AGGLUTINATION

TESTS BASED ON AG/AB REACTIONS

•All tests based on Ag/Ab reactions will have to depend on lattice formation or they will have to utilize ways to detect small immune complexes

•All tests based on Ag/Ab reactions can be used to detect either Ag or Ab.

COMPLEX MAY BE DIRECTLY VISIBLE OR INVISIBLE:

-Directly visible - agglutination

-Invisible

• requires specific probes (enzyme-labelled antiimmunoglobulin, isotope-labelled antiimmunoglobulin, etc.)

• binds Ag-Ab complex and amplifys signals

• signals can be measured by naked eyes or specific equipment e.g. in ELISA, RIA, IFA

METHODS FOR AG-AB DETECTION

- Precipitation
- Agglutination
- Hemagglutination and hemagglutination inhibition
- Viral neutralization test
- Radio-immunoassays
- ELISA
- Immunoflourescence
- Immunoblotting
- Immunochromatography

-AGGLUTINATION

- 1- Active (direct) agglutination.
- 2- Passive (Indirect) agglutination.
- 3- Reverse Passive agglutination.
- 4- Hemagglutination
- 5- Hemagglutination inhibition.
- 6- Viral Hemagglutination.

-PRECIPITATION(IMMUNODIFFUSION)

A-Immunodiffusion in gel (Passive e.g RID- Active e.g electroimmunodiffusion)

B- Fluid phase Immunoprecipitation e.g. nephelometry and turbidimetry

Agglutination: The interaction between antibody and a particulate(Insoluble) antigen results in visible clumping called agglutination.

Particulate antigen include:

- bacteria,
- white blood cells,
- red blood cells,
- latex particles



Phases of Agglutination

• Agglutination is a two-Phase reaction that results in the formation of a stable lattice network

• Primary Phase (Sensitization)

- Ab reacts with a single antigenic determinant on the surface of Ag.

• Secondary Phase (Lattice formation)

- Ab must be able to bridge the gap between particles so that at least one Fab portion is attached to an antigenic determinant on each of two adjacent particles, is dependent on environmental conditions and the relative concentrations of antigen and antibody.

This represents what occurs during stage one of agglutination: Sensitization



Antibody molecules attach to their corresponding Antigenic site (epitope) on the red blood cell membrane. There is no visible clumping.

This represents what occurs during stage 2 of agglutination: Lattice Formation



Antibody molecules crosslink RBCs forming a lattice that results in visible clumping or agglutination.

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AGGLUTININ AND AGGLUTINOGEN

• An **agglutinin** is an antibody that interacts with antigen on the surface of particles such as erythrocytes, bacteria, or latex particles to cause their agglutination.

• An **agglutinogen** is an antigen on the surface of particles such as red blood cells that react with the antibody known as agglutinin to produce agglutination.

• The most widely known agglutinogens are those of the ABO and related blood group systems.

The following examples of agglutination reactions :

1. Rheumatoid factor latex agglutination

- 2. Bacterial latex agglutination
- 3. Coombs test
- 4. Blood typing

AGGLUTINATION

IN THIS TEST THE ANTIGEN IS PARTICULATE (VISIBLE, BIG AND INSOLUBLE) (E.G. BACTERIA AND RED BLOOD CELLS) OR AN INERT PARTICLE (LATEX BEADS) COATED WITH ANTIGEN.

ANTIBODY IS DIVALENT AND CROSS LINKS THE MULTIVALENT ANTIGEN TO FORM A LATTICE NETWORK OR CLUMPS (AGGLUTINATION).

□ THIS REACTION CAN BE PERFORMED IN A TUBE OR ON A GLASS SLIDE E.G. ABO BLOOD GROUPING.

FACTORS INFLUENCING THE REACTION:

• ELEVATION OR DECREASE OF TEMPERATURE.

• MOTION (SHAKING, STIRRING, CENTRIFUGATION).

• PH.

• CLASS OF ANTIBODY (IGM/IGG).

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When Abs & Ags are present in equimolar ratios They form insoluble complexes that

Ppt (ZONE OF EQUIVALENCE)

• Decreased amounts of ppt are formed in zones of Ag or Ab excess.

PROZONE PHENOMENA

In an agglutination or precipitation reaction, the zone of relatively high antibody concentrations within which no reaction occurs. As the antibody concentration is lowered below the prozone, the reaction occurs.(solve by dilution).

This phenomenon may be due simply to antibody excess or it may be due to blocking antibody or to nonspecific inhibitors in serum.

A)Qualitative agglutination tests

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody.

The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen .

B) Quantitative Agglutination Test

The Ab titre can be determined using serial dilution of the patient serum.

Serial Dilution

A *serial dilution* is simply a series of simple dilutions which amplifies the dilution factor quickly beginning with a small initial quantity of material (i.e., bacterial culture, a chemical, orange juice, etc.). The source of dilution material for each step comes from the diluted material of the previous. In a serial dilution the *total dilution factor* at any point is the *product* of the individual dilution factors in each step up to it.

Final dilution factor (DF) = DF1 \times DF2 \times DF3 etc.

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DIRECT AGGLUTINATION

Principle

• combination of an insoluble particulate antigen with its soluble antibody

- forms antigen-antibody complex

- particles clump/agglutinate
- used for antigen detection Examples:

- bacterial agglutination tests for sero-typing and sero-grouping e.g., Vibrio cholerae, reeî Salmonella spp



POSITIVE

PASSIVE AGGLUTINATION

• Definition - agglutination test done with a soluble antigen coated onto a particle (Ag is fixed to a solid surface)

• Applications

- Measurement of antibodies to soluble antigens

PASSIVE (INDIRECT) AGGLUTINATION

Principle

- coating antigen onto the surface of carrier particles like red blood cells, latex, gelatin, bentonite or charcoal.

-background clears

Examples of types

- latex agglutination

- passive hemagglutination (treated red blood cells made resistant)

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Examples of tests

- agglutination for leptospirosis

-Widal test (typhoid fever)

REVERSE PASSIVE AGGLUTINATION

Antibody attached to carrier particle instead of antigen. (The Ab is fixed to a solid surface)

Principle:

- antigen binds to soluble antibody coated on carrier particles and results in agglutination are

-detects antigens.

Example

- detecting cholera toxin

Agglutination: Performance, applications

ADVANTAGES

- sensitive for antibody detection

LIMITATIONS

- Prozone phenomenon:

• requires the right combination of quantities of antigen and antibody

· handled through dilution to improve the match

Time taken

-10-30 minutes

ADVANTAGES:

-Portable.

-Rapid.

-efficient.

-quick and simple.

N.B: they are semiquatitative assays.

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APPLICATIONS:

More than 100 infectious disease.

More than 60 chemical analyte e.g. hCG, CRP,

ASO, fecal occult blood.

HEMAGGLUTINATION

Principle

- It is a type of agglutination test performed on RBCs.

- many human viruses have the ability to bind to the surface structures on red blood cells from different species thereby causing agglutination

Example

- influenza virus binds to fowl's red blood cells

HAEMAGGULTINATION TESTS:

It has two types:

 \Box Active: the antigen is the RBC itself.

-Viruses can clump red blood cells from one species or another (active hemagglutination)

- Example is the test used in ABO grouping.

 \Box **Passive:** the antigen here is not the RBC. The RBC absorbs it and expresses it on the surface.

- It will form clumps when mixed with antibodies.

- i.e. red cells are passive carriers .

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HEMAGGLUTINATION INHIBITION

Principle

Antibodies to the virus in the patient serum bind to the virus; blocks binding sites on the viral surfaces

12.

- prevents the virus from agglutinating the red cells

Example

- detecting antibodies to influenza and dengue viruses

HEMAGGLUTINATION:

Performance, applications

ADVANTAGES

- highly specific
- can be used as gold standard

LIMITATIONS

- technically demanding
- time consuming
- cannot distinguish IgG from IgM

Time taken

– 1 day

APPLICATIONS

- Blood typing
- Bacterial infections
- -Fourfold rise in titer
- Practical considerations
- Easy
- Semi-quantitative