Wake Forest Baptist Medical Center	**ABORh Protocol** BB.Routine.1043.6	**Dept:**	324311
		Dept Name	Blood Bank
		Effective Date:	3/26/01
		Revised Date:	
Name & Title: CLIA Laboratory Medical Director		**Contact:**	Julie Simmons/ Christina Warren
Signature: G. Pomper		**Date:**	**5/9/2019**

1. General Protocol Statement:

A. Purpose:

The ABO blood group system is the most important blood group system in transfusions and organ transplants. The ABO antigens are found on red blood cells, platelets and other proteins found in the blood. It is the only system where antibodies are naturally formed (believed to be due to exposure to gut and environmental bacteria. i.e. *Enterobacteriaceae*) to the missing RBC antigen. Due to these antibodies the transfusion of incompatible red cells can cause immediate red cell lysis (acute intravascular hemolysis) which can lead to renal failure and death. One of the leading causes of death as reported to the FDA is due to transfusion of ABO incompatible blood.

The Rh system is very complex consisting of over 50 different antigens. It was first discovered in Hemolytic Disease of the Newborn that was caused by a paternal antigen inherited by the newborn. These antigens are found on red cells but not platelets. These antigens are very immunogenic with the D antigen being the most immunogenic of the system. Thus the need for routine testing of the D antigen.

B. Responsible Department/Scope:

i. Protocol owner/Implementer: Julie H. Simmons/ Christina S. Warren
ii. Protocol prepared by: Julie Jackson
iii. Who performs protocol: Department staff/management

C. Definitions:

NOGR: No Group, unable to determine the ABO group

RHU: Unable to determine the Rh (D) type

SCC: Soft Computer Systems, Blood Bank Information System

PCW: Patient Caution Window in SCC

List of SCC tests that contain ABORh testing:

SCC Computer Code	Description
GTX	Group and Type
OBX	Obstetrics Prenatal Panel
TRX1 & TRX2	Transfusion Reaction (Phase 1 &2)
TSX	Type and Screen
XMBM	Bone Marrow Crossmatch
GTXBM	Group and Type of BMT product
TSXBM	Type and Screen on donor peripheral blood samples
CORDP	Cord Blood Panel
TSXN	Type and Screen Neonate
GTXN	Group and Type Neonate
TPLCK	ABO Recheck for BMT Transplant DONOR
ABOCK	ABORh Recheck
KDX	Kidney Patient Blood Type and Screen for non-surgical patients
KATSX	Kidney ABOi Type and Screen

2. Protocol:

1.0 ABO Forward typing: in the forward ABO typing, the presence or absence of ABO antigens is determined by testing cells with anti-A, anti-B, and/or anti-A,B and observing for agglutination. Absence of agglutination is a negative test result which indicates the absence of the corresponding antigen on the red cells. Agglutination of ≥2+ with a given reagent is a positive test result, which indicates the presence of the antigen.

2.0 ABO Reverse Typing: reverse typing is demonstrated by the presence or absence of agglutination of the expected, reciprocal ABO antibodies in the plasma or serum of A, B, and O patients with reagent A1 and B cells. Absence of agglutination is a negative test result which indicates that the antibody corresponding to the red cell is not present. Agglutination of ≥2+ with a given cell is a positive test result, which indicates the presence of the antibody in the patient's plasma/serum.

NOTE: **On ECHO only, a '1+' reverse reaction can be considered a positive reaction.**

3.0 Rh typing: Rh typing tests for the presence or absence of the D red cell antigen. Rh typing is demonstrated by the presence or absence of agglutination when testing cells with reagent anti-D antibody. Absence of agglutination is a negative test result while agglutination of ≥2+ is a positive test result.

4.0 Weak D testing: Weak D phenotypes are defined as having a reduced amount of D antigen, requiring an indirect antiglobulin test (IAT) for detection. Red blood cells that appear to be D negative or are reacting <2+ by direct test methods may be further tested for the weak D antigen by the weak D test. Weak D testing is performed on all OB patients, Rh Immune Globulin candidates, Bone Marrow and organ donors and recipients, and recipients of directed donor units.

