COOMB'S TEST
(THE ANTIGLOBULIN TEST)

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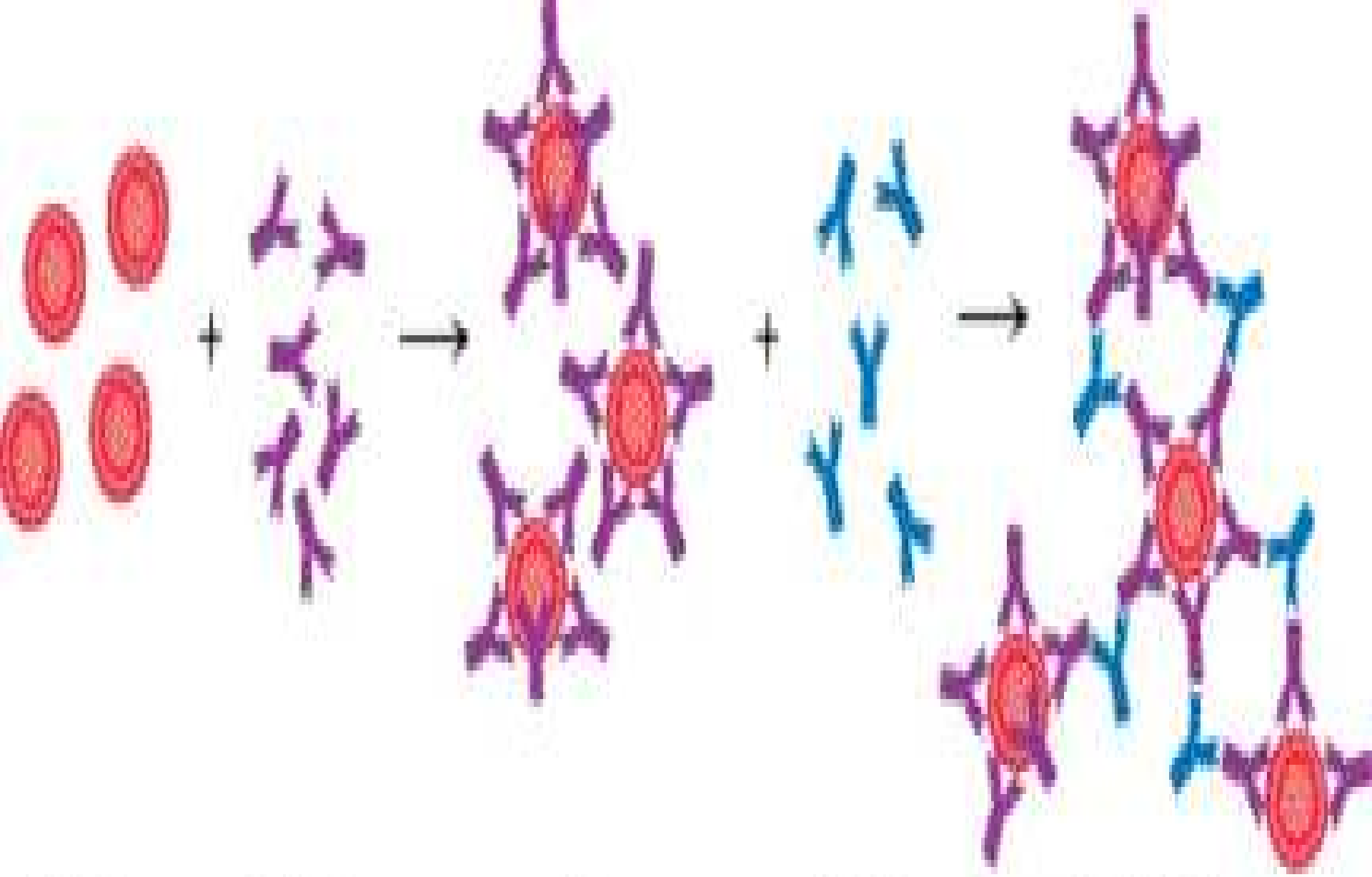
Introduction

- The anti-globulin test also called Coomb's test in honor of one of the investigator who developed the test for laboratory use in 1945 for detecting attachment of antibodies that didn't produce agglutination.
- This test uses antibodies to human globulins.
- It was first used to demonstrate antibodies in serum but later the same principle was used to demonstrate in –vivo coating of red cells with antibody or complement components.

