Wake Forest Baptist Health	**DOCUMENT TYPE:** ☒ Procedure	**ORIGIN DATE IN TITLE 21** 3/31/2020
CLIA Lab Director: Gregory Pomper, MD	**LAB DEPARTMENT:** Blood Bank	**CONTACT:** Blood Bank Management

APPLICABLE LABORATORY(S)):

☒ North Carolina Baptist Hospital (NCBH)
☐ Lexington Medical Center (LMC)
☐ Davie Medical Center (DMC)
☐ Wilkes Medical Center (WMC)
☐ High Point Medical Center (HPMC)
☐ Westchester
☐ Clemmons

PROCEDURE STATEMENT

ABO typing is determined by testing the patient's red cells for antigens (forward typing) and plasma for antibodies (reverse 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.
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.
ABO testing is required for all blood products.
A second ABO (ABO Recheck) is required for computer crossmatches if no history is found or history is before February 2006.

SCOPE

i. Procedure Owner/Implementer: Blood Bank Management
ii. Procedure Prepared by: Julie Jackson
iii. Who Performs Procedure: Blood Bank Staff

DEFINITIONS

A. Procedure: A process or method for accomplishing a specific task or objective.
B. WFBH Lab System: Wake Forest Baptist Lab System is a health system that includes Wake Forest Baptist Medical Center and all affiliated organizations including Wake Forest University Health Sciences (WFUHS), North Carolina Baptist Hospital (NCBH), Lexington Medical Center (LMC), Davie Medical Center (DMC), Wilkes Medical Center (WMC), High Point Medical Center (HPMC), Lab at Westchester and Lab at Clemmons.

C. MR#: Medical Record number
D. Blood Bank requisition or equivalent: Antibody ID summary, Wake Blood Bank Order requisition

E. NOGR: No Group= when a patient's forward and reverse type do not match and cannot be resolved.
F. IS: Immediate Spin
G. RT: Room Temperature
H. SS: Same Sample, SCC result for ABO2/ABOEO for documentation of sample type/used
I. NS: New Sample, SCC Result for ABO 2 for documentation of sample type/used
J. CS: Core Lab Sample, SCC Result for ABO2 for documentation of sample type/used
K. Cord blood: sample of blood collected from the umbilical cord when a baby is born
L. Wharton's jelly: A gelatinous substance that provides insulation and protection within the umbilical cord that can interfere with blood bank testing.
M. PUBS: Percutaneous Umbilical cord Blood Sampling (also called cordocentesis): a blood sample from an unborn infant

Sections:

Section I: Tube testing
Section II: ABO/Rh Gel testing

POLICY GUIDELINES

1. **Refer to: *ABORh Protocol, BB.Routine.1043***

I. Tube Testing

Chemical Risk Assessment: None
Biological Risk Assessment: None
Protective Equipment: Lab coat, gloves
Supplies: 10x75 or 12x75 test tubes
Transfer pipets
Blood Bank requisition or equivalent

Reagents: Anti-A antisera/Anti-B antisera
A1 cells/B cells
Saline

Equipment: Agglutination lamp
Specimen centrifuge: Plasma Prep centrifuge or EBA 20 centrifuge
Testing centrifuge: Sero-fuge or cell washer
Refrigerator or ice bath for 4°C incubation if needed
Blood Bank Computer System

Specimen Requirements:
A properly labeled EDTA tube:
6.0 mL pink top is preferred
3.0 mL or 10.0 mL lavender top is also acceptable
A properly labeled clot tube, no serum separator, may be used if EDTA is not available.
Refer to Specimen Labeling Requirements and BBID Numbers- BB.FD.1001

STEPS	INSTRUCTIONS
1.0	**Centrifuge the specimen tube:** 1.1 Preferred method: 3 minutes at 7200 RPM in the "*Plasma Prep"* centrifuge 1.2 Alternative method: 7 minutes at 3150 – 3350 RPM in the "EBA 20" centrifuge
2.0	**Compare all labels and identification numbers on specimen, Blood Bank requisition or equivalent.** 2.1 Identifying information must be identical on ALL items. See Front Desk: Specimen Labeling Requirements and BBID Numbers; BB.FD.1001
3.0	**Label 10x75 test tubes with a minimum of the first 3 letters of the patient's last name and testing to be performed.** 3.1 Patient test tubes must be labeled with – ***at minimum*** – the first three letters of the patient's last name AND the test being performed in the tube (see chart that follows). 3.2 Refer to the chart for required testing by patient category:

