

# Beaumont

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## Newborn Blood Bank Work Up and Cord Blood Evaluation - Blood Bank

Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

This document will provide the Blood Bank staff with instructions for testing cord blood.

### II. CLINICAL SIGNIFICANCE:

- A. A newborn cord blood may be tested to determine if there is a serologic evidence for Hemolytic Disease of the Newborn (HDN) that any antibodies of maternal origin may cause or to determine possible Rh Immune globulin candidacy for the mother.

### III. PRINCIPLE:

- A. With HDN, red blood cells (RBCs) of the fetus become coated with IgG alloantibody of maternal origin. This alloantibody is directed against an antigen of paternal origin that is present on the fetal RBCs. The sensitized RBCs undergo accelerated destruction before and after birth.
- B. Clinical severity of the disease is extremely variable, ranging from intrauterine death to a condition that can be detected only by serologic tests. The most severe cases of HDN are usually caused by antibodies in the Rh system (primarily Anti-D), while those cases that are only detectable serologically are most often due to ABO incompatibility. Sometimes the offending maternal antibody is directed at a paternal antigen of low frequency in the general population and is, therefore, difficult to detect and identify. The CORDE is designed to determine whether serologic evidence of HDN exists.

## IV. SCOPE:

- A. The cord blood evaluation (CORDE) or Newborn Blood Bank Workup (NEWBRNBES) shall be performed whenever this test is ordered by the caregivers; ultimately, it is the responsibility of the patient's physician to order the cord evaluation. However, a cord evaluation is usually indicated in the following situations:
1. Cord Blood specimens of babies born to Rh negative mothers are routinely collected and tested to determine respective RhIG candidacy
  2. When the current maternal sample has a positive antibody screen
  3. When the physician requests it due to possible ABO incompatibility between the mother and infant
  4. When unexpected antibodies are detected in the neonatal antibody screen. Note that a neonatal antibody screen is usually not indicated but is indicated if a transfusion is required and maternal sample is unavailable.
- B. Exceptions / when the Cord Blood evaluation (CORDE) or Newborn Blood Bank Workup is not indicated:
1. The CORDE is not indicated if the unexpected maternal antibody is Anti-Lea, Anti-Leb, Anti-I, Anti-H, Anti-P1, Anti-A<sub>1</sub>, a cold reacting antibody of unknown specificity, a cold autoantibody (CLD), or reactions due to reagent preservatives (NSG). These antibody specificities are generally not associated with causing HDN because the corresponding neonatal antigens are not well formed at birth, and because the antibodies are usually IgM and therefore do not cross the placenta.

## V. DEFINITIONS:

- A. **HDN:** also referred to as HDFN, HDNB; Hemolytic Disease of the Fetus / Newborn. The destruction of fetal or newborn red blood cells by maternal alloantibodies specific for inherited paternal red cell antigens.
- B. **Unexpected antibodies:** any antibody (other than naturally occurring Anti-A or Anti-B that is regularly found in normal serum or plasma) that is currently or was historically present in a patient's sample.
- C. **Passively acquired antibodies:** antibodies that are transferred from the donor(s) to a recipient through the transfusion or administration of plasma-containing components (i.e., RhIG administration).
- D. **Alloimmunization:** the process whereby a recipient forms antibody in an immune response to foreign antigens on donor RBCs.
- E. **Neonates:** neonates from birth through 4 months of age.
- F. **Clinically significant antibody:** an antibody that:
1. is known to cause Hemolytic Disease of the Newborn (HDN) or shortened survival of antigen positive RBCs, and
  2. requires transfusion of antigen negative red cells, and

3. is usually IgG and best detectable with antihuman globulin (AHG).
- G. **Clinically insignificant antibody:** an antibody that;
1. does not cause shortened red cell survival of antigen positive RBCs, and
  2. does not require transfusion of antigen negative red cells, and
  3. is usually IgM and reacts best below 37C.
- H. **HIS:** Hospital Information System; the hospital-wide computer system, patient electronic health record.
- I. **LIS:** Laboratory Information System
- J. **Standard panel:** a commercially prepared panel that usually consists of 11 vials of human RBCs. It is usually performed on patients who do not have a historical antibody record.
- K. **Selected cell panel:** a panel that is pre-selected based on the antigenic profile of the test RBCs.

