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3 rd. year

Module : Clinical Immunology

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Session : 3 (Practical)

Lecture title : Serology part 1

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What is the serological diagnosis of infections?

- It is the diagnosis of infectious diseases based upon the Antigen-Antibody (Ag-Ab) reactions.
- Ag – Ab reaction is the union of an Ag with its **specific Ab**, best detected when each is in optimal concentration.
- The type of such reaction depends upon the nature of both Ag and Ab used.
- These reactions are mostly used in the laboratory (*In Vitro*) to detect either the **Ag (Direct diagnosis)** or the **Ab (indirect diagnosis)**.



Types of Ag-Ab Reactions (*In Vitro*):

Agglutination.

Precipitation.

Enzyme linked immunosorbent assay
(ELISA).

Immunofluorescence Assay (IFA)

Immuno-chromatography (ICT) .

Immuno-blotting assays.



Sensitivity of different serologic tests in detecting antibody levels in Ug/mL in the unknown sample:

SN	Test	Sensitivity level (Ug/mL)
1	Gel-diffusion	30
2	Ring Precipitation	18
3	Bacterial agglutination	0.05
4	Passive Hemagglutination	0.01
5	Hemagglutination inhibition test	0.005
6	Immunofluorescence	0.005
7	ELISA	0.0005
8	Bacterial neutralization	0.00005

N.B. The lower the level of detection the higher the sensitivity of the test.



Application of Ag-Ab Reactions (Serology):

1. **Diagnosis** of many infectious diseases (direct antigen detection or measuring Immunity to infection) especially for **non-culturable** or delayed growing microorganisms.
2. Estimation of the Severity or **stage** of diseases.
3. Determination of the **Response to treatment**.
4. **Epidemiological** studies.
5. Diagnosis of **congenital** infections.
6. Screening **donation** of blood and tissues.
7. **Non-infectious** diagnostic applications (Tumors, Autoimmune diseases, endocrinology, ...etc.



Agglutination

- Is the visible **clumping** of particulate (**insoluble**) Ag with its specific Ab forming visible lattice. The Ab is the **divalent** agglutinating one either **IgG** or **IgM** type.
- The reaction can be either on **slide** or **tube** agglutination.
- It can be **direct** or via the use of carrier (**Passive or indirect**) e.g. Latex, RBCs (hemagglutination), or Staph protein A (Coagglutination). **Indirect more easily visible.**
- The reaction can be **qualitatively** or **quantitatively** expressed.



Examples of agglutination reactions:

