University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 01-30-2017 Revision Effective Date: 05-23-2022	Number: PC-0060.02
TITLE: **ABO/Rh Discrepancy Resolution**		

PURPOSE:
To provide guidance for resolving ABO and Rh testing discrepancies. A discrepancy exists when:

- Results of the forward group do not agree with the reverse group
- Unexplained weak or mixed field reactivity
- Current testing is not in agreement with previous records

PRINCIPLE & CLINICAL SIGNIFICANCE:
The test methods employed depend on the type of discrepancy detected. Failure to resolve a blood typing discrepancy accurately can lead to transfusion of incompatible blood components which can result in hemolytic transfusion reactions.

- Discrepancies may arise from patient identification or labeling errors, intrinsic problems with red cell (forward type), serum (back type), patient disease, treatment, or technical errors in test performance.
- Mixed field (MF) reaction and other discrepancies may arise due to transfusion of ABO compatible but non-identical blood components and or allogeneic BMT
- Weak D positive donors considered Rh positive can lead to Rh discrepancies between the patient and transfused circulating red blood cells.

POLICIES:
- Difficulty in typing and resolution must be documented in the LIS as a BBCS comment.
- Mixed field reactions seen with cord blood samples may be due to **c**ontamination from maternal blood**,** Warton's jelly and weak expression of RBC antigens. Follow the instructions in section **Cord Blood Mixed Field Reaction** for resolution and action to take if not resolved.

SPECIMEN REQUIREMENTS:
EDTA is preferred and if not tested soon after collection, should be stored at 1-6°C.
Red top tubes are also acceptable. See SOP *Specimen Acceptability and Order Receipt*

REAGENTS/SUPPLIES/EQUIPMENT:
Refer to the SOP specific for the test being performed.

QUALITY CONTROL:
Refer to the SOP specific for the test being performed.

INSTRUCTIONS:

TABLE OF CONTENTS:

All Discrepancies

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Review the patient’s history in the Laboratory Information System (LIS) and/or Electronic Medical Record (eMR) for indications of the cause of the discrepancy

NOTE: It may be necessary to contact other facilities where the patient may have been treated to obtain transfusion and/or transplant history.
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Patient has received a BMT or other type of HSCT</td><td>• If the discrepancy is explained by a mixed population of donor and recipient cells and/or antibodies -report as No Type Determined (NTD).
• Go to section Reporting Results</td></tr>
<tr><td>Patient received out of group blood</td><td>• If the discrepancy is explained by a mixed population including the donor RBCs, report as the patient’s original blood type
• Go to section Reporting Results</td></tr>
<tr><td>Current does not match historical</td><td>Request a new sample and repeat testing.
<table>
<tr><th>If new sample</th><th>Then</th></tr>
<tr><td>Agrees with historical</td><td>• Proceed to interpretation and reporting results. Reject first sample as MISLABEL
• Look for a reciprocal mislabeled specimen
• Notify a manager or lead
• Write a QI</td></tr>
<tr><td>Disagrees with historical</td><td>• Examine historical for misinterpretation
• Ask RN to verify photo-ID
• Notify department management and obtain Manager/Lead approval before issuing any products to patient if current and new samples both disagree with historical type</td></tr>
</table></td></tr>
<tr><td>Discrepancy not resolved</td><td>Go to next step</td></tr>
</table></td></tr>
</table>

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>2</td><td>Prepare a new 3-4% patient cell suspension and repeat the ABO/Rh testing using the manual test method
<table>
<tr><th>If discrepancy is</th><th>Then</th></tr>
<tr><td>Resolved</td><td>Go to section Results Reporting</td></tr>
<tr><td>NOT resolved</td><td>• Save reaction results, but do not enter an interpretation in Sunquest
• Go to next step</td></tr>
</table></td></tr>
<tr><td>3</td><td>Review patient’s transfusion/transplant/age history for cause of the discrepancy. The following sources may be used to obtain relevant patient information:
• Sunquest BBI
• Epic
• Contact the patient, patient’s physician or caregiver
• Contact other medical facilities where the patient was provided care</td></tr>
<tr><td>4</td><td>Select the type of discrepancy below and follow the suggested technique as appropriate to resolve the discrepancy
• Unexpected positive reactions in the forward type (patient cells)
• Weak or unexpected negative reaction in the forward type (patient cells)
• Unexpected positive reactions in reverse type (patient serum)
• Weak or unexpected negative reaction in the reverse type (patient serum)
• Rh Discrepancy
• Cord Blood Mixed Field Reaction</td></tr>
</table>