Patient Category	Forward typing		Reverse typing	
	Anti-A	Anti-B	A_1 cells	B cells
Adult/Child (>4 months old)	✓	✓	✓	✓
Neonate (≤4 months old)	✓	✓		
Cordblood/PUBS ABOCK (Neonates)	✓	✓		
ABO Recheck ABOEO or ABO2 (Adults)	✓	✓	✓	✓
Label tubes with test ⇨	**A**	**B**	**AC**	**BC**

STEPS	INSTRUCTIONS
	3.3 NOTE: When testing patient with same last three letters use First name and if the same as other patient use MRN.
4.0	**Place all test tubes in a test tube rack, labeling facing forward.** 4.1 A maximum of 3 patient specimens may be in a test tube rack at any given time a. There must be at least one empty row left in the rack between each patient
5.0	**Read vial reagent name and add antisera.** 5.1 Add one drop of anti-A antisera to the tube labeled A 5.2 Add one drop of anti-B antisera to the tube labeled B R. NOTE: Read vial label and tube label before dropping antisera to confirm that they match.
6.0	**Read plasma or serum tubes and add two drops of patient plasma or serum to tubes labeled AC and BC.**
7.0	**Read vial product name and mix vial and dropper well and add reagent red cells.** 7.1 Add one drop of A1 cells to the tube labeled AC 7.2 Add one drop of B cells to the tube labeled BC NOTE: Read vial label and tube label before dropping cells to confirm that they match.
8.0	**Prepare a 3-5% cell suspension of the patient's red cells** 8.1 Label a 10x75 or 12x75 test tube for the patient's red cell suspension with the patient's full name and MRN either written or using a label. 8.2 Add 1-2 drops of packed patient red cells into a properly labeled tube. 8.3 Add 0.85% saline to produce a red cell suspension. 8.4 Mix red cell suspension. 8.5 Visually compare color of suspension with that of a 3-5% commercial reagent red cell suspension. a. If it appears <3%, add patient red cells to achieve a 3-5% suspension. b. If it appears >5%, add saline to suspension to achieve a 3-5% suspension. 8.6 **Cordblood** samples should be reamed prior to loading on the instruments. They may be washed a **minimum of 6 times** with saline prior to testing to remove Wharton's Jelly (a jelly-like soft connective tissue in the umbilical cord) if all forward reactions and control are positive. a. Mixed field reactions may indicate contamination of sample with Mom's blood. The sample must be rejected and a heel stick collected.
9.0	**Read, mix and add one drop of 3-5% patient red cell suspension to the tubes labeled A and B.**

STEPS	INSTRUCTIONS
10.0	**Mix tube gently, centrifuge immediately at room temperature, 3400-3600 RPM for the immediate spin (IS) calibrated time as noted on centrifuge.** 10.1 Only Two ABO/Rh types on two different patients may be spun in the same centrifuge head and the same time. 10.2 Tubes for patient 1 must be placed in holes 1-5. Tubes for patient 2 must be placed in holes 7-11 Refer to protocol: *Blood Bank Work Organization; BB.PROTOCOL.1001*
11.0	**Carefully remove 2-3 tubes from centrifuge at a time, observe tubes for hemolysis** 11.1 Only one set of patient's tubes may be removed from the centrifuge at a time for reading. 11.2 Hemolysis may a. Indicate a positive test result in the presence of an antigen/antibody reaction b. Be the consequence of hemolyzed reagent red cells or patient red cell suspension 11.3 Make note of any hemolysis present in the absence of hemolyzed red cell suspensions in the computer. 11.4 Complete or partial hemolysis must be interpreted as a positive reaction if the original serum/plasma and/or reagent red cell suspension was free of hemolysis.

<table>
<tr><th>STEPS</th><th>INSTRUCTIONS</th></tr>
<tr><td>12.0</td><td>
Dislodge / resuspend cell button from bottom of tubes gently over an agglutination lamp.