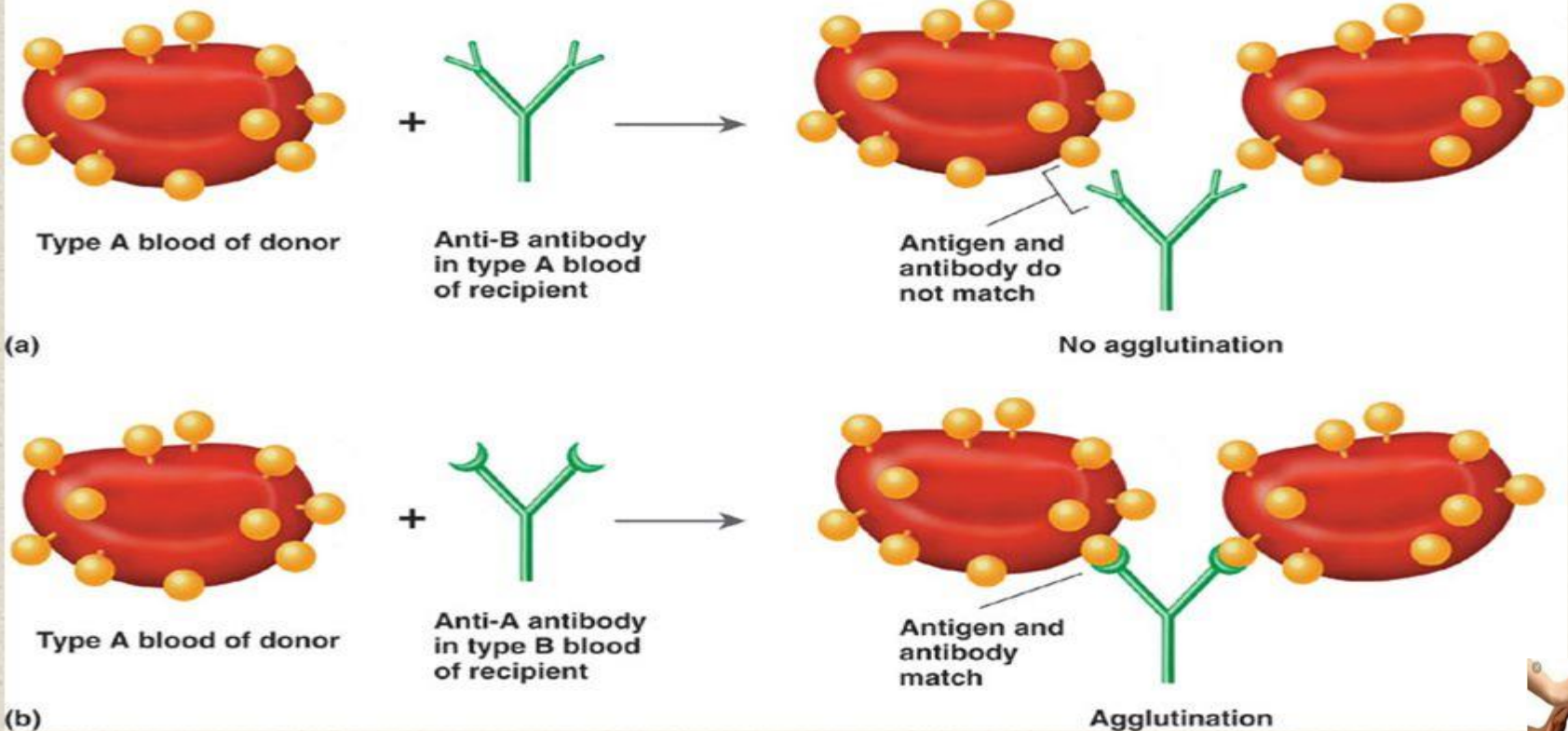
1. Direct **slide** detection of a bacterial or viral **antigens** in a lesion or culture.
2. Direct **tube** agglutination (**Classical Widal** test) for diagnosis of typhoid fever (replaced now by Latex)
3. Indirect **Latex** tests commonly used in most microbiology laboratories now adays.
4. Indirect **passive hemagglutination** tests e.g. Treponema Pallidum Hemagglutination (**TPHA**) for diagnosis of syphilis which is more specific.
5. **Brucella slide or tube agglutination** test (Prozone phenomenon).



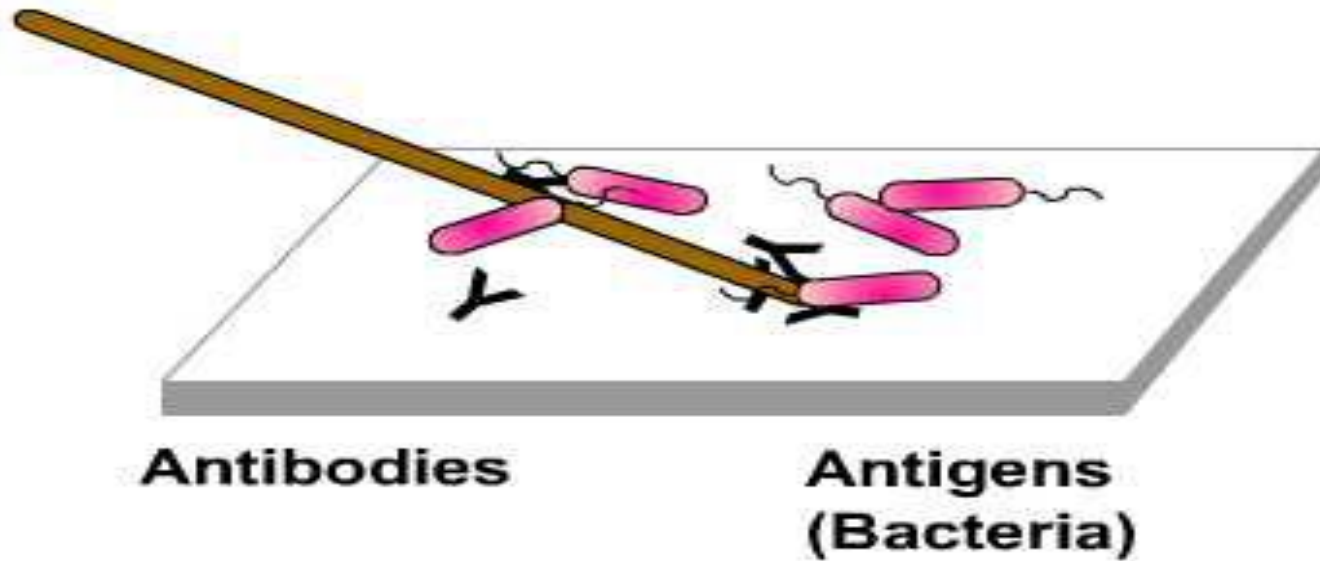
Direct agglutination for blood grouping:

Agglutination Reaction

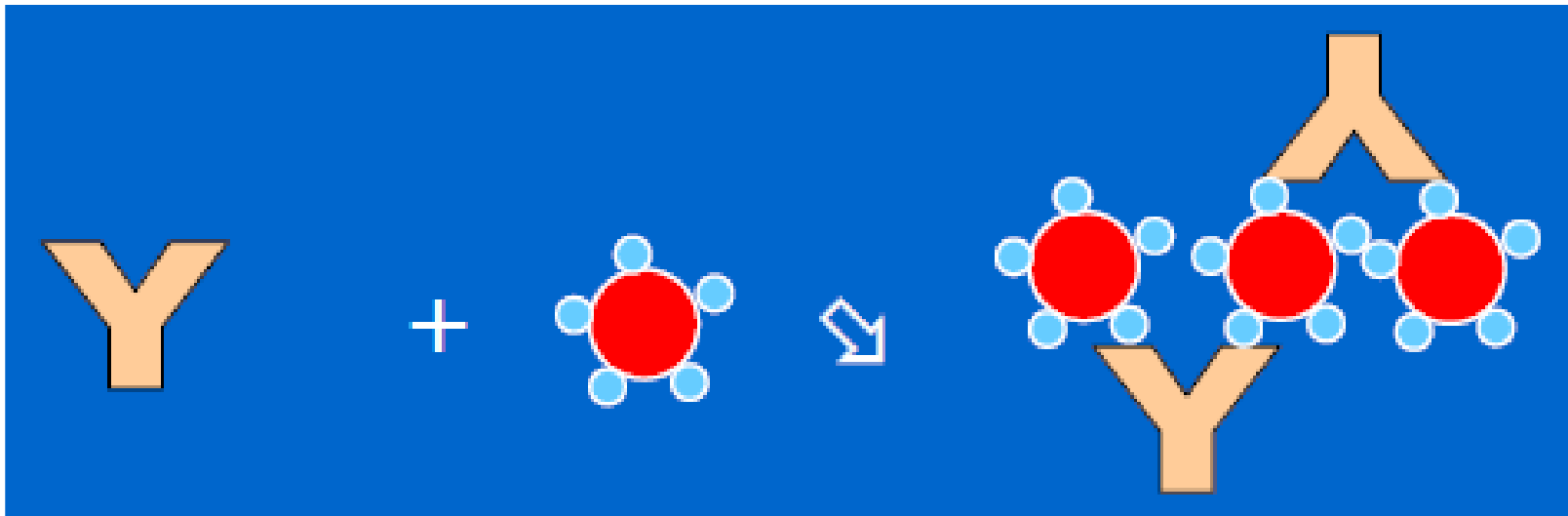
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Direct agglutination



