The need is constant. The gratification is instant. Give blood.

Direct Antiglobulin Test
The simple test that helps solve complex problems

History of the DAT
- DAT performed in laboratories for 50 years
- Originally described in 1908 (Moreschi)
- Remained obscure until rediscovered by Coombs (1945)
- Coombs did give credit to Moreschi in his 1945 paper.

Understanding the DAT
- "The direct antiglobulin test is a simple test used to determine if red cells have been coated in vivo with immunoglobulin (Ig), complement, or both."

Robin Coombs

Understanding the DAT
- DAT vs IAT
- DAT
  - Diagnosis of immune hemolytic anemia
  - Hemolytic transfusion reaction
  - Hemolytic disease of the fetus and newborn
  - Autoimmune hemolytic anemia
  - Drug induced IHA

AHG (Coombs Serum)
- Originally made by injecting human globulins into laboratory animals
- Garraty and Petz recommended guidelines for reagents
- Today it is regulated by the FDA as a variety of reagents
**AHG (Coombs Serum)**

- Today there is a wide selection of various AHG reagents ranging from monoclonal to traditional rabbit AHG.
- They are different! Read package inserts and follow manufacturers directions.

**Polyspecific vs Monospecific**

- Polyspecific AHG must contain anti-IgG and anti-C3d.
  - IgG antibodies are generally clinically significant.
  - Anti-C3d is important to study AIHA.

**Monospecific Anti-IgG**

- Available in a number of preparations.
- Heavy chain specific contains only anti-heavy-chain-specific (gamma) antibodies.
  - Eliminates any reactivity with light chains of other immunoglobulins (e.g., IgA or IgM)
- Monoclonal anti-IgG also only reacts with IgG

**Monospecific Anticomplement Reagents**

- Available in preparations that contain anti-C3b, -C3d, -C4b, or -C4d separately or in combination.
- NO IgG activity.
- Detection of C3d bound to red cells is the most important function of this test
- Monoclonal C3d requires room temp incubation

**DAT Procedure**

- Specimen – EDTA anticoagulated sample preferred. (manufacturers insert)
- Visual inspection – look for indications of autoagglutination.
- Test within 48 hours.
- Need a quality specimen.
- False positives from silicone gel separator tubes.

**DAT Procedure**

- Use fresh packed red cells. Do not use cells that have been resuspended in saline for an extended period of time.
- Wash red cells three or four times with PBS. Resuspend to create 2%-5% suspension. Wash 1 drop of cell suspension.
- Fully decant after last wash
- Add reagent to dry button (not a suspension drop)
DAT Procedure

- After addition of reagent, spin and read immediately!
- 5 minute RT incubation (Complement)
- As a general rule complement gets stronger after incubation, IgG gets weaker.
- This is not intended as a replacement for monospecific AHG testing. May help on initial evaluation.

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DAT Procedure

- Donors (149) with POS DAT (Allan and Garrity, 1980)

<table>
<thead>
<tr>
<th>Strength</th>
<th>RBC Bound Protein</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>IgG</td>
<td>32</td>
</tr>
<tr>
<td>3+</td>
<td>IgG + C3</td>
<td>34</td>
</tr>
<tr>
<td>2+</td>
<td>C3</td>
<td>34</td>
</tr>
<tr>
<td>1+</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>W+</td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>

14

DAT Procedure

- This test is detecting a relatively small amount of IgG molecules.

<table>
<thead>
<tr>
<th>DAT Strength</th>
<th>mols/RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25-120</td>
</tr>
<tr>
<td>W+</td>
<td>120</td>
</tr>
<tr>
<td>1+</td>
<td>200</td>
</tr>
<tr>
<td>2+</td>
<td>300-500</td>
</tr>
<tr>
<td>3+/4+</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

*Taken from Garratty lecture (08-2012)

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DAT Procedure

- Final step in preliminary evaluation of a positive DAT is preparation of an eluate if:
  - Clinical evidence of hemolysis
  - Transfusion or pregnancy in past 3 months
  - History of pos DAT that is significantly different from the current DAT results,

AABB Technical Manual recommendation: "When no unexpected antibodies are present in the serum, and if the patient has not been recently transfused, no further serologic testing of and autoantibody is necessary.