Unexpected positive reactions in the forward type (patient cells)

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Perform an IgG DAT
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Positive</td><td>Treat with EGA and repeat testing with EGA treated cells</td></tr>
<tr><td>Negative</td><td>Wash patient cells with warm saline 4X and repeat testing</td></tr>
</table></td></tr>
<tr><td>2</td><td>
<table>
<tr><th>If resolved</th><th>Then</th></tr>
<tr><td>Yes</td><td>Go to section Results Reporting</td></tr>
<tr><td>No</td><td>Consult with TSL management or submit to IRL</td></tr>
</table></td></tr>
</table>

Weak or unexpected negative reaction in the forward type (patient cells)

STEP	ACTION	
1	**If**	**Then**
	Weak subgroup of A or B or depression of antigen expression	Try the following in order and discontinue additional testing once the discrepancy is resolved: • Incubate patient's cells with anti-A, anti-B, 7% albumin and anti-A,B (if applicable) for 15-20 minutes at RT and repeat testing • Incubate the tubes from repeat ABO/Rh testing (along with 1 drop group O screen cells and two drops of patient's plasma as a control) at 4°C for 10 min • Try using a different manufacturer of antisera if available
	Suspect neutralization of blood group antisera	Wash patient cells 3-4 times in blood bank saline and repeat testing
2	**If resolved**	**Then**
	Yes	Go to section Results Reporting
	No	Consult with TSL management or submit to IRL

Unexpected positive reactions in reverse type (patient serum)

STEP	ACTION	
1	Perform a microscopic evaluation of the reaction to look for rouleaux	
	If rouleaux is	**Then**
	Observed (retractile stacking of red cells resembling a stack of coins)	Perform Saline Replacement and repeat testing
	Not Observed	Go to next step
2	Perform an antibody screen at IS phase	
	If Antibody screen is	**Then**
	Positive	Suspect possible cold antibody: • Perform a cold panel to detect cold-reacting antibodies – If identified repeat testing with antigen negative reverse cells • Prewarm reverse typing for cold agglutinin
	Negative	Suspect isoantibodies such as anti-A_1 • Type patient cells with A_1 lectin and test patient plasma with A_2 cells
3	**If resolved**	**Then**
	Yes	Go to section Results Reporting
	No	Consult with TSL management or submit to IRL

Weak or unexpected negative reaction in the reverse type (patient serum)

STEP	ACTION
1	Suspect depressed antibody production and try the following in the order listed and stop once discrepancy is resolved: • Incubate patient's plasma with A_1 and B cells, screen cells and an auto control for 15-30 minutes at room temperature (RT) • Incubate at 4°C for at least 10 minutes • Increase the serum to cell ratio by using 3-4 drops of patient's plasma and one drop of reagent A_1 and B cells and repeat testing
2	**If resolved** / **Then** Yes / Go to section Results Reporting No / Consult with TSL management or submit to IRL

Rh Discrepancy

STEP	
1	Repeat ABO/Rh using alternate method - refer to SOPs *ABO/Rh Manual Tube Method* or *Ortho Vision Patient and Donor Testing* and the appropriate package insert **If** / **Then** Resolved / Go to section Results Reporting Not Resolved / Go to next step
2	Perform Weak D testing per SOP ***Weak D Tube Testing Procedure*** **If** / **Then** Resolved / Report results according to SOP ***Weak D Tube Testing Procedure*** Not Resolved / Consult with TSL management

Cord Blood Mixed Field Reactions

STEP	ACTION
1	Wash cord blood specimens 3 times with saline
2	Repeat ABO/Rh front type
3	**If mixed field is resolved** / **Then** Yes / Go to section Results Reporting below No / Go to next step
4	**If cord blood ABO/Rh is** / **Then** For maternal Rh immunoglobulin evaluation (mother is Rh negative) / • Reject specimen • Notify the RN a new specimen is required

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td></td><td>
<table>
<tr><td></td><td>
• Cancel the BBCORD test in SQ and create a QI
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>First collected specimen</td><td>• Reject specimen
• Notify the RN a new specimen is required
• Cancel the BBCORD test in SQ and create a QI</td></tr>
<tr><td>Repeat specimen</td><td>Go to section Results Reporting below</td></tr>
</table>
</td></tr>
<tr><td>NOT for Maternal Rh immunoglobulin evaluation</td><td>Go to section Results Reporting below</td></tr>
</table>
</td></tr>
</table>

CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES

Results Reporting

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>
Record all reactions immediately in Sunquest or the appropriate manual result form.
<table>
<tr><th>If discrepancy is</th><th>Then</th></tr>
<tr><td>Resolved</td><td>Refer to interpretation and reporting results according to SOP ABO/Rh Testing by Tube Method</td></tr>
<tr><td>Due to a bone marrow transplant (BMT) with discrepant forward/reverse reactions or in between typing</td><td>Report the ABO/Rh interpretation as NTD

NOTE: In some cases, the patient may not make the new isoantibody following conversion. Testing may still be reported with the new blood group with TSL MD approval on file.</td></tr>
<tr><td>Due to recent transfusion of blood components that were not identical to the patient’s own ABO/Rh</td><td>Result the patient’s actual blood type and add a BBCS comment explaining the cause of the mixed cell population</td></tr>
<tr><td>Resolved by an Immunohematology Reference Lab (IRL)</td><td>TSL manager or designee will enter the results</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td></td><td>
<table>
<tr><td>Unresolved cord blood specimen mixed field where the source is not contaminated</td><td>• Report the ABO/Rh interpretation as NTD
• Document the following on the cord blood log
o ABO: as “NTD”
o Rh: record as POS or NEG based on anti-D reaction results – refer to SOP ABO/Rh Manual Tube Method</td></tr>
</table>
</td></tr>
<tr><td>2</td><td>Add a PB comment indicating the cause of the discrepancy in SQ for future reference

NOTE: If the cause of the discrepancy is already noted in the BAD file, the PB comment is not required</td></tr>
<tr><td>3</td><td>Determine if the new blood type is discrepant with the type of record in the BAD file
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Does match the historical type or the historical type is NTD</td><td>No action needed</td></tr>
<tr><td>Does NOT match the historical type</td><td>Refer the discrepancy to TSL management for evaluation for record correction.

NOTE: BMT patient’s historical record should not be updated following type conversion until the conversion is verified to be complete and approved by the TSL MD</td></tr>
</table>
</td></tr>
</table>

CALIBRATION:
NA

PROCEDURE NOTES AND LIMITATIONS:
- Refer to reagent manufacturer’s package insert for instructions and limitations for use of reagent

REFERENCES:
- Standards for Blood Banks and Transfusion Services, Bethesda, MD; AABB, current edition
- Technical Manual, Bethesda, MD; AABB, current edition

RELATED DOCUMENTS:
FORM *Extended Workup*
FORM *Cord Blood Log*
SOP *Specimen Acceptability and Order Receipt*
SOP *Quality Control for Manual Testing Reagents*
SOP *Labelling for Manual Testing*
SOP *ABO/Rh Manual Tube Method*
SOP *Antibody Identification*
SOP *Grading Reactions*
SOP *Saline Replacement*
SOP *Weak D Manual Tube Testing*
SOP *EGA Treatment of Red Blood Cells*
SOP *DAT (Direct Antiglobulin Test) by Tube Method*
SOP *Cold Panel*

APPENDIX: Possible Causes of ABO Typing Discrepancies

TYPE OF DISCREPANCY	POSSIBLE CAUSES
Mixed-field forward type	• Recent transfusion • Transplantation • Fetomaternal hemorrhage • Twin or dispermic (tetragametic) chimerism

Extra reaction in the forward type (includes positive control)	• Autoagglutinins/excess protein coating red cells
	• Unwashed red cells:
	  ○ plasma proteins
	  ○ antibody in patient's serum to reagent constituent
	• Transplantation
	• Acquired B antigen
	• B(A) phenomenon
	• Out-of-group transfusion

Negative and/or weak forward type	• ABO subgroup • Leukemia/malignancy • Recent transfusion • Intrauterine fetal transfusion • Transplantation • Excessive soluble blood group substance
Extra reaction in the reverse type	• Cold autoantibody • Cold alloantibody • Serum antibody to reagent constituent • Excess serum protein • Recent transfusion of plasma components • Transplantation • Infusion of intravenous immune globulin
Negative, weak reverse type	• Age related (<4-6 months old, elderly) • ABO subgroup • Hypogammaglobulinemia • Transplantation
Rh does not match historical Type	• Weak D antigen • Differences in antisera specificity • Recent transfusion • Transplantation

UWMC SOP Approval:			
UWMC CLIA Medical Director	Andrew Bryan, MD	Date	
Transfusion Service Manager	Nina Sen	Date	
QA Manager	Tayler Reeves	Date	
Transfusion Service Medical Director	Monica Pagano, MD	Date	
UWMC Biennial Review:			
		Date	
		Date	

REVISIONS:

07/21/2020: Add instructions for resolution of mixed field reactions from cord blood and neonatal specimens