Passive hemagglutination



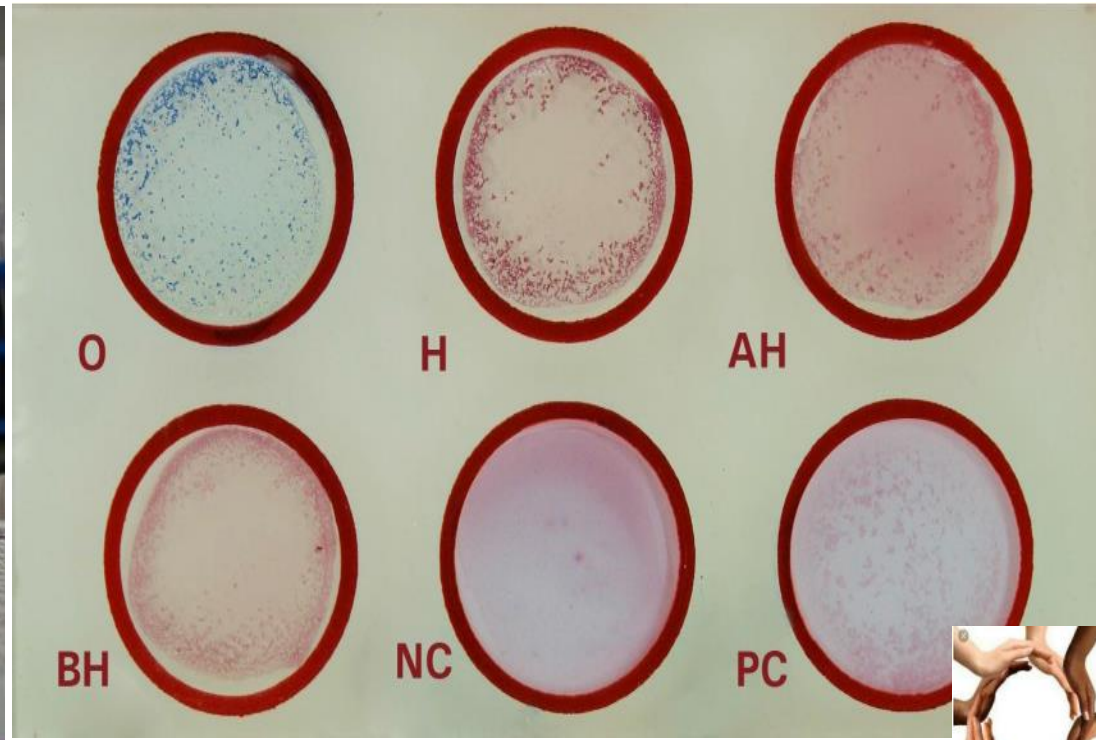
Ab

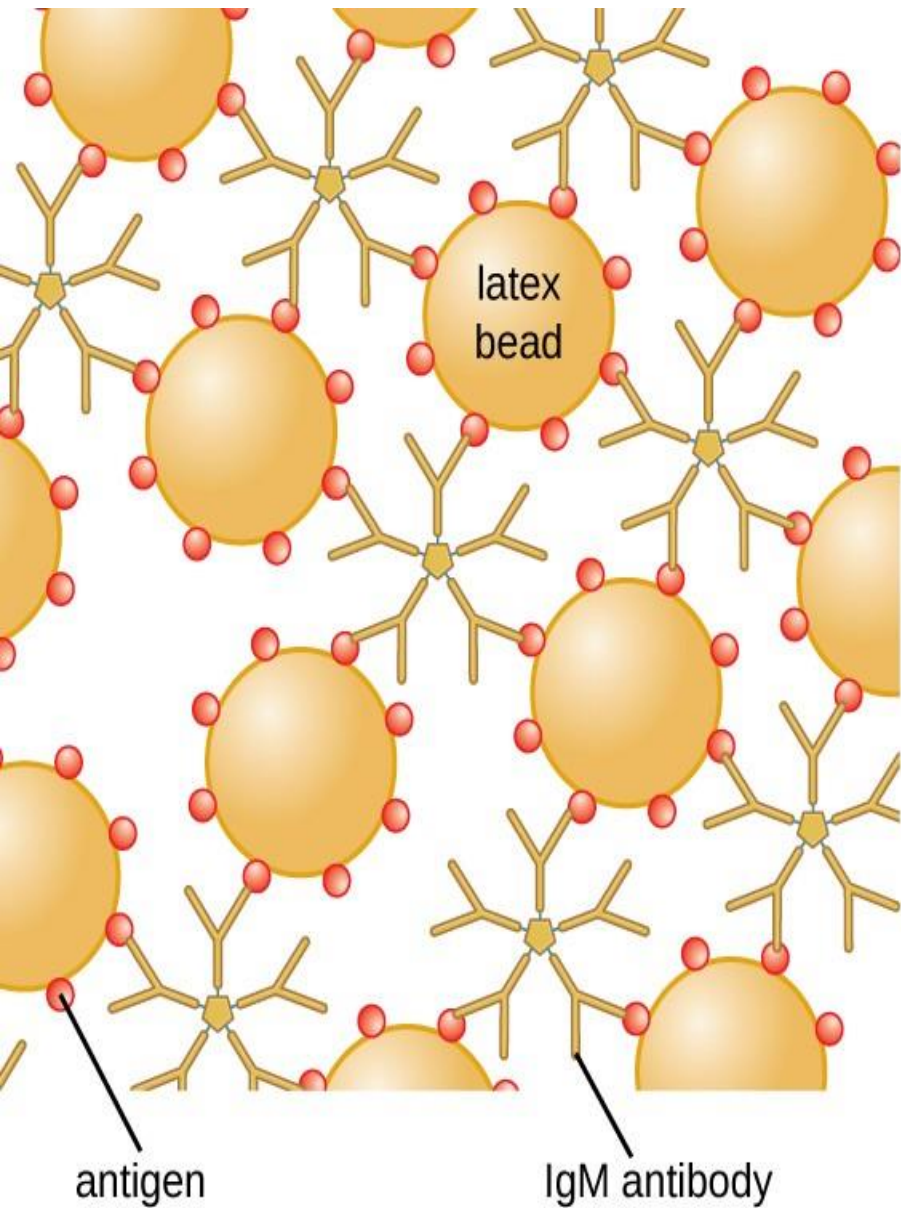
