at room temperature or 15-30 minutes at 4°C to increase the association of antibody with antigen. 3. Result is invalid if the AC is reactive. 4. If the discrepancy is not resolved, check patient's diagnosis, transfusion history, and transplant history. 5. Transfusion History: Obtain transfusion dates, the blood products transfused, the number of units transfused, patient's historical and donor cell's blood types. 6. BMT/HSCT History: Obtain transplant date(s), auto versus allo transplant, patient's historical and donor's blood types. 7. Reference Lab: Adsorption (at room temperature or at 4°C) and elution method using human anti-A or anti-B can be used to detect weak ABO antigens.
	Leukemia/malignancy results in weakened antigen expression	
	Transfusion massive transfusion of non-type specific group compatible blood	
	Intrauterine Fetal Transfusion	
	Bone Marrow or Hematopoietic Stem Cell Transplant ABO mismatched transplants	
	Excessive soluble blood group substance in plasma neutralizes anti-A or anti-B	

E. **Interpretation for Unresolved ABO Discrepancy**

If the ABO discrepancy cannot be resolved at the conclusion of the investigation, interpret the current blood type **and** change the historical blood type to **ABO 'Indeterminate'** and add **Blood Bank Comments: ABO Discrepancy. Give Group O red cells and AB plasma**. **NOTE:** For HSCT patients, please refer to SFOWI-1309 Transfusing Patient Post-HSCT for detailed instructions.

F. **Selection of Blood if Discrepancy is Due to an Antibody**
Refer to SFOWI-0088 Antibody Identification and SFOWI-0089 Compatibility Testing.

G. **Sources of Technical Error**

1. **False Negative Results:**
   a. Specimen mix-up.
   b. Red cell suspension too light.
   c. Under-centrifugation of tests.
   d. Use of contaminated reagents, specimen or saline.
   e. Too vigorous shaking of cell button.
   f. Failure to add reagent or test plasma/serum to tube(s).
   g. Failure to identify hemolysis as a positive reaction.
   h. Failure to use the appropriate ratio of plasma (or reagent) to red cells.
   i. Incorrect interpretation or recording of test results.
   j. Failure to follow manufacturer's instructions.
2. **False Positive Results:**
   a. Specimen mix-up.
   b. Red cell suspension too heavy.
   c. Over-centrifugation of tubes.
   d. Use of contaminated reagents, specimen or saline.
   e. Use of dirty glassware.
   f. Incorrect interpretation or recording of test results.
   g. Failure to follow manufacturer's instructions.

REFERENCES

A. AABB Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.
B. AABB Technical Manual, current edition, Bethesda, MD.
C. Manufacturer's Anti-sera Reagent inserts.
D. Manufacturer's Red Blood Cell Reagent inserts.

Associated Documents:

External Documents

SFOWI-0088 Antibody Identification
SFOWI-0089 Compatibility Testing
SFOWI-0104 Cord Blood Testing
SFOWI-0105 Neonatal Transfusion
SFOWI-1309 Transfusing Patient Post-HSCT
Associated Quality System Documents - None

Documents Generated:

Check As Applicable (X or NA)	Format History	New Format Requirements
	A document created before September 1, 2005 was written before the new document format template and electronic approving process were implemented. Documents were copied from another document database and pasted on the QSI Quality Management System in order to be included in the Kaiser Permanente San Francisco Laboratory electronic document control database.	This document will be re-written to conform to the new Kaiser Permanente San Francisco Laboratory document format template whenever this document is revised.
Comments:	Documents created after QSI implementation have been directly entered in the QSI environment.	

Document Revision History:

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Document Author: Cara H Lim/CA/KAIPERM	**Document Manager:** Richard Chui/CA/KAIPERM

Reason for Change:

Revision:	Sec/Para Changed	Change Made:	Date
1	N/A	Initial Issue of Document	
2	Procedure	Change Lifeline to RILIS	5/12/07
3	Approver	New Lab Director	7/1/07
4	Procedure D. Investigation of discrepancies	Interpret the ABORH I as "* " .	8/3/08
5	Procedure D. Investigation of discrepancies	Interpret the ABORH I as "NOTE " .	9/4/09
6	Approver	New Lab Director	6/11/11
7	Procedure D. NOTE. Whole document	Changed numbering from 1 to NOTE. Changed from "NOTE" to blank for ABO interpretation. Deleted reference to RILIS.	11/29/12
8	Approver Procedure A.	New Lab Director. New.	3/26/13
9	Approver Table - Serum extra reactivity - Unexpected Reaction with A1 cells Table - Serum extra reactivity - Unexpected alloantibodies that react at room temperature Table - Extra red cell reactivity Procedure D. Associated Documents Procedure D. Note	New BB Medical Director. Revised. Deleted O cells and added 'it may be an unexpected alloantibody that reacts at room temperature if patient is A1'. Revised. Added 'by performing an IS or RT ABSC with autocontrol'. Added instructions for excess proteins and cold autoagglutinins. Deleted section for Problem with Forward Typing - moved instructions to table as Excess proteins and Cold autoagglutinins. Added SFOWI-0088 & SFOWI-0089. Deleted 'to leave interpretation blank'.	10/31/13
10	Procedure D. Note. Procedure E.	Revised. Added 'perform serological', 'and give AB plasma'. New. Added instructions for ABO discrepancy interpretation.	12/9/13
11	Table 'Possible Causes and Solutions for ABO Typing Discrepancies', Plasma weak/missing reactivity.	Revised. Moved instruction to increase plasma to reagent cell ratio as first option. Clarified inclusion of AC and ABSC (as 'O cells') for RT and 4^{0}C testing. Added suggestion to include group O cord cells to rule out/in anti-I/i.	1/5/16
12	Whole Document	Revised as the standardized ABO SOP (reference version) for KP NCAL.	1/18/17

Notification List:

Approvals:

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Document History Section