PROCEDURE
Corewell Health East - Performing Neonatal Direct Antiglobulin Test (DAT) by the Gel Method - All Beaumont Hospitals

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Grosse Pointe Hospital, Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital, Corewell Health Farmington Hills Hospital, Corewell Health Taylor Hospital, Corewell Health Trenton Hospital, Corewell Health Wayne Hospital, Corewell Health William Beaumont University Hospital (Royal Oak)

Applicability Limited to: N/A

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Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Blood Bank

1. Principle

A. This document will provide policies and procedures relating to neonatal Direct Antiglobulin Tests (DATs). All neonatal DATs are performed by the gel method.
B. Antihuman globulin reagent (AHG) is used to detect antibodies and complement components bound to red blood cells (RBCs).
C. Neonatal DATs are performed when specifically ordered by the patient's physician when there is suspicion of Hemolytic Disease of the Newborn.
D. In Hemolytic Disease of the Newborn (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.

2. Responsibility

Personnel who have completed the competency requirements will perform this testing.

3. Specimen

A. Preferred sample is a Microtainer® (heel stick), cord blood, or peripheral blood EDTA sample affixed with identifying label.
B. All samples must be collected and labeled in accordance with requirements found in Corewell Health East - Triaging And Identifying Acceptable Samples For Testing- Blood Bank - All Beaumont Hospitals.
C. Minimum acceptable volume is 0.5 mL.
D. Samples should be tested as soon after collection as possible but may not be tested greater than 48 hours after collection.

E. If EDTA is unavailable, specimens drawn into ACD, CPD, or CPDA-1 are preferable to non-anticoagulated clotted specimens. For additional information, refer to the Micro Typing Systems Instruction for Use for the MTS™ Anti-IgG Card.

4. Reagent/Equipment Needed

A. Ortho MTS™ Anti-Human Globulin Anti-IgG (Rabbit) Anti-IgG Card
B. Ortho MTS Diluent 2™ (a hypotonic buffered saline solution)
C. 12x75mm test tube
D. Automated Method Equipment
   1. ORTHO VISION™ Analyzer
E. Manual Gel Method Equipment
   1. MTS Centrifuge or Ortho Workstation
   2. Calibrated pipette (electronic or manual)

5. Quality Control

A. Quality control (QC) of the diluent and IgG MTS gel cards must be performed on each day that testing is performed. This QC testing is performed on the ORTHO VISION™ as described in the Transfusion Medicine policy, Corewell Health East - ORTHO VISION Analyzer QC - All Beaumont Hospitals. If this QC is not performed on the ORTHO VISION™, then this QC testing must be performed by the manual gel method as described in site specific Transfusion Medicine policies, 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. All refrigerated reagents or gel cards must be brought to room temperature (18ºC-25ºC) before use.
C. Do not use reagents or gel cards beyond their expiration date.
D. Do not use gel cards that do not pass visual inspection. Each well of the gel card should have a clear liquid layer on top of the opaque gel. Additionally, do not use the gel card if any of the following are true:
   1. the gel matrix is absent
   2. the liquid level in the microtube is at or below the top of the gel matrix
   3. the cards show signs of drying, discoloration, bubbles, crystals, or other artifacts
   4. foil seal appears damaged or opened.
E. If the centrifuge 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 equipment that has passed applicable quality control and maintenance requirements for use.
G. All equipment used should be up to date on required preventative maintenance and quality control.

6. Procedure

A. Receive the applicable specimen as described in Transfusion Medicine procedure Corewell Health East - Triaging And Identifying Acceptable Samples For Testing- Blood Bank - All Beaumont Hospitals.
   1. Cord Blood Specimens –
      a. Gel DAT is completed as part of the Cord Blood Evaluation Test
         1) DATIGGGEL is selected in the tests tab in the BBIS during triage of the specimen (along with ABORhFWDES)
         2) Primary method of testing is automated on the ORTHO VISION
         3) Secondary method, if specimen is QNS for automated testing or the analyzer(s) are unavailable for testing, is manual gel.
      b. Proceed to the procedure steps below for automated testing.
   2. Microtainer® (heel stick) or peripheral blood EDTA specimens –
      a. Gel DAT is completed using one of the following test codes:
         1) Newborn Blood Bank Workup

A. DATIGGGEL is selected in the test tab in BBIS during triage of the specimen (along with ABORhFWDES).

2) DATGL (Newborn Direct Antiglobulin Test-IgG-Gel)

A. Note: If the caregiver orders DAT (Direct Antiglobulin Test) – tube method, the Gel DAT test can be added on to the specimen to replace the DAT.

b. Proceed to the procedure steps below for manual gel method.

3. Automated on the ORTHO VISION™ with Cord Blood Specimen

Note: If the sample to be tested is a heel stick drawn in a microtainer, the DAT gel testing must be performed using the manual gel method.

a. Rim out the patient sample using wooden applicator sticks to remove any clots that may be present.

b. Centrifuge the patient sample for 10 minutes at the centrifuge's calibrated speed to obtain packed red blood cells.

c. Load the patient sample onto the ORTHO VISION™. Testing should begin automatically, once the samples are scanned by the instrument.

1) Specimen caps must be removed prior to loading on the ORTHO VISION™.

