Status Scheduled PolicyStat ID 12372735

Beaumont

| | |
|---|---|
| Origination | 9/8/2021 |
| Last Approved | 10/7/2022 |
| Effective | 10/22/2022 |
| Last Revised | 10/7/2022 |
| Next Review | 10/6/2024 |

| | |
|---|---|
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| Area | Laboratory-Blood Bank |
| Applicability | All Beaumont Hospitals |

# Determining The ABO and RhD Of Patients Who Are At Least Four Months Old

Document Type: Procedure



# I. PURPOSE AND OBJECTIVE:

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

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

# II. SCOPE:

A. This document applies to all patients who are at least four months old.

B. For patients less than four months old, refer to Transfusion Medicine policy, Forward Typing Determination of Neonatal ABO and RhD.

# III. DEFINITIONS / ACRONYMS:

A. **MTS**: Micro Typing System

B. **BBCDM**: Blood Bank Computer Documentation Manual

C. **HIS**: Hospital Information Services, the hospital-wide computer system

D. **Patient test identification**: Sufficient correlating letters or numbers to associate patient sample, requisition, tubes for testing and recording device.

E. **Interface**: The computer process by which test results are sent from the Blood Bank computer system to the HIS.

F. **RBCs**: Red Blood Cells

G. **DB**: Beaumont Dearborn

H. **FH**: Beaumont Farmington Hills

I. **GP**: Beaumont Grosse Pointe

J. **RO**: Beaumont Royal Oak

K. **TY**: Beaumont Taylor

L. **TN**: Beaumont Trenton

M. **TR**: Beaumont Troy

N. **WA**: Beaumont Wayne

O. **NPR**: (No Previous Record), the Blood Bank test code that is ordered when a banded patient does not have a previous ABO/Rh test result in the Blood Bank computer.

P. **ABO/Rh Discrepancies**: An ABO or RhD discrepancy occurs when:

1. The ABO or RhD of the current sample is not in agreement with the ABO or RhD of a historical sample, or
2. ABO or RhD graded reactions are not valid (see the *Interpretation* section), or
3. Graded reactions do not yield a valid interpretation (see the *Interpretation* section).

Q. **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 Transfusion Medicine policy, Forward Typing Determination of Neonatal ABO and RhD.

R. **BSA**: Bovine Serum Albumin



# IV. PRINCIPLE:

A. 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.

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

C. 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 Transfusion Medicine policy, Forward Typing Determination of Neonatal ABO and RhD. ABO discrepancies related to low levels of ABO antibodies are sometimes encountered in young pediatric patients.

D. If an ABO or RhD discrepancy is encountered on any patient (including pediatric patients) refer to Transfusion Medicine policy, Resolution of ABO and RhD Discrepancies.

# V. 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.

A. **Historical Record Check**

1. 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 Transfusion Medicine polic,y Historical Record Check for additional information.

B. **ABO and RhD Discrepancies**

1. If an ABO or RhD discrepancy exists, then before entering the interpretations in the Blood Bank computer system the technologist must refer to Transfusion Medicine policy, Resolution of ABO and RhD Discrepancies and attempt to resolve the discrepancy.

C. **Requirement for Two Separate ABO/Rh Typings**

1. All patients must have two complete, separate sets of ABO/Rh results in the Blood Bank computer system before crossmatching a non-Group O red blood cells (RBCs) to the patient. The source of these two separate typings may be:
    a. Two manual typings of the current sample, performed by two different technologists, or
    b. Repeat testing of the same sample on the ORTHO VISION™, or
    c. One manual typing and one ORTHO VISION™ typing of the current sample, or
    d. Testing of both the current sample and testing of a historical sample by any method, or
    e. Testing of the current sample by any method and testing of a separate sample (ABOCN) by any method.

D. **NPR Test Code**

1. The results of the NPR test code do not cross the interface. Due to the potential for human error, when performing a manual typing (by the tube or gel method) where both the TYPE and NPR tests codes are ordered, the NPR should be resulted first.

E. **Royal Oak and Dearborn: Donor ABO Testing for Potential Organ Donors**

1. 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 $A_1$ 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 Transfusion Medicine policies *Antigen Typing Procedures* and *Antigen Typing Policies*.

