ABSORPTION AND ELUTION

I. Definitions

Absorption: The removal of antibodies from serum/plasma through the addition of the corresponding antigen (usually found on red blood cells).

Adsorption: The uptake of antibody onto specific receptors on the red blood cell (RBC) surface.

Elution: The removal of antibody from the RBC surface. Total elution removes the antibody coating the RBCs and destroys the antigens to which they were attached. Partial elution removes the antibody, but allows the antigen to remain intact.

Eluate: A fluid medium containing the antibodies that have been deliberately removed from RBCs, allowing for antibody identification.

II. Elution

A. Performed to remove antibodies (usually IgG) that are sensitizing RBCs. The elution method serves to concentrate and purify the antibody. Applications include:

1. Investigation of a positive DAT. Generally requires total elution, in which the RBCs are completely destroyed. Once removed from the RBCs, the antibody solution (eluate) is tested with panel cells for antibody identification. RBCs with a positive DAT due to complement will have no antibody reactivity in the eluate.
   
   a. HDFN - the elution confirms that IgG antibody from the mother is coating the RBCs of the newborn
   
   b. HTR - the elution of antibody from the recipient’s RBCs containing transfused donor RBCs confirms the antibody specificity responsible for the reaction
   
   c. Acquired hemolytic anemia: immune or drug induced - the elution of autoantibody or antibody to certain drugs from the patient’s red blood cells.

2. Preparation of antibody-free RBCs for use in phenotyping or autologous adsorption studies.
B. Methods: Alteration of thermodynamics, membrane structure or reversal of attractive forces between antigen and antibody causes the release of antibody from the RBC surface.

1. Temperature
   a. Heat: Changes the thermal motion of molecules and causes conformational changes. Used in the investigation of ABO HDFN, and the elution of IgG antibodies from RBCs. Easy to perform, yet limited applications.
      1) Gentle heat 45°C- release of IgG from RBC surface, allowing for antigen typing (partial elution).
      2) 56°C- RBCs destroyed
   b. Lui Freeze: Used in HDFN investigations. RBCs are frozen and then rapidly thawed to cause lysis. Easy to perform, but limited applications.

2. pH: Antibody attachment to the RBC membrane is dependent on many factors, one being pH of the surrounding medium (normal range for pH is 6.5-7.5). Acidification (pH 3.0) has proven to be an efficient and elution method for routine use in the laboratory
   a. Gamma Elu-Kit II
      1) Composition:
         a) Eluting solution - a low pH glycine buffer designed to dissociate bound antibody from washed RBC
         b) Buffering solution, a Tris (hydroxymethyl) -aminomethane with bovine albumin which neutralizes acidity of eluting solution
      2) Action: Total elution
         a) Washed RBCs are suspended in glycine solution at pH 3.0 to dissociate bound antibody
         b) Supernatant (eluate containing antibody) is neutralized by buffer
         c) Eluate is then ready for antibody identification. Additional potentiators are not necessary since the eluate is already a low ionic strength substrate
         d) RBCs older than 72 hrs may yield a less potent eluate
   b. EGA - EDTA Glycine- Acid: Uses acid pH to dissociate antibody from RBCs. Kell antigens are destroyed, but most other antigens remain intact (partial elution). Useful to prepare RBCs for antigen typing in patients with a positive DAT.
3. Chemical elution – Most are partial elution methods capable of splitting immune complexes without denaturing the red blood cell membrane. The most commonly used chemicals for obtaining antibody-free intact RBCs are:

a. ZZAP (a.k.a. W.A.R.M.)

1) Most commonly used to prepare RBCs for use in autoabsorption procedures. Removes bound autoantibody in order to free antigen sites, allowing additional autoantibody to be adsorbed out of plasma by the treated RBCs.

2) Composition – Dithiothreitol (a reducing agent), cysteine and activated papain (an enzyme) in a phosphate buffer

3) Action - Reduces inter-chain disulfide bonds of immunoglobulin molecule rendering molecules more susceptible to digestion by enzyme. Enzyme also alters RBC membrane. Enhances Rh antigens, denatures K, and destroys Fy, M, N, and S.

4) Not used for RBCs that need to be antigen typed

b. Chloroquine diphosphate (a.k.a. Gamma Quinn)

1) Will dissociate IgG antibody without destroying the RBCs but will not cleave complement components, therefore, must be used with anti-IgG AHG

2) Use - Allows RBCs to be antigen typed and to be used for autoadsorption
   a) May weaken Rh antigens, but usually stay strong enough to be detected with antisera employing the modified tube method.
   b) Treated RBCs give weaker reactions with saline-reactive reagents; stronger reactions with AHG testing

4. Organic solvents: Rarely used in the clinical laboratory. Solvents involved are often unstable, flammable or toxic. Method is relatively simple and produces a potent eluate.

a. The red blood cell membrane, which is comprised of 43.6% lipids, will be disrupted by organic solvents capable of dissolving lipids. Antibody dissociation may occur as a result of antigen site denaturation when the membrane is destroyed.

b. Solvents are also used to decrease surface tension of the liquid medium and disrupt van der Waal forces that hold antigen and antibody together.