2) Refer to Transfusion Medicine Policy, Corewell Health East - Routine Testing on the ORTHO VISION Analyzer - All Beaumont Hospitals for additional information.

d. If the ORTHO VISION™ is unable to perform testing, testing should be performed using manual gel method described below.

e. Upon completion, the test results will either interface to the Blood Bank computer system or require manual review.

f. Negative results will be auto-verified in the BBIS and automatically interfaced to the laboratory's LIS for reporting in the patient chart.

g. All other test results require manual review and acceptance of results, proceed as described in Transfusion Medicine Policy, Corewell Health East - ORTHO VISION Analyzer Manual Card Review - All Beaumont Hospitals.

1) Positive results accepted on the analyzer will be sent to Interface Manager in the BBIS for manual completion of resulting.

4. **Manual Gel Method Procedure**

a. Centrifuge the patient sample for 10 minutes at the centrifuge's calibrated speed to obtain packed red blood cells. (Note: for cord blood specimens tested with manual method, use wooden applicator sticks to remove any clots that may be present prior to centrifugation.)

1) If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before testing.

2) All cord blood samples must be adequately washed before manual testing due to potential contamination with Wharton's jelly.

b. Label a test tube with neonate's last name. In situations where there are multiple specimens use additional identifiers as necessary including MRN, and/or neonate's first name etc.

c. Prepare a 0.8% suspension of the neonate's RBCs in the labeled test tube as described in Transfusion Medicine Policy, Corewell Health East - Making a Test Red Cell Suspension - All Beaumont Hospitals.

d. Visually inspect the gel card before use.

e. Label one well of the IgG gel card to identify the sample on which the gel DAT will be performed.

f. Remove the foil from the well that will be used for testing.

1) Foil should be removed immediately before testing or within one hour of testing. Once opened, the gel may begin to dry out which could affect testing.

g. Add 50µL of the 0.8% cell suspension to the correspondingly labeled well of the IgG gel card. The pipette tip should not touch the gel card. Erroneous results due to carryover may occur.

h. Centrifuge the gel card(s) in the MTS Centrifuge™ or Ortho Workstation™ centrifuge at the calibrated speed for ten (10) minutes.

1) MTS Centrifuge™ = 895 ± 25 RPM.
2) Ortho Workstation™ = 1032 ± 10 RPM

i. Read both front and back of each well for agglutination and grade the reactions. Refer to Transfusion Medicine Policy, Corewell Health East - Reading, Grading, and Recording Test Reactions - Blood Bank - All Beaumont Hospitals or the ID-Micro Typing Systems Interpretation Guide.
j. Record and interpret the graded reactions in the Blood Bank computer system or the appropriate downtime form.

7. Results/Interpretation

A. Valid Graded DAT IgG Reactions in Gel Testing
   1. Negative Result – No agglutination and no hemolysis of the RBCs is a negative test result. A complete sedimentation of all RBCs is present in the bottom of the well.
   2. Positive Result – Agglutination and/or hemolysis of the RBCs is a positive test result. RBCs may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions.

B. If testing is completed as part of a Newborn Blood Bank Workup or Cord Blood Evaluation order, refer to Transfusion Medicine Procedure Corewell Health East - Newborn Blood Bank Work Up and Cord Blood Evaluation - Blood Bank - All Beaumont Hospitals for more information on applicable testing and result comments including eluate testing and HDN test code for result comments following positive DAT results.

8. Limitations

A. Adherence to the procedure is critical to test performance. Variations in RBC concentration can markedly affect the sensitivity of test results. If RBC suspensions are too concentrated, they can give weaker results due to the increase in the antigen/antibody ratio. In addition, RBCs may fail to completely migrate to the bottom of the well and could cause a false positive interpretation. When the RBCs are too low in concentration, they become difficult to visualize and, in extreme cases, a weak positive can fail to be detected.
B. Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may result in some red blood cells layering at the top of the gel. Mixed-field reactions should only be observed in tests containing a dual population of red blood cells, such as a transfused patient, bone marrow recipient, or when a pooled red blood cell sample is used for testing. However, not all mixed cell situations have a sufficient minor population to be detected.
C. False positive or false negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test samples.
D. Anomalous results may be caused by fresh serum, fibrin, or particulate matter in serum or plasma, or red blood cells that stick to the sides of the well. Anomalous results (i.e. a line of RBCs on the top of the gel) may be observed with serum samples and can be minimized by the use of specimens collected in EDTA.
E. False positive results may occur if a card that shows signs of drying is used in testing.
F. Negative DAT results do not necessarily rule out hemolytic disease of the newborn, especially if ABO incompatibility is suspected.
G. All testing profiles may not be validated and/or in use at every Corewell Health location for all methods. Only testing and methods that have been implemented and properly quality controlled in each individual Corewell Health Blood Bank shall be performed at that location.

9. Revisions

Corewell Health reserves the right to alter, amend, modify, or eliminate this document at any time without prior written notice.

10. Resources

Entities will reference associated Documentation contained within this document as applicable
Printouts of this document may be out of date and should be considered uncontrolled.

A. Micro Typing Systems Instruction for Use/MTSTM Anti-IgG Card, REF MTS 084024.

11. References

A. AABB Technical Manual, current edition.
B. AABB, Standards for Blood Banks and Transfusion Services, current edition.

12. Procedure Development and Approval

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13. Keywords

Not Set