BASIC ANTIBODY IDENTIFICATION TECHNIQUES

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Enhancement Techniques

- Investigation of ABO discrepancies
- Enhancement reagents
- Serum techniques
- Red cell techniques
- Elutions
- Titrations

Investigation of ABO Discrepancies

- Incubation at room temperature
- Decreasing the temperature
  - 18°C / 4°C
  - Include control cells
    - Group O cells
    - Auto control

Investigation of ABO Discrepancies

- Use of lectins
  - Made from plant seeds
  - React with specific carbohydrate antigens
  - Control cells

Use of Lectins

- Ulex europaeus
  - Anti-H specificity
  - Identifies Bombay phenotype
    - hh genotype
    - Produce potent anti-H
      - Reacts with all red cells

Use of Lectins / Additional Reagent Red Cells

- Dolichos biflorus
  - Anti-A_1 specificity
  - Identifies A_1 phenotype
- Reagent A_2 red cells

Enhancement Reagents

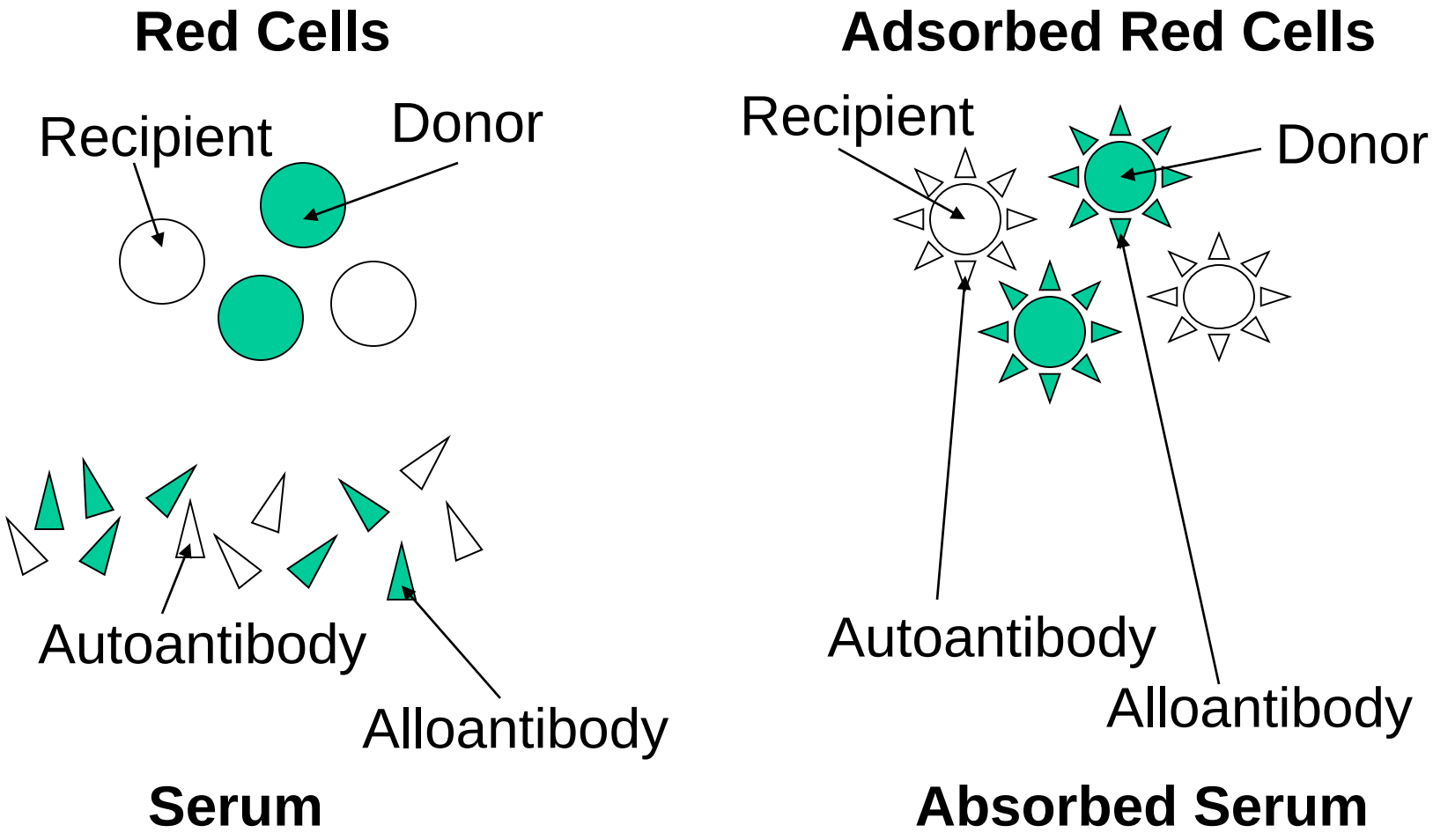
LISS Albumin PEG

- May see nonspecific reactivity
- Clinically significant antibodies are excluded
- Repeat with different reagent

