1. **Principle**

To investigate the possible causes of a positive Direct Antiglobulin Test (DAT) result.

Investigation of a positive DAT will assist in establishing a diagnosis and/or transfusion therapy.

1. **Scope and Related Policies**
   1. If there is insufficient plasma from the neonate, a maternal sample may be used for crossmatching and antibody screening.9.1
   2. Antiglobulin reagent used for Direct Antiglobulin testing must contain anti-IgG and anti-C3d. The exception is that is for neonates. For neonates a reagent containing only anti-IgG may be used.9.1
   3. If the DAT test is performed on a clotted sample identifies complement, the result shall be verified using an EDTA sample.9.1
   4. Investigation of a suspected hemolytic transfusion reaction must include a DAT.9.2
2. **Specimens – N/A**
3. **Materials – N/A**
4. **Quality Control – N/A**
5. **Procedures**

|  |  |
| --- | --- |
| 1. Send Samples to reference laboratory if DAT investigation is not performed on site | 1. Follow procedure for sending samples to a reference laboratory. Obtain the following information:  * Clinical history (including mother if patient is neonate) * Medication history * Transfusion(s) in the past three months (including intrauterine)   It may be necessary to ask the patient, the patient’s family, the attending nurse and/or the physician to obtain an accurate history. |
| 1. Consider the possibility of a delayed hemolytic transfusion reaction if DAT investigation is performed on site | * + 1. Consider the possibility of a delayed hemolytic transfusion reaction if the patient has been transfused in the past three months (or if the history of transfusion is unsure or unknown). See Procedural Notes 8.1 |
| * + 1. Find out where the transfusion occurred and contact the hospital to inquire about the pre-transfusion antibody screen and DAT status |
| * + 1. Perform an antibody screen or a group and screen on the current specimen.  |  |  | | --- | --- | | ***If*** | ***then*** | | the antibody screen is positive | perform an antibody identification. See NRT.007 – Antibody Identification of Warm Reactive Antibodies | | if all cells (screening and panel) are positive | consider performing an allogeneic red cell adsorption or sending specimens to a reference laboratory for investigation. See Procedural Notes 8.2. | |
| * + 1. Prepare an eluate or send the specimen to a reference laboratory for an elution See NRT.010 – Acid Elution. An elution may be omitted if all the criteria in procedural note 8.3 are met or if directed by a laboratory physician  |  |  | | --- | --- | | ***If*** | ***then*** | | the patient has not been transfused in the past three months | an eluate is not required. See Procedural Notes 8.4 | | the the DAT is positive with anti-C3 and negative with anti IgG | an eluate is not required. Cold Agglutinin Disease (CAD) or Paroxysmal Cold Hemoglobinuria (PCH) should be considered.  Usually results are strongly positive in these cases.9.2 If these clinical conditions are suspected, the following test(s) may be performed if available or sent to a reference laboratory   * Cold agglutinin screen to confirm CAD. See SP.003 – Cold Autoagglutinins Screen Test * Donath-Landsteiner (D-L) test to confirm PCH. See SP.006 – Donath Landsteiner Test for PCH. | |
| 1. Evaluate the Patient for evidence of hemolysis | 1. Whether or not the patient has been transfused, it may be necessary to evaluate the patient for evidence of hemolysis caused by premature destruction of the coated red cells. This will depend on clinical presentation and investigation and additional testing must be requested by a physician. See Procedural Notes 8.5 |
| 1. The following additional tests may be ordered by the TS Medical Director or designate  * Hemoglobin * Red cell morphology * Additional tests such as a reticulocyte count, bilirubin, LDH, etc  |  |  | | --- | --- | | ***If*** | ***then*** | | patient does not show evidence of hemolysis as determined by a physician, | see Procedural Notes 8.4 and report as in 7.1 – Reporting. | | a transfusion is not anticipated, | see Procedural Notes 8.4 and report as in 7.1 – Reporting. | | the patient shows evidence of hemolysis because of the risks of aggravating the hemolysis and/or alloimmunizing the patient | refer any request for transfusion to the TS Medical Director or designate  See Procedural Notes 8.6 and 8.7. | |
| 1. Neonates with a Positive DAT | 1. If the patient is a neonate and has a positive DAT or is jaundiced and has a negative DAT, the physician should consider hemolytic disease of the fetus or newborn (HDFN). |
| 1. Perform the following test:  * ABO grouping on the maternal and neonatal specimens * Antibody screen on the maternal specimen  |  |  | | --- | --- | | ***If*** | ***then*** | | the antibody screen is positive | perform antibody identification. See NRT.007 – Antibody Identification of Warm Reactive Antibodies | | the antibody screen is negative and the baby is ABO incompatible with the mother | perform an antibody screen for Immune A/B. See RT.014 - Antibody Screen Immune Anti -A and/or -B | |
| 1. Interpret Results | 1. Refer to section 7.0 – Reporting for interpretation |
| 1. Perform a clerical check | 1. Ensure that the specimen label information for each specimen tested coincides with the information on the corresponding test tubes and request form |
| 1. Initial | 1. Initial or sign and record the completion time and date on the request   form or in the computer. |
| 1. Verification of results must be recorded. See 7.0 Reporting. |