12.1 Interpret agglutination strength
<table>
<tr><th>Testing</th><th>If the Reaction is</th><th>Then the Interpretation is</th><th>Also</th><th>Note</th></tr>
<tr><td rowspan="3">Forward reactions:
Anti-A
Anti-B
Anti-D</td><td>2+ to 4+</td><td>Positive</td><td colspan="2">If mixed field, select the correct result in SCC
(ex. M4= 4+ mixed field reaction)</td></tr>
<tr><td>Hemolysis present</td><td>Positive</td><td colspan="2">Verify patient red cell suspension is free of hemolysis</td></tr>
<tr><td>Weak+ to 1+</td><td>Positive but will need to be Interpreted as NOGR or RHU (No Group/Rh Unknown)</td><td>If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field reaction)</td><td>May be enhanced to 2+ or stronger by RT incubation for 20 min*</td></tr>
<tr><td></td><td>0 and no hemolysis</td><td>Negative</td><td>-</td><td>-</td></tr>
<tr><td rowspan="4">Reverse reactions:
A1 cells
B cells</td><td>2+ to 4+</td><td>Positive</td><td>-</td><td>-</td></tr>
<tr><td>Hemolysis present</td><td>Positive</td><td colspan="2">Verify specimen, patient red cell suspension and reagent cells are free of hemolysis</td></tr>
<tr><td>Weak+ to 1+</td><td>Positive but will need to be Interpreted as
NOGR
(No Group)</td><td>To enhance to 2+ or stronger: Incubate at RT for 15-30 min*</td><td>If reactions are still weak incubate at 4°C for 15-30 min with auto control and screening cells*</td></tr>
<tr><td>0 and no hemolysis</td><td>Negative</td><td>-</td><td>-</td></tr>
</table>
* See Attachment 1: Resolving ABO Discrepancies

12.2 Saline Control: If both A and B tubes are positive and the D is positive a saline control must be done to rule out polyagglutination.

Go to: Rh Testing Procedure; BB.Routine.1028

12.3 For further instructions on grading of reaction strength,

Go to: Grading of Positive and Negative Reactions; BB.Routine.1018
</td></tr>
<tr><td>13.0</td><td>
Document reactions in SCC or during downtime on Blood Bank requisition or equivalent immediately, discard tubes only after documentation complete.

13.1 Confirm patient identification on test tube matches the requisition and computer screen.

13.2 Only one patient’s requisition should be in the work area at the time of reading, entering, and interpretation of reactions.

13.3 Serologic reactions must be entered or recorded on the Blood Bank requisition or equivalent BEFORE tubes are discarded.
</td></tr>
</table>

<table>
<tr><th>STEPS</th><th>INSTRUCTIONS</th></tr>
<tr><td>14.0</td><td>
Document results in computer as tubes are being read or during downtime on Blood Bank requisition or equivalent.
14.1 Select appropriate Worksheet to result in SCC
a. Using the chart below find the Main worksheet based on the test to be resulted.
i. Go to SCC>Results
ii. Select main worksheet
iii. Click Build.
b. Select the Test worksheet from the drop down list. refer to chart below based on the test to be resulted.
<table>
<tr><th>MAIN WORKSHEET</th><th>TEST WORKSHEET</th><th>TEST TO BE RESULTED</th></tr>
<tr><td>Joint Worksheet</td><td>TSXM</td><td>TSX, XMIS, ABOEO, ABO2</td></tr>
<tr><td rowspan="9">Patient Test worksheets</td><td>ABOCK</td><td>ABOCK, ABOEO, ABO2</td></tr>
<tr><td>CORD</td><td>CORDP</td></tr>
<tr><td>GTX</td><td>GTX</td></tr>
<tr><td>KIDNY</td><td>KDX, HRT1, HRT2, HRTDN</td></tr>
<tr><td>OBX</td><td>OBX, WEAKD</td></tr>
<tr><td>PUBS</td><td>PUBS</td></tr>
<tr><td>TSX</td><td>TSX</td></tr>
<tr><td>TSXN</td><td>TSXN</td></tr>
</table>
14.3 F12 to accept worksheet.
14.4 Click Ctrl+O to select by order number.
a. Scan order number (Beaker label).
b. F12 and click Yes to accept
14.5 Select rack#, unless already defaulted.
14.6 Confirm patient identification on the requisition or specimen matches what is in computer.
14.7 Enter reaction results obtained for Anti-A, Anti-B, Control (if applicable) and A1, B cells.
NOTE: Results may also be entered in Patient > Orders > Results by clicking on each test and entering results.
</td></tr>
<tr><td>15.0</td><td>
Interpret ABO Group– refer to chart below

15.1 Forward and reverse reactions must agree. Any discrepancies must be resolved prior to interpretation.
Refer to Attachment 1: Resolving ABO Discrepancies
</td></tr>
</table>

STEPS	INSTRUCTIONS

15.2 Reactions that are 1+ or weaker require that the ABO group Interpretation be NOGR (No Group).

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

i. 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.

