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Origination	5/6/2022	Document Contact	Kelly Sartor
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Direct Antiglobulin (DAT) Test by Tube Method - Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide policies and procedures related to Direct Antiglobulin Testing (DAT) for patients greater than four months old by the tube method.

II. CLINICAL SIGNIFICANCE:

A. Antihuman globulin reagent (AHG) is used to detect antibodies and complement components bound to red blood cells (RBCs). AHG reagents may be polyspecific (containing both anti-IgG and anti-C3b,-C3d) or monospecific (containing only anti-IgG or anti-C3b,-C3d).
   1. The DAT is useful in the diagnosis of:
      a. Autoimmune hemolytic anemia,
      b. Warm auto-immune hemolytic anemia (WAIHA),
      c. Drug induced hemolytic anemia, and
      d. Transfusion reactions.

III. DEFINITIONS/ACRONYMS:

A. Comp: Anti-Human Globulin, Anti-C3b,-C3d
B. DAT: Direct Antiglobulin Test
C. IgG: Anti-Human Globulin Anti-IgG (Rabbit)
D. Poly: Anti-Human Globulin (Rabbit and murine Monoclonal) BioClone®, Anti-IgG, -C3d polyspecific
E. AHG: Anti-Human Globulin

IV. POLICIES:

A. The DAT is initially performed with polyspecific AHG. If the DAT with polyspecific AHG is positive, then a differential DAT shall be performed with anti-IgG and anti-C3b,-C3d AHG. If the polyspecific, anti-IgG, and anti-C3b,-C3d are all reactive, then a room temperature saline control must be run as well. The differential DAT indicates whether the RBCs are coated with immunoglobulin, complement, or both.

B. Any DAT performed with AHG that contains complement (e.g., the polyspecific and complement AHG reagents) must include a room temperature incubation, as described in the Procedure section.

C. The test RBCs must be thoroughly washed before the addition of AHG. Washing removes unbound human protein that may neutralize the AHG.

D. DAT results are documented as described in Blood Bank CDM- Direct Antiglobulin Test (Tube Method).

E. **Notification of Patients' Caregivers**
If the DAT of a patient greater than four months old is positive with a strength of 2+ or greater, then the patient's caregiver must be notified. This notification will be documented as an external comment to the polyspecific DAT in the Blood Bank computer. This notification must occur regardless of the reason the DAT was ordered; for example, regardless of whether the physician ordered the DAT, or whether the Blood Bank ordered the DAT due to a positive autocontrol, etc. The caregiver must be notified each time a positive DAT is observed, for each subsequent sample. If you are unable to contact a physician on your shift, the patient's information and result must be placed on the Communications bench so that the next shift may follow up with notification.

V. SPECIMEN COLLECTION AND HANDLING:

A. No patient preparation is required prior to specimen collection

B. The preferred specimen is a 6ml EDTA sample with affixed identifying label. Refer to, Triaging And Identifying Acceptable Samples For Testing- Blood Bank.

C. Blood should be drawn in EDTA and must be tested within 48 hours of collection.

D. Samples that have been refrigerated at 2°C to 8°C for up to 48 hours may be tested. However, the risk of false positive results due to spontaneous agglutination or autoagglutination may increase for samples that have been refrigerated. Refer to VII, B *Saline Control.*

E. Unrefrigerated, clotted blood may also be used, however, if DAT testing performed on a clotted specimen is positive it must be repeated with EDTA anti-coagulated blood to rule out misleading reactions due to complement bound in vitro sensitization.

F. For additional information, refer to the manufacturers' inserts for the AHG reagents that are listed in section VI below.

VI. REAGENTS AND EQUIPMENT AND SUPPLIES:

A. AHG Reagents

1. Ortho® Anti-Human Globulin (Rabbit and murine Monoclonal) BioClone®, Anti-IgG, -C3d polyspecific

2. Ortho® Anti-Human Globulin Anti-IgG (Rabbit)
3. Immucor/Gamma® Anti-Human Globulin, Anti-C3b,-C3d (Murine Monoclonal) Gamma-clone®, or Ortho® Anti-Human Globulin Anti-C3b,-C3d (Murine Monoclonal)
4. Check Cells
    a. Hemobioscience® Coombs Control IgG Coated Reagent Red Blood Cells or Ortho® Coombs Control IgG Coated Reagent Red Blood Cells (Pooled cells)
    b. Immucor/Gamma® Complement Control Cells

B. Normal saline
C. 10 x 75 or 12 x 75 mm test tubes
D. Water resistant marking pen
E. Disposable pipette
F. Table top centrifuge
G. Automated Cell Washer

VII. QUALITY CONTROL (QC):

A. Check Cells
    1. IgG-coated RBCs are used as a control for negative result that are tested with Anti-IgG polyspecific/monospecific. The reaction must be positive to validate the test, if negative with IgG coated cell the test is invalid and must be repeated.
    2. Complement coated RBCs are used as a control for negative result that are tested with anti-C3b,-C3d. The reaction must be positive to validate the test, if negative with complement coated RBCs, the test is invalid and must be repeated.

