Northern Arizona Healthcare	LABORATORY DEPARTMENT POLICY AND PROCEDURES	Department: BLOOD BANK Number: 726.0-Blbk-gu-rev04/15

ABO AND ~~Rh~~ D TESTING

POLICY

~~1. The ABO and Rh blood group systems are two of the most antigenic systems known.~~
~~a. All intended transfusion recipients are typed for ABO and Rh antigens for this reason.~~
~~b. The results of direct typing must always be confirmed by reverse typing using red cells of known ABO groupings if results are to be utilized for transfusion or treatment.~~
~~c. The results of such typing determine an individual's "blood type".~~
~~2. The ABO system is unique in that the blood of individuals lacking one or both of the A/B antigens possess the corresponding antibody(ies).~~
~~a. Because of this, the results of the ABO typing may be confirmed by looking for the corresponding antibodies in the plasma or serum.~~
~~3. The Rh system lacks the natural occurrence of antibodies.~~
~~a. Rh antibodies are typically the result of an exposure to the antigen during pregnancy or through transfusion.~~
~~b. Donor cells are typically typed for the weak D to prevent transfusion of D positive cells to D negative recipients.~~
~~c. There being no danger of exposure by transfusing D positive individuals with D negative units, recipients are not typically typed for the weak D.~~
~~1) This may lead to a discrepancy between records maintained by a donor blood bank and records maintained by a transfusion blood bank.~~
~~2) An individual may have been typed as D positive (because of the presence of weak D) as a donor and typed D negative as a potential recipient.~~
~~d. Prior records are checked prior to all patient testing.~~

The ABO and Rh blood group systems are two of the most antigenic systems known. All intended transfusion recipients must have an ABO and D typing performed with each admission prior to any non-emergent transfusions.

Patients are classified as A, B, AB, or O by the presence or absence of two inheritable antigens (forward typing). The ABO system is also characterized by the presence or absence of naturally occurring antibodies directed against the A and B antigen(s) not expressed by the patient/donor. As a result, the ABO typing is confirmed by looking for the corresponding antibody(ies) in the plasma or serum (reverse typing). The forward and reverse typing must agree for a blood type to be determined.

The Rh system lacks the natural occurrence of antibodies. Antibodies to the Rh system are typically the result of an exposure to the corresponding antigen during pregnancy or through transfusion. Donor cells are typed for weak D to prevent transfusion of D positive cells to D negative recipients. There is no danger in transfusion of D positive individuals with D negative units; as a result, recipients are not typically typed for weak D. This may cause a discrepancy between records maintained by a donor blood bank and records maintained by a transfusion service blood bank.

PROCEDURES

SPECIMEN REQUIREMENTS ~~COLLECTION~~

~~1. The preferred specimen is a pink top EDTA sample, although a red top tube is acceptable. The 4 ml tubes utilized in Hematology are appropriate for the blood bank if the specimen is appropriately labeled also (See procedure "Collection of Blood Bank Specimens").~~

~~2. The minimum amount is governed by the testing to be performed. Typically one 6 ml tube is sufficient for blood typing and antibody screen and crossmatching several units. For ABO/Rh testing alone, 1 ml or less is sufficient.~~

~~3. **Properly labeled** specimens may be utilized up to 72 hours 3 days if stored at 1-6°C. ABO/Rh type re-checks may be performed on specimens >72 hours when add-on orders for FFP, Platelets or Cryo are received for patients if the patient is still wearing the current Blood Bank armband.~~

The preferred specimen is an EDTA sample, although a red top tube is acceptable. Tubes with neutral gel separators are unacceptable.

The minimum sample volume is governed by the testing to be performed. The minimum volume for a type and crossmatch is 3 mL. For ABORh testing alone, 1 mL or less is sufficient.

Properly labeled specimens for red cell transfusion may be utilized for three days if stored at 1-6°C. Specimens may be used up to 10 days for issuing of plasma, platelets or cryoprecipitate AHF.

If testing is delayed, specimens should be stored at 1-6°C. Samples may be tested up to 10 days post collection, if gross hemolysis or contamination is observed specimen should not be used.

Donor segments may be tested until the expiration of the unit.