F. **Weak D Testing**

1. Weak D testing is not routinely performed unless it is needed to assess maternal RhIG candidacy or to investigate an Rh discrepancy. See Transfusion Medicine policy, Resolution of ABO and Rh Discrepancies.

# VI. SPECIMEN COLLECTION AND HANDLING:

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

# VII. REAGENTS:

A. **Manual Gel Card Method**

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

B. **Tube Method**

1. Ortho BioClone Anti-A
2. Ortho BioClone Anti-B
3. Ortho BioClone Anti-D
4. 7% BSA
5. 3% AFFIRMAGEN® Reagent Red Blood Cells



# VIII. EQUIPMENT:

A. **Tube Method**

1. Table top centrifuge
2. Lighted agglutination viewer

B. **Manual Gel Card Method (alternative method)**

1. MTS Centrifuge
2. Ortho Workstation
3. Calibrated pipette (electronic or manual)

# IX. SUPPLIES:

A. Pipette tips
B. Test tubes, 10 x 75mm or 12 x 75mm, plastic or glass
C. Disposable pipettes
D. Gauze
E. 0.9% Normal Saline

# X. QUALITY CONTROL:

A. Quality control (QC) of the manual gel card ABO and RhD 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 Transfusion Medicine policy, 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 Transfusion Medicine policy, *Quality Control of the Manual Gel System Reagents*. This shall be documented in the Blood Bank computer system or on paper per site procedure.

B. Daily quality control of ABO and RhD tube testing is performed as described in site specific and documented in the Blood Bank computer system or on paper per site procedure.

C. All refrigerated reagents and gel cards must be brought to room temperature (18C - 25C) before use.

D. Do not use reagents or gel cards beyond their expiration date.

E. If the centrifugation phase is interrupted, then all affected specimens must be retested.

F. If the speed of centrifugation is not at an acceptable level, then all affected specimens must be retested using different equipment if necessary.

G. ABO testing is also controlled by obtaining correlating test results for forward and reverse blood grouping tests.



# XI. BOVINE SERUM ALBUMIN (BSA) CONTROL AND DOCUMENTATION IN THE BLOOD BANK COMPUTER

A. In order to interpret the ABO or RhD of a patient who appears to be AB positive (RBCs react with the Anti-A, Anti-B, **and** Anti-D reagents), 7% BSA must be tested and must be non-reactive.

B. The 7% BSA should be visually inspected prior to use. Product should be clear, slightly yellow-yellowish brown in color, and free from particulates.

    1. Unopened and refrigerated (2-8°C), the stability is until expiration date.
    2. Opened and refrigerated (2-8°C), the stability is ≤ 28 days when tightly sealed.

C. The purpose of the control described below is to prevent potential false positive results with the Anti-A, Anti-B, and Anti-D reagents.

    1. 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 7% BSA shall be performed as described in Step 10 of the *Tube Method Procedure* section below.

        a. If testing with this control is reactive, the ABO and RhD cannot be interpreted; refer to Transfusion Medicine policy, Resolution of ABO/Rh Discrepancies.

D. 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 7% BSA 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 "NT"

# XII. BEFORE YOU BEGIN:

A. Perform the following before starting this procedure:

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

# XIII. PROCEDURE:

A. **Tube Method Procedure**



1. Verify the requirements in the *Before You Begin* section of this document have been met.
2. Label six test tubes with the patient first initial and last name (name may be truncated based on length) 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 6 – [Name] "3%"
3. Add 2 drops of patient plasma to the corresponding test tubes labeled "a" and "b". Note: 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.
   Note: Refer to Transfusion Medicine policy 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"
Note: 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.
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.
Note: Refer to Transfusion Medicine policy, Reading, Grading, and Recording Test Reactions if necessary
10. Determine whether testing with the 7% BSA control is indicated by evaluating the forward typing reactions, and proceed as follows:
    a. 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 test tube with the patient's last name and "C" for the control.
        ii. Add 1 drop of the 7% BSA control to the corresponding test tube.
        iii. Repeat steps 8 and 9 for the control.
    b. If the patient's RBCs do not appear to be AB positive, testing with the 7% BSA control is not indicated. Document the control result field as "NT".
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 site specific Transfusion Medicine policies, *Storing and Disposing of Patient Samples*.

