

National university Sudan
Faculty of Medical Laboratory Sciences
Advanced Hematology MLS –HEMA-324

Direct &

*Indirect Antiglobulin test (AHG)
Coombs' test*

U. Elham Ibrahim Mohammed
Lecture(35)

- There are two major types of blood group antibodies; **IgM** & **IgG**.
- IgM have a large pentamer structure so bind to the corresponding antigen and directly agglutinate RBCs suspended in saline.
- IgG antibodies have a monomer structure, so can not agglutinate RBCs directly.

Sensitization & Agglutination

- **Sensitization:** binding of the antibody to the antigen on the red cell surface.
- **Agglutination:** clumping together of sensitized red blood cells.

IgG antibodies cause sensitization (invisible), so we must convert it to agglutination in order to be visible.....

The addition of AHG
reagent (contain anti-IgG) to
RBCs sensitized with IgG
antibodies allows for
agglutination for these
sensitized cells....

Principle

Antihuman globulins (AHGs) obtained from immunized nonhuman species bind to human globulins either free in serum or attached to antigens on RBCs.

Preparation of AHG

1. Human serum is injected to a laboratory animal such as rabbits.
2. The human globulin behaves as foreign antigen.
3. The rabbit's immune system is triggered.
4. Antibodies to human globulin are produced.

AHG reagent either...

- **(polyspecific)**: prepared by combining anti-IgG & anti complement. The reagent may also contain antibodies of other specificities such as anti-IgM, anti-IgA, anti C-3, or anti C-4.
- **Monospecific**: contains only a single antibody: anti-IgG or only anticomplement.

AHG tests

- Either
 - Direct AHG test (**DAT**)

OR

- Indirect antiglobulin test (**IAT**)

Direct AHG test (dat)

- Clinical conditions that can result In in-vivo coating of RBCs are:
 1. Haemolytic disease of the newborn (HDN): maternal Ab coating fetal RBCs.
 2. Haemolytic transfusion reaction (HTR): recipient Ab coating donor RBCs.
- DAT detect in vivo sensitization of RBCs.
 1. Autoimmune haemolytic anaemia: auto Ab coating individual's RBCs.

Method of DAT

1. Into a test tube (12x75), add 1 drop of 2-3% suspension of the test RBCs.
2. Wash the cells three times with saline (ensure that all saline is completely decanted after the last wash).
3. Add 2 drops of AHG reagent.
4. centrifuge, resuspend the cells, and read the result.

Indirect antiglobulin test (IAT)

- IAT detect in-vitro sensitization of RBCs.
- IAT used in the following situations:
 1. RBC phenotype, e.g. weak D (D^u method).
 2. Antibodies screening, identification, and titration.

Method of IAT

1. Into a test tube (12x75), add 2 drops of the test serum and 1 drop of 2-3% suspension of Oscreening RBCs.
2. Mix, and incubate for 30mins in 37°C.
3. Wash the cells three times with saline (ensure that all saline is completely decanted after the last wash).
4. Add 2drops of AHG reagent.
5. centrifuge, resuspend the cells, and read the result.