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Technical Considerations

- Very simple test, but there are ways to get incorrect results or clinically insignificant results:

<table>
<thead>
<tr>
<th>False Positives</th>
<th>False Negatives</th>
<th>Insignificant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous agglutination</td>
<td>Improper washing</td>
<td>Passive acquired AB</td>
</tr>
<tr>
<td>Improper Saline Storage</td>
<td>Poor Technique</td>
<td>Normal Patients</td>
</tr>
<tr>
<td>Overcentrifugation</td>
<td>Delayed Testing</td>
<td>Normal Patients</td>
</tr>
<tr>
<td>Wrong Specimen</td>
<td>Cryoglobulin</td>
<td>Normal Patients</td>
</tr>
</tbody>
</table>

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Normal Patients

- Most blood donors with positive DAT appear perfectly healthy.
- However new research is interesting:


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Clinical Conditions Associated with a Positive DAT

- It is important to remember that a positive DAT caused by in-vivo sensitization does not indicate that the patient has IHA, nor does a negative DAT prove the opposite.\(^{12(p188),39,40}\)
- We have to look at the clinical conditions to see if a hemolytic process is ongoing.

Problem Solving Pathway for Pos DAT

1. Polyspecific AHG
2. Monospecific AHG (IgG, C3, Control)
3. Serum Studies
4. Elution procedures (if indicated)

*Remember to keep clinical and laboratory data in consideration.

Things to Consider When Dealing With Positive DAT

Information to consider
1. Evidence of in-vivo hemolysis?
2. Degree of anemia and transfusion requirements?
3. Recent Transfusions?
4. Prior Transfusions?
5. Unexpected antibodies in serum?
6. Medications?
7. Special Procedures? (transplantation)
8. Diagnosis?

ALLOIMMUNE HEMOLYTIC ANEMIAS

- Hemolytic Transfusion Reaction
  – Delayed hemolytic transfusion reaction
- Hemolytic Disease of the Fetus and Newborn

Delayed Hemolytic Transfusion Reactions

- Positive DAT (may be mixed field)
  – In POSTTRANSFUSION specimen of recently transfused patient.
- Can occur with or without appearance of alloantibody in serum studies of pretransfusion specimen.
- Transfusion provides stimulation for an anamnestic response with a rise in antibody titer in as little as 2 days. (Peaking in 10-20 days)
- Rarely DAT can be negative
  – If sensitized cells are destroyed rapidly.
Delayed Hemolytic Transfusion Reactions

- Serum testing will usually detect alloantibodies from post transfusion specimen.
- There are times when only eluate studies can identify the presence of a clinically significant alloantibody.
- Why?

Hemolytic Disease of the Fetus and Newborn (HDFN)

- Incidence rate for positive DAT in cord blood samples can be as high as 4%.
- Roughly 13% of infants demonstrating positive DATs require treatment for ABO HDFN.
- DAT is a poor predictor of HDFN cause by ABO incompatibility.

Why?

- If the alloantibody is present only in eluate, not in the IAT → Our alloantibody has high affinity for red cells, binding entirely. This keeps the alloantibody from circulating in the plasma/serum.

Hemolytic Disease of the Fetus and Newborn (HDFN)

- However, DAT results on cord cells are, in fact, considered reliable predictors of HDFN when it is cause by non ABO, IgG antibodies.
- Alloantibody ID in maternal serum = No eluate on DAT pos cord cells
- Maternal sample unavailable with neonate sample Pos DAT = Eluate performed
- Maternal AB screen neg and neonate sample Pos DAT = think low incidence antigen.

HDFN Testing Protocols

- Madlon-Kay studied 301 infants from mothers with group O blood.
  - Automatic DAT on 113
  - 188 selectively tested on basis of clinical status
  - No clinically significant differences
- Kaplan and Garrity suggest using automatic DAT testing to screen newborns sent home, to identify those who might require monitoring and possibly testing.
HDFN with negative DAT?

- In rare instances negative DAT on cord blood cells does not eliminate the possibility of mild to severe HDFN in ABO compatible infants when a clinically significant alloantibody is present.
- Conversely babies can have positive DAT without HDFN severe enough to require treatment (e.g., 40% D+ babies born to mothers with anti-D and many A/B babies born to group O mothers.)