c. Chemicals used include ether, xylene, chloroform, and dichloromethane (DCM).
III. Technical factors affecting elution

A. Incorrect technique
   1. Temperature should be optimal for antibody removal
   2. Volume of cells to eluate = 1:2
   3. Wash should be removed immediately after centrifugation to decrease the possibility of antibody dissociation from RBC membrane due to low protein concentration
   4. Stromal debris

B. Incomplete washing
   1. Incomplete removal of organic solvent
   2. Test the supernatant saline from the last wash in parallel with the eluate to ensure that there is no residual unbound antibody activity contaminating the eluate

C. Binding of protein to glass
   - As antibodies are proteins, they can be adsorbed nonspecifically onto the surface of a glass test tube. When sensitizing RBCs with high-titer antibodies *in vitro*, as in confirmation studies for weak antigens, if the same test tube is used for both sensitization and elution, antibody that may have adsorbed onto the tube during sensitization could dissociate and appear in the eluate. Transfer the washed sensitized RBCs from the tube used for sensitization to a clean tube before the eluate is prepared.

D. Storage changes of solvents
   - Solvents may become acidic upon storage

E. Dissociation of antibody before elution
   - Eluates that are prepared in saline may be unstable due to low levels of protein. These eluates should be tested as soon as possible after preparation to ensure maximum activity. The addition of small volumes of albumin will help maintain antibody activity.

F. Nonspecific antibody uptake
   - Although a rare occurrence, it has been shown that antibody can non-specifically adsorb to RBCs. Therefore, RBCs may adsorb and elute an antibody even though the cells do not possess the corresponding antigen. When an unexpected antibody is encountered it is usually weaker in reactivity than the expected antibody.
IV. Absorption / Adsorption

A. Method

1. RBCs with known antigen are incubated with serum/plasma to remove a suspected antibody from the plasma. Alternatively, known antibody is incubated with unknown RBCs to see if the cells will take up the antibody.

   a. Temperature of incubation depends on the optimum temperature of reactivity, i.e. 4°C for cold antibodies and 37°C for warm antibodies.

   b. If RBCs show agglutination during the incubation period, all antigens sites have been saturated. The plasma is harvested and placed on a fresh aliquot of cells to continue the absorption.

   c. RBCs are often pretreated to remove any autoantibodies coating them and/or to enhance reactivity.

2. The absorbed plasma is tested to detect any remaining antibodies, which had previously been masked by the antibody that was absorbed out.

B. Clinical applications

1. Separation of antibodies in serum

   a. Autologous or, in the case of a recently transfused patient, homologous red blood cells are used to remove autoantibody from the serum; the absorbed serum is then tested for any underlying alloantibodies. (#1 use of adsorption)

   b. Known red blood cell antigens are incubated with serum/plasma to absorb out a suspected antibody, e.g., antibody to a high incidence antigen. Then the absorbed serum is retested for the presence of other antibodies.

2. Preparation of typing reagents

   a. Absorbed anti-A\textsubscript{1} (human): absorb group B plasma with A\textsubscript{2} cells

   b. Removal of ABO antibodies from sera used for antigen typing reagents, e.g., anti-Kell typing reagent

C. Reagents

1. Eluting reagents to prepare RBCs – ZZAP and chloroquine
2. **RESSt - Rabbit Erythrocyte Stroma**

   a. **Action** - rabbit stroma, which possesses structures resembling I, IH and H, absorbs cold reacting autoantibodies. Anti-B and anti-P1 also bind to rabbit erythrocytes; therefore, serum absorbed with RESSt cannot be used for ABO grouping or crossmatch.

   b. **Uses**
      1) To absorb recently transfused patients which are not able to be autoadsorbed
      2) When complement is bound to RBCs by cold agglutinin, and stays reactive at the AHG phase, therefore, possibly masking clinically significant antibodies
      3) If during the prewarm technique, the use of anti-IgG monospecific AHG does not prevent potent cold antibodies from reacting at the AHG phase

3. **HPC - Human Platelet Concentrate** made from pool of platelets

   a. HPC absorbent that carries HLA antigens removes unwanted HLA antibodies from serum. These HLA antibodies may cause hemagglutination which interferes with the detection of significant antibodies

   b. May also absorb antibodies to ABH, I, Le\(^a\), Fy\(^a\), Pp\(_1\)P\(_k\)

**V. Absorption / Adsorption - Elution**

**A. Method:**

1. Known red blood cells are incubated with serum/plasma to absorb out a suspected antibody **OR** a known antibody reagent is incubated with RBCs suspected of possessing the corresponding antigen, allowing antibody to adsorb onto the RBCs.

2. Elution is performed to allow antibody removal

3. Eluate is tested to identify the antibody

**B. Clinical Applications:**

1. Confirmation of antibody specificity - Known RBC antigens are incubated with patient’s serum to absorb out a suspected antibody, then the antibody is eluted from the cells. The eluate is tested to confirm the identity of the antibody.

2. Confirmation of the presence of weak antigen on RBCs - e.g., used to confirm weak subgroups of group A or B. RBCs are incubated with known anti-sera. If the RBCs possess the corresponding antigen, the antibody can be eluted and identified.