4.1 Weak D results of any strength on an OB patient should be sent for weak D analysis at Blood Center of Wisconsin.

4.2 The tests that were performed by BCW will be charged under the appropriate previously tested specimen.

4.3 The RHINT (Rh Geno Interp) should be added to the specimen in SCC and then resulted based on the BCW genotype results. Test appears as RH(D) Interpretation in Beaker.

a. Result as POS if patient should be considered RH positive. The following comment will be added to the result in Beaker.
   - *Based on the genotype of this patient, this patient is to be considered Rh Positive. See the pathologist's Blood Bank note.*

b. Result as NEG if patient should be considered RH negative. The following comment will be added to the result in Beaker.
   - *Based on the genotype of this patient, this patient is to be considered Rh Negative. See the pathologist's Blood Bank note.*

5.0 Frequencies of ABO and Rh in the population:

Refer to Attachment 1: ABO Frequencies

6.0 Interpretation of ABO and Rh results

Refer to Attachment 2: Interpretation of ABO and RH Results

6.1 In SCC, if forward type is mixed field and ≥2+, then result the numerical grade from drop down and mixed field in comments. If ≤ 1+ (1+, w+) and mixed field, select mixed field from drop down.

a. Mixed field reaction choices in SCC are as follows:

Reaction	SCC Result
Weak mixed field	MW
1+ mixed field	M1
2+ mixed field	M2
3+ mixed field	M3
4+ mixed field	M4

6.2 **No Group (NOGR)**: When the forward ABO typing does not match the reverse ABO typing the interpretation must be No Group (NO GR). When the Rh results are invalid such as when the saline control or Weak D control is positive the Rh interpretation must be RHU. When the Rh results are valid but the ABO results do not correlate, the Rh interpretation may be entered as Positive or Negative.

6.3 **No Group (NOGR):** If the ABO forward and reverse types do not match and there is no prior history or the patient is a kidney or solid organ transplant, then a green sheet should be complete to flag management for review.

a. If the No Group is due to a missing forward or reverse then test with anti-A,B and anti-A1 Lectin

b. If there is a discrepancy on a **Kidney patient**- notify management.

- If the forward type is ≥2+ and the back type doesn't match-result the ABO interpretation based on the forward type. Do Not result as NOGR.
- This will require a SCC supervisor override. If no one is available to do this leave for management to result.

6.4 Check the patient demographic window in SCC. Management needs to remove any prior ABO if present since the ABO does not automatically update to NOGR. The same is true when the Rh is interpreted as RHU. Notify management to update. Technologist should enter a "Note to tech" comment in PCW that the patient is NOGR or RHU.

a. When an interpretation of NO GR is entered the patient must receive the following component blood types, see following chart.

i. Patients on BMT protocol who type NOGR or RHU may have specific product ABO/Rh requirements noted in the PCW.
Refer to BM/HPC Procedures and Protocols, BB.Specials.1005.

ABO interp	Rh interp	Platelets to give*	Plasma to give	RBCs to give**
NO GR	POS	AB Pos or Neg	AB	O Pos or Neg
NO GR	NEG	AB Neg	AB	O Neg
NO GR	RHU	AB Neg	AB	O Neg

*Refer to Platelet Protocols for options if AB Neg platelets are not available.

****NOTE:** Group AB patients that have received multiple out of group red cells and meet the criteria for No Group should continue receiving Group A red cells. Management may approve the interpretation for AB with an additional ABO recheck so that group A red cells can be issued without a supervisor override in SCC.

7.0 Quality control

7.1 **Refer to Daily Reagent Quality Control BB.QC.1006**

8.0 The Blood Bank Work Organization Protocols must be followed throughout all steps of testing.

8.1 **Refer to Blood Bank Work Organization Protocol, BB.PROTOCOL.1001**

9.0 The following tests will be performed as part of the following test codes:

Test	**Test Code**							
				Prenatal	**BMT**	**Kidney**	**Neonate**	**ABORh rechecks**
	GTX	**TSX**	**TRX1 TRX2**	**OBX**	**GTXBM TSXBM XMBM**	**KDX KATSX**	**CORDP TSXN GTXN**	**TPLCK & ABOCK**
ABORh (forward and reverse)	**X**	**X**	**X**	**X**	**X**	**X**		
ABORh (forward only)							**X**	**X**
Weak D (DU)				**X**			**X**	

10.0 An ABO/Rh typing performed within the last 12 months is required for any patient receiving non-red cell containing blood products. If products are needed emergently, issue components on emergency release.

11.0 **ABO Recheck**: Patient specimens with no previous ABO/Rh and/or those with interpretations on file prior to February 2006 must have an ABO Recheck performed.