Auto Immune Hemolytic Anemia

- Presence of autoantibodies in patient’s serum can provide the greatest challenge for the blood banker.
  - Warm AIHA
  - Cold Agglutinin syndrome
  - Mixed Type AIHA
  - Paroxysmal cold hemoglobinuria

Warm Autoimmune Hemolytic Anemia

- 70% of all AIHA cases
- Warm reactive autoantibodies primarily IgG – react optimally at 37°C.
- Need to identify the immunoglobulin causing the hemolysis.
  - 67% IgG demonstrated on red cell
  - 13% show only C3 on red cell

Warm Autoimmune Hemolytic Anemia

- Warm autoantibodies may mask alloantibodies.
- AIHA may cause pos DAT but neg IAT
- May mimic alloantibodies
- Branch et al suggests that this may be removing senescent RBC’s

Cold- Reactive Autoantibodies

- 16% of all AIHA classified as cold hemagglutinin syndrome.
  - Acute form- often transient. Seen in children and young adults with lymphoproliferative disorders or infectious diseases (EBV or M. pneumoniae).
  - Chronic form- most often elderly patients with mild to moderate hemolysis.

Cold Reactive Autoantibody

Taken from "Blood Journal 28 OCTOBER 2010 I VOLUME 116, NUMBER 17"
Cold Reactive Autoantibodies

- IgM antibodies bind to red cells in peripheral circulation. Activates complement.
- Antibody dissociates when it returns to warm areas, leaving bound complement
- C3d is generally only protein detected on red cells.
- Pathological cold agglutinins = titer can be >1000 at 4C. (Does not correlate with severity)

Drug Induced AIHA

- Rare
- Only if all previously discussed etiologies eliminated
- Methyldopa -15% patients will develop pos DAT, 1% will develop immune hemolysis
- Cephalosporins (Cefotan)

Red Cell Antigen Typing with Pos DAT

- Antigen typing is used to determine or confirm the presence of alloantibodies an individual may produce or has produced
- If typing sera uses IAT method, DAT pos cells cannot be used
- DDT, CDP or EGA
- Full phenotype

Causes for RBC-Bound Complement

- Autoantibody
- Alloantibody
- Drug Induced
- Old RBC’s
- C’ activation remote from RBC’s
- Membrane modification

Significance of RBC-Bound Complement Components

- Intravascular lysis
- Sequestration in reticuloendothelial system leading to phagocytosis
- Temporary sequestration with normal survival
- Essentially normal survival
Case Study 1

• A 65 year old male patient, LH, presents with diagnosis of possible heart attack and anemia.
• Hgb: 9 gm/dL    Hct: 29 %
• Orders for 2 RBC’s
• Historical Record: 5 RBC units 1 year ag.

Case Study 1

• Is an eluate indicated?
• Next step? Antibody panel
• ADSORPTION?
Case Study 1

- LH was previously prescribed procainamide for coronary artery disease
- Lookback - warm autoantibody (probably caused by procainamide) with two clinically significant underlying alloantibodies.
- How did DAT help us arrive at this conclusion?

Case Study 2

BH, one day old infant with increased bilirubin and jaundice. Hgb low. DAT positive on cord blood at hospital. Mother suspected placental abruption.

<table>
<thead>
<tr>
<th>Poly</th>
<th>Igs</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+</td>
<td>2+</td>
<td>0</td>
</tr>
</tbody>
</table>

Mother's type: O negative
Baby's type: A negative

What information do we need to continue? What are we thinking right away?

Case Study 2

- ABO HDFN? Eluate on the cord cells reveals Anti-A and Anti-A,B.
- Are we done?

Case Study 2

Maternal Antibody Screen

<table>
<thead>
<tr>
<th>IS 37C AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI 0 0 0</td>
</tr>
<tr>
<td>SCI 0 0 0</td>
</tr>
<tr>
<td>SCI 0 0 0</td>
</tr>
<tr>
<td>SCI 0 0 1+</td>
</tr>
<tr>
<td>AC 0 0 0</td>
</tr>
</tbody>
</table>

What is that?

Case Study 2

- When working through a HDFN it is important to have a recent antibody screen on the mother.
- Lab has no AB screen on this mother's most current specimen
- Blood banker performs AB screen

Case Study 2

- Looking back to the infant's specimen. Did we test any cells with our first eluate that were Wr{a+}?  
  - In this case we did not
- New eluate on cord cells. Test with Wr{a}+ cells \( \rightarrow \) REACTIVITY!
- Type infant for Wr{a} \( \rightarrow \) POS (<0.01%)
Case Study 3

- Patient SP, 77 year old male. Trauma!!!
- Hgb 8.6 gm/dL HCT 25.4%
- Orders for two units
- Not all information on request form is filled out

A trauma patient with mixed field? Two red cell populations? Something is wrong.

"Well SP was a trauma patient a week ago out of state, he came in to us because he wasn’t feeling well"
"SP did receive two units 7 days ago"

Case Study 4

- A 30 year old female trauma patient with multiple injuries from MVA.
- Hgb: 13.5%
- Orders for 2 RBC’s
- No history (yet....)
Case Study 4

- With no clinical history (yet…) three probable causes for initial testing reactivity
  - Only warm autoantibodies present
  - Both warm autoantibodies and alloantibodies are present
  - Patient has been recently transfused and is experiencing a DHTR.
- After questioning patient, AL states she has never been pregnant or transfused.

Case Study 4

- Blood banker decides to perform warm autoadsorption and repeat antibody screen

<table>
<thead>
<tr>
<th>Antibody screen with 2x Adsorbed serum</th>
<th>IS</th>
<th>37°C</th>
<th>Anti-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Adsorbed serum crossmatched with 2 donor units, found to be compatible.

Case Study 5

- Patient NM, 43 y.o. male lymphoma patient.
- Hgb: 9.4 gm/dL  Hct: 26.6%
- Two units ordered
- Long list of prescriptions attached to request form.

<table>
<thead>
<tr>
<th>ABO/RH</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A cells</th>
<th>B cells</th>
<th>Anti-D</th>
<th>Rh control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>0</td>
</tr>
</tbody>
</table>

Antibody Screen (IAT)

<table>
<thead>
<tr>
<th>Antibody Screen (IAT)</th>
<th>IS</th>
<th>37°C</th>
<th>Anti-IgG</th>
<th>Anti-C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AC</td>
<td>0</td>
<td>0</td>
<td>3+mf</td>
<td>0</td>
</tr>
</tbody>
</table>

DAT Profile

<table>
<thead>
<tr>
<th>Poly</th>
<th>Anti-IgG</th>
<th>Anti-C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+mf</td>
<td>3+mf</td>
<td>0</td>
</tr>
</tbody>
</table>

Mixed Field!
One unit transfused 1 month prior.

Case Study 6

- A 54 year old male, SL, admitted for hip surgery
- 4 RBC units ordered. Patient able to donate 2 autologous preoperatively.
- Day 9 after surgery, dramatic drop in Hgb/Hct (6 gm/dL and 18%)
- 2 additional units ordered. Serum on new crossmatch grossly hemolyzed.

Case Study 5

Antibody Screen in PEG reveals weakly positive cells matching anti-E

Eluate in PEG reveals reactivity with all cells tested → reactivity stronger with double dose E cells.

Antigen type patient for E → 3+mf

Closer look at prescriptions → Patient is getting IV IgG every three weeks

What more can we do?

*Due to recent transfusion and administration of IV IgG, it cannot be determined at this time if the anti-E is an autoantibody, alloantibody or a passively acquired antibody from the IV IgG.*
Case Study 6

ABO/RH

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A cells</th>
<th>B cells</th>
<th>Anti-D</th>
<th>Rh control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransfusion</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>Posttransfusion</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
</tr>
</tbody>
</table>

Hgb (gm/dL) | Hct(%) | Haptoglobin (mg/dL) | Pretrans | Posttrans |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>15.5</td>
<td>36</td>
<td>&gt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>18</td>
<td>&gt;3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DAT Profile

<table>
<thead>
<tr>
<th></th>
<th>Poly-AHG</th>
<th>Anti-IgG</th>
<th>Anti-C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretrans</td>
<td>3+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Posttrans</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

Case Study 6

• Patients chart – cefotetan.
• This sample will definitely go to reference lab
  – Distinguish antibody to high incidence antigen from antibody to cefotetan.
  – Test pre and post samples against untreated and cefotetan coated cells.

Case Study 6

• Investigation reveals SL received 2 autologous units, followed by 2 allogeneic units.
• Urine sample – free hemoglobin, no intact red cells.
• 2 allogeneic units found to be incompatible with post-transfusion sample.

Case Study 6

• Conclusion ➔ After extensive antibody testing and antigen phenotyping it was discovered that this was indeed a DHTR due to anti-cefofetan.
• Not a very common result, but a great example of how DHTR’s present.

References

• Garratty G. Interpreting the Results of a Positive Direct Antiglobulin Test. Southern California Region Blood Transfusion Medicine Lecture Series. 08-08-2012.
Title goes here

• Lorem ipsum dolor sit amet, consectetur adipiscing elit. Morbi lorem eros, pellentesque ut sagittis in, congue ac lacus. Pellentesque ornare tristique sem eget laoreet. Aenean elit tellus, dictum