Beaumont Laboratory
Transfusion Medicine

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DETERMINING THE ABO AND Rh(D) OF PATIENTS WHO ARE AT LEAST FOUR MONTHS OLD

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Purpose

The purpose of this document is to provide Blood Bank staff with policies and stepwise, manual testing instructions for determining the ABO and Rh(D) of patients who are at least four months old by the tube method and the manual gel card method.

Routine ABO/Rh testing of patients who are at least four months old is performed using the ORTHO VISION™ Analyzers as indicated in P717, *Routine Testing of the ORTHO VISION™ Analyzer*.

Scope

- This document applies to all patients who are at least four months old.
- For patients less than four months old, refer to P514, *Forward Typing Determination of Neonatal ABO and Rh(D) by the Tube Method.*

Principle

Landsteiner's Law applies to ABO testing. It states that if ABO antigens are present on the test red blood cells, then the test plasma should lack the corresponding antibodies. If ABO antigens are absent from the test red blood cells, then the corresponding antibodies are expected in the test plasma.

Samples from adult patients with normal immune systems generally follow Landsteiner's Law. An ABO or Rh(D) discrepancy occurs when the ABO or Rh(D) of the current sample is not in agreement with the ABO or Rh(D) of a historical sample, or when graded reactions are not valid, or when graded reactions do not yield a valid interpretation.

ABO antibodies are not present at birth but continue to rise during early childhood and achieve adult levels by 5 to 10 years. Therefore, the ABO of neonatal patients is performed by forward typing only; refer P514, *Forward Typing Determination of Neonatal ABO and Rh(D) by the Tube Method*. ABO discrepancies related to low levels of ABO antibodies are sometimes encountered in young pediatric patients.

If an ABO or Rh(D) discrepancy is encountered on any patient (including pediatric patients) refer to P623, *Resolution of ABO and Rh(D) Discrepancies*.

Policies

As with all manual tests, batch testing must be limited to 6 tests per batch. If workload becomes excessive, supervisory staff must be notified immediately.

Reagents must be dispensed into the test tubes and gel cards immediately before testing as described in the *Procedure* section; they may not be pre-dispensed in anticipation of testing.

Historical Record Check

Before testing, a technologist must perform a historical record check on each sample. After testing, a technologist must verify all test results before they are saved. Refer to P121, *Historical Record Check* for additional information.

ABO and Rh(D) Discrepancies

If an ABO or Rh(D) discrepancy exists, then before entering the interpretations in the Blood Bank computer system the technologist must refer to P623, *Resolution of ABO and Rh(D) Discrepancies* and attempt to resolve the discrepancy.

Requirement for Two Separate ABO/Rh Typings

All patients must have two complete, separate sets of ABO/Rh results in the Blood Bank computer system before results are sent/interface to the HIS or before RBCs are crossmatched. The source of these two separate typings may be:

- Repeat testing of the same sample by two different technologists or on the ORTHO VISION™, or
- Testing of both the current sample and testing of a historical sample.

Note that one or both of these typings may be performed by the manual gel card method or tube method as described in this document.

Appropriate Blood Bank Computer Test Code

For computer documentation of ABO/Rh results, it is important to select the correct test code: TYPE or NPR. The correct code is important because:

- The results for the TYPE code will interface to the HIS; the results of the NPR test will not.
- Due to the potential for human error, if a TYPE typing is performed manually (by the tube or manual gel method) then the result should not interface unless a historical type is on record or until the second type is complete.

Donor ABO Testing for Potential Organ Donors

For transplant purposes, a Donor ABO with A Subgroup test is ordered as part of the donor evaluation process by the transplant team for living donors. This is a test of record ordered by transplant, and tested by the blood bank. Any potential organ donor that types as group A or AB, will have an A1 antigen typing performed on the sample. The donor's recent transfusion history will be provided by the transplant team through an order comment in the HIS. Antigen typing cannot be performed if the patient has been transfused within the last 90 days. Refer to P605, *Antigen Typing Procedures* and P606, *Antigen Typing Policies*.