12.0 Results of ABO recheck must be identical to initial results

12.1 **When historical or present ABO/Rh does not match the current specimen, a new suspension from the same source specimen tube must be tested by a second technologist.**

If Historical ABO/Rh is	and Current is	Comment	RBCs to give**	Platelets to give*	Plasma to give
Present	NO GR RHU, NO GR NEG		O Neg	AB Neg	AB
Present	NO GR POS		O Pos or Neg	AB Pos or Neg	AB
Present	ABO/Rh different than historic	Obtain another blood specimen and notify management	O Neg	AB Neg	AB
NOGR RHU, NOGR NEG NOGR POS	Conclusive ABO/Rh	Current ABO and Rh type must be 2+ or greater	Group Specific	Group Specific	Group Specific

*Refer to Platelet Protocols for options if AB Neg platelets are not available.

**Refer to NOTE in section 6.4a for AB patients.

12.2 Testing for patient ABO rechecks include forward ABO and RhD testing only.

12.3 Manual testing for ABO recheck is appropriate for ABO recheck when initial testing is done by automation or manually by another technologist.

12.4 ABO Recheck testing must be performed by a second technologist using a new cell suspension made from the same source specimen tube

a. An ABO recheck on automation with initial on automation is not acceptable.

12.5 An ABO recheck must be completed before any group specific blood products can be allocated or issued. When unable to perform ABO recheck:

a. For Pediatrics: give group O neg red cells, AB Neg platelets and AB plasma products.

b. For Adults: give group O red cells according to the Emergency Release Protocol.

12.6 A saline control is not required when performing an ABO recheck on AB positive donor units.

12.7 It is not required to repeat weak D testing as part of an ABO recheck.

12.8 If the historical ABORh is NOGR RHU, NOGR NEG or NOGR POS while the current sample is conclusive or the historical does not match the current ABORh **and** the patient has had a BMT transplant in the past, the patient's historical blood type may be changed. This must be reviewed and OK'd by the Medical Director or management of the Blood Bank.

13.0 Performance of electronic crossmatch requires that there are concordant results of at least two determinations of the recipient's ABO type on record, one of which is from a current sample.

14.0 Discrepancies:

14.1 All forward and reverse reactions must be ≥2+ positive in order to be valid. Reactions which are <2+ must be investigated further.

NOTE: **On ECHO only, a '1+' reverse reaction can be considered a positive reaction.**

14.2 All forward and reverse reactions must agree before an interpretation of ABO group can be made.

14.3 If an interpretation cannot be made after investigation the interpretation should be resulted as: NOGR (No Group) for ABO and RHU for Rh type.

a. Refer to Steps 6.2 and 6.4.

b. Check the patient demographic window in SCC. Management needs to remove any prior ABO if present since the ABO does not automatically update to NOGR. The same is true when the Rh is interpreted as RHU. Notify management to update. Technologist should add a "Note to Tech" in PCW that the patient is NOGR or RHU.

c. The exception to this is ABORhs performed on Neonates (0-6 months) when the patient is too young to have developed antibodies yet. Neonates do not require reverse testing. The ABO interpretation is resulted based on forward typing only.

d. If there is a discrepancy on a Kidney patient- notify management.

e. **See ABO Testing; BB.Routine.1001b**

14.4 All ABO discrepancies (Forward and Reverse) must be resolved and documented before an ABO interpretation can be made.

a. See Common Causes of false-negative and false-positive results in ABO testing/ Solving Discrepancies; BB.Routine.1022

14.5 Patients who have cold (RT) reactive allo- or naturally occurring antibodies should have reverse typing performed with reagent reverse cells (A1 cells and B cells) found to lack the offending antigen for which the patient has the antibody.

15.0 ABO/Rh reconfirmation of donor units:

15.1 For incoming donor units only the forward typing is required for ABO reconfirmation of donor units. Testing with Anti-A,B is acceptable to reconfirm ABO type on O donor units. The Rh must be tested on all Rh negative units. ABO/Rh reconfirmation must be performed on the following:

a. Red blood cells
b. Granulocyte products
c. Bloody platelets that contain more that 2mls of red blood cells
d. The following Red cells that have been processed at WFBMC Blood Bank:
    - Deglyced red cells
    - Washed red cells
        - The RETYP test must be manually ordered on the washed red cell in Inventory > Orders > New add

16.0 Cord blood specimens received for ABO testing must be washed thoroughly (≥6 times) to remove Wharton's Jelly from the red cells. A reverse typing is not performed.