Principles of AHG test

- All antibody molecules are globulins. Animals injected with human globulin produce antibodies to the human globulin. After the animal serum is absorbed to remove unwanted agglutinin it will reacts specifically with human globulins and can be called AHG.
- IAT : Two stage technique
- DAT : One stage technique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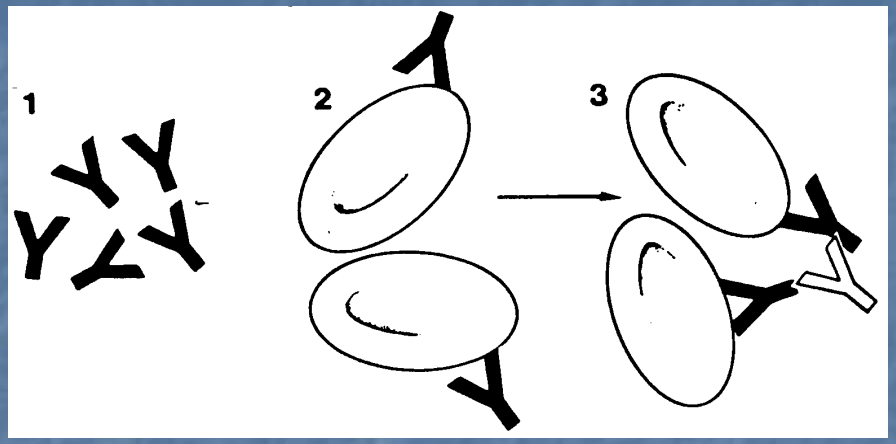
Antibody
No
Anti-Ig
Agglutination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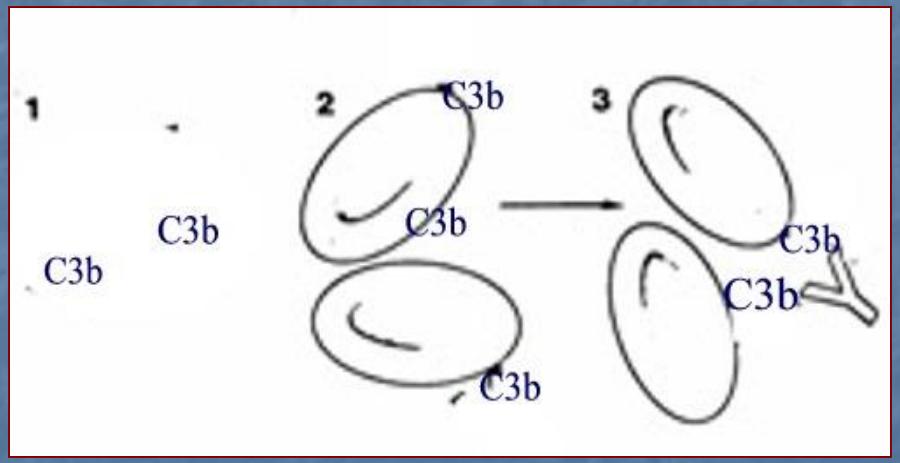
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The Role of Immunoglobulins in Antiglobulin Reaction.

Reaction between anti-IgG and IgG coated red cells

- In the detection of alloantibodies by AHG test anti-IgG is clearly essential but anti IgM is not required.
- Using radio-labeled antiglobulin serum the maximum number of anti-IgG molecules that can combine with an anti D(IgG) molecule on a red cell surface was estimated to be 6-9.
- Near saturation of the antigen sites of an IgG(anti D) molecule is obtained by having a free equilibrium concentration of 10-15 µg anti IgG/ml ; that is to say, after the uptake by antiD of the maximum number of anti-IgG molecules 10-15 µg anti-IgG/ml should remain in solution.

Prozones

- If an access of anti IgG serum(AHG) is added to a sample of IgG sensitized red cells, agglutination is inhibited because all the IgG molecules attach to the red cells are coated with anti IgG, so that no ‘bridges’ can be formed by particular anti IgG molecules between two or more red cells.