Weak D Testing

Weak D testing is not routinely performed unless it is needed to assess maternal RhIG candidacy, as described in P505, *Rh(D) Typing of Neonatal Samples to Assess Maternal*

RhIG Candidacy. For additional information involving Weak D interpretations, refer to P623F, *Resolution of Rh(D) Discrepancies.*

Rh(D) Negative Results (Applies to Patients with No Previous Blood Type in the Blood Bank Computer System)
When a patient does not have a previous blood type in the Blood Bank computer, the NPR test should be ordered. If the patient appears to be Rh(D) negative by the tube method, the technologist should repeat the Rh(D) typing before accepting the test results. The purpose of this repeat Rh(D) typing is to prevent an erroneous result from being entered in the computer (e.g. if the Anti-D reagent was mistakenly omitted). The Rh(D) type should be repeated using a new cell suspension. This repeat Rh(D) type will be entered as an internal comment to the NPR test, for example ”Repeat Rh negative.”

Definitions / Abbreviations

- **MTS:** Micro Typing System
- ~~**BBCDM:** Blood Bank Computer Documentation Manual~~
- **HIS:** (Hospital Information Services) The hospital-wide computer system
- **Interface:** The computer process by which test results are sent from the Blood Bank computer system to the HIS.
- **NPR:** (No Previous Record) The Blood Bank test code that is ordered when a patient does not have a previous ABO/Rh test result in the Blood Bank computer.
- **ABO/Rh Discrepancies:** An ABO or Rh(D) discrepancy occurs when:
  - the ABO or Rh(D) of the current sample is not in agreement with the ABO or Rh(D) of a historical sample, or
  - ABO or Rh(D) graded reactions are not valid (see the *Interpretation* section), or
  - Graded reactions do not yield a valid interpretation (see the *Interpretation* section).
- **Complete ABO/Rh Typing:** ABO/Rh typing that includes both a forward and a reverse typing. A neonatal typing is not a *complete* typing because a reverse typing is not performed; see P514, *Forward Typing Determination of Neonatal ABO and Rh(D) by the Tube Method*.

Specimen Collection and Handling

The preferred specimen is a 6 ml EDTA sample with affixed identifying label. See P101, *Identifying and Triaging Acceptable Samples for Testing* for acceptable alternatives.

Forms

- F-120f, *Downtime Form: QC of Manual Gel ABORh Testing*

Reagents

Tube Method

- Ortho BioClone Anti-A
- Ortho BioClone Anti-B

- Ortho BioClone Anti-D
- 6 – 8% bovine albumin control
- 3% AFFIRMAGEN® Reagent Red Blood Cells

Manual Gel Card Method

- MTS™A/B/D Monoclonal and Reverse Grouping Cards
- 0.8% AFFIRMAGEN® Reagent Red Blood Cells
- MTS™ Diluent 2 Plus

Equipment

Tube Method

- Table top centrifuge
- Lighted agglutination viewer

Manual Gel Card Method

- MTS Centrifuge
- Ortho Workstation
- Calibrated pipette (electronic or manual)

Supplies

- Pipette tips
- 10 x 75 mm test tubes
- 12 x 75 mm test tubes
- Disposable pipettes
- Gauze
- 0.9% Normal Saline