PATIENT HISTORY CHECK

1. Prior Blood Bank history records must be checked prior to all patient testing.
   a. If prior records are available, the patient retyping must be checked against the prior records to ensure consistency of results.
      1) Review the patient history in Cerner ~~and in the Blood Bank History Card File~~ for any previous typing problems, special requirements, or discrepancies previously encountered.
      ~~2) If the patient has a previous history in the Blood Bank Card File but not in Cerner, add a "BB Hist. ABORh" test to the accession # and document the patient's historical blood type in Cerner (See "***Accession Add On***" section under "***Result Entry Blood Bank***" in the Cerner Manual".~~
      ~~3) If the patient has a history of antibodies, the "BB Hist. ABID" and "BB Hist. Antigen Type" tests should be added and resulted as well.~~
   b. If no prior records are available, **Patients requiring non-emergent red blood cell transfusion will have confirmatory ABORh testing performed.** The confirmatory testing will include both a forward and reverse type. The specimen for confirmatory testing will be on a second sample collected by a separate phlebotomy, a different draw time, a different draw site or a different phlebotomist. This sample may be from a previous or subsequent sample utilizing an acceptable anticoagulant, if available. The technologist will be responsible for ordering the confirmatory ABORh if necessary. The purpose of confirmatory testing is to reduce the risk of mistransfusion due to misidentification of the recipient at the time of sample collection.
      ~~1) At the time of specimen collection, to verify the identity of the patient, the patient is identified by two people.~~
      ~~a) Both people identifying the patient will document their Cerner ID's on the specimen.~~
      ~~b) The person who receives the specimen into the Lab in the LIS documents the second~~

identifying person's Cerner ID in Comments at specimen receipt.

c) The information can be viewed in Cerner under comments in the application "Container Inquiry". See procedure "***Collection of Blood Bank Specimens***" for further information.

1) The tech must repeat the ABO forward grouping and Rh typing a second time to confirm the blood type of the patient.

a) Perform the repeat testing on a new 2-5% red cell suspension.

b) Perform the repeat testing on a second properly labeled specimen if available.

c) Using the Accession Add On function in Cerner, add the "BB ABORH Confirm" test onto the original Accession number and result as usual (See the "***Result Entry Blood Bank***" and "***Accession Add On***" sections in the Cerner manual).

2. When performing all add-on orders it is required to perform an ABO forward and Rh type recheck on the involved specimen (add-on orders for FFP, PLT's and CRYO are included).
   a. Using the Accession Add On function in Cerner, add the "BB ABORH Confirm" test onto the original Accession number and result as usual (See the "***Result Entry Blood Bank***" and "***Accession Add On***" sections in the Cerner manual).
   b. Reverse typing (back type) is not required when performing type rechecks.
   c. If previous testing was performed by you during the current work shift, the type recheck may be omitted.
   d. Previous reactions must be reviewed prior to starting add on testing.

REAGENTS AND SUPPLIES

Anti-A
Anti-B
Anti-A,B (optional)
Anti-D
Monoclonal Control
A_1 and B reagent red cells
Anti-IgG (if applicable)
Coombs Checkcells (if applicable)
Pipettes
Test Tubes

QUALITY CONTROL

1. Typing reagents are QC'd daily.
2. Specimens positive with Anti-A, Anti-B, and Anti-D are tested with the monoclonal control to ensure that spontaneous agglutination of cells is not responsible for the positive results. The test with the Monoclonal control must be negative. If the Monoclonal control is positive then a valid interpretation of the results cannot be made.

DIRECT OR FORWARD GROUPING (PATIENT'S RBCs)

1. Verify the patient name and medical record number (MRN) or financial identification number (FIN) on the specimen tube matches the requisition and computer screen. Ensure the correct collection date and time are recorded on the tube.
2. Resuspend several drops of ~~the~~ patient's red cells to a 2-5% suspension with normal saline.
3. Label two (2) test tubes for anti-A and anti-B antisera (anti-A,B is optional) and with patient identifying information.
4. Place one drop of each reagent in the appropriately labeled tube. If reagent is colorless, verify

reagent has been added prior to proceeding to next step.
5. Add one drop of the ~~patient~~ red cell suspension to each tube.
6. Mix each tube gently and ~~place the tubes in the~~ centrifuge for the appropriate calibrated spin time.~~15-30 seconds at approximately 3500 RPM.~~
7. Examine for hemolysis and then gently and completely resuspend the cell button. Using the agglutination viewer, examine for macroscopic agglutination.
8. Grade the reactions and record the results immediately ~~onto the Blood Bank Worksheet~~ in Cerner, prior to discarding the tubes. If the computer system is unavailable, record results concurrently on the Blood Bank Worksheet.

REVERSE OR SERUM GROUPING (PATIENT'S PLASMA OR SERUM)

Infant plasma or serum may lack the corresponding antibodies. Therefore, reverse typing is omitted on infants.