ii. This will require a SCC supervisor override. If no one is available to do this leave for management to result.

iii. Refer to *ABORh Protocol; BB.Routine.1043*

FORWARD GROUP Red cells			REVERSE GROUP Plasma		ABO group Interpretation
Anti-A	**Anti-B**	**Saline Control**	**A1 cells**	**B cells**	
Neg	Neg		2+ to 4+	2+ to 4+	O
2+ to 4+	Neg		Neg	2+ to 4+	A
Neg	2+ to 4+		2+ to 4+	Neg	B
2+ to 4+	2+ to 4+	Neg	Neg	Neg	AB

15.3 Patients >6 months of age and adults

a. If forward and reverse do not agree and cannot be resolved, report as NOGR.

15.4 Infants ≤6 months of age

a. ABO results may be reported based on the forward group only if reactions are ≥2+. Reverse may be reported as "not done". This will require a Supervisor override in SCC.

15.5 Kidney Patients

a. If the forward and reverse do not agree

i. Notify Management

ii. Report forward only

iii. Resolve discrepancy

15.6 F12 to accept.

a. May continue entering additional results and interpretations and then F12.

2. Procedure: II. ABO/RH Gel Testing

Chemical Risk Assessment: None
Biological Risk Assessment: None
Protective Equipment: Lab coat, gloves
Supplies: 10x75 or 12x75 test tubes
Transfer pipets
MLA Pipette
Blood Bank requisition or equivalent

Reagents: MTS A/B/D Monoclonal and Reverse Grouping Gel Card (A/B/Drev)
MTS Diluent 2 Plus

Equipment: Specimen centrifuge: Plasma Prep centrifuge or EBA 20 centrifuge
Ortho Workstation
Blood Bank Computer System

Specimen Requirements:
A properly labeled EDTA tube:
6.0 mL pink top is preferred
T. 3.0 mL or 10.0 mL lavender top is also acceptable
A properly labeled clot tube, no serum separator, may be used if EDTA is not available.
Refer to Specimen Labeling Requirements and BBID Numbers- BB.FD.1001

STEPS	INSTRUCTIONS
1.0	**Centrifuge the specimen tube:** 1.1 Preferred method: 3 minutes at 7200 RPM in the "*Plasma Prep"* centrifuge 1.2 Alternative method: 7 minutes at 3150 – 3350 RPM in the "EBA 20" centrifuge
2.0	**Compare all labels and identification numbers on specimen, Blood Bank requisition or equivalent.** 2.1 Identifying information must be identical on ALL items. See Front Desk: Specimen Labeling Requirements and BBID Numbers; BB.FD.1001
3.0	**Label a 10 x 75 or 12 x75 tube with patient's full name and MRN or use taglet and prepare a 4% ± 1% cell suspension.** 3.1 Dispense 0.5 mL of MTS Diluent 2 Plus to the test tube. 3.2 Pipette 25uL of centrifuged cells into the same tube. 3.3 Mix gently.

<table>
<tr><th>STEPS</th><th>INSTRUCTIONS</th></tr>
<tr><td>4.0</td><td>Label A/B/D rev Gel card with a minimum of the first 3 letters of the patient's last name and/or a taglet with patient information.
3.1 Select the appropriate ABO gel card based on testing to be performed.
<table>
<tr><th rowspan="2">Patient Category</th><th colspan="2">Forward typing</th><th>Rh typing</th><th colspan="2">Reverse typing</th></tr>
<tr><th>Anti-A</th><th>Anti-B</th><th>Anti-D</th><th>A_1 cells</th><th>B cells</th></tr>
<tr><td>Adult/Child (>4 months old)</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td></tr>
<tr><td>Neonate (≤4 months old)</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
<tr><td>Cordblood/PUBS/ABOCK(neonates)</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
<tr><td>ABO Recheck (ABOEO/ABO2)</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td></tr>
</table></td></tr>
<tr><td>5.0</td><td>Place gel card upright in rack.</td></tr>
<tr><td>6.0</td><td>Read vial reagent name.
6.1 Pipette 50uL of 0.8% Affirmagen (A and B) cells to each microtube labeled "BUF" in the MTS A/B/D Monoclonal and Reverse Grouping Card.