Quality Control

- Quality control (QC) of the manual gel card ABO and Rh(D) testing must be performed on each day that manual gel testing is performed. This QC testing is performed on the ORTHO VISION™ as described in P716, *ORTHO VISION™ Analyzer QC*. If this QC is not performed on the ORTHO VISION™, then this QC testing must be performed by the manual gel card method as described in P328, *Quality Control of the Manual Gel System Reagents*. This shall be documented on F-120f, *Downtime Form: QC of Manual Gel ABORh Testing.* When resulting out manual gel card ABO and Rh(D) results in the Blood Bank computer system, the QCVIS QC rack should be used.
- Daily quality control of ABO and Rh(D) tube testing is performed as described in P305, *Routine Quality Control of Blood Bank Reagents* and documented in the Blood Bank computer system.
- All reagents and gel cards must be brought to room temperature (18°C - 25°C) before use.
- Do not use reagents or gel cards beyond their expiration date.
- If the centrifugation phase is interrupted, then all affected specimens must be retested.
- If the speed of centrifugation is not at an acceptable level, then all affected specimens must be retested using different equipment if necessary.
- ABO testing is also controlled by obtaining correlating test results for forward and reverse blood grouping tests.

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Bovine Albumin Control and Documentation in the Blood Bank Computer

In order to interpret the ABO or Rh(D) of a patient who appears to be AB positive using the tube testing method (RBCs react with the Anti-A, Anti-B, and Anti-D reagents), a 6 - 8% bovine albumin control must be tested and must be non-reactive. This control is prepared as described in P122, *Preparing the 6 – 8% Bovine Serum Albumin control.*

- The purpose of the control described below is to prevent potential false positive results with the Anti-A, Anti-B, and Anti-D reagents.
- If the patient's RBCs appear to be AB positive (RBCs are reactive with the Anti-A, Anti-B, and Anti-D reagents) then potential false positive results are a concern. Testing with the 6 - 8% bovine albumin control shall be performed as described in Step 10 of the *Tube Method Procedure*.
    - If testing with this control is non-reactive, then the control field in the Blood Bank computer system shall be documented as "0". A test comment shall be added indicating that the bovine albumin control was used, and the lot number of the bovine albumin control.
    - If testing with this control is reactive, the ABO and Rh(D) cannot be interpreted; refer to P623, *Resolution of ABO and Rh(D) Discrepancies*. A test comment shall be added indicating the bovine albumin control was used, the lot number of the bovine albumin control, and that the result was reactive.
- If the patient's RBCs do not appear to be AB positive (RBCs are non-reactive with the Anti-A, Anti-B, or Anti-D reagents) then testing with the 6 - 8% bovine albumin control is not indicated. False positive results are not a concern, as demonstrated by the non-reactivity with the Anti-A, Anti-B, or Anti-D reagent. To indicate that this testing is not indicated, the control field in the Blood Bank computer shall be documented as "0." It is not necessary to add a comment in this case.

Before You Begin

Perform the following before starting this procedure:

- Verify the patient specimen satisfies all labeling requirements as described in P101, *Triaging and Identifying Acceptable Samples for Testing*. Verify all patient information from the specimen match the information in the Blood Bank computer system.
- Centrifuge specimens to obtain clear plasma for 10 minutes at the calibrated RPM of the centrifuge as described in P101, *Triaging and Identifying Acceptable Samples for Testing*.
- Verify that all QC requirements have been completed as indicated in the *Quality Control* section of this document.

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Procedure

Tube Method Procedure

1. Verify the requirements in the *Before You Begin* section of this document have been met.

2. Label six 10 x 75 mm test tubes with the patient name and the intended use of the tube, including the corresponding reagents or the patient's 3% red cell suspension. See the example below:
    a. Tube 1 – [Name] "A"
    b. Tube 2 – [Name] "B"
    c. Tube 3 – [Name] "D"
    d. Tube 4 – [Name] "a"
    e. Tube 5 – [Name] "b"
    f. Tube 5 – [Name] "3%"

3. Add 2 drops of patient plasma to the corresponding test tubes labeled "a" and "b".
    Patient plasma must be added to the test tubes prior to adding the 3% AFFIRMAGEN® RBC reverse cells.

4. Prepare a 2 – 4% red cell suspension in the tube labeled "3%" using the patient's own RBCs.
    Refer to P060, *Making a Test Red Cell Suspension* for additional information.