Ag coated RBC



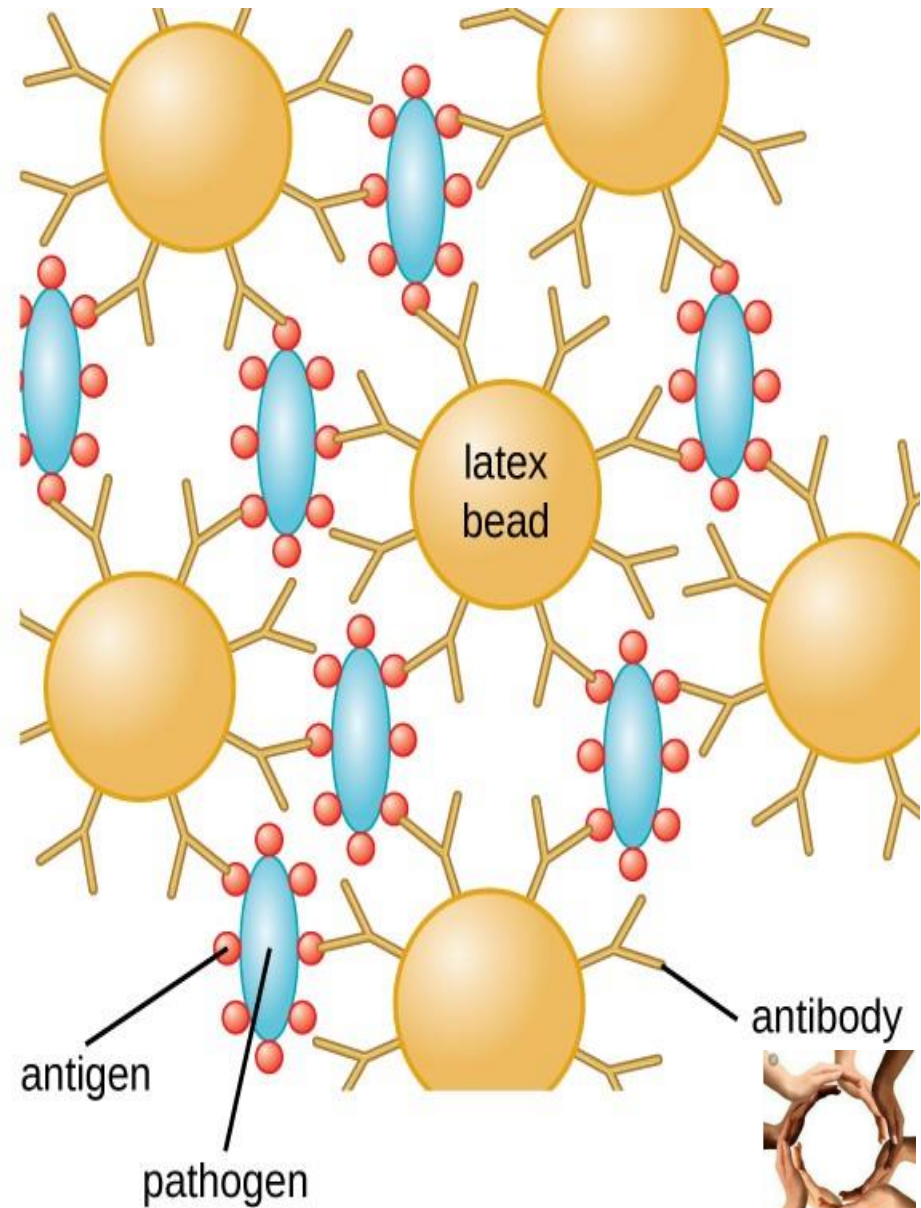
Widal latex slide agglutination tests for diagnosis of typhoid fever.