Optimal concentration of anti IgG

- A single dilution of a particular AHG reagent is usually optimal for detecting almost all examples of anti D.

Diluent for antiglobulin reagent

- AHG reagents are usually provided at their optimal dilution and for storage at 4°C.
- Various diluents are employed like

  0.1 mol/L NaCl;0.05 mol/L Phosphate pH 7.2;

  1g/L Na_4 EDTA $2H_2O$;1g/L Sodium azide.
- Dye may be added to AHG reagents to provide a means of checking that the reagent has been added to tubes.

Relationship between number of bound IgG molecules and reactions with anti IgG

- Using the spin- antiglobulin test the minimum number of IgG anti D molecules per cell detectable with anti IgG is between 100-150.
- The minimum detectable number of IgG, anti A and anti B molecules per red cell is also about 150.
- In normal subjects with a negative DAT the number of IgG molecules per red cell was found to be in the range 5-90.
- A correlation exists between agglutination strength and the number IgG molecules bound per cell in both DAT and IAT.
- The number of IgG molecules per cell required for maximum agglutination with anti IgG is in the range of 500-2000.

Inhibition of anti IgG by IgG in serum

- When using an AHG reagent containing about 10µg anti IgG/ml obvious weakening of the reaction between anti IgG and IgG coated red cells is not likely to occur.
- Normal serum contains about 10mg IgG/ml so it must be diluted at 1000 times during washing of antibody coated red cells to avoid false negative results.
- A better safety margin is a dilution of about 5000.

Reactions of Anti IgM

- Certain IgM antibodies are active at temperature upto about 25° or 30°C, but at 37°C they may act as incomplete antibodies.(Lewis antibodies, anti HI, anti P_1 and alloantiI)
- In addition to these cold antibodies few examples of warm incomplete IgM antibodies were found like one of three examples of anti-K and three of 15 examples of anti-Jk^a.
- The agglutination produced by anti IgM is weak compared with that produced by anti IgG because of the small numbers of IgM molecules attached to the red cells.
- As all incomplete IgM antibodies describe so far bind complements and it is always easier to detect complement by anti complement antibodies.

Reactions of anti IgA

- Some blood group antibodies (eg. anti-A, anti-B, anti-D and Lutheran antibodies) may be partly IgA, but are then always also partly IgG so that anti IgG can be used for their detection.

Role of Complement in Antiglobulin Reactions

- Complement components may attach to red cells in vivo or in vitro by one of two mechanism.

1. Complement binding antibodies may cause attachment of complement to the cell surface.
2. Immune complexes of various specificity unrelated to red cell antigen may be present in plasma and active complement components adsorb onto red cells in a non-specific manner.

- "Innocent bystander" complement coating.

Complement as the only coating Globulin

- Complement alone without immunoglobulin may be present on washed red cells in certain situations.

1. IgM antibodies reacting in vitro occasionally attach to red cell antigen without agglutinating the cells.

   IgM coating is difficult to demonstrate in AHG test partly because IgM molecules tend to dissociates during the washing process and partly because polyspecific AHG contains little anti IgM activity.

   However several hundred C3 molecules bound to the cell membrane near the site of antibody attachment.

2. About 10-20% of patients with warm AIHA have red cells with a positive DAT due to C3 coating alone. No IgG, IgA or IgM coating is demonstrable with routine procedures.
3. Cold hemagglutinin disease – complement component usually detected by AHG reagents is C3dg.
4. "Innocent bystander" complement coating.

Which Anticomplement Components to be included in AHG Reagents

- Anti-C3 to anti-C4
- The choice of particular anti-C3 component is more difficult.
- Anti-C3b, anti-C3d & anti-C3dg are essential anticomplement components of AHG reagents.

