1. **Principle**

To detect in vivo red blood cell sensitization and to determine which protein is coating red cells.

A 3% saline suspension of the red cells, to be tested, is washed with normal saline. Antiglobulin reagents, polyspecific (anti-IgG and C3) and/or monospecific (anti-IgG and anti-C3) antisera, are added to the washed red cell button, mixed, centrifuged and then examined microscopically.

1. **Scope and Related Policies**
	1. The Direct Antiglobulin Test (DAT) may be performed for investigation of:
* hemolytic disease of the fetus and newborn (HDFN)
* autoimmune hemolytic anemia
* transfusion reactions
* sensitization caused by drugs
	1. A DAT is required:
* if performing antibody identification and an auto control cannot be done (e.g., limited volume of plasma)
* if performing antigen typing by indirect antiglobulin test
	1. The antiglobulin reagent used for a direct antiglobulin test shall contain antibodies to IgG and the C3d component of complement. The only exception is cord blood testing that may be performed with a monospecific anti-IgG reagent.9.1 See- Procedural Notes 8.4
	2. If a direct antiglobulin test performed on a clotted specimen identifies complement on the red cell surface, the result shall be verified using an EDTA sample.9.1 SeeProcedural Notes 8.1
	3. All reagents shall be used and controlled according to the supplier’s recommendations and procedures.9.1
1. **Specimens**

EDTA anticoagulated blood

* 1. Cells from a clotted specimen may be used. However, if the test is positive with anti-C3, it must be repeated on an EDTA specimen. See Procedural Notes 8.1.
	2. A positive DAT on a clotted cord blood specimen is a valid result. Confirmation with an EDTA specimen is not required.
1. **Materials**

**Equipment:** Serological centrifuge

 Cell washer

 Microscope

**Supplies:** Test tubes – 10 x 75 mm

 Serologic pipettes

**Reagents:** Polyspecific AHG (anti-IgG, -C3)

 Monospecific anti-IgG

 Monospecific anti-C3 (anti-C3b, -C3d)

 IgG-coated cells

 C3 coated cells

 6% BSA (Bovine Serum Albumin)

1. **Quality Control**

Test polyspecific and anti-IgG reagents against IgG coated cells, C3 coated cells and unsensitized cells at time of use or once daily as applicable.

1. **Procedure**

|  |  |
| --- | --- |
| **STEP** | **ACTION** |
| 1. Check the suitability of the patient specimen(s)
 | 1. See PA.002 – Determining Specimen Suitability.
 |
| 1. Perform a patient history check
 | 1. See PA.003 – Patient History Check.
 |
| 1. Label tubes
 | 1. Label 2 tubes with the patient name and corresponding reagent. Patient name may be abbreviated to the first 3 letters of the family name:
* Label the 1st tube: Poly (for polyspecific)
* Label the 2nd tube: Con (for control)
 |
| 1. Prepare a 3% red cell suspension
 | 1. Mix the patient specimen and. See RT.003- Preparation of a 3% Red Cell Suspension.
2. Dispense 1 drop of the patient 3% red cell suspension to each labeled tube from 6.3.
 |
| * 1. Perform the antiglobulin test
 | 1. Wash the tubes 4 times with normal saline. See RT.002 – Cell Washing Automated and Manual.
2. Add 2 drops of polyspecific reagent to the tube labeled “Poly”.
3. Add 2 drops of 6% BSA to the tube labeled “Con”.
4. Mix the tubes immediately and centrifuge at 3400 rpm for 10-15 seconds.
5. Immediately after centrifugation resuspend the cells and read macroscopically. If negative, read microscopically. See Procedural Notes 8.2.
6. Grade and record the result. See RT.001– Reading and Recording Hemagglutination Reactions.

|  |  |
| --- | --- |
| ***If*** | ***Then*** |
| tube containing the reagent polyspecific is negative | * Incubate at room temperature for 5 minutes. See Procedural Notes 8.3Proceed to step 6.5
 |
| tube containing reagent polyspecific is positive | * interpret and report results (see 7.1- Interpretation)
 |

 |
| * 1. 5 min Room temp incubation
 | * + 1. Incubate all tubes containing polyspecific reagent with no agglutination for 5 min at room temperature
1. 6.6.2 Centrifuge, resuspend, read macroscopically and  microscopically
2. 6.6.3 Grade and record results. See RT.001– Reading and  Recording Hemagglutination Reactions

|  |  |
| --- | --- |
| ***If*** | ***then*** |
| tube containing the polyspecific reagent is negative | add 1 drop of IgG-coated cells. Centrifuge, resuspend cells, read macroscopically and record results.Agglutination (grade 2) must be present or the test must be repeated. |
| the result with the polyspecific reagent is negative | interpret and report results (see 7.1- Interpretation) |
| result with the polyspecific reagent is positive and the control is negative on a neonatal specimen | see Procedural Notes 8.4, interpret and report results (see 7.1 Interpretation ) |
| result with the polyspecific reagent is positive and the control is negative on a specimen other than a neonatal specimen; testing with monospecific anti-IgG and anti-C3 must be performed: | Label 2 tubes with patient name and “IgG, “C3”. Add 1 drop of the patient 3% red cell suspension to each tube.Proceed to step 6.6 |

 |
| * 1. Perform the antiglobulin test using Monospecific anti-IgG and anti-C3
 | * + 1. Wash the tubes 4 times with normal saline
		2. Add 2 drops of anti-IgG to the tube labeled “IgG” and mix
		3. Add 2 drops of anti-C3 to tube labeled “C3” and mix
		4. Centrifuge immediately at 3400 rpm for 10-15 seconds; resuspend; read macroscopically, if negative, read microscopically
		5. Grade and record results for the tube containing anti-IgG. See RT.001– Reading and Recording Hemagglutination Reactions

|  |  |
| --- | --- |
| ***If*** | ***Then*** |
| tube containing anti-IgG is negative | add 1 drop of IgG-coated cells to the tube labeled “IgG”; centrifuge, resuspend, read macroscopically and record results*Agglutination (grade 2) must be present or the test must be repeated* |
| Tube containing anti-IgG is positive | interpret and report results (see 7.1- Interpretation) |

1. Grade and record the results for the tube containing anti-C3

|  |  |
| --- | --- |
| ***If*** | ***Then*** |
| tube containing anti-C3 is negative or weak positive,  | * incubate at room temperature for 5 minutes. See Procedural Notes 8.3
* centrifuge the tube containing anti-C3, resuspend, read macroscopically and microscopically, grade and record results
* After incubation:

|  |  |
| --- | --- |
| ***If*** | ***Then*** |
| tube containing anti-C3 is negative, | * add 1 drop of C3 coated cells to the tube labeled C3; centrifuge, resuspend, read macroscopically and record results

*Agglutination (grade 2) must be present or the test must be repeated* |
| tube containing anti-C3 is positive | * interpret and report results (see 7.1- Interpretation)
 |

 |
| Tube containing anti-C3 is positive | * interpret and report results (see 7.1- Interpretation)
 |

 |
| * 1. Interpret results
 | * + 1. See 7.1 – Interpretation.
 |
| * 1. Perform Clerical Check
 | * + 1. When the procedure is complete, perform a clerical check. For each specimen tested, check that:
* The patient name and identification number are identical on all specimens and on the request form
* The patient name is the same on all test tubes and on the request form
* The test results have been interpreted and reported correctly
 |
| * 1. Complete paperwork
 | * + 1. Initial or sign and record the completion time and date on the request form or in the computer.
		2. Verification of results must be recorded. See 7.0 Reporting.
 |