Weil-Felix Test used for the diagnosis of rickettsial disease e.g., epidemic typhus which is non-specific test using the Heterophil Ag of proteus species.





(a) positive agglutination test for antibodies



(b) positive agglutination test for antigens

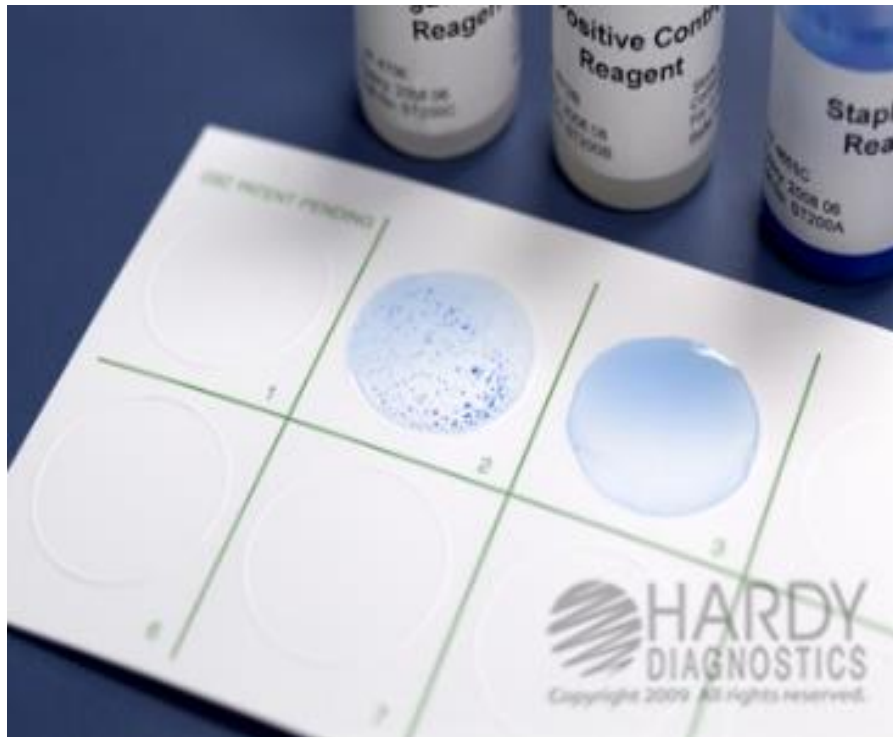
Why latex test are more common?

Easy to manufacture, to use, cheap, clearly visible.

No instruments

Can be coated with any Ag (**Soluble**) to detected Ab

Can be coated with the Ab to detect the Ag.



HCG Latex Agglutination Test



Pregnancy Test latex



MRSA Latex



Toxoplasmosis - Latex Agglutination



Cryptococcal Antigen Latex



Examples of diseases that can be detected using agglutination tests:

1. Typhoid Fever: Detected using the Widal test, which identifies antibodies against *Salmonella Typhi*.
2. Rheumatic Fever: Detected by measuring antibodies against *Streptococcus pyogenes* using the Lancefield classification.
3. Bacterial Infections: Various bacterial infections can be detected through agglutination tests, including those caused by *Staphylococcus aureus* (e.g., the coagulase test).



Examples of diseases that can be detected using agglutination tests:

4. Hemolytic Disease of the Newborn (HDN): Detected using the Coombs test, which identifies antibodies against red blood cells.
5. Leptospirosis: Detected using the microscopic agglutination test (MAT) to identify antibodies against *Leptospira* species.
6. Brucellosis: Detected using agglutination tests to identify antibodies against *Brucella* species.



Precipitation Tests:

It the reaction between **soluble** Ag and its specific Ab.

It can be Precipitation in **solution** or in **gel**.

Example of Precipitation in solution include the **Ring test** for detection of infectious Ag in CSF.

Another example of precipitation **in solution** is the slide test for detection of **pneumococcal capsule**.

Precipitation in **gel** is used for the quantification of Immunoglobulins in patient serum (less common) in form of single, double radial immuno-diffusion or immuno-electrophoresis.



Flocculation tests

Flocculation is an antigen-antibody reaction that occurs if the antigen is neither cellular nor soluble, but is an insoluble particulate.