Different types of AHG Reagents

Reagent	Definition
Polyspecific (Rabbit polyclonal; rabbit/murine monoclonal blend; and murine monoclonal)	Rabbit polyclonal contains anti-IgG and anti-C3d(may contain other anticomplement and other anti-immunoglobulin antibodies); rabbit/murine monoclonal blend contains a blend of rabbit polyclonal antihuman IgG and murine monoclonal anti-C3b and –C3d; murine monoclonal contains murine monoclonal anti-IgG, -C3b and –C3d.
Anti-IgG (rabbit polyclonal; IgG heavy chains; monoclonal IgG)	Rabbit polyclonal contains anti-IgG with no anticomplement activity (not necessarily gamma chain specific); IgG heavy chains contain only antibodies reactive against human gamma chains; monoclonal IgG contains murine monoclonal anti-IgG.
Anti-C3d and anti-C3b, (rabbit polyclonal) and anti-C3d, -C4b, -C4d (rabbit polyclonal)	Contain only antibodies reactive against the designated complement component(s), with no anti-immunoglobulin activity.
Anti –C3d (murine monoclonal) And anti-C3b, -C3d (murine monoclonal)	Contains only antibodies reactive against the designated complement component, with no anti-immunoglobulin activity.

Direct Antiglobulin Test(DAT)

The direct antiglobulin test(DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.

In vivo coating of red cells with IgG and/or complement may occur in:

1. Hemolytic disease of new born (HDN)
2. Autoimmune hemolytic anemias (AIHA)
3. Drug induced hemolytic anemias (AIHA)
4. Hemolytic transfusion reaction (HTR)

Control Cells for AHG Tests

- Positive control cells
- Negative control cells
- The best control for the DAT & IAT is the addition of IgG sensitized O Rh (D) positive cells to any AHG test that is non-reactive. If there is agglutination the test is valid.

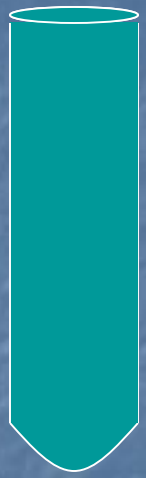
Procedure of DAT

N

1 drop of 3-5% cell suspension of ORh (D) positive unsensitized cells

+

1 drop of AHG reagent

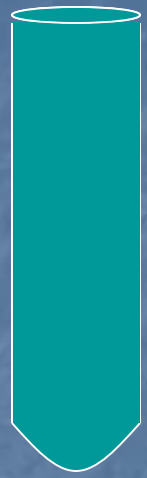


P

1 drop of 3-5% cell suspension of ORh (D) positive sensitized cells

+

1 drop of AHG reagent



T

1 drop of 3-5% cell suspension of ORh (D) positive test cells

+

1 drop of AHG reagent

Patterns of Reactivity in Autoimmune Hemolytic Anemia

Anti-IgG	Anti-C3d	Type of AIHA
+	+	WAIHA (67%)
+	-	WAIHA (20%)
-	+	CHD,PCH,WAIHA (13%)

WAIAH – warm autoimmune hemolytic aneamia, CHD – cold hemagglutinin disease, PCH – Paroxysmal cold hemoglobinuria.

Indirect Antiglobulin Test (IAT)

Indications

The IAT is done to determine the presence of sesitization of red cells with IgG and/or complement in vitro in the following conditions:

1. Compatibility testing.
2. Screening and detection of unexpected antibodies in serum.
3. Determination of red cells phenotype K, Le^a, Fy^a, Fy^b, Jk^a,Jk^b and sub-group of Rh etc by using known sera.

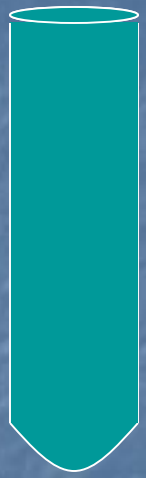
Procedure of IAT

N

1 drop of pooled 3-5% cell suspension of ORh (D) positive cells

+

2 drops of NS or AB Neg plasma

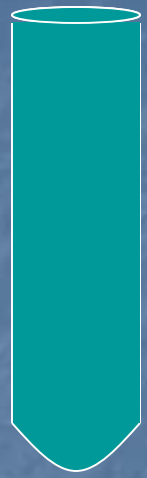


P

1 drop of pooled 3-5% cell suspension of ORh (D) positive cells

+

2 drops of diluted Anti-D (Blend)