Cold Autoantibodies

- Cold autoadsorption techniques
  - Adsorb autoantibody onto autologous red cells
  - Re-test absorbed serum for underlying clinically significant alloantibodies
  - **Cannot be performed on recently transfused recipient**

Autoadsorption of Recently Transfused Recipient



Prewarmed Techniques

- Can be performed on recently transfused recipient
- Results used to characterize antibody
- Results may be used to exclude alloantibodies
- May be only method to find compatible donor red cell units

Prewarmed-IAT

- Warm serum / cells
- No enhancement reagent
- Extended incubation
- Wash / warm saline
- Anti-IgG

- Warm serum / cells
- Enhancement reagent
- Incubation
- Wash / warm saline
- Anti-IgG

No reactivity at AHG--no underlying clinically significant antibodies

Settle Technique

- Warm reagent cells and serum
- Incubate at 37^{o}C until red cells settle
- Do not centrifuge
- No AHG reagent
- No reactivity at 37^{o}C
  - Antibody is probably not clinically significant

Inhibition / Neutralization

- Performed on specific antibodies
  - Lewis, P1, Sda, Chido/Rogers
- Specific substance can neutralize specific antibody
- Re-test neutralized serum
  - Look for underlying clinically significant antibodies

Inhibition / Neutralization Test

- Add neutralizing substance to serum aliquot
- Allow to incubate
- Re-test neutralized serum
- Need for control
  - Dilutional effect of technique
  - Saline and serum aliquot

Inhibition / Neutralization Interpretation

Test	Control	Interpretation
0	+	No additional antibodies
+	+	Additional antibodies or high-titered antibody
0	0	Invalid results (weak Ab)

Rouleaux

- Due to abnormal serum proteins
- May cause pseudoagglutination
- May be detected at room temp / 37^{o}C
- Not observed at AHG phase
  - Saline disperses rouleaux
- Observe serum:cells microscopically
  - Stacked coin appearance

Saline Replacement

- Centrifuge serum:cell mixture
- Remove serum
- Add 2 drops of saline
- Re-centrifuge
- If negative----rouleaux
- If positive-----true agglutination

Enzyme Treatment

Untreated cells	**Enzyme-treated cells**	**Interpretation (presumptive)**
Fy(a+) Jk(a+)	Fy(a+) Jk(a+)	
2+	0	Anti-Fy^a
2+	3+	Anti-Jk^a

DTT / AET / ZZAP

- Can dissolve disulfide bonds
- Can modify various high incidence antigens
- Procedure
  - Treat reagent cells and re-test with serum

Red Cell Treatment for Antigen Typing

- Use of specific reagents
  - DTT / AET / CDP / EDTA/acid
- Can dissociate antibodies from coated red cells
  - Positive DAT
- Frees antigens for antigen typing--IAT

Indications for Elution

- Removes coating IgG antibody
  - Warm autoantibody
  - Alloantibody in recently transfused recipient
    - Delayed or immediate transfusion reaction
- Allows identification of coating IgG antibody

Eluting Mediums

- Coating IgG antibody is removed
  - Thermal conditions
    - Heat, cold, freeze-thaw
    - ABO antibodies
  - Organic solvents
    - Acid, ether
    - IgG antibodies

Elution Procedure

- Wash the red cells
  - Removes unbound antibody
- Treat red cells with eluting medium
  - Antibody is released into eluate
- May require phosphate buffer
  - Stabilize pH for further testing

Elution Control

- Wash steps are critical
  - Ensures antibody identified in eluate is the coating antibody
- Test aliquot of last wash step along with eluate
- If control negative--valid eluate results
- If control positive--invalid eluate results

Antibody Titration Procedure

- Prepare Master Dilution
  - Prepare 2-fold dilutions of serum
- Add 2 drops of each dilution
- Add 1 drop of appropriate red cells
- Incubate all tubes / centrifuge
- Observe for agglutination beginning with largest dilution

Titration Terms

- Titer
  - Reciprocal of the last dilution that exhibits 1+ agglutination
- Endpoint
  - Reciprocal of last dilution that exhibits any agglutination
- Score
  - Addition of assigned values to each agglutination grade

Titration Score

12	4+ agglutination
10	3+ agglutination
8	2+ agglutination
5	1+ agglutination
2	w+ agglutination
0	No agglutination

Titration Example #1

1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
2+	1+	1+	w+	0	0	0	0	0
8	5	5	2	0	0	0	0	0

Titer 4
Endpoint 8
Score 20

Titration Example #2

1:1	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**
3+	2+	2+	1+	1+	1+	w+	w+	0
10	8	8	5	5	5	2	2	0

Titer 32
Endpoint 128
Score 45

Antibody Titration Interpretation

	Baseline	Second sample
Titer	4	32
Score	20	45

Titer > 3 dilutions
Score >10

Are these results clinically significant?

Indications for Antibody Titrations

- Monitoring maternal IgG antibody
  - HDN
- Assessing thermal amplitude of cold autoantibody
- Identifying antibodies with HTLA characteristics
- Determining ABO secretor status