B. Saline Control
    1. When the DAT is reactive with all three AHG reagents (polyspecific AHG, anti-IgG, and anti-C3b,-C3d), then the RBCs must be tested with a room temperature saline control. Lack of agglutination with this control provides some assurance that the DAT is not falsely positive due to spontaneous agglutination or autoagglutination. A warm saline control shall be performed if the room temperature saline control is positive.
    2. For directions to test the room temperature and warm saline control, see the Procedure section.
    3. Refer to section IX. *Interpretation*.

C. Reagent QC
    QC testing must be performed on the all reagents as described in your site specific *Quality Control of Blood Bank Reagents* procedures.

VIII. PROCEDURE:

The DAT is initially performed with polyspecific AHG. If the DAT with polyspecific AHG is positive, then a differential DAT shall be performed with anti-IgG and anti-C3b,-C3d AHG reagents and a room

temperature saline control.

A. Verify that the reagent QC has been satisfactorily tested on the date that the DAT is performed. Refer to Section VII, C.
B. Prepare a 3 – 4 % saline suspension of the patient's RBCs in a test tube labeled with the patient's name. Refer to Transfusion Medicine procedure, Making a Test Red Cell Suspension.
C. Label test tubes with the patient's last name and the type of AHG reagent or saline that will be used for the DAT.
    1. For example, label with the patient's name and:
        a. Poly" or "P" for the DAT with polyspecific AHG.
        b. "IgG" or "I" for the DAT with Anti-IgG AHG.
        c. "Comp" or "C" for the DAT with complement AHG.
        d. "Saline" or "S" for the DAT saline control.
D. Add one drop of the patient's RBC suspension to the correspondingly labeled test tube.
E. Wash the tube(s) 3 – 4 times in the automatic cell washer or manually, decanting completely after the last wash.
F. Add 2 drops of the applicable AHG reagent or saline to the dry cell button.
G. Mix well and centrifuge according to calibrated time.
H. Gently resuspend the tube's contents and read, grade, and record the reactions. Refer to Transfusion Medicine procedure, Reading, Grading, and Recording Test Reactions - Blood Bank; observe any agglutination pattern for mixed-field appearance.
I. If performing the DAT with the polyspecific AHG reagent or with the Anti-C3b,-C3d AHG reagent, then incubate the tubes for 5 minutes at room temperature. Repeat steps G and H, and record the reactions observed after the room-temperature incubation.
    1. Incubation at room temperature and re-centrifugation may increase the sensitivity of complement/anti-complement reactivity. Incubation at room temperature should be observed with both positive and negative polyspecific DAT results.
J. Add the appropriate check cells to all DATs that are negative.
    1. Mix well and centrifuge.
    2. Gently resuspend the tube's contents and read, grade, and record the reactions of the check cells.
    3. The reaction must be positive with the check cells, or the test is invalid and must be repeated.
K. Evaluate and interpret the DAT results.
    1. If the polyspecific DAT is positive, then a differential DAT shall be performed with anti-IgG and anti-C3b,-C3d AHG reagents.
    2. If the DAT is reactive with all three AHG reagents (polyspecific, Anti-IgG, and complement) then a room temperature saline control should be performed.
    3. If the room temperature saline control is positive then a warm saline control must be tested as per step L.
    Refer to IX. *Interpretations* section.

L. **If the room temperature saline control is reactive, repeat testing using an aliquot of test RBCs that have been warmed to 37°C and washed with warm saline.** Test the warm saline control as described in this step only if the DAT with all three AHG reagents (polyspecific, Anti-IgG, and complement) and the room temperature saline control are reactive.