The flocculation reaction is a special type of **precipitation** reaction.

Example of the flocculation reaction in current use are the Venereal Disease Research Laboratory (**VDRL**) test and the rapid plasma regain (**RPR**) used for the diagnosis of syphilis.

These tests are **non-specific** (non-treponemal) or called standard test of syphilis (**STS**).



The antigen used is **cardiolipin**, a hapten from normal beef heart those cross-reacts with a **heterophile** (Heterogenetic) antigen of the spirochete of syphilis so to detects antibodies to *Treponema pallidum*.

Cholesterol particles with water-insoluble cardiolipin on their surface are used in the test.

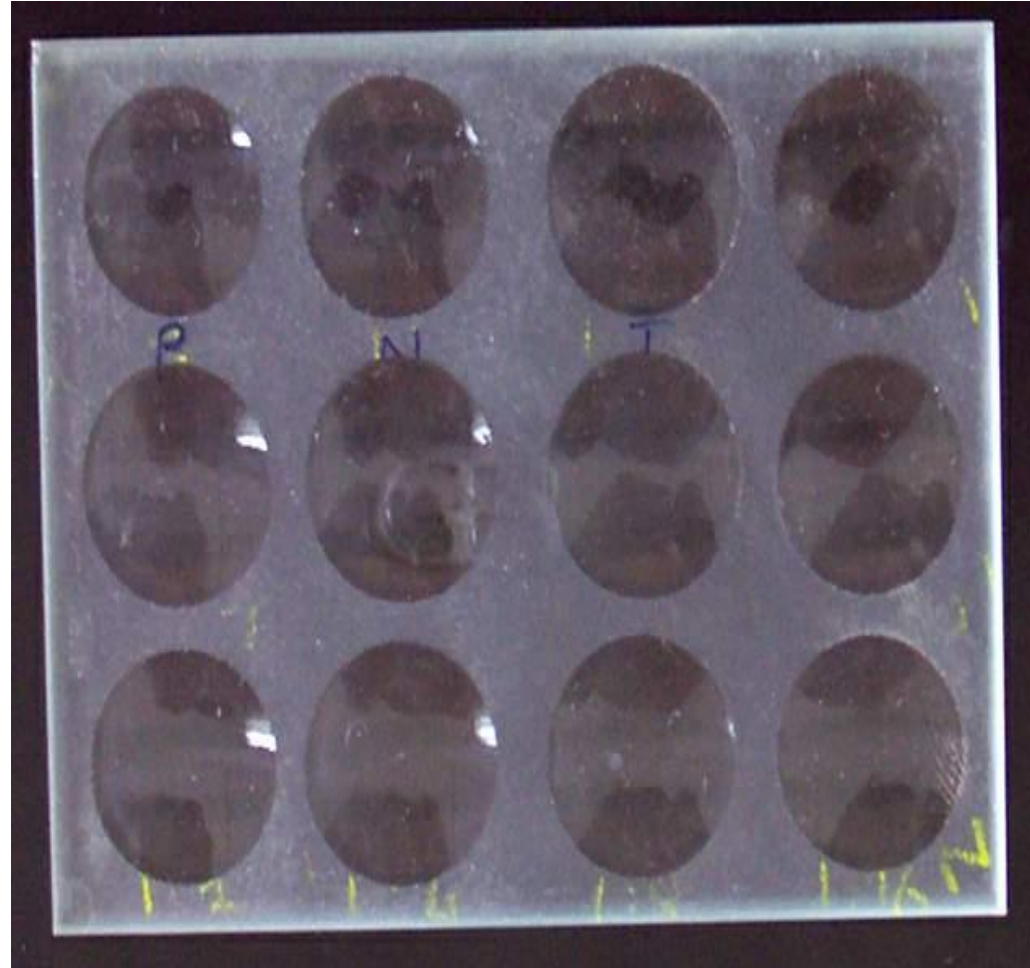
Visible aggregates form in the presence of an antibody (regain) in the serum of patients with syphilis.

