Exercise 8  Direct Antiglobulin Test (DAT)

Objectives:

1. State the purpose for performing the DAT.
2. State what a positive DAT indicates.
3. List the reagents which are used for performing the DAT.
4. State the sample of choice for a DAT.
5. State the reason clotted samples should not be used for the DAT.
6. List four conditions for which the DAT may be used to assist in diagnosing.
7. List three items of the patients medical history which are crucial for determining the clinical significance of a positive DAT.
8. State the source of the sample used for DAT on an infant.
9. Describe Wharton’s jelly.
10. State the problems that contamination with Wharton’s jelly will cause.
11. State the three tests which are performed when a positive DAT is obtained on a cord blood.
12. Given the results of cord blood and maternal blood bank testing results state the correct ABO/D type and any additional antigens the donor unit must be negative for.

Introduction

The Direct Antiglobulin Test (DAT) is a simple test used to determine if red cells have been coated in vivo with immunoglobulin, complement or both, which are specific types of proteins. This test may be performed on any patient as part of differential diagnosis when the presence of hemolysis has been established or as part of a work up to determine the cause of hemolysis or anemia.

A positive DAT indicates that the red cells have been coated in-vivo with protein. Both immunoglobulin and complement are proteins. It is important, in most cases, to test for the presence of both. The test should be performed with polyclonal antiglobulin reagent which contains antibodies to IgG and the C3d component of complement. The only exception to this is cord blood testing. Cord blood is tested with monospecific anti-IgG reagent since this is the only coating protein expected.

The specimen of choice for the DAT is an EDTA specimen. EDTA prevents in-vitro fixation of complement by chelating the calcium needed for C1 activation. If a clotted specimen is used a false positive result may be obtained due to non-specific binding of complement to the red blood cells in-vitro. The clotted sample can be used but if a positive reaction is obtained with anti-C3b it must be verified with a test performed on an EDTA sample.

The DAT is used to assist in the diagnosis of hemolytic disease of the fetus and newborn (HDFN), autoimmune hemolytic anemia, sensitization of red cells due to drugs, investigation of transfusion reactions and hemolysis due to ABO-incompatible platelet transfusions or ABO- mismatched organ transplants.
1. **Diagnosis of hemolytic disease of the fetus and newborn (HDFN).** A pregnant woman may form IgG antibodies directed against antigens on baby's cells while it is in utero. These have the ability to cross the placenta and attach to the antigens present on the fetus's red blood cells. IgG is the only antibody class capable of crossing the placenta.

2. **Diagnosis of autoimmune hemolytic anemia.** A patient forms antibodies directed against their own red blood cells causing decreased cell survival.

3. **Investigation of red blood cell sensitization caused by drugs.** Drugs sometimes induce the formation of antibodies, either against the drug itself or against intrinsic red blood cell antigens. Drugs act as haptens eliciting antibodies only after they have been firmly bound to a protein carrier. Once formed, the antibodies can react with the small hapten independent of any protein attachment. Drug-induced antibodies may cause positive DATs and sometimes hemolytic anemias.

4. **Investigation of transfusion reactions.** A patient may form antibodies against transfused cells, causing the transfused cells to be coated with antibodies in-vivo.

A positive DAT alone is not diagnostic. The interpretation of the possible clinical significance requires knowledge of the patient’s history which includes:

- The patient's diagnosis,
- Transfusion and pregnancy history
- Recent/current medications the patient is taking in the hospital and at home

These are vital pieces of information which must be obtained when working up a positive DAT.

Dialogue with the attending physician is important. Clinical considerations together with laboratory data should dictate the extent to which a positive DAT is evaluated.

**Principle**

The principle of the procedure is based on the test developed by Coombs, Mourant and Race for the detection of antibodies which attach to red cells but that do not produce direct agglutination. A drop of red cells is thoroughly washed. Anti-human globulin (AHG) and/or anti-C3 are added to the washed red cells. If the red cells have been coated the AHG or anti-C3 will bind producing visible agglutination.

**Discussion**

This lab will determine whether or not an infant's red blood cells have been sensitized in-vivo by maternal antibody by performing the direct antiglobulin test (DAT). Please remember that the DAT is a procedure that is performed on cord bloods, infants, adults and donor units. The purpose of the test in this student laboratory session is to detect sensitization of fetal cells by maternal antibody, but keep in mind that this test is performed in many other situations.