1. Verify the patient name and medical record number (MRN) or financial identification number (FIN) on the specimen tube matches the requisition and computer screen. Ensure the correct collection date and time are recorded on the tube.
2. Label two (2) test tubes for A1 and B cells ~~(A2 cells are optional)~~ and with the patient identifying information.
3. To each tube, add two (2) drops of the patient's plasma or serum, ensure plasma/serum has been added prior to proceeding.
4. Place one (1) drop of A1 and B cells into the respective tubes.
5. Mix each tube gently and ~~place the tubes in the~~ centrifuge for the appropriate calibrated spin time. ~~15-30 seconds at approximately 3500 RPM.~~
6. Examine for hemolysis and then gently and completely resuspend the cell button. Using the agglutination viewer, examine for macroscopic agglutination.
7. Grade the reactions and record the results immediately ~~onto the Blood Bank Worksheet~~ in Cerner, prior to discarding the tubes. If the computer system is unavailable, record results concurrently on the Blood Bank Worksheet.

Rh (D) TYPING

1. Verify the patient name and medical record number (MRN) or financial identification number (FIN) on the specimen tube matches the requisition and computer screen. Ensure the correct collection date and time are recorded on the tube.
2. Resuspend several drops of the patient's red cells to a 2-5% suspension with normal saline.
3. Label two (2) test tubes for anti-D and Monoclonal control ~~D control and~~ with the patient identifying information.
4. Place one (1) drop each of anti-D and ~~D~~ Monoclonal control into the respective tubes, ensure antisera has been added prior to proceeding.
5. To each tube, add one (1) drop of the patient cell suspension to be tested.
6. Mix each tube gently and ~~place the tubes in the~~ centrifuge for the appropriate calibrated spin time. ~~15-30 seconds at approximately 3500 RPM.~~
7. Examine for hemolysis and then gently and completely resuspend the cell button. Using the agglutination viewer, examine for macroscopic agglutination.
8. Grade the reactions and record the results immediately ~~onto the Blood Bank Worksheet~~ in Cerner, prior to discarding the tubes. If the computer system is unavailable, record results concurrently on the Blood Bank Worksheet.
9. If a weak D is required, the Rh typing tubes may be converted to the weak D test.

WEAK D TESTING ~~(PREVIOUSLY D^{U})~~

Weak D testing is only required when performing the Cord Blood Work-up/ABORh on a baby to determine if the mother is a Rhogam candidate.

Repeat donor unit testing on Rh negative units does not require performance of the weak D, as this is performed by the blood provider.

1. Prepare red cells as in steps 1-7 in Rh typing above, or utilize the tubes previously used in the above Rh testing.
2. Mix the tubes gently and incubate both tubes at 37°C ± 1°C for a minimum of 15 minutes and a maximum of 60 minutes. ~~for 15-30 minutes at 37°C~~.
3. After incubation, wash the cells 3-4 times with isotonic saline (use of the cell washer is preferable, but cells may be washed by hand if the cell washer is unavailable for use). Decant completely after the last wash. ~~the Cellwasher (See procedure "*Sorvall Cellwasher 2 Plus (CW2 plus)*" or manually with saline (fill the tube), centrifuging and decanting between each wash.~~
4. After washing, add two (2) drops of Anti-IgG ~~either polyspecific or IgG antiglobulin (Coombs)~~ to each tube.
5. Mix each tube gently and ~~place the tubes in the~~ centrifuge for the appropriate calibrated spin time. ~~15-30 seconds at approximately 3500 RPM.~~
6. Examine for hemolysis and then gently and completely resuspend the cell button. Using the agglutination viewer, examine for macroscopic agglutination.
7. ~~Grade the reactions and record immediately~~. Grade the reactions and record the results immediately in Cerner, prior to discarding the tubes. If the computer system is unavailable, record results concurrently on the Blood Bank Worksheet.
8. Add one (1) drop of Coombs Control Cells to each negative reaction.
9. Mix gently and centrifuge the tubes for the appropriate calibrated spin time. ~~15-30 seconds at 3500 RPM.~~
10. Gently and completely resuspend the cell button. Using the agglutination viewer, examine for macroscopic agglutination.
11. ~~Grade the reactions and record immediately~~. Grade the reactions and record the results immediately in Cerner, prior to discarding the tubes. If the computer system is unavailable, record results concurrently on the Blood Bank Worksheet.

INTERPRETATION OF RESULTS:

1. ABO GROUPING
   a. Agglutination of tested patient red cells and agglutination or hemolysis in the reverse typing constitutes a positive test result.
   b. A Smooth cell suspension after resuspension of the cell button is a negative test result.
   c. Any discrepancy between the forward and reverse typing should be resolved before an interpretation is recorded.
   d. ~~ABO GROUPING: Interpret the ABO Group based on this chart~~ The interpretation of forward and reverse typing is given in the table below:

~~Anti-A~~	~~Anti-B~~	~~Anti-A,B~~	~~A1 Cells~~	~~B Cells~~	~~A2 Cells~~	~~ABO Group~~
~~0~~	~~0~~	~~0~~	~~+~~	~~+~~	~~+~~	~~O~~
~~+~~	~~0~~	~~+~~	~~0~~	~~+~~	~~0~~	~~A~~
~~0~~	~~+~~	~~+~~	~~+~~	~~0~~	~~+~~	~~B~~
~~+~~	~~+~~	~~+~~	~~0~~	~~0~~	~~0~~	~~AB~~

Anti-A	Anti-B	Anti-A,B (optional)	A_1 Cells	B Cells	Interpretation
+	0	+	0	+	**A**
0	+	+	+	0	**B**
0	0	0	+	+	**O**

+	+	+	0	0	AB
"0" negative "+" positive					

2. Rh (D) TYPING
   a. Agglutination in the anti-D tube and no agglutination in the control tube constitutes a valid test. The red cells are interpreted as D positive.
   b. No agglutination in both the anti-D tube and the control tube constitutes a valid test. The red cells are interpreted as D negative.
   c. If the control tube shows agglutination, repeat the procedure.
      1) If agglutination is still present on the repeat, no valid interpretation of the D test or weak D test can be made.
      2) In this situation, if the red cells are from a patient, D negative blood products should be transfused.
      3) If the red cells are from a donor unit, the product must be returned to the supplier and not used for transfusion.
   d. ~~Du Positive (~~Weak D Positive~~)~~ patients are reported as Rh Positive. ~~Document clearly on the patient's Blood Bank History Card that they are Du Positive.~~Add a Blood Bank comment to identify the patient as weak D positive.
   e. The interpretation of D typing is given in the table below:

Anti-D	Control	Coombs Control Cells	Interpretation
+	0	+	Positive / Weak D – sample is D positive
+	+	NP	Invalid Results – unable to determine D type
0	0	+/+	Negative Weak D – sample is D negative
0	+	NP	Invalid Results – unable to determine D type
0	0	0	Testing invalid – repeat testing
"0" negative "+" positive "NP" not performed			

3. Hemolysis and/or agglutination is considered a positive test result.
4. If a discrepancy is encountered during testing, see procedure "Test Result Discrepancies". If a discrepancy between the forward group and the reverse group exists, and transfusion is required before the discrepancy can be resolved, type O red blood cells and AB plasma must be given.
   ~~a. Washing of the patient/donor red blood cells may be necessary to obtain an accurate type when typing some patients to remove excess protein and other contaminating substances.~~
   ~~b. Inadequate washing of the patient's red cells may cause false positive results.~~
   ~~c. Rouleaux formation, bacterial contamination, or certain disease states may cause false positive results.~~
   ~~d. Improper incubation time or temperature may cause false negative results.~~
   ~~e. Contamination of the antiglobulin test with human protein may cause false negative results.~~
   ~~f. Inadequate or excessive centrifugation, incorrect red cell suspension, or improper reading technique may cause false results.~~
   ~~g. The concentration of the patient red cell suspension may be compared to the 3% reagent red cell suspension to assist in estimating the 2-5% solution.~~
5. Some subtypes of group A may develop anti-A1. This would create a discrepancy between the forward typing and the reverse typing. (Forward type as Group A and back type as Group O). These individuals should be transfused with group O cells and group A plasma.
6. ~~Direct typing may be performed for informational purposes only at health fairs, or as a public~~

~~service. In this instance, finger stick collection of sufficient RBCs to prepare the cell dilution and direct typing is sufficient.~~

7. The reagents may not detect very weak subgroups of A or B.

~~**REPORTING OF RESULTS:**~~

~~After interpretation, the ABO/Rh results are entered into Cerner. See "***Result Entry Blood Bank***" section in the Cerner Manual.~~

REFERENCES

1. American Association of Blood Banks, ~~25~~30th Ed. (2015) ~~(2008)~~. *Standards for Blood Banks and Transfusion Services*. Bethesda, Maryland: AABB.
2. American Association of Blood Banks, 18th ~~16th~~ Ed. (2014) ~~(2008)~~. *Technical Manual*. Bethesda, Maryland: AABB.

Prepared by/Title/Date:		**Dates Reviewed/Revised:**
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Signature on File in Lab		03/06 04/15
		04/07 12/14/2016