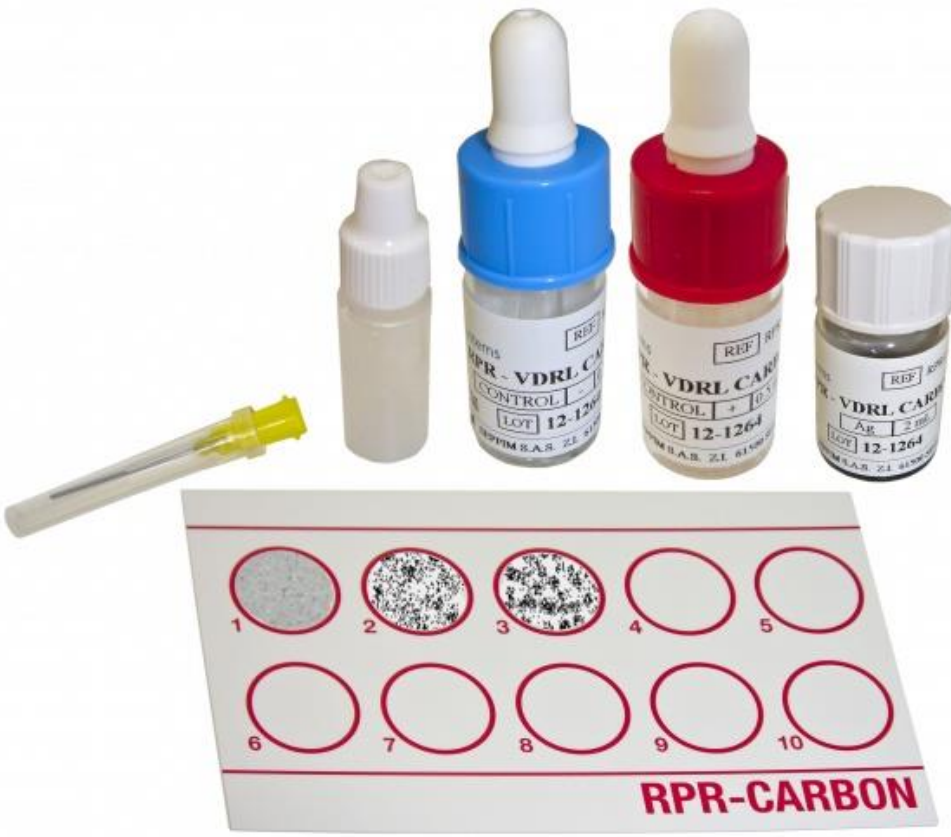
VDRL has many limitation and not in current use nowadays and the in current use is the RPR.





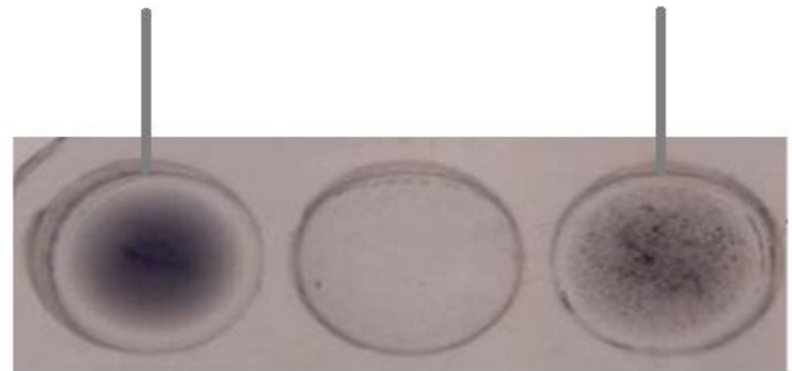
VDRL Slides





Non-Reactive

Reactive



RPR Test



Here are some diseases that can be detected using precipitation tests:

1. Rheumatoid Arthritis: The rheumatoid factor (RF) test helps detect antibodies associated with this autoimmune condition.
2. Systemic Lupus Erythematosus (SLE): Tests that detect antinuclear antibodies (ANAs) can indicate the presence of SLE.
3. Syphilis: The Wassermann test, although less common now, is a historical method for detecting syphilis through the identification of antibodies against *Treponema pallidum*.
4. Infectious Mononucleosis: The heterophile antibody test (Monospot test) detects antibodies related to the Epstein-Barr Virus (EBV) infection.



Here are some diseases that can be detected using precipitation tests:

5. Fungal Infections: Certain fungal infections can be diagnosed by detecting specific antibodies that form precipitates when mixed with fungal antigens.
6. Hepatitis: Some hepatitis infections can be identified through precipitation tests for specific antibodies in the serum.
7. Coccidioidomycosis: Precipitation tests can be used to detect antibodies against *Coccidioides immitis*, the fungus causing this disease.
8. Allergic Reactions: Certain precipitation tests can be used to identify specific IgE antibodies associated with allergies.





THANKS