B. **Manual Gel Method Procedure (not performed at FH or GP)**

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 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.
Note: If multiple patients are being tested at the same time, include additional information such as first name and/or medical record number.
3. Remove the foil seal from the gel card.
Note: 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.
    Note: 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 Sartorius/BioHit Pipette |
|---|---|
| i. Dispense 0.5 ml of MTS™ Diluent 2 Plus into the labeled test tube.<br>ii. Add 25 µl of packed RBCs from the patient's sample to the test tube.<br>iii. Mix the contents. | i. Program the BioHit pipette to program # 7.<br>ii. Aspirate 200 µl of MTS™ Diluent 2 Plus.<br>iii. Aspirate 15 µl of air into the pipette tip.<br>iv. Aspirate 10 ul packed RBCs and wipe the outside of the tip.<br>v. Purge all contents from the tip into the test 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 Sartorius/BioHit Pipette |
|---|---|
| Add 10-12.5 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.
    a. MTS Centrifuge = 895 +/- 25 RPM
    b. Ortho Workstation = 1032 +/- 10 RPM

8. Read both the front and back of the gel card for agglutination. Grade the reactions in the microtubes.
    Note: Refer to Transfusion Medicine policy, 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.

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

# XIV. INTERPRETATION:

A. **Valid Graded ABO and RhD Reactions in Tube Testing**

1. Valid graded ABO and RhD 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+ |
| RhD typing | 0, 1 - 3+, or 4+ |
| Control | 0 |
| Reverse ABO grouping | 0 or 2 - 4+ |

   a. 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.

   b. 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.

   c. The test cannot be interpreted if the bovine serum albumin control is reactive.

   d. Note that a mixed-field reaction is considered an invalid graded reaction and must be investigated. Refer to *Invalid Graded Reactions*, below.

B. **Valid Graded ABO and RhD Reactions in Manual Gel Card Testing**

1. Valid graded ABO and RhD 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+ |
| RhD typing | 0, 1 - 3+, or 4+ |
| Control | 0 |
| Reverse ABO grouping | 0 or 2 - 4+ |

   a. Negative Result – No agglutination and no hemolysis of the red blood cells is a negative test result.

   b. 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.

   c. Gel method results of 1 -3+ are weak D positive for RhD typings.

   d. The test cannot be interpreted if agglutination occurs in the control well.

   e. Note: Interpretations are the same for tube and VISON™/gel methodology, with the exception of RhD testing.

f. Note: Mixed-field reactions are considered invalid graded reactions and must be investigated. Refer to the section *Invalid Graded Reactions*, below.

C. **Invalid Graded Reactions**

1. **Reactive Bovine Albumin Control**

a. The 7% BSA must be tested and must be non-reactive in order to interpret the ABO or Rh 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 RhD cannot be interpreted; refer to Transfusion Medicine policy, Resolution of ABO/Rh Discrepancies.

2. **Reactive Monoclonal Control**

a. 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 RhD type cannot be established. Additional testing will be necessary to resolve this false positive reaction; refer to Transfusion Medicine policy, Resolution of ABO and RhD Discrepancies.

D. **ABO/Rh Discrepancies**

1. An ABO or RhD discrepancy may occur if:
   a. The ABO or RhD graded reactions are not valid, or
   b. The graded reactions do not yield a valid interpretation, or
   c. The 7% BSA or monoclonal gel control is reactive, or
   d. The current type does not match the historical type.
2. If an ABO or RhD discrepancy exists, then before entering the interpretations in the Blood Bank computer system the technologist must refer to Transfusion Medicine policy, Resolution of ABO and RhD Discrepancies.
3. **ABO and RhD 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 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 1 – 3+ | 0 | | | Rh weak D positive |
| + or 0 | + or 0 | + or 0 | + | + or 0 | + or 0 | Cannot interpret; refer to *Invalid Graded Reaction* section. |

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

b. Any gel RhD reaction that is 3+ or weaker will be interpreted as weak D positive.

4. **ABO and RhD Interpretation by the Tube Method**

| **Forward Grouping** | | | | **Reverse Grouping** | | **Interpretation** |
|---|---|---|---|---|---|---|
| **Anti-A** | **Anti-B** | **Anti-D** | **7% BSA** | **$A_1$ 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. |

a. "+" 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 Transfusion medicine policy, Resolution of ABO and Rh Discrepancies.

# XV. REFERENCES:


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


## Approval Signatures

| Step Description | Approver | Date |
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