## VI. SPECIMEN COLLECTION AND HANDLING:

- A. The preferred specimen is a 6 ml EDTA sample with affixed identifying label. Heel stick specimens collected in EDTA obtained direct from the neonate are also acceptable. See Transfusion Medicine policy Identifying and Triaging Acceptable Samples for Testing for acceptable alternatives.

## VII. POLICIES:

- A. **Collection of a Cord Blood Sample**
1. It is the policy of the Labor and Delivery department that a cord blood specimen is collected on all deliveries.
- B. **Cord Blood Labeling Policies / Not Acceptable for Transfusion Purposes**
1. Cord blood samples are labeled according to Transfusion Medicine policy Triaging and Identifying Acceptable Samples for Testing-Blood Bank
  2. Cord blood samples are not acceptable for transfusion purposes. If a neonatal transfusion is required, a direct sample from the infant is required. **See Neonatal Transfusion**
- C. **Neonatal Microtainer® Samples / Labeling Policies**
- A neonatal microtainer sample should be collected for testing when a neonatal transfusion is needed, or when a cord sample was not collected, and neonatal testing is indicated. This sample should be labeled with an ID label, which includes the neonate's name, Medical Record Number (MRN), wristband number (N number), date/time, and collectors' identification. This information may be handwritten on the label.
- D. **Neonatal ABO/Rh Testing**
- The preferred method of ABO/Rh testing of cord blood samples is in gel on the Ortho Vision. The alternate method of testing these samples is manually. Refer to Transfusion Medicine policy Forward Typing Determination of ABO and Rh for Patients Less Than Four Months of Age by Tube Method.

**E. Neonatal Direct Antiglobulin Test (DAT) Performed by the Gel Method**

The preferred method for DAT testing of cord blood samples is in gel on the Ortho Vision. The alternate method of testing these samples is manually in gel (not the tube method). Refer to Transfusion Medicine policy [Performing Neonatal Direct Antiglobulin Test \(DAT\) by Gel Method](#).

**F. Notification of Positive DAT (Critical Value)**

The Blood Bank shall notify the nursing staff if the neonatal gel DAT is positive, regardless of the strength of the DAT, as described in Transfusion Medicine policy [Critical Value Notification Policy for Transfusion Medicine](#).

**G. Antigen Testing**

As described in Transfusion Medicine policy, [Antigen Typing](#), the following factors must be considered:

1. the neonatal transfusion history (if applicable), and
2. the neonatal DAT results. RBCs with a positive DAT result cannot be accurately tested with typing reagents that require an Indirect Antiglobulin Tests (IAT).

**H. ABO Incompatibility**

The mother and neonate are considered ABO incompatible when the maternal plasma contains ABO antibodies that correspond to ABO antigens present on the neonatal RBCs. For example, when the mother is group O (Anti-A and Anti-B are present in her plasma) and the baby is group A or B. Many positive neonatal DATs can be explained by an ABO incompatibility. The Blood Bank will not perform a neonatal eluate due to ABO incompatibility, unless

1. The neonatologist specifically requests an eluate.
2. The Blood Bank is already preparing an eluate due to unexpected maternal antibodies.

**I. Eluate Testing**

1. An eluate of the neonatal RBCs shall be performed when indicated (see the Procedure section of this document for eluate indications).
2. If an unexpected antibody has been identified in the maternal sample or history, then the eluate should be tested against a selected cell panel. The test cells should include the following:
  - a. 3 cells that are positive for the antigen corresponding to the maternal antibody, and
  - b. Appropriate cells to perform antibody exclusions (rule-outs).
    - i. If unexpected antibody (ies) have been detected in the maternal sample or history, but have not been identified (i.e. weak unidentified) then the eluate should be tested against a standard cell panel.
    - ii. If the Blood Bank is already performing an eluate due to unexpected maternal antibodies, and the neonatal DAT is positive with an ABO incompatibility, then the eluate should also be tested against 2 examples each of A1 and B cells (use a set of reverse cells, an A-negative donor cell, and a B-negative donor

cell).

- iii. If an unexpected antibody is identified in the eluate (one that was not previously identified in the maternal sample or maternal history) then the Medical Director should be consulted.

#### J. Special Situations

1. **Suspected Maternal Antibody to a Low Prevalence (paternal) Antigen**

This could happen when the father passes an antigen to a low prevalence antigen to the fetus, and the mother develops the corresponding antibody to this fetal antigen. The neonate's DAT may be strongly positive, but the maternal antibody screen may be negative (because the screen cells do not include test cells with the low prevalence antigen). If a maternal antibody to a low prevalence antigen is suspected, consult the supervisor or Medical Director. Testing with the paternal sample may be required to confirm the presence of a maternal antibody to a low prevalence antigen.

