Serological