1. **Reporting**
	1. Interpretation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Polyspecific** | **Control** | Anti-IgG | **Anti-C3** | **DAT Interpretation** |
| Negative | Negative | Not tested | Not tested | Negative |
| Weakly Positive | Negative | Negative | Negative | Negative - See 7.3 |
|  |  |  |  |  |
| Positive | Negative | Positive  | Positive  | Positive - See 7.2 |
| Positive | Negative | Positive  | Negative | Positive - See 7.2 |
| Positive | Negative | Negative | Positive  | Positive - See 7.2 |
| Positive | Positive  | Positive  | Positive  | Unable to report. See Procedural Notes 8.5 |
| Neonatal reporting - only |
| Positive | Negative | Not Tested | Not Tested | Positive  |
| Not Tested | Negative | Positive | Not Tested | Positive |
| Not Tested | Negative | Negative | Not Tested | Negative |

* 1. If the DAT is positive with polyspecific AHG, anti-IgG and/or anti-C3 and the control is negative, obtain a patient medication and recent (past 3 months) transfusion history. It may be necessary to ask the patient or patient’s family, nurse and/or physician to obtain an accurate history. See NRT.005 – Investigation of a Positive Direct Antiglobulin Test (DAT).
	2. If the DAT is weakly positive with polyspecific AHG but negative with anti-IgG, anti-C3 and the control, repeat testing and ensure correct technique, addition of reagents and incubation times. If the results are reproducible, report the DAT as negative.
1. **Procedural Notes**
	1. False positive results due to in-vitro coating with complement may be detected if testing is done on a clotted specimen.
	2. Tests should be read immediately after centrifugation. Delay may cause bound IgG to dissociate from red cells and either leave too little IgG to detect or neutralize AHG reagent causing false negative results.
	3. To enhance weak anti-complement reactions, the tubes containing red cells/polyspecific AHG or red cells/anti-C3 are incubated for 5 minutes at room temperature after initial reading of the test. They are then centrifuged and read again.
	4. If cord blood or neonate samples are to be tested, it is appropriate to use anti-IgG only, as HDFN results from fetal red cells sensitization with maternally derived IgG antibody, and complement activation rarely occurs.9.1
	5. A positive control could be due to a strong cold agglutinin present in the patient’s plasma. In this case, repeat the DAT but wash the cells with 37° C normal saline before adding the reagents.
	6. See Table 1 on page 8 for various drugs that are known to be associated with a positive DAT.
	7. **References**
	8. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine, 5.3.6.1, 5.3.6.2, 5.3.1.1.
	9. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 497-499; 905-906.
	10. Judd WJ. Methods in Immunohematology, 3rd ed. Bethesda, MD: American Association of Blood Banks, 2008; 418-420.
2. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revisions** |
| January 31, 2014 | * Revised name of manual
* Revised procedure number to RT.007 and updated references to other related procedures
* Section 2.2 - add a reference to “Procedural Notes 8.4”
* Section 6.0 – corrected 6.6 reference to 6.4; 6.9 and 6.10 corrected reference to interpretation table 7.1
* Revised the wording of section 8.4 to include the appropriate use of anti-IgG in cord and neonate samples; corrected page reference for Table 1
* Updated references to incorporate the latest versions/ editions and correct page references; updated references for Table 1
 |

Table 1

Various drugs are known to be associated with a positive DAT, e.g.,

|  |  |  |  |
| --- | --- | --- | --- |
| MECHANISM | DRUG | IMMUNOGLOBULIN CLASS | ACTIVITY |
| Drug Adsorption | Penicillins,Cephalosporins | IgG (sometimes C3 also) | React with drug-coated RBCs but not untreated RBCs |
| Immune Complex | Phenacetin, quinidine, third generation cephalosporins antihistamines | C3 (sometimes IgG also) | Serum reacts with RBCs only in the presence of the drug; eluate nonreactive |
| NonimmunologicProteinAdsorption | Cephalothin | IgG + C3 + albumin, etc. | Serum may contain low titre anti-drug antibody; eluate nonreactive |
| Autoimmunity | α-methyldopa(Aldomet), procainamide | IgG (rarely C3 also) | React with normal RBCs in absence of the drug |

References specific for drug induced positive DAT.

1. Reid M, Lomas-Francis C, The Blood Group Antigen Facts Book 2nd Edition, Academic Press, 2004.
2. Roback JD, ed. AABB, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 5519-522.