17.0 Post-delivery and OB patients: weak D reactions showing agglutination ≤2+ or showing a mixed field reactions may indicate a mixture of Rh negative maternal blood with Rh positive fetal blood. Consult with BB medical director and/or management to determine if performance of a quantitative fetal hemoglobin test is indicated.

a. All Weak D positive samples (weak or strong reactions) on OB patients must be sent to Blood Centers of Wisconsin for a Weak D analysis.

b. Order BCW tests: Weak RHD analysis and Partial RHD analysis.

c. Complete the Reference Lab Tracking Form.

d. The RHINT (Rh Geno Interp) should be added to the specimen in SCC and then resulted based on the BCW genotype results. Test appears as RH(D) Interpretation in Beaker.

i. Result as POS if patient should be considered RH positive. The following comment will be added to the result in Beaker.
    - *Based on the genotype of this patient, this patient is to be considered Rh Positive. See the pathologist's Blood Bank note.*

ii. Result as NEG if patient should be considered RH negative. The following comment will be added to the result in Beaker.
    - *Based on the genotype of this patient, this patient is to be considered Rh Negative. See the pathologist's Blood Bank note.*

18.0 Manufacturer's directions should be consulted for performance characteristics and limitations of all reagents used.

19.0 Limitations of ABO and Rh Testing

Refer to package inserts for reagents for limitations.

3. Review/Revised/implemented:

All protocols must be reviewed as stated in the Document control protocol.
All new protocols that have major revisions must be signed by the CLIA Director.
All reviewed protocols with minor revisions can be signed by the designated section medical director or designee.

4. Related Protocols:

Common Causes of false-negative and false-positive results in ABO testing/ Solving Discrepancies; BB.Routine.1022
Blood Bank Work Organization Policies BB.PROTOCOL.1001
Grading of Positive and Negative Reaction BB.ROUTINE.1018
Selection of Blood and Blood Products BB.ROUTINE.1022
Specimen Labeling Requirements BB.FD.1001
ABO Testing; BB.Routine.1001b
BM/HPC Procedures and Protocols, BB.Specials.1005

5. References:

AABB Technical Manual. Revised periodically.
Reid, Marion E. et al. THE BLOOD GROUP ANTIGEN FACTS BOOK. Revised periodically.
Blood Grouping Reagents [package insert]. BIO-RAD Medical Diagnostics GmbH.
Blood Grouping Reagents [package insert]. Immucor Gamma
Modern Blood Banking and Transfusion Practice, Harmening; revised periodically.

6. Attachments:

Attachment 1: ABO Frequencies
Attachment 2: Interpretation of ABO and Rh Results

7. Revised/Reviewed Dates and Signatures:

See Archived Document Change Control

Attachment 1: ABO and Rh(D) Frequencies

ABO (% Occurrence)				
Phenotype	**Caucasians**	**Blacks**	**Asian**	**Mexican**
A_1	**33**	**19**	**27**	**22**
A_2	**10**	**8**	**Rare**	**6**
B	**9**	**20**	**25**	**13**
O	**44**	**49**	**43**	**55**
A_1B	**3**	**3**	**5**	**4**
A_2B	**1**	**1**	**Rare**	**Rare**
Rh (D) (% Occurrence)				
Phenotype	**Caucasians**	**Blacks**	**Asian**	**Native American**
D	**85**	**92**	**99**	**99**

Attachment 2: Interpretation of ABO Results

Reactions with Red Cells: Forward Type			Reactions with Plasma: Reverse Type**		ABO Interpretation	
Anti-A	Anti-B	SC	A1 cells	B cells		
≥2+	0	ND	0	≥2+	A	Conclusive interpretation
0	≥2+	ND	≥2+	0	B	
0	0	ND	≥2+	≥2+	O	
≥2+	≥2+	0	0	0	AB	
0-1+	0	ND	0	≥2+	NO GR	Inconclusive interpretation
≥2+	0	ND	0	0-1+	NO GR	
0	0-1+	ND	≥2+	0	NO GR	
0	≥2+	ND	0-1+	0	NO GR	
0	0	ND	0-1+	0-1+	NO GR	
0-1+	0-1+	ND	0	0	NO GR	
1+	1+	ND	≥2+	≥2+	NO GR	
1+-4+	1+-4+	1+-4+	0-4+	0-4+	NO GR	

SC= saline control NO GR= No ABO Group Type

NOTE: **On ECHO only, a '1+' reverse reaction can be considered a positive reaction.

Attachment 3: Interpretation of RH Results

Immediate spin		AHG		Rh (D) Interpretation	
Anti-D	SC	Wk D	Wk D control		
≥2+	ND	ND	ND	POS	Conclusive interpretation
0-1+	ND	≥2+	0	POS	
0	ND	0	0	NEG	
0	ND	ND	ND	NEG	
1+-4+	1+-4+	ND	ND	RHU	Inconclusive interpretation
0-1+	ND	0-1+	0	RHU	
0-1+	ND	0-4+	1-4+	RHU	

SC= saline control
NO GR= No ABO Group Type
RHU= No Rh Type (Rh Unknown)