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# Work Instruction

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| **Title:** | **TS-Direct Antiglobulin Test** | **WI Number** SFOWI-0118<br>**Revision:** 11 |
| **Department:**<br>Immunohematology<br>**Area:**<br>2425 Geary Blvd SFO Hospital Lab | ***Document is in the Final Approval Process. 2 - approvals are required*** | |
| **Type of Document:**<br>Work Instruction | | **Review Period -** 340 **Days** |

## PURPOSE

The direct antiglobulin test (DAT) is used for the detection of in vivo red cell sensitization. Washed red blood cells from the patient are directly tested with antihuman globulin. Indications for DAT are:

a. Diagnosis of Hemolytic Disease of the Newborn (HDN)
b. Diagnosis of Autoimmune Hemolytic Anemia (AIHA)
c. Investigation of red cell sensitization caused by drug.
d. Investigation of possible delayed transfusion reactions when the patient has received transfusion within the previous 3 months.

## REAGENTS

Antihuman globulin (AHG)
Anti-IgG, -C3d (Polyspecific)
Anti-IgG
Anti-C3b,-C3d
Isotonic saline
Check cells
IgG Coombs control cells
Complement Coombs control cells.

## EQUIPMENT

12x75 mm test tubes
Serologic centrifuge
Cell washer
Transfer pipettes
Agglutination viewer
Microscope

## SPECIMEN

Refer to SFOWI-0079 ***Blood Bank Specimen and Requisition*** for DAT sample requirement.

**QUALITY CONTROL**

1. Agglutination of Coombs Control Cells will show that the anti-IgG or anti-complement reagent is active.
2. Reagents are tested as part of Daily Reagent QC.

**PROCEDURE**

1. Place one drop of the 5% suspension in a labeled 12 x 75 mm tube.
2. Wash 3-4 times with saline manually or in the cell washer, without interruption.
3. Make sure to decant to a dry button before testing.
4. Perform testing immediately after washing. Do not delay.

**5. Polyspecific AHG**

a. Add two drops of polyspecific AHG.
b. Mix well and centrifuge at calibrated speed and time.
c. Resuspend the cells by gentle shaking. (Refer to 'Reading and Grading Hemagglutination' protocol).
d. Immediately, read macroscopically and microscopically.
e. The characteristic of mixed field agglutination should be noted as it may provide valuable clue in the investigation of transfusion reaction.
f. If negative, incubate at RT for 5 minutes. This allows for maximal sensitivity for complement detection.
g. Centrifuge and read again.
h. If negative, add one drop of IgG coated check cells to confirm the negative reaction.
i. Centrifuge and read.
j. DAT results is invalid if no agglutination is seen with check cells.
k. If DAT is positive, perform differential DAT with anti-IgG, anti-C3 and saline.

**6. Monospecific anti-IgG**

a. Add two drops of anti-IgG
b. Mix well and centrifuge at calibrated speed and time.
c. Resuspend the cells by gentle shaking. (Refer to 'Reading and Grading Hemagglutination' protocol).
d. Immediately, read macroscopically and microscopically. Take note of mixed-field reaction.
e. If negative, add one drop of IgG coated check cells.
f. Centrifuge and read.
g. DAT results is invalid if no agglutination is seen with check cells.

**7. Anti-C3 and Saline Control**

a. Add two drops of anti-C3 or saline.
b. Mix well and centrifuge at calibrated speed and time.
c. Resuspend the cells by gentle shaking.
d. Immediately, read macroscopically and microscopically.
e. If negative, incubate at RT for 5 minutes. This allows for maximal sensitivity for complement detection.
f. Centrifuge and read again.
g. If negative, add one drop of complement check cells to the anti-C3 tube only.
h. Incubate at RT for 5 minutes.
i. Centrifuge and read.
j. Negative check cells and/or Positive Saline control invalidates the DAT results.
k. Positive saline control can be caused by cold agglutinin. Wash cells with warm saline

and repeat poly AHG and differential DAT.

8. **Cord Blood / Neonate (< 4 months old) DAT**
   a. Refer to 'CORD BLOOD TESTING' procedure for testing instructions.
   b. Elution is performed only per MD's request or if the positive DAT cannot be explained by the mother's ABORh /ABSC.

9. **Results Entry**
   a. Enter results in the computer as it is being read.

**CAUSES OF NEGATIVE CHECK CELLS**

1. Inadequate washing results in neutralization of AHG by patient's serum protein.
2. Omission of AHG.
3. Inactive AHG due to contamination or improper storage.
4. Never report a negative result if the check cells give negative reaction.

**SOURCES OF FALSE POSITIVES**

1. Potent cold- or warm-reactive autoantibodies.
2. Refrigerated clotted samples.
3. Tubes with silicone gel.
4. Over centrifugation.

**Summary Table**

| Saline control | DAT |
|---|---|
| NEGATIVE | VALID |
| POSITIVE | INVALID |

**INVESTIGATION OF POSITIVE DAT**

1. Obtain patient history including medications, diagnosis, and previous transfusions. If recently hospitalized elsewhere, call the Blood Bank at that hospital for the patient's transfusion history.
2. Elution
   a. Perform an elution if the differential DAT is positive with anti-IgG and the patient has been transfused in the last 3 months or when the transfusion history is unknown but there is suspicion of a transfusion reaction.
   b. If appropriate, initiate delay transfusion reaction workup. Refer to 'Transfusion Reaction Workup' and 'Delayed Transfusion Reaction' protocols.
   c. Elution is not required if DAT is positive with anti-C3 only.
   d. Elution should be performed if there is evidence of a hemolytic reaction even when the DAT result is negative. Refer to 'Transfusion Reaction Workup' and 'Delayed Transfusion Reaction' protocols.

**CAUSES of POSITIVE DAT (with or without shortened red cell survival)**

1. Autoantibodies either cold or warm.
2. Alloantibodies reacting with recently transfused donor cells.
3. Transfusion of non type specific platelets.
4. Infusion of WinRho or IVIG.
5. Maternal alloantibodies directed against fetal red cells.
6. Drug induced antibodies. Refer to the latest edition of the AABB Technical Manual for the list of culpable drugs.
7. Nonspecifically adsorbed proteins, including immunoglobulins, associated with hypergammaglobulinemia.
8. Red-cell-bound complement. This may be due to complement activation by alloantibodies,

autoantibodies, drugs, or bacterial infection.

9. Antibodies produced by passenger lymphocytes in transplanted organs or hematopoietic components.

**REFERENCES**

A. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.

B. AABB Technical Manual, current edition, Bethesda, MD.

## Associated Documents:

External Documents

Associated Documents:

DAT FLOWCHART

Click to Open an Associated Document

## Documents Generated:

## Document Revision History:

| | | |
|---|---|---|
| **Revision:** 11 | **Date Created:** 09/22/2005<br>**Date of Last Revision:** 02/12/2019 | **Last Approval Date:** 10/05/2016 |

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| **Document Author:**<br>Cara H Lim/CA/KAIPERM | **Document Manager:**<br>Richard Chui/CA/KAIPERM |

## Reason for Change:

| Revision: | Sec/Para Changed | Change Made: | Date |
|---|---|---|---|
| 1 | N/A | Add perform saline control if DAT-IgG is positive | 8/6/2006 |
| 2 | All sections<br>PROCEDURE<br>Results Entry<br>Elution | Corrections of multiple spelling errors.<br>Divide procedure into subsections for easy referral.<br>Added section.<br>Remove criteria other than transfusion within 3 months or unknown transfusion history. | 8/21/2006 |
| 3 | Cord Blood/Neonate DAT | Do not need to perform 5 min RT incubation for saline control. Positive DAT in the 1st week of life is critical. | 10/31/2006 |
| 4 | Investigation of Positive DAT<br>Causes of Positive DAT<br>N/A | Added Elution<br>Made into a new section<br>Approver | 1/16/07 |
| 5 | PROCEDURE<br><br><br>Approver | Deleted procedure steps for Cord Blood/Neonate DAT.<br>Deleted RILIS results entry for Cord Blood Testing as RILIS is now the main BB LIS.<br>New Medical Director | 7/17/07 |
| 6 | Procedure & Causes of Positive DAT | Corrected spelling | 7/03/08 |
| 7 | Approver | Changed Medical Director | 6/1/11 |
| 8 | Approver | New Medical Director. | 4/18/13 |
| 9 | Approver<br>Investigation of Positive DAT:<br>2.a.<br><br>2.d. | New BB Medical Director.<br><br>Revised. Added "but there is suspicion of a transfusion reaction'.<br>New. Added instructions to perform elution when hemolytic reaction is suspected regardless of DAT results. | 11/26/13 |
| 10 | Approver | New CLIA Director. | 9/28/16 |
| 11 | Specimen | Revised to refer to SFOWI-0079 Blood Bank Specimen and | 2/8/19 |

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|---|---|---|---|
| | | Requisition. | |

## Notification List:

## Approvals:

**First Approver's Signature**

**Name:** Maria F Serrano/CA/KAIPERM
**Title:** Transfusion Service Medical Director

**Second Approver's Signature**

**Name:** Eric Suba/CA/KAIPERM
**Title:** Chief of Pathology; CLIA Director

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**Document History Section**

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