T

1 drop of pooled 3-5% cell suspension of ORh (D) positive cells

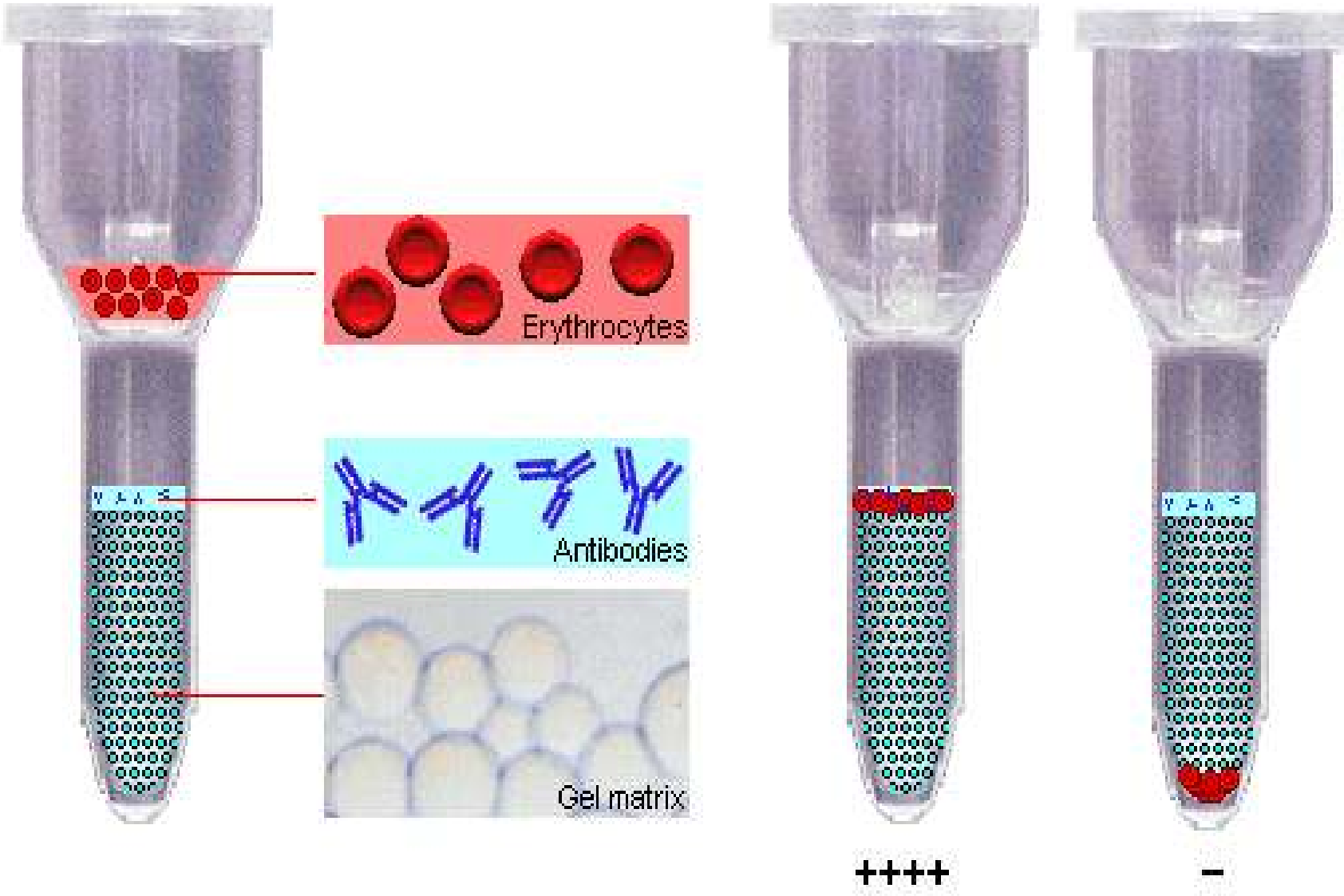
+

2 drops of Pt's serum

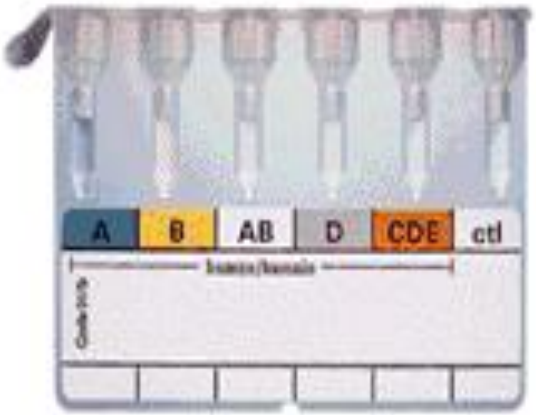
Other Methods of IAT

- Bovine albumin (22%) – IAT
- Enzyme – IAT
- LISS – IAT
- Gel and Glass Microbeads - IAT

Principle of the Gel Test

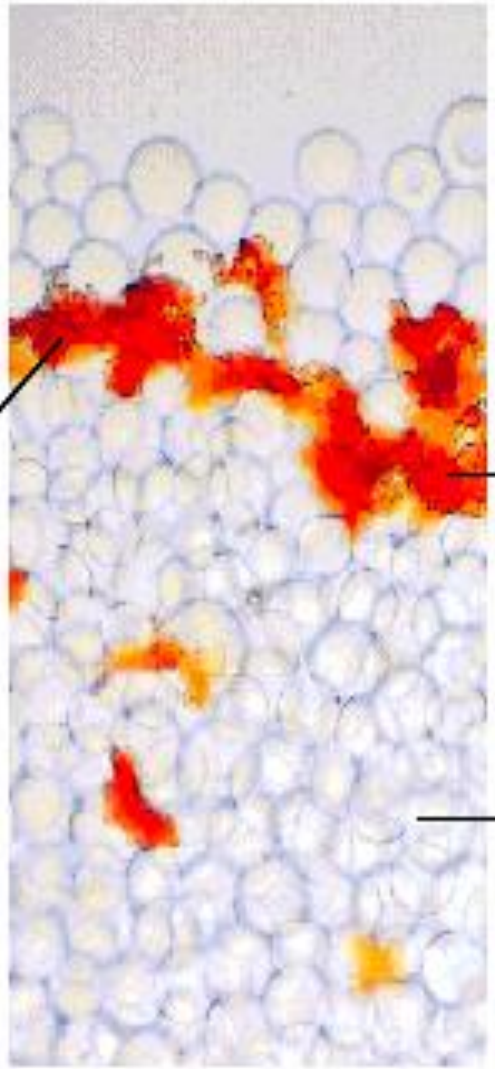


Gel Technique



Gel card for blood group determination

Microtube



For Detecting Antigens LEWIS, Kell, Duffy, Kidd etc.

1. Take known reagent serum corresponding to the antigen .
2. Add 1 drop of 3-5% suspension of washed test cells .
3. Follow all steps as in IAT.

Factors Affecting AHG Test

- Effect of ratio of serum to red cells
- Effect of period of incubation
- Effect of temperature
- Effect of low ionic strength solution

Sources of Error in Antiglobulin Testing -- False- Negative Results

- **Neutralization of Antihuman Globulin (AHG) Reagent**
- **Interruption in Testing**
  - Bound IgG may dissociate from red cells and either leave too little IgG to detect or may neutralize AHG reagent.
  - Agglutination of IgG-coated cells will weaken. Centrifuge and read immediately.
- **Improper Reagent Storage**
- **Improper Procedures**
- **Complement**
  - Rare antibodies, notably some anti-JK^a , -JK^b, may only be detected when polyspecific AHG is used and active complement is present.
- **Saline**

False- Positive Results

- **Prior to Cells Agglutinated Washing**
  - If potent agglutinins are present, agglutinates may not disperse during washing. Observe cells prior to addition of antihuman globulin (AHG) or use control tube substituting saline for AHG, reactivity prior to addition of AHG or in saline control invalidates AHG reading.
- **Particles or Contaminants**
- **Improper Procedures**
- **Cells Have Positive Direct Antiglobulin Test (DAT)**
  - Cells that are positive by DAT will also be positive in any indirect antiglobulin test.
- **Complement**

References

- AABB Technical Manual; 13th edition.
- Mollison's Blood Transfusion in Clinical Medicine; 10th edition.
- Transfusion Technical Manual; DGHS; 2nd edition.


Thank You