2. **Blocking: Possible False Negative Antigen Typing**

Anti-D and Anti-Kell have the potential to cause blocking. Blocking may occur when the mother has Anti-D or Anti-Kell, and the neonatal RBCs become heavily coated with maternal antibody. Testing of the neonatal RBCs may yield a false negative result. Therefore, if the maternal antibody is allo Anti-D or Anti-Kell, and the neonatal DAT is positive, an eluate will be performed regardless of the neonatal antigen typing (as described in the Procedure section of this document).

## VIII. REAGENTS:

- A. Ortho BioClone Anti-A
- B. Ortho BioClone Anti-B
- C. Ortho BioClone Anti-D
- D. Ortho 7% BSA
- E. Immucor Gamma Clone Anti-D
- F. Immucor Gamma Clone Control
- G. MTS™ A/B/D Monoclonal and Reverse Grouping Cards
- H. 0.8% AFFIRMAGEN® Reagent Red Blood Cells
- I. MTS™ Diluent 2 Plus
- J. Ortho MTS™ Anti-Human Globulin Anti-IgG (Rabbit) Anti-IgG Card
- K. Ortho MTS Diluent 2™ (a hypotonic buffered saline solution)

## IX. EQUIPMENT:

- A. Automated Method
  1. ORTHO VISION™ Analyzer
- B. Manual Gel Method

1. MTS Centrifuge
  2. Ortho Workstation
  3. Calibrated pipette (electronic or manual)
- C. Table top centrifuge
- D. Lighted agglutination viewing mirror

## X. SUPPLIES:

- A. Disposable pipettes
- B. Pipette Tips
- C. Gauze
- D. Test Tubes, 12x75mm, plastic or glass
- E. Blood Bank Isotonic Saline

## XI. QUALITY CONTROL (QC)

### A. Ortho Vision™ and Manual Gel Testing

1. Daily quality control for the Ortho Vision™ instruments are performed as described in Transfusion Medicine policy [Ortho Vision Analyzer QC](#).
2. Daily quality control for the Manual Gel reagents are performed as described in Transfusion Medicine policy, [Quality Control of Blood Bank Reagents](#).

### B. Manual Tube ABO/Rh(D) and Weak D Testing

1. Daily quality control of ABO and Rh testing is performed as described in Transfusion Medicine Policy, [Quality Control of Blood Bank Reagents](#).
2. Weak D testing should not be performed on samples with a positive direct antiglobulin test (DAT); false-positive weak D results may be obtained.
3. IgG-coated RBCs are used as a control if the graded reaction of the weak D test is negative. The graded reaction after the addition of the IgG-coated RBCs must be positive. If the requirements are not met, then the weak D test is not valid and must be repeated.

## XII. PROCEDURE:

### A. Specimen Receipt

1. Receive the specimen in BBIS in accordance with the Transfusion Policy, [Triaging and Identifying Acceptable Samples for Testing](#).
  - a. For Cord Blood orders it is necessary to select the testing to be performed on the specimen:
    - i. Go to **Tests Tab**
    - ii. Check box for Pending **CORD E S** for your specimen ID

- iii. Select **Results** Button
- iv. Click **Add** → **Test**
- v. Mark the checkboxes for **ABORhFWDES** and **DATIGGGEL** test codes.  
Note: It is very important to add the tests in that order to prevent duplicate testing on the automation. Do not select ABORhFWDG as this test code is reserved for Corewell South Blood Banks.
- vi. Click **Select**.
- vii. Click **Cancel** to exit screen.