1. Warm Saline Control
    a. Prewarm several drops of the patient's RBC suspension.
    b. Label test tubes with the patient's last name and the type of AHG reagent or saline that will be used for the DAT.
    c. Add one drop of the patient's warm RBC suspension to the labeled test tubes.
    d. Wash the tubes 3 - 4 times with warm saline manually, decanting completely after the last wash.
    e. Add 2 drops of the applicable AHG reagent or warm saline to the dry cell button.
    f. Mix well and centrifuge according to calibrated time.
    g. Gently resuspend the tube's contents and read, grade, and record the reaction as an internal test comment.
        i. Refer to, Transfusion Medicine procedure, *Reading, Grading and Recording Reactions;* observe any agglutination pattern for mixed-field appearance.
    h. If performing the DAT with the Anti-C3b,-C3d AHG reagent, then incubate the tubes for 5 minutes at room temperature. Repeat steps f and g, and record the reactions observed after the room-temperature incubation.
    i. Control all negative DATs
        i. Add appropriate check cells to all DATs that are negative.
        ii. The reactions must be positive , or the test is invalid and must be repeated.
    j. Record all results in the Blood Bank computer.
    k. Evaluate and interpret the DAT results.

The saline control must be non-reactive in order to interpret a DAT that is reactive with all three AHG reagents; refer to the *Interpretation* section.

IX. INTERPRETATIONS:

A. **Positive test result**: Agglutination of the test RBCs in the immediate-spin or room temperature (if applicable) phase of the DAT.

B. **Negative test result**: No agglutination of the test RBCs in the immediate-spin or room temperature (if applicable) phase of the DAT.

C. **Quality Control must pass before the DAT is interpreted**
All QC requirements (including check cells, the saline control, and routine QC of the reagents) must be met in order to interpret the DAT.

1. If the check cell requirements are not met, then the DAT must be repeated. If these requirements are not met after repeat testing, then the DAT is invalid.
2. A reactive saline control may indicate the presence of a strong cold agglutinin or spontaneous

agglutination. If the room temperature saline control and warm saline control are reactive, then the DAT is invalid.

D. **Complimentary Reactivity Expected with AHG Reagents**

1. Similar to ABO testing where forward and reverse testing of a sample are expected to give appropriate complimentary reactions,the use of polyspecific and monospecific AHG reagents should yield complimentary test results. For example:
    a. If the DAT with polyspecific AHG is reactive, then the DAT with Anti-IgG or complement AHG is expected to be reactive. If complementary results are not observed, then the DAT may be invalid.

E. **Invalid DAT / Failing QC**

1. If the validity of the DAT is in doubt for any reason (e.g., if the check cell or saline control requirements were not met, or if complimentary reactivity with the AHG reagents was not observed) or if the routine QC of the DAT reagents failed for any reason, then do not enter the DAT results in the computer. Document and submit a variance form, for review by the Medical Director or designee.

X. NOTES:

A. Accurate DAT testing is sensitive to the use of an appropriate RBC suspension. Too heavy or too light of a suspension may result in difficulty in reading and weakened test reactions.

B. A mixed field agglutination pattern may be seen in a hemolytic transfusion reaction as only antigen positive donor cells are coated with the allo-antibody and are subsequently agglutinated by the AHG. In HDN, WAIHA and drug-induced hemolytic anemia all the RBCs are expected to be coated by some antibody and, therefore, a more even agglutination pattern is seen.

C. The incubation and re-centrifugation of the DAT with the polyspecific AHG reagent:

1. May increase the sensitivity of complement/anti-complement reactivity.
2. May decrease the sensitivity of anti-IgG reactions.
3. Therefore, it is important to record the results of the DAT with the polyspecific AHG reagent at both the immediate-spin phase and after the room-temperature incubation.

XI. REFERENCES:

1. American Association of Blood Banks, Technical Manual, current edition.
2. College of American Pathologist Accreditation Program, current edition.

Approval Signatures

Step Description	Approver	Date
	Vaishali Pansare: Chief, Pathology	5/6/2022

	Jeremy Powers: Chief, Pathology	4/22/2022
	John Pui: Chief, Pathology	4/22/2022
	Ryan Johnson: OUWB Clinical Faculty	4/22/2022
	Craig Fletcher: System Med Dir, Blood Bank	4/21/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	4/21/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	4/21/2022
	Karrie Torgerson: Supv, Laboratory	4/21/2022
	Teresa Lovins: Supv, Laboratory	4/21/2022
	Kelly Sartor: Supv, Laboratory	4/20/2022
	Kelly Sartor: Supv, Laboratory	4/20/2022