1. **Reporting**
   1. Interpret and report all tests performed. Refer to relevant procedures
2. **Procedural Notes**
   1. A positive DAT developing after a recent transfusion should always alert one to the possibility of a delayed hemolytic transfusion reaction. Recovery of alloantibody in a red cell eluate suggests a hemolytic (delayed) transfusion reaction. The alloantibody may or may not be present in the plasma. When an alloantibody can be demonstrated in the plasma, sometimes a second alloantibody can be found in the eluate.
   2. Usually warm autoantibodies mimic a multiple complex Rh specificity but autoantibodies against other blood group systems may also be present. Patients with previous transfusion or pregnancy histories may have alloantibody(ies) that are masked by the presence of the autoantibody. Adsorption procedures may be required to determine whether an alloantibody is present.
   3. If the patient has been transfused within the past three months, but not within the last three weeks, an elution may be omitted if all of the following apply:
      1. The DAT was positive on the last specimen tested (i.e. collected more than three weeks ago) and was investigated, and
      2. The positive DAT (i.e. strength of the reaction) on the current specimen is not stronger than the DAT performed on the last specimen, and
      3. The antibody screen (i.e. strength of the reaction) on the current specimen is the same as on the last specimen.

**Note:** Clinical circumstances, evaluation of transfusion, test result history and/or specimen history may override the above criteria and elution may be desirable for selected patients.

* 1. Between 1 in 1000 individuals and up to 1 in 14,000 blood donors and 1% to 15% of hospital patients have a positive DAT with anti-IgG and/or anti-C3. Healthy individuals can have 5 to 90 IgG molecules/red cell and 5 to 40 C3d molecules/red cell. These levels are below the thresholds of detection in routine testing. The significance of the positive DAT in healthy individuals is unknown.9.2
  2. A positive direct antiglobulin test may result from:
     1. Hemolytic Transfusion Reactions; Alloantibodies in a patient’s circulation reacting with antigens on recently transfused donor cells.
     2. Passively acquired alloantibodies 9.2 (donor plasma, platelets, or derivatives (e.g. IVIg, RhIg etc.) and maternal antibodies in a neonate).
     3. Non-specifically adsorped proteins (e.g. hypergammaglobulinemia or modification of red cell membrane by some drugs).
     4. Autoantibodies to intrinsic red cell antigens.9.2
     5. Complement activation due to bacterial infection, autoantibodies or alloantibodies.9.2
     6. Antibodies produced by passenger lymphocytes (Hematopoietic components) or Transplant organs.9.2
     7. Drug Induced antibodies 9.2
  3. Transfusion of patients with AIHA is a clinical decision balancing risk and/or clinical need. Transfusion should not be withheld solely because of serological incompatibility. The volume transfused should be the smallest amount required to maintain oxygen delivery, not to reach an arbitrary hemoglobin level.9.2 Patients with little or no evidence of hemolysis tolerate transfusion quite well. The risk is usually associated with the difficulties in pretransfusion testing. In patients with active hemolysis, transfusion may increase hemolysis. Destruction of transfused cells may increase hemoglobinemia and hemoglobinuria. DIC can develop with these patients.
  4. In the presence of a warm autoantibody, transfusion should be avoided if possible and should only be given for severe life-threatening anemia. Medical treatment ordered by a physician should be the mainstay of treatment when possible (e.g. steroids).
  5. When a maternal antibody has been identified as the cause of a positive DAT on a neonatal specimen (as determined by eluate), exchange transfusion should be anticipated. If the neonate’s clinical condition indicates the possibility of exchange transfusion as determined by a physician, the transfusion service should make arrangements to obtain suitable donor units. See CSP.001 – Selection of Blood Components for Transfusion.

1. **References**
   1. Standards for Hospital Transfusion Services Version 3 – February 2011. Canadian Society for Transfusion Medicine. 5.9.4.1, 5.3.6.
   2. Roback JD, ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:497-522,735.
   3. Judd WJ. Methods in immunohematology, 3rd ed. Durham, NC: Montgomery Scientific Publications, 1998:418-421.
2. **Revision History**

|  |  |
| --- | --- |
| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised name of manual * Updated reference cited from 9.1 to 9.2 in sections 2.4 and 6.2.4 * Changed title from Medical Chief to TS Medical Director in section 6.3.2 * Changed referral to section 7.3 to 7.1- Reporting in section 6.4.2 * Changed procedure number in 6.4.2 and 6.4.3 from RT.008 to RT.014 * Renumbered section 8.0 and made slight grammatical changes * Updated section 8.4 based on updated reference * Inserted sections 8.5.3 and 8.6 * Updated list of references to include latest editions/versions |