## B. Cord Blood Testing using the Gel Method on the Ortho Vision Analyzer

1. Cord Blood Samples drawn in EDTA tubes are required when performing neonate testing on Ortho Vision. If a heel stick is drawn in a microtainer or if the cord specimen is received in a red top (no anticoagulant) tube, then testing must be performed using alternative manual methods.
2. Rim out the patient sample using wood applicator sticks to remove any clots that may be present.
3. Centrifuge the patient sample for 10 minutes at the centrifuge's calibrated speed to obtain packed red blood cells.
4. Load the patient samples onto the Ortho Vision. Testing should begin automatically once the samples are scanned by the instrument.
  - a. Specimen caps should be removed prior to loading on the Ortho Vision
  - b. Refer to Transfusion Medicine policy, Routine Testing on the Ortho Vision Analyzer for additional information.
  - c. If the Ortho Vision is unable to perform the testing, the ABO/Rh testing is performed by Manual Tube Method and the DAT should be performed using Manual Gel Method.
5. Upon completion, the test results will interface to the BBIS or require manual review.
  - a. If the test results interface automatically, they should be verified in the BBIS as described in
  - b. If the test results require manual review, proceed as described in Transfusion Medicine policy, Ortho Vision Analyzer Manual Card Review.
6. Evaluate for further testing taking into consideration the following:
  - a. DAT negative: No further testing indicated.
  - b. DAT positive: Order a Type & Screen in LIS on the mom if a recent test has not already been performed.
    - i. If mom's antibody screen is negative, suspect ABO incompatibility, no further testing is indicated unless Medical Director or physician requests special studies.
    - ii. If mom's antibody screen is positive, perform an antibody ID.

- iii. Consult the Medical Director. An eluate may also be performed on the cord blood cells to confirm the specificity of the antibody coating the infant's cells if requested by the Medical Director.

7. The technologist will perform the testing described in the table below, based on the maternal antibody which was detected.

| Maternal Antibody/<br>Examples                                 | Neonatal Antigen Typing  | Neonatal Eluate   |
|--|--|---|
| C, E, c, e, Jk <sup>a</sup> ,<br>Jk <sup>b</sup> ,<br>Little s | Yes  | Yes, when neonatal DAT and antigen type are both positive<br><br>No, when either neonatal DAT or antigen type is negative |
| *S, Fy <sup>a</sup> , Fy <sup>b</sup>                          | Yes, when neonatal DAT is negative<br><br>No, when neonatal DAT is positive since antigen typing invalid with positive DAT | Yes, when neonatal DAT is positive<br><br>No, when neonatal DAT is negative   |
| D, Kell*   | Yes (possible blocking)  | Yes, when neonatal DAT is positive<br><br>No, when DAT is negative  |
| WRM, wkU,<br>Kp <sup>a</sup> , Js <sup>a</sup>                 | NA<br>Corresponding anti-sera not available  | Yes, when neonatal DAT is positive<br><br>No, when DAT is negative  |

**Note:** If the maternal antibody is passive Anti-D due to recent documented RhIG administration then eluate is not indicated even if the current antibody screen is positive.

**\*Antigen testing cannot be performed with antisera that require an IAT (indirect antiglobulin test) if the neonatal DAT is positive.**

**C. Cord Blood Testing Using the alternative Manual Methods**

1. Perform the ABO/Rh (Forward Type Only) in accordance with Transfusion Medicine policy Forward Determination of ABO/Rh for Patients Less than 4 months old.



- a. Note: To prevent erroneous results due to Wharton's jelly, all cord blood samples are to be washed 4 times with saline prior to testing by tube methods. Cord bloods that appear Rh negative require further confirmation before results should be reported. See procedure step 3.
  - b. Record results in BBIS using the Transfusion Medicine Policy, Safetrace (Blood Bank) Application.
2. Perform Manual Gel DAT in accordance with Transfusion Medicine policy Performing Neonatal Direct Antiglobulin (DAT) by the Gel Method. Record results in BBIS using the Transfusion Medicine Policy, Safetrace (Blood Bank) Application
3. Cord bloods that appear to be Rh negative must be repeated for Rh(D) type using the Immucor Gammaclone Anti-D reagent and Gamma clone Rh Control. [Use the ABO Rh Repeat (ABOR) test for computer documentation of these results] If the results with the 2 different Anti-D reagents are different, refer to Transfusion Medicine policy Resolution of ABO Rh Discrepancies.
4. Weak D: Cord bloods that appear to be Rh negative must be tested for weak D antigen if the mother is Rh negative. An auto control must be tested along with the weak D test. The weak D results are not valid if the specimen has a positive DAT. Report Rh as Rh negative with free text result comment "Unable to determine infant's Rh. Infant will be considered Rh for Transfusion Purposes. Infant's mother should receive a post-partum dose of RhIG."
5. Evaluate for further testing taking into consideration the following:
  - a. DAT negative: No further testing indicated.
  - b. DAT positive: Order a Type & Screen in LIS on the mom if a recent test has not already been performed.
    - i. If mom's antibody screen is negative, suspect ABO incompatibility, no further testing is indicated unless Medical Director requests special studies.
    - ii. If mom's antibody screen is positive, perform an antibody ID.
    - iii. Consult the Medical Director. An eluate may also be performed on the cord blood cells to confirm the specificity of the antibody coating the infant's cells if requested by the Medical Director.
6. The technologist will perform the testing described in the above table, based on the maternal antibody which was detected.

