Beaumont Laboratory
Royal Oak

Effective Date: 06/17/2020
Supersedes: 11/02/2017
Related Documents: ~~P060,~~ P061, ~~P101~~, P305, P340, P512, Making a Test Red Cell Suspension, Triaging and Identifying Acceptable Samples for Testing

DIRECT ANTIGLOBULIN TEST (DAT) FOR PATIENTS GREATER THAN FOUR MONTHS OLD

RC.BB.SP.PR.611.r03.03.00

Purpose

The purpose of this document is to provide policies and procedures related to Direct Antiglobulin Testing (DAT) for patients greater than four months old by the tube method.

Scope

- This document applies to DAT testing for patients greater than four months old by the tube method.
- For neonates, refer to P512, *Performing Neonatal Direct Antiglobulin Test (DAT) by the Gel Method.*

Principle

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). The DAT is useful in the diagnosis of hemolytic disease of the newborn (HDN), warm auto-immune hemolytic anemia (WAIHA), drug induced hemolytic anemia, and transfusion reactions.

Policies

- 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 ran as well. The differential DAT indicates whether the RBCs are coated with immunoglobulin, complement, or both.
- 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.
- The test RBCs must be thoroughly washed before the addition of AHG. Washing removes unbound human protein that may neutralize the AHG.
- DAT results are documented in the Blood Bank computer system or on applicable downtime worksheets.

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 F-008c, *Blood Bank Communications and Daily Blood Rounds Log* so that the next shift may follow up with notification.

Specimen Collection and Handling

- The preferred specimen is a 6ml EDTA sample with affixed identifying label. See Transfusion Medicine policy, ~~P101~~, *Triaging and Identifying Acceptable Blood Samples for Testing.*
- Blood should be drawn in EDTA and must be tested within 48 hours of collection.
- 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 the *Quality Control* section / *Saline Control* for additional information.
- For additional information, refer to the manufacturers' inserts for the AHG reagents that are listed in the following *Reagents* / *Equipment* / *Supplies* section.

Reagents / Equipment / Supplies

- AHG Reagents
  - Ortho® Anti-Human Globulin (Rabbit and murine Monoclonal) BioClone®, Anti-IgG, -C3d polyspecific
  - Ortho® Anti-Human Globulin Anti-IgG (Rabbit)
  - Immucor/Gamma® Anti-Human Globulin, Anti-C3b,-C3d (Murine Monoclonal) Gamma-clone®, or Ortho® Anti-Human Globulin Anti-C3b,-C3d (Murine Monoclonal)
- Check Cells
  - Ortho® Coombs Control IgG Coated Reagent Red Blood Cells (Pooled cells)
  - Immucor/Gamma® Complement Control Cells, or Hemo bioscience C3 Control Cells
- Normal saline
- 10 X 75 mm
- Disposable pipettes
- Table top centrifuge
- Automated Cell Washer

Quality Control (QC)

Check Cells

- IgG-coated RBCs are used as a control for DATs that are tested with polyspecific or Anti-IgG AHG, if the graded reaction of the DAT is negative. The ~~graded~~ reaction after the addition of the IgG-coated RBCs must be positive (any strength) ~~at least 2+.~~ If these requirements are not met, then the DAT is not valid and must be repeated.
- Complement coated RBCs are used as a control for all DATs that are tested with anti-C3b,-C3d AHG, if the graded reaction of the DAT is negative. The reaction after the addition of the complement coated cells must be positive (any strength). If these requirements are not met, then the DAT is not valid and must be repeated.

Saline Control

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.

- For directions to test the room temperature and warm saline control, see the *Procedure* section.
- Refer to the *Interpretation* section for additional information.

Reagent QC

QC testing must be performed on the AHG reagents and check cells as described in P305, *Routine Quality Control of Blood Bank Reagents.*

Forms

F-008c, *Blood Bank Communications and Daily Rounds Log*

DAT 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.

1. Verify that the reagent QC has been satisfactorily tested on the date that the DAT is performed.
   Refer to the *Quality Control* section of this document and to P305, *Routine Quality Control of Blood Bank Reagents.*

2. 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 policy, ~~P060~~, *Making a Test Red Cell Suspension.*

3. Label test tubes with the patient's last name and the type of AHG reagent or saline that will be used for the DAT.
   For example, label with the patient's name and:
   - "Poly" or "P" for the DAT with polyspecific AHG.
   - "IgG" or "I" for the DAT with Anti-IgG AHG.
   - "Comp" or "C" for the DAT with complement AHG.
   - "Saline" or "S" for the DAT saline control.

4. Add one drop of the patient's RBC suspension to the correspondingly labeled test tube.

5. Wash the tube(s) 3 - 4 times in the automatic cell washer or manually, decanting completely after the last wash.

6. Add 2 drops of the applicable AHG reagent or saline to the dry cell button.

7. Mix well and centrifuge according to calibrated time.
   Refer to P340, *Calibration of Serologic Centrifuges*.

8. Gently resuspend the tube's contents and read, grade, and record the reactions.
   Refer to P061, *Reading, Grading, and Recording Test Reactions*; observe any agglutination pattern for mixed-field appearance.

9. 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 7 and 8, and record the reactions observed after the room-temperature incubation.

   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.

10. Quality Control all DATs that are negative as follows:
    a. Add the appropriate check cells:
       - For DATs using the polyspecific or Anti-IgG AHG reagent, add 1 drop of IgG coated check cells.
       - For DATs using the complement AHG reagent, add 1 drop of complement coated check cells.
    b. Mix well and centrifuge.
    c. Gently resuspend the tube's contents and read, grade, and record the reactions of the check cells.
       - IgG coated check cells must ~~react~~ be positive (any strength) ~~2+ or greater~~ to be acceptable.
       - Complement coated check cells must be reactive (any strength) to be acceptable.
       - Refer to the *Quality Control* and *Interpretation* sections for additional information.

11. Evaluate and interpret the DAT results.
    - If the polyspecific DAT is positive, then a differential DAT shall be performed with anti-IgG and anti-C3b,-C3d AHG reagents.
    - If the polyspecific, anti-IgG, and anti-C3b,-C3d are all reactive, then a room temperature saline control must be ran as well.
    - If the DAT is reactive with all three AHG reagents (polyspecific, Anti-IgG, and complement) and the room temperature saline control, then a warm saline control must be tested (see step 12).
    - Refer to the *Interpretation* section of this document.

12. 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.
    a. Warm Saline Control
       i. Prewarm several drops of the patient's RBC suspension
       ii. Label test tubes with the patient's last name and the type of AHG reagent or saline that will be used for the DAT
       iii. Add one drop of the patient's warm RBC suspension to the labeled test tubes
       iv. Wash the tubes 3 - 4 times with warm saline manually, decanting completely after the last wash.
       v. Add 2 drops of the applicable AHG reagent or warm saline to the dry cell button.
       vi. Mix well and centrifuge according to calibrated time.

           Refer to P340, *Calibration of Serologic Centrifuges*.

vii. Gently resuspend the tube's contents and read, grade, and record the reaction as an internal test comment.
Refer to P061, *Reading, Grading and Recording Test Reactions;* observe any agglutination pattern for mixed-field appearance.

viii. If performing the DAT with the Anti-C3b,-C3d AHG reagent, then incubate the tubes for 5 minutes at room temperature. Repeat steps vi and vii, and record the reactions observed after the room-temperature incubation.

ix. Quality Control all DATs that are negative, see step 10.

x. Evaluate and interpret the DAT results, see step 11.

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.

Interpretation

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

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.

- 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.
- 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.
- The reagent QC must have been satisfactorily tested on the date that the DAT is performed. If the reagent QC has been tested but the QC failed for any reason, then the DAT must not be performed. Refer to P305, Routine *Quality Control of Blood Bank Reagents,* for additional information.

Complimentary Reactivity Expected with AHG Reagents

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:
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.

Invalid DAT / Failing QC

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 at the next daily rounds.

Notes

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.

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.

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

- May increase the sensitivity of complement/anti-complement reactivity.
- May decrease the sensitivity of anti-IgG reactions.
  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.

References

- AABB, *Technical Manual*, current Edition.
- College of American Pathologist Accreditation Program, version 06/17/2010.
- Manufacturer's inserts for the AHG reagents and check cell reagent that are used in this procedure, as listed in the *Reagents/Equipment/Supplies* section of this document.

Authorized Reviewers

Chief, Pathology and Laboratory Medicine
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Document Control

Location of Master: Master electronic file stored on the Beaumont Laboratory server under S:/ Master printed document stored in the section Supervisor's office.
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Jennifer Sarhan	08/01/2011	r03.00.00	• Complete revision. • Samples may be refrigerated but must be tested within 48 hours. • Added the requirement to test the saline control if the DAT is reactive with all 3 AHG reagents.	
Additional Review by: Heather Asiala	11/02/2011			
Supervisor: Judy Easter	01/05/2012			
QA: Louisa Serafimovska	11/02/2011			
Approved by: Peter Millward, MD	12/28/2011			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Reviewed by: Peter Millward, MD	08/01/2012			
Reviewed by: Peter Millward, MD	08/15/2013			
Revised by: Jennifer Sarhan, QA	04/17/2014	r03.01.00	Revised the policy *Notification of Patients' Caregivers,* page 1	
Supervisor: Judy Easter	04/18/2014			
Approved by: Peter Millward, MD	04/18/2014			
Reviewed by: Peter Millward, MD	03/11/2015			
Revised by: Ashley Wilson	03/17/2015	r.03.02.00	Revised the policy *Notification of Patients' Caregivers,* revised Saline Control steps.	
Validated by: Heather Asiala	05/13/2015			
QA: Jennifer Sarhan	05/13/2015			
Supervisor: Judy Easter	05/13/2015			
Approved by: Peter Millward, MD	05/13/2015			
Approved by: Peter Millward, MD	03/09/2016			
Approved by: Peter Millward, MD	05/08/2017			
Revised by: Christopher Ferguson	11/02/2017	r03.02.01	Corrected when a saline control should be used in the policy section. Updated template header.	
Approved by: Peter Millward, MD	11/02/2017			
Approved by: Elizabeth Sykes, MD	02/22/2018			

Document Control, continued

Reviewed by: (Signature)	Date	Revision #	Modification	Related Documents Reviewed/ Updated
Approved by: Peter Millward, MD	02/26/2019			
Approved by: Craig Fletcher, MD	05/21/2019			
Revised by: Christopher Ferguson	05/01/2020	r03.03.00	Check cells are only added to DAT results that are negative instead of negative or less that 2+. Updated authorized reviewers and references. Added Hemo bioscience C3 Control cells to reagents. Removed F-303, *Variance Report*.	
Manager: Billie Ketelsen	05/05/2020			
Approved by: Craig Fletcher, MD	05/06/2020			
Approved by: Craig Fletcher, MD	07/23/2020			
Revised by: Samantha Maynard	09/08/2021		Any strength check cells are acceptable. Updated policy name changes.	
Manager:				
Approved by: Craig Fletcher, MD				

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