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Performing Neonatal Direct Antiglobulin Test (DAT) by the Gel Method

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide policies and procedures relating to neonatal Direct Antiglobulin Tests (DATs). All neonatal DATs are performed by the gel method.

II. CLINICAL SIGNIFICANCE:

A. Antihuman globulin reagent (AHG) is used to detect antibodies and complement components bound to red blood cells (RBCs).

B. Neonatal DATs are performed when specifically ordered by the patient's physician when there is suspicion of Hemolytic Disease of the Newborn.

C. 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.

III. SPECIMEN COLLECTION AND HANDLING:

Preferred sample is microtube (heelstick), cord blood or peripheral blood EDTA sample affixed with identifying label.

A. All samples must be collected and labeled in accordance with requirements found in Triaging and Identifying Acceptable Samples

B. Minimum acceptable volume is 0.5 mL.

C. Samples should be tested as soon after collection as possible but may not be tested greater than 24 hours after collection.

D. 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 / MTS^{TM} Anti-IgG Card.

IV. REAGENTS:

A. Ortho MTS™ Anti-Human Globulin Anti-IgG (Rabbit) Anti-IgG Card

B. Ortho MTS Diluent 2™ (a hypotonic buffered saline solution)

V. EQUIPMENT:

A. Automated Method

   1. ORTHO VISION™ Analyzer

B. Manual Gel Method

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

VI. SUPPLIES:

A. Pipette Tips

B. Test tubes, 10x75 or 12x75, plastic or glass

C. Disposable Pipettes

D. Gauze

VII. EQUIPMENT MAINTENANCE:

Verify that all equipment maintenance is performed (if applicable). Refer to Transfusion Services Policies, Ortho Vision Analyzer Maintenance and *Maintenance of the Manual Gel Workstations.*

VIII. QUALITY CONTROL (QC):

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, 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 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.

   1. Each well of the gel card should have a clear liquid layer on top of the opaque gel. Do not use gel cards if

      a. the gel matrix is absent

      b. the liquid level in the microtube is at or below the top of the gel matrix

      c. the cards show signs of drying, discoloration, bubbles, crystals, or other artifacts

      d. foil seal appear damaged or opened.

D. If the centrifuge phase is interrupted, then all affected specimens must be retested.

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

IX. BEFORE YOU BEGIN:

A. Verify the patient specimen satisfies all labeling requirements as described in Transfusion Medicine Policy, Triaging and Identifying Acceptable Samples For Testing. Verify all specimen patient information from the specimen match the information in the Blood Bank computer system.

B. Verify that all QC requirements have been completed as indicated in the Quality Control Section of this document

X. PROCEDURE:

A. Automated on the ORTHO VISION™

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

1. Verify the requirements of the BEFORE YOU BEGIN section of this document have been met.
2. Rim out the patient sample using wooden applicator sticks to remove any clots that may be present.
3. Centrifuge the patient sample for 10 minutes at 3400RPM to obtain packed red blood cells.
4. Load the patient sample onto the ORTHO VISION™. Testing should begin automatically, once the samples are scanned by the instrument.
    a. Specimen caps must 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 testing, testing should be performed using manual gel method.
5. Upon completion, the test results will either interface to the Blood Bank computer system or require manual review.
    a. If the test results interface automatically, they should be verified in the Blood Bank computer system as described in the *Blood Bank CDM - Completing Vision™ Results.*
    b. If the test results require manual review, proceed as described in Transfusion Medicine Policy, Ortho Vision Analyzer Manual Card Review

B. Manual Gel Method Procedure

1. Verify the requirements of the BEFORE YOU BEGIN section of this document have been met.
2. Centrifuge the patient sample to obtain packed cells.
    a. If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before testing.
    b. All cord blood samples must be adequately washed before manual testing due to potential contamination with Wharton's jelly.
3. 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.

4. Prepare a 0.8% suspension of the neonate's RBCs in the labeled test tube as described in Transfusion Medicine Policy, Making a Test Red Cell Suspension
5. Visually inspect the gel card before use.
6. Label one well of the IgG gel card to identify the sample on which the gel DAT will be performed.
7. Remove the foil from the well that will be used for testing.
   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.
8. 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.
9. Centrifuge the gel card(s) in the MTS Centrifuge™ or Ortho Workstation™ centrifuge at the calibrated speed for ten (10) minutes.
   a. MTS Centrifuge™ = 895 ± 25 RPM.
   b. Ortho Workstation™ = 1032 ± 10 RPM
10. Read both front and back of each well for agglutination and grade the reactions. Refer to Transfusion Medicine Policy, *Reading, Grading and Recording Test Reactions* or the *ID-Micro Typing Systems Interpretation Guide.*
11. Record and interpret the graded reactions in the Blood Bank computer system or the appropriate downtime form. Refer to Blood Bank CDM - Neonatal DAT by Gel and to the *Interpretation* section of this document.
    Note: A positive DAT on an inpatient neonate is a critical value, regardless of the strength of the DAT. Refer to Transfusion Medicine Policy, *Critical Value Notification Policy - Transfusion Medicine*. The CVDAT canned message is used to document the notification in the blood bank computer system. Refer to Blood Bank CDM, Neonatal Gel DATs.

XI. INTERPRETATIONS:

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.

XII. LIMITATIONS:

A. Adherence to the procedure is critical to test performance. Variations in the 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 EDTA plasma.

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.

XIII. REFERENCES:

1. Micro Typing Systems Instruction for Use / MTS^{TM} Anti-IgG Card, REF MTS 084024.
2. AABB Technical Manual, current edition.
3. AABB, Standards for Blood Banks and Transfusion Services, current edition.

XIV. NOTES:

All testing profiles may not be validated and/or in use at every Beaumont location for all methods. Only testing and methods that have been implemented and properly quality controlled in each individual Beaumont Health Blood Bank shall be performed at that location.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
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Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	11/8/2021

Step Description	Approver	Date
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Applicability

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