#### **D. Result Entry**

1. The neonatal ABO, Rh, gel DAT, and antigen typing results will be entered in the BBIS.
2. Eluate results will be documented on an antigram copy and should be ordered and resulted in the computer.
3. The HDN final report will be entered using additional comments described in the interpretation section.

### XIII. INTERPRETATION:

- A. An interpretation code or comment will be entered for each of the following: maternal antibody, neonatal antigen type, neonatal gel DAT, neonatal eluate, ABO incompatibility, and HDN interpretation if applicable.

| Interpretation  | Code   | Descriptions of Interpretation Codes   |
|---|--------|--|
| Neonatal Antigen type codes                                     | HDAN   | Neonate is neg for ag corresponding to maternal ab.  |
|   | HDAP   | Neonate is pos for ag corresponding to maternal ab and free text the maternal antibody specificity                                 |
|   | HDAUNC | Cannot type neonate for ag corresponding to maternal ab.   |
|   | HDAAP  | Neonate RBCs assumed to be pos for ag corresponding to maternal ab, based on eluate results.                                       |
|   | HDAUB  | Unable to type neonate for ag corresponding to maternal ab; antigen sites may be blocked by maternal ab.                           |
| Gel DAT codes   | HDATN  | Neonate DAT negative.  |
|   | HDATP  | Neonate DAT positive.<br><b>This is a Critical Result for inpatient neonates, check that the result was called and documented.</b> |
| Neonatal eluate codes (add if applicable)                       | HDENR  | Neonate RBC eluate is non-reactive with test RBCs.   |
|   | HDENS  | Neonate RBC eluate is non-specific with test RBCs.   |
|   | HDESM  | Neonate RBC eluate has same specificity as maternal ab and free text the maternal antibody specificity.                            |
|   | HDEA   | Neonate RBC eluate has Anti-A specificity.   |
|   | HDEB   | Neonate RBC eluate has Anti-B specificity.   |
|   | HDEAB  | Neonate RBC eluate has Anti-A, B specificity.  |
|   | HDEN   | Non-specific eluate reactivity may be due to drugs, warm autoantibody reactivity, or an antibody to a high incidence antigen.      |
| Neonatal ABO Codes (add only if ABO incompatibility is present) | HDABO  | Neonate is ABO incompatible with maternal plasma.  |
|   | HDA    | Neonate RBCs are group A.  |
|   | HDB    | Neonate RBCs are group B.  |
|   | HDAB   | Neonate RBS are group AB.  |
| HDN Survey Interpretation Codes                                 | HDNN   | No serologic evidence for HDN.<br>Add if DAT and antigen type are both negative.   |
|   | HDNP   | Serologic evidence for potential HDN; clinical correlation required.<br>Add if only one of the two (DAT or antigen type) is        |

|  |      |  |
|--|------|--|
|  |      | positive.  |
|  | HDNS | Serologic evidence of HDN exists; clinical correlation required.<br>Add if both DAT and antigen type are positive. |

## XIV. LIMITATIONS:

- A. In order to minimize the chance of missing a Rh Immune Globulin candidate, newborns are to be linked to the mother under the baby's demographic information in the LIS. This will result in an exception report being generated when the mother is a candidate. These reports are then reviewed to verify that a RhIG Evaluation has been ordered.
- B. Eluates may be more sensitive in detecting the presence of antibody coating red cells than the DAT.
- C. If a maternal sample/history is unavailable, then an antibody screen will be performed on the neonatal sample. Because alloimmunization is rare during the neonatal period, unexpected antibodies detected in the neonatal sample are generally assumed to be of maternal origin.

## XV. REFERENCES:

1. American Association of Blood Banks and Transfusion Services, current edition.
2. AABB Technical Manual, current edition.
3. Ortho manufacturer's inserts: Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG card, revision date October 2019.

## Approval Signatures

| Step Description | Approver                                    | Date      |
|------------------|---|-----------|
|                  | Ann Marie Blenc: System Med Dir, Hematopath | 9/6/2024  |
|                  | Kristina Davis: Staff Physician             | 9/5/2024  |
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## Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne