How to Approach Antibody Identification  
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**Overview**

- Antibody screen/identification are mainly performed on:
  - Potential transfusion recipients to prevent hemolytic transfusion reactions
  - Pregnant women to evaluate the risk for HDFN
- The screen:
  - Patient’s serum/plasma is mixed with a set of (2-3) reagent red blood cells
  - Reagent RBCs chosen such that various significant antigens are expressed and represented, so that commonly formed alloantibodies can be detected.
  - Reagent RBCs are group O to avoid “interference” by ABO antibodies.
  - Look for agglutination or hemolysis at three phases,
    - Immediate spin (IS) at room temp- this is often optional, because this usually detects IgM, non-ABO antibodies that are reactive at room temp, the majority of them are clinically insignificant
    - After incubation at 37C, this facilitates agglutination by warm reactive antibody, which are often IgG
    - After adding AHG (anti-human IgG) to further enhance agglutination by IgG antibodies
  - If screen positive, a more expanded panel is done with 10-12 reagent cells, representing various antigens in the same manner, to allow determination of antibody specificity.
  - Autologous control (patient’s own RBCs) done in parallel: to pick up autoantibodies.

**What makes an alloantibody clinically significant?**

- An antibody is considered significant if its specificity has been associated with
  - Hemolytic disease of the fetus and newborn (HDFN)
  - Hemolytic transfusion reactions
  - Notable decreased survival of transfused red cells.
- Some specificities are well known to be clinically significant, for e.g., Anti-Rh, Kidd, Kell, Duffy, S-s
- For less well known ones, you can consult the Antigen Facts Book by Marion and Reid, or check the current literature.

Generally…

- An antibody that reacts at the 37C or the AHG phase is more likely to be clinically significant –usually a warm reactive IgG antibody
- If the antibody is only reactive at the room temperature/immediate spin (IS) phase, then it is not very likely to be significant, usually a “cold” reactive, non-ABO, IgM antibody (Part of the reason that some labs have abandoned the IS phase of antibody screen/identification.)

In summary:
Clinically significant antibodies tend to be IgG and warm-reacting.
- IgM tend to be cold-reacting and clinically insignificant.  
(Mnemonic for IgM antibodies: LIPMAN: Lewis, I/little-i, P1, M, ABO, N )
- Important Exceptions: IgM ABO antibodies, which are often warm reactive and very clinically significant

How do you approach an antibody screen?
Things to consider:
1. At what phase did the reaction occur? (IS, 37°C, or AHG) Is it cold, or warm?
   - If cold reactive, think Le^a, Le^b, I/i, M, N, P1. (LIPMAN)
   - Most IgG are detectable at AHG phase only, except Rh antibodies, often detectable at 37°C as well
   - If 37°C reactive, and weaker reactivity during AHG, think IgM with high thermal amplitude.

2. Is the auto-control (AC) positive or negative? This will change the interpretation of the panel.
   - If AC negative, and panel is positive, there is an alloantibody
   - If screen and AC both positive and patient has not been transfused recently – there is an autoantibody. Need to perform an autoadsorption to rule out underlying alloantibody.
   Adsorption: Use patient’s own (autoadsorption) or specific RBC’s (known as alloadsorption, performed when patient has been transfused recently) with known phenotype to remove cold or warm auto from the serum to see if there is an underlying alloantibody

3. How many cells reacted?
   - Some positive, some negative: most likely alloantibody present, may see a pattern
   - All reactive, and auto-control is negative: suspect hi frequency antigen
   - All reactive, and auto-control is positive – could be cold or warm auto, the phase at which the reactions occur will help you.

4. Does the strength of reactivity vary? If it does, could be due to:
   - Dosage effect due to homozygous vs. heterozygous expression of the offending antigen on the reagent RBCs
   - More than one antibody

Steps to follow:
1. Check autocontrol
2. Look at which phase the + reactions occurred- cold vs. warm, IgG vs. IgM
3. Look at how many cells reacted, see if there is variability in strength
4. Perform rule-outs using non-reactive cells
   - Only try to use homozygous cells to rule out antigens, except Kell system (due to paucity of K+k- reagent RBCs), and those systems that do not have two alleles.
• For the boards, you can probably ignore the high freq or low freq antigens

5. Use positive cells to try to fit a single antibody (if all warm or all cold reactive), if one antibody won’t explain all the reactions, or there seems to be a mixture of warm and cold-reactive antibodies, move on to two, three, etc.

6. Special techniques that can be used:

• Enzymes (include bromelin, papain, ficin, trypsin): destroy or expose antigens, therefore can enhance or weaken certain antibody-RBC antigen reactions:
  1. Enhances: **Lewis P Is A Rhotten Kidd!** And others.
  2. Destroys: M/N, Duffy, Lutheran (Mnemonic: **My Dog Lutheran**) etc
  3. Does not affect: Kell

• Chemical modifications:
  1. Reducing reagents (DTT) treated serum to distinguish IgG from IgM: DTT cleaves the bonds between IgM subunits and inactivated IgM. Useful when distinguishing warm reactive IgG from warm reactive IgM (rare)
  2. Chemical reagents (DTT, chloroquine) can destroy certain antigens on RBC
    a. DTT destroys Kell antigen, by reducing the disulfide bonds to –SH groups
    b. Chloroquine weakens Bg

• Phenotype the patient: by definition, you can’t form an allo against antigen you have. Caveat is that patient should not have been transfused recently.

Question: How do you phenotype the RBC’s if the DAT is positive? The bound IgG will “block” the antigens from the monoclonal antibodies used in phenotyping:

*Answer: Elute antibodies with chloroquine diphosphate, this will leave RBC antigens relatively intact and accessibly to typing monoclonal antibodies. Acid elution can also be done, but this might be too harsh.*

• Neutralizing or inhibitor substances: will eliminate reactivity of antibodies with certain specificities. This can be used to confirm the suspected specificity of some alloantibodies. Some examples are:
  1. saliva from secretors: anti-Lewis
  2. hydatid cyst fluid: anti-P1
  3. serum: anti-Chido, Rodgers
  4. urine from “super Sid” individuals, guinea pig urine: anti-Sd
  5. saliva: anti-H

• Adsorption: Use patient’s own or specific RBC’s with known phenotype to remove cold or warm auto, or allogeneic cells with certain specificity from the serum to see if there is something else
• Acid elution (with glycine acid, digitonin): Remove (“elute”) antibodies from DAT positive RBCs by acid, heat, or other organic solvent. The resulting “eluate” will give you a more concentrated solution of antibodies and may allow you to finally identify the specificity when the serum reactivity is not strong enough.

7. Final rule in: positive reaction with three different cells positive for the specific antigen to confirm the specificity

Pattern recognition: Always know the following

1. Warm auto
2. Cold auto
3. HTLA antibodies vs. anti-high freq

HTLA (Hi titer, low avidity)

• Specificities: Chido/Ridgers, John Milton Hagen, Knops, McCoy York, Cost-Sterling, Bg, Sda,
• Limited clinical significance
• Weak reactivity (at most 1+), hence low avidity, but high titer, and reactivity persists after serial dilutions