5. Add 1 drop of each 3% AFFIRMAGEN® RBC reverse cell into the corresponding tubes labeled "a" and "b".

6. Add 1 drop of the following forward typing antisera into the corresponding test tubes labeled "A", "B", and "D".
    a. Ortho BioClone Anti-A for tube "A"
    b. Ortho BioClone Anti-B for tube "B"
    c. Ortho BioClone Anti-D for tube "D"
    Forward typing antisera must be added to the test tubes prior to adding the patient's red cell suspension.

7. Add 1 drop of the patient's 2 – 4% red cell suspension to the corresponding test tubes labeled "A", "B", and "D".

8. Gently agitate the test tubes to mix the contents. Centrifuge the test tubes according to the calibrated time of the centrifuge.
    If necessary, refer to P340, *Calibration of Serologic Centrifuges* for additional information.

9. Observe the supernate in the test tubes for hemolysis. Gently resuspend the cell button of each tube. Read, grade, and record the reactions in the Blood Bank computer system or on an appropriate downtime form.
    Refer to P061, *Reading, Grading, and Recording Test Reactions* if necessary.

10. Determine whether testing with the 6-8% bovine serum albumin control is indicated by evaluating the forward typing reactions, and proceed as follows:

a. If the patient's RBCs do not appear to be AB positive, testing with the 6 – 8% bovine serum albumin control is not indicated. Document the control result field as "0".
b. If the patient's RBCs appear to be AB positive (reactive with Anti-A, Anti-B, and Anti-D reagents), then test the patient's RBCs with the bovine serum albumin control.
    i. Label a 10 x 75mm test tube with the patient's last name and "C" for the control.
    ii. Add 1 drop of the 6 – 8% bovine serum albumin control to the corresponding test tube.
    iii. Repeat steps 8 and 9 for the control.

Refer to the *Quality Control* section / *Bovine Albumin Control* and *Documentation of the Bovine Albumin Control in the Blood Bank Computer*.

11. Interpret the graded reactions and document this in the Blood Bank computer system or on an appropriate downtime form.
    Refer to the *Interpretation* section of this document.

12. If testing is complete and no additional actions are required, ensure the sample is capped and stored as directed in P062, *Storing and Disposing of Patient Samples*.

Manual Gel Method Procedure

1. Verify the requirements in the *Before You Begin* section of this document have been met.

2. Label an A/B/D Monoclonal and Reverse Grouping Card and a 12 x 75 mm test tube with patient information. A computer-generated accession label may be used for the gel card, but the patient's last name should be used as a minimum
    If multiple patients are being tested at the same time, it may be necessary to include additional information such as first name and/or medical record number.

3. Remove the foil seal from the gel card.
    Foil should be removed immediately before testing, not more than one hour before testing.

4. Set up and dispense the components for the reverse typing as follows:
    a. Add 50 µl of the 0.8% AFFIRMAGEN® A1 RBCs to the first (left) buffered well.
    b. Add 50 µl of the 0.8% AFFIRMAGEN® B RBCs to the last (right) buffered well.
    c. Add 50 µl of patient plasma to both of the buffered wells.

    Ensure the pipette tip does not touch the gel card.

5. Prepare a 4% ±1% RBC suspension of the patient's cells as follows:

If using the Manual Pipette	**If using the Electronic Pipette**
i. Dispense 0.5 ml of MTS™ Diluent 2 Plus into the labeled 12 x 75mm test tube. ii. Add 25 µl of packed RBCs from the patient's sample to the 12 x 75 mm test tube. iii. Mix the contents.	i. Program the electronic pipette to program # 7. ii. Aspirate 200 µl of MTS™ Diluent 2 Plus. iii. Aspirate 15 µl of air into the pipette tip. iv. Aspirate 10 µl packed RBCs and wipe the outside of the tip. v. Purge all contents from the tip into the 12 x 75 mm tube and mix.