For diagnosis of HDFN the specimen for testing is obtained from the baby's umbilical cord immediately following birth. The preferred method for sample collection is to use a syringe to withdraw the sample from the umbilical cord to prevent contamination with Wharton's jelly. Wharton's jelly is a gelatinous substance which protects the umbilical cord from collapsing and gives it an elastic and cushion affect. If the cord specimen becomes contaminated with this substance it will cause non-specific agglutination of the red cells (false positives). False positive reactions are usually discovered when the ABO/D typing is performed. All forward typing tubes are positive, an Rh control is run, and it is also positive, invalidating the test. It is a
When a **positive cord blood DAT** is obtained a series of tests are required to determine the specificity of the offending antibody.

1. **An ABO/D type is performed on the cord blood.** This information, when compared with the maternal ABO type, will determine whether or not ABO antibodies are involved. *Please remember that a reverse type cannot be performed on babies, as the ABO antibodies present in their serum/plasma are of maternal origin*

2. **Type and Screen is performed on the maternal sample** to identify the specificity of the maternal antibody coating the cord cells. If the mother is group O, the antibody screen is negative and the infant is an A or a B then ABO HDN is suspected. If the antibody screen is positive and a panel study reveals the presence of an unexpected antibody, the clinical significance of the alloantibody must be determined. The results of the antibody screen on the mother will determine which type of elution to perform, as some elution procedures are best at recovering alloantibodies, while others are best at recovering immune ABO antibodies.

3. **An elution may be performed on the cord cells** to identify the specificity of the coating antibody.
   a. If the mother's antibody screen is positive, a panel study is done on the mother's serum. If a clinically significant antibody is identified an acid elution must be performed on the infant's cord cells to verify that the antibody in the mother's serum is, indeed, coating the baby's cells.
   b. If the mom's screens are negative and she is ABO incompatible with her infant then a heat elution is performed on the infant's cord cells to verify the presence of maternal ABO antibodies coating the cord cells.

The results of the DAT on the cord blood, ABO/D type of infant, ABO/D type of maternal sample, results of maternal antibody screen and, if necessary, results of panel identification, will determine the classification of HDFN. HDFN is classified as follows:

1. **ABO** - most common and mildest form of HDFN. Most commonly detected in group A or B infants born of group O mothers. The key findings are a positive cord blood DAT, an A or B infant, group O mother and a negative antibody screen on the mother.

2. **“Other”** - May cause mild to severe HDFN. This type of HDFN is due to immune antibodies OTHER THAN anti-D. The key finding is positive cord blood DAT, a positive antibody screen on the mother, identification of a clinically significant antibody in the maternal sample and elution of the clinically significant antibody from the cord cells. IMPORTANT: If the mother is ABO incompatible with the infant the eluate must be run against A and B cells as ABO antibodies may be coating the cord cells as well.

3. **Anti-D** - This is the most severe form of HDFN. It may be due to anti-D alone or anti-D plus other Rh antibodies. The key findings are a positive DAT on the cord blood, D positive infant, D negative mother with a positive antibody screen, identification of immune anti-D in the maternal sample and elution of anti-D from the cord cells.
If the hemolytic disease is severe at birth this may necessitate an exchange transfusion on the infant. The blood must be **compatible with the baby and the mother and lack all antigens to which the mother has antibodies.**

**Examples:**

<table>
<thead>
<tr>
<th>Mom ABO/D</th>
<th>Antibodies</th>
<th>Baby ABO/D</th>
<th>Donor Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>O pos</td>
<td>anti-A, -B and A,B</td>
<td>B pos</td>
<td>O pos</td>
</tr>
<tr>
<td>B neg</td>
<td>anti-A and anti-D</td>
<td>A pos</td>
<td>O neg</td>
</tr>
<tr>
<td>A pos</td>
<td>anti-B, anti-K</td>
<td>A pos</td>
<td>A pos, K neg</td>
</tr>
<tr>
<td>O pos</td>
<td>anti-A, -B, -A,B, anti-E</td>
<td>A neg</td>
<td>O neg, E neg</td>
</tr>
</tbody>
</table>
Please keep the following flow chart handy as you perform the cordblood DAT testing.

