Absorption/Adsorption and Elution

Definitions

- Absorption: To remove antibodies from serum through the action of the target antigen.
- Adsorption: The uptake of antibody by the target antigen.
- Elution: The removal of antibody from the surface of the red blood cell.

Absorption/adsorption is like a sponge wiping water off a desktop. The water is absorbed off the desktop, and adsorbed into the sponge.

Elution would be like squeezing the sponge to remove the water.

Elution is performed when the DAT is positive with anti-IgG Coombs serum.
Elution Procedure

- Antibody-coated RBCs are treated to free the antibody from the red blood cell surface.
  - Disrupts Ag/Ab bonds.
- Methods include:
  - Temperature
  - pH
  - Chemicals

Elution Procedure

- The antibody-containing supernatant is called the eluate.
- The eluate may be tested against a panel of RBCs to determine the antibody specificity.

Total Elution

- A total elution is one in which the red blood cells are completely destroyed.
- The cells cannot be used for phenotyping or autoadsorption.
- Methods include:
  - 56°C heat
  - Lui freeze
  - Acid

Partial Elution

- A partial elution removes the antibody while leaving the red blood cell membrane intact.
- The treated RBCs may be used for phenotyping or autoadsorption.
- Methods include:
  - Gentle heat 45ºC
  - ZZAP
  - Chloroquine
  - EGA

Absorption / Adsorption

- To perform an absorption, serum containing the antibody is incubated with the target antigen, usually antigen positive red blood cells.
- If the patient has not been transfused in the last 3 months, autologous RBCs may be used for removal of autoantibodies. Otherwise, a different antigen source will be necessary.
  - Homologous absorption
  - Differential absorption
  - RESI
In this example, the blue antibodies are adsorbed onto the RBCs (or absorbed from the plasma) by attaching to the antigen , leaving the pink antibodies behind in the plasma.

Select the best RBC to use for absorption:

- Imagine that you are trying to prepare anti- from a donor who is A positive (anti- in plasma).
- What would be the phenotype of the RBC used for absorption?

The Best RBC

- The best RBC would be a Group B, Fy negative cell.
- The anti-B would be adsorbed onto the B antigens on the RBC.
- The anti- Fy would not be adsorbed; it would remain free in the plasma.

Applications

- The most common use of absorption is to remove autoantibodies from serum, and then test the absorbed serum for underlying alloantibodies.
  - 38% of patients with a warm autoantibody also have an alloantibody.
- Removal of unwanted ABO antibodies in human source reagent preparation is also an application of absorption/adsorption.

Warm Autoantibody Removal

In this example, there is a mixture of autoantibody and alloantibody in the patient's plasma. The patient has autoantibody coating the red blood cells. The RBCs are washed, then treated to remove the coating antibody (partial elution).
The treated RBCs are incubated with the patient’s plasma at 37°C. Autoantibody reattaches to the red blood cells, leaving behind any alloantibodies present in the plasma.

The plasma is harvested and tested to determine if alloantibodies are present, and if so, determine the specificity of the alloantibody.

Absorption and Elution

- Used to separate a mixture of antibodies.
- For example: How do you know if a patient has anti-D \( \text{Y} \) and anti-C \( \text{Y} \), or if they have anti-G \( \text{Y} \)?

Using an RBC sample that is D negative and C positive, the RBCs are incubated with the antibody-containing plasma.

- If both anti-D and anti-C are present, only anti-C will be absorbed.
- Anti-D will remain free in the plasma.
- If an eluate is made from the absorbing RBCs, anti-C will be present in the eluate.

If anti-G is present, it will adsorb to the RBC membrane.

The remaining plasma will not contain any antibodies.

An eluate made from the adsorbing RBCs will have anti-D and anti-C activity (i.e. anti-G)
Absorption and Elution

- Can be used to confirm the presence of subgroups in the ABO system.
  - Suspect RBCs are incubated with >A or >B.
  - An elution is performed on the RBCs following incubation.
  - The eluate is tested for presence of >A or >B that had adsorbed to the red blood cell antigens during incubation.

Reagents

**REST**

- Rabbit Erythrocyte Stroma
  - Used to absorb cold reacting autoantibodies.
  - May be used if patient has been transfused.
  - Absorbed plasma should not be used for ABO determinations or crossmatching, as >B may be removed.

*It is difficult to tell from this photo, but there are white solid particles at the bottom of this tube. These particles (the stroma) are suspended in a preservative solution. The preservative is replaced with patient’s plasma, before incubation.*

**WARM**

- Commercial form of ZZAP.
- Contains lyophilized preparation of DTT, cysteine, and papain.
- Destroys enzyme sensitive antigens, & denatures Kell.
- Used to remove autoantibody coating RBCs to prepare them for use in autoadsorption.

**HPC**

- Human Platelet Concentrate
  - HLA antigens present on platelets are used to absorb anti-Bg-like antibodies that may be masking clinically significant antibodies.
  - Patient’s serum is added to lyophilized platelet reagent.

**EGA**

- pH method (acid)
- Kit contains EDTA, glycine and a buffer.
- Used to quickly perform a partial elution, allowing for antigen typing.
EluKit

- Acid elution (total elution)
- Reagents include:
  - Wash solution to create a low ionic environment
  - Acid to lower pH
  - Buffer to restore pH of eluate to neutral before testing with reagent RBCs.

The End

Please complete the reading assignments, then take the Self Assessment Quiz.