6. Add the patient's RBCs to the gel card for the forward typing as described below:

If using the Manual Pipette	**If using the Electronic Pipette**
Add 12.5 µl of the 4% ±1% RBC suspension to the Anti-A, Anti-B, Anti-D, and control wells.	Add 10 µl of the 4% ±1% RBC suspension to the Anti-A, Anti-B, Anti-D, and control wells. Note that program # 9 will aspirate 60 µl, and will dispense 10 µl six times.

7. Centrifuge the gel card in the MTS Centrifuge or Ortho Workstation for 10 minutes at the calibrated speed of the gel centrifuge.
   - MTS Centrifuge = 895 ± 25 RPM
   - Ortho Workstation = 1024 ± 10 RPM

8. Read both the front and back of the gel card for agglutination. Grade the reactions in the microtubes.
   Refer to P061, *Reading, Grading, and Recording Test Reactions*, or the *ID-Micro Typing Systems™ Interpretation Guide*.

9. Record and interpret the graded ABO/Rh reactions in the Blood Bank computer system or on an appropriate downtime form.
   Refer to applicable *BBCDM / Type & Screen* for documentation in the Blood Bank computer system. Refer to the *Interpretation* section of this document.

10. If testing is complete and no additional actions are required, ensure the sample is capped and stored as directed in P062, *Storing and Disposing of Patient Samples.*

Interpretation

Valid Graded ABO and Rh(D) Reactions in Tube Testing

Valid graded ABO and Rh(D) reactions in tube testing are defined in the following table:

If the test is:	Then the graded result must be:
Forward ABO grouping	0 or 3 - 4+
Rh(D) typing	0 or 2 - 4+
Bovine albumin control	0
Reverse ABO grouping	0 or 2 - 4+

- Negative Result – No agglutination and no hemolysis of the red blood cells is a negative test result, indicated by a smooth cell suspension after resuspension of the cell button.
- Positive Result – Agglutination and/or hemolysis of the red blood cells is a positive test result. Agglutination must be of the strength listed in the table above to be considered a valid graded reaction. Refer to *Invalid Graded Reactions*, below, if applicable.
- The test cannot be interpreted if the bovine serum albumin control is reactive.
- Note that a mixed-field reaction is considered an invalid graded reaction and must be investigated. Refer to *Invalid Graded Reactions*, below.

Valid Graded ABO and Rh(D) Reactions in Manual Gel Card Testing

Valid graded ABO and Rh(D) reactions in manual gel card testing are defined in the following table:

If the test is:	then the graded result must be:
Forward ABO grouping	0 or 3 - 4+
Rh(D) typing	0 or 4+
Control	0
Reverse ABO grouping	0 or 2 - 4+

- Negative Result – No agglutination and no hemolysis of the red blood cells is a negative test result.
- Positive Result – Agglutination and/or hemolysis of the red blood cells is a positive test result. Agglutination must be of the strength listed in the table above to be considered a valid graded reaction. Refer to Invalid Graded Reactions, below, if applicable.
- The test cannot be interpreted if agglutination occurs in the control well.
- Note that a mixed-field reaction is considered an invalid graded reaction and must be investigated. Refer to *Invalid Graded Reactions*, below.

Invalid Graded Reactions

Reactive Bovine Albumin Control in the Tube Testing Method

The 6 – 8% bovine albumin must be tested and must be non-reactive in order to interpret the ABO or Rh(D) of a patient who appears to be AB positive (the patient's RBCs react with the Anti-A, Anti-B, and Anti-D reagents). If this control is reactive, then the ABO and Rh(D) cannot be interpreted; refer to P623, *Resolution of ABO and Rh(D) Discrepancies*.

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Reactive Monoclonal Control in the Manual Gel Card Method

The control must be non-reactive to interpret the ABO/Rh. If false positive reactions (e.g. Rouleaux, red blood cells coated with immunoglobulins, etc.) occur in the control well, the ABO and Rh(D) type cannot be established. Additional testing will be necessary to resolve this false positive reaction; refer to P623, *Resolution of ABO and Rh(D) Discrepancies*.