**DAT results on cord cells**

**NEGATIVE**
- No elution necessary

**POSITIVE**
- Perform ABO/D typing on cord blood
  - Perform Type and Screen on mom
- Rh on cord cells
  - If mom's screens are positive
    - Test mom's serum against panel cells
    - Perform acid elution on cord cells and test eluate against panel cells
  - If mom's screens are negative and infant is ABO incompatible with mom
    - Perform Lui freeze or heat elution. Test the eluate and last wash against screen and reverse cells.
PROCEDURE

1. Label a tube for each cord specimen using the mother's full name and hospital number.
2. As soon as you have labeled the tube place it in the test tube rack and immediately pick up the appropriate cord blood and place 3-5 drops of red cells in the labeled tube. DO NOT label your next tube until you have placed red cells in the tube you have just labeled.
3. Prepare a washed 2-5% suspension of cord cells in a properly labeled test tube.
4. Place one (1) drop of cell suspension to be tested in a properly labeled test tube.
5. Wash three (3) times with saline, decanting completely after each wash.
6. After last wash, blot tube with gauze to obtain a dry cell button.
7. Add two (2) drops of AHG reagent.
8. Gently shake tube to mix and centrifuge for 10-15 seconds.
10. Record the GRADED reaction.
11. To all negative tests or tests with 1+ or less agglutination add one drop of IgG – sensitized red blood cells (check cells).
12. Gently mix, centrifuge for 15 seconds.
13. Resuspend the cell button, grade and record results and interpretation.

NOTE: If the initial washing adequately removed all unbound globulin, the antiglobulin serum will still be reactive and capable of agglutinating the sensitized control red blood cells. Thus, the negative test result obtained for the patient can be considered valid. This is an important control because it is the only way to monitor the adequacy with which each test is washed.

INTERPRETATION

1. Negative, no in-vivo sensitization detected.
2. Positive, in-vivo sensitization detected; elution studies should be performed. The type of elution done will be determined by the results of the mother's type and screen. *At this time, perform the Type and Screen on the maternal specimen according to your previous exercise.*

While the type and screen on the mother is incubating, perform an ABO/D on the baby's cells. A reverse type cannot be performed on an infant since all antibodies in the baby are of maternal origin. After all results are in, look at the flow chart to determine which eluate will be performed next week.
## Cord Blood DAT
### Recording Results

**IMPORTANT**: Record the GRADED reaction for the DAT

<table>
<thead>
<tr>
<th>Mother’s Name</th>
<th>ID Number</th>
<th>Mother’s ABO/D</th>
<th>DAT</th>
<th>anti-A</th>
<th>anti-B</th>
<th>anti-D</th>
<th>D ctrl</th>
<th>Wk D</th>
<th>Wk D Ctrl</th>
<th>Interp</th>
<th>RhIg?</th>
<th>Completion</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td>✓</td>
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</table>

**EXERCISE 8**

Name_________________________________ Date_________________________________
EXERCISE 8

Direct Antiglobulin Test (DAT)

Study Questions

1. What is the purpose of performing the DAT procedure? (1 point)

2. What does a positive DAT indicate? (1 point)

3. What three reagents may be used in the DAT procedure? (1.5 point)
   a. 
   b. 
   c.

4. What is the sample of choice for the DAT? Why? (2 points)

5. The DAT is used to assist in hemolysis associated with what four (4) clinical conditions. (2 points)
   a. 
   b. 
   c. 
   d. 

6. What 3 items in the patient history are crucial in determining the clinical significance of a positive DAT? (3 points)
   a. 
   b. 
   c. 

7. State the principle of the DAT procedure. (1 point)

8. What antibody class is involved in HDFN? (0.5 point)

9. What is the source of the specimen used for the DAT on newborns? (1 point)

10. What is Wharton’s jelly? (1 point)

11. What problems will contamination of the cord blood sample with Warton’s jelly cause? (1 point)
12. State the three additional tests which must be performed when an infant has a positive DAT. Be sure to state which test is performed on mom and which on baby. (3 points)
   a. 
   b. 
   c. 

13. List the three types of HDFN from mildest to severe AND state the antibody specificity involved in each. (3 points)
   a. 
   b. 
   c. 

14. State the reason a reverse type cannot be performed on an infant. (1 point)

15. What must be done for the infant if the HDFN is severe at birth. (1 point)

16. Fill in the shaded columns in following chart:

<table>
<thead>
<tr>
<th>Mom ABO/D</th>
<th>ABO Antibodies</th>
<th>Immune Antibody Identified</th>
<th>Baby ABO/D</th>
<th>Donor Blood</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>O pos</td>
<td></td>
<td>None</td>
<td>B pos</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>A neg</td>
<td></td>
<td>anti-D</td>
<td>B pos</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>B pos</td>
<td></td>
<td>anti-Fy^a</td>
<td>B pos</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>O pos</td>
<td></td>
<td>anti-S</td>
<td>A neg</td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>