NOTE: Read vial label and microtube label before dropping to confirm that they match.</td></tr>
<tr><td>7.0</td><td>Read plasma or serum tubes.
7.1 Pipette 50uL of test plasma/serum into each microtube labeled "BUF."</td></tr>
<tr><td>8.0</td><td>Pipet 10-12.5uL of the 4%±1% patient/donor cell suspension into the A,B,D and control microtubes.
NOTE: Read vial label and tube label before dropping cells to confirm that they match.</td></tr>
<tr><td>9.0</td><td>Centrifuge the gel card in the Ortho Workstation centrifuge.</td></tr>
<tr><td>10.0</td><td>Carefully remove gel card from centrifuge one at a time, observe for hemolysis
10.1 Only one patient gel card may be removed from the centrifuge at a time for reading.
10.2 Hemolysis may
a. Indicate a positive test result in the presence of an antigen/antibody reaction
b. Be the consequence of hemolyzed reagent red cells or patient red cell suspension
10.3 Make note of any hemolysis present in the absence of hemolyzed red cell suspensions in the computer.
10.4 Complete or partial hemolysis must be interpreted as a positive reaction if the original serum/plasma and/or reagent red cell suspension was free of hemolysis.</td></tr>
</table>

STEPS	INSTRUCTIONS
11.0	**Interpret agglutination strength.**

Testing	If the Reaction is	Then the Interpretation is	Also	Note
Forward reactions: **Anti-A Anti-B Anti-D**	2+ to 4+	Positive	If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field reaction)	
	Hemolysis present	Positive	Verify patient red cell suspension is free of hemolysis	
	Weak+ to 1+	Positive but will need to be Interpreted as **NOGR or RHU** (No Group/Rh Unknown)	If mixed field, select the correct result in SCC (ex. M4= 4+ mixed field reaction)	May be enhanced to 2+ or stronger by RT incubation for 20 min*
	0 and no hemolysis	Negative	-	-
Reverse reactions: **A1 cells B cells**	2+ to 4+	Positive	-	-
	Hemolysis present	Positive	Verify specimen, patient red cell suspension and reagent cells are free of hemolysis	
	Weak+ to 1+	Positive but will need to be Interpreted as NOGR (No Group)	To enhance to 2+ or stronger: Incubate at RT for 15-30 min*	If reactions are still weak incubate at 4°C for 15-30 min with auto control and screening cells*
	0 and no hemolysis	Negative	-	-

* This will need to be performed in Tube. Refer to Section I.

See *Attachment 1: Resolving ABO Discrepancies*

11.1 For further instructions on grading of reaction strength,

Go to: *Grading of Positive and Negative Reactions; BB.Routine.1018*

12.0	**Document reactions in SCC or during downtime on Blood Bank requisition or equivalent immediately, discard only after documentation complete.** 12.1 Confirm patient identification on test tube matches the requisition and computer screen. 12.2 Only one patient's requisition should be in the work area at the time of reading, entering, and interpretation of reactions. 12.3 Serologic reactions must be entered or recorded on the Blood Bank requisition or equivalent BEFORE gel card is discarded.

STEPS	INSTRUCTIONS
13.0	**Interpret ABO Group.** *Refer to Section I: Step 15.*
14.0	**Interpret the Rh type.** *Refer to Rh Testing and weak D typing.*

REFERENCES

Reagent package inserts, updated periodically
AABB Technical Manual, updated periodically
Modern Blood Banking and Transfusion Practice, Harmening; updated periodically.
Merriam-Webster Dictionary, updated periodically
Ortho manufacturer's inserts

RELATED PROCEDURES/POLICIES (NAVEX)

Grading of Positive and Negative Reactions; BB.Routine.1018
Front Desk; Specimen Labeling Requirements; BB.FD.1001
Routine ABO/Rh Computer Entry; BB.R.1030
ABO Recheck Computer Entry; BB.R.1035
Rh Testing Procedure; BB.Routine.1028
Blood Bank Work Organization; BB.PROTOCOL.1001

ATTACHMENTS/LINKED DOCUMENTS (TITLE 21)

Attachment 1: Resolving ABO Discrepancies

REVISION DATES: REVIEW CHANGE SUMMARY AS REPRESENTED IN TITLE 21.

Attachment 1: Resolving ABO Discrepancies

Common Causes of False-Negative and False-Positive Results in ABO Testing

False-Negative Results	False-Positive Results
Reagent or test serum not added to tube	Overcentrifugation
Hemolysis not identified as a pos. reaction	Use of contaminated reagents, red cells or saline
Inappropriate ratio of serum or reagent to red cells	Use of dirty equipment
Tests not centrifuged sufficiently	Incorrect interpretation or recording of results
Tests incubated at temps. Above 20-24˚C	
Incorrect interpretation or recording of results	

Strict adherence to procedures is critical to avoid false positive and negative results.
In all cases, general considerations when resolving ABO discrepancies

a. Repeat testing on the same sample
b. Obtain a new sample
c. Obtain patient's history of diagnosis, previous transfusions, marrow transplantation and medications.
d. Wash red cells at least 3 times with 0.9% saline.

SOLVING DISCREPANCIES – REVERSE GROUPING

Problem – unexpected (extra) reactions	**Solution**
Anti-A_1 in serum	*Refer to Routine: A_1 Lectin Testing and Test for anti-A_1 procedure*
Cold antibodies	• Cold adsorption *Refer to Specials: Adsorption and Prewarm Techniques*
High concentrations of serum proteins	• If rouleaux, perform saline replacement *Refer to Specials: Antibody Identification. Section VII: Saline Replacement*
Small fibrin clots that look like agglutination.	Respin and repeat test.
Weak or missing reactions	**Solution**
High concentrations of anti-A and anti-B in plasma/serum causing a neg. reaction (prozone reaction)	Dilute plasma/serum (Consult with management before diluting specimen). *Refer to Specials: Titrations*
Negative or weak reactions from elderly patients or immunodeficient patients	• Check age and diagnosis • Incubate reactions at room temperature for 15-30 mins. • Incubate at 4˚C with autocontrol and screening cells for 15-30 mins *Refer to Specials: Antibody Identification: Section VIII: Cold Antibody Identification*
Negative or weak reactions from infants under 4-6 months	• No action required
Miscellaneous	
Forward and reverse do not match	• Obtain history (possible BMT patient, transfusion with out of group platelets, subgroup)

SOLVING DISCREPANCIES - Forward Grouping

Problem – weak or missing reactions	**Solution**
Failure to obtain expected reactions • due to disease states such as leukemia • newborn	• Incubate at room temperature for 30 mins. • Extended incubation at 4˚ requires autocontrol and screening cells • Wash red cells 3 times with saline and repeat testing. • Cells may be treated with enzymes. This increases antigen-antibody reaction with anti-A or anti-B *Refer to Specials: Antibody Identification, Section V. Testing with Enzymes*
• subgroups	Test with anti-A,B- some weak subgroups may react Test with A_1 lectin *Refer to Routine: Anti-A1 lectin and testing for Anti-A1*
If patient's rbc are suspended in serum/plasma, high concentrations of A or B blood group substances in serum/plasma can neutralize reagent antibodies to give neg. reactions	1. Wash rbc 3 times with saline and suspend patient's rbc in saline. 2. Repeat testing.
Problem – unexpected or extra reactions	**Solution**
Abnormal concentrations of proteins - example, Wharton's jelly	Wash cells 3-4 times and retest
Inherited or acquired abnormalities of the red cell membrane that can lead to a polyagglutinable state	Test with monoclonal antisera or lectins to detect polyagglutination. Consult with management.
If patient's rbc are suspended in serum/plasma, antibodies in the serum/plasma can give false agglutination to dyes in anti-A and anti-B	Wash patient's rbc 3 times with saline and suspend patient's rbc in saline and repeat testing.
Potent cold-reactive autoagglutinins	Incubate cell suspension at 37˚C and wash with warm saline and repeat testing.
If patient's serum/plasma contains a pH or diluent dependent autoantibody, false positive reactions will occur if rbc are suspended in serum/plasma	Wash patient's rbc 3 times with saline and suspend patient's rbc in saline and repeat testing.
Circulating rbc of more that one group: - Bone marrow transplant - Out of group transfusion (group O to A patient)	Observe carefully for mixed field agglutination. Obtain history and document.
- Acquired B Phenotype Check direction circular for monoclonal anti-B to see if it reacts with acquired B.	Test patient's plasma/serum with autologous rbc (will not agglutinate auto rbc)
Acquired A like antigens	To differentiate from the A produced by the A-gene transferase - treated red cells with enzymes *Refer to Specials: Antibody Identification, Section V. Testing with Enzymes*
Antibody-coated rbc	• Gentle elution at 45˚C can remove antibody from cells heavily coated with IgG such as infants with HDN. • Chloroquine treatment *Refer to Specials: Antibody Identification, Section XI: Chloroquine Treatment* • Incubating cell suspensions briefly at 37˚C and then washing several times with warmed (37˚C) saline can remove IgM autoagglutinins