ABO/Rh Discrepancies

An ABO or Rh(D) discrepancy may occur if:

- the ABO or Rh(D) graded reactions are not valid, or
- the graded reactions do not yield a valid interpretation, or
- the bovine serum albumin control or monoclonal gel control is reactive, or
- the current type does not match the historical type.

If an ABO or Rh(D) discrepancy exists, then before entering the interpretations in the Blood Bank computer system the technologist must refer to P623, *Resolution of ABO and Rh(D) Discrepancies.*

ABO and Rh(D) Interpretation by the Tube Method

Forward Grouping				Reverse Grouping		Interpretation
Anti-A	**Anti-B**	**Anti-D**	**Albumin Control**	**A_1 Cell**	**B Cell**	
0	0	2 - 4+	Not indicated	2 - 4+	2 - 4+	O positive
0	0	0	Not indicated	2 - 4+	2 - 4+	O negative
3 - 4+	0	2 - 4+	Not indicated	0	2 - 4+	A positive
3 - 4+	0	0	Not indicated	0	2 - 4+	A negative
0	3 - 4+	2 - 4+	Not indicated	2 - 4+	0	B positive
0	3 - 4+	0	Not indicated	2 - 4+	0	B negative
3 - 4+	3 - 4+	2 - 4+	0	0	0	AB positive
3 - 4+	3 - 4+	0	Not indicated	0	0	AB negative
		Wk – 1+	0 or Not indicated			See the note below*
+ or 0	+ or 0	+ or 0	+ any strength	+ or 0	+ or 0	Cannot interpret; refer to *Invalid Graded Reaction* section.

"+" indicates the presence of agglutination "0" indicates the absence of agglutination

Note: If a patient types Wk – 1+ with Anti-D during tube testing, they are likely weak D positive. Refer to P623F, *Resolution of Rh(D) Discrepancies.*

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ABO and Rh(D) Interpretation by the Manual Gel Card Method

Forward Grouping				Reverse Grouping		Interpretation
Anti-A Microtube	Anti-B Microtube	Anti-D Microtube	Control Microtube	Buffered Gel A_1 Cell Microtube	Buffered Gel B Cell Microtube	
0	0	4+	0	2 - 4+	2 - 4 +	O Positive
0	0	0	0	2 - 4 +	2 - 4 +	O Negative
3 - 4+	0	4+	0	0	2 - 4 +	A Positive
3 - 4+	0	0	0	0	2 - 4 +	A Negative
0	3 - 4+	4+	0	2 - 4 +	0	B Positive
0	3 - 4+	0	0	2 - 4+	0	B Negative
3 - 4+	3 - 4+	4+	0	0	0	AB Positive
3 - 4+	3 - 4+	0	0	0	0	AB Negative
		Wk - 3+	0			Weak D
+ or 0	+ or 0	+ or 0	+	+ or 0	+ or 0	Cannot interpret; refer to *Invalid Graded Reaction* section.

"+" indicates the presence of agglutination "0" indicates the absence of agglutination

Note: If a patient types Wk – 3+ in the Rh(D) microtube during manual gel card testing, they are likely weak D positive. Refer to P623F, *Resolution of Rh(D) Discrepancies.*

References

- AABB, *Technical Manual*, current edition.
- AABB, *Standards for Blood Banks and Transfusion Services*, current edition.

Authorized Reviewers

Chief, Pathology and Laboratory Medicine
Medical Director and/or Designee, Blood Bank
Manager, Blood Bank

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Revised by: Christopher Ferguson	08/04/2020	r05.00.00	Combined this procedure with P103A. This policy now applies to tube and manual gel ABO/Rh testing. Updated Weak D testing information. A 3+ reaction in manual Rh gel is now considered weak D; no reflex tube testing required. Updated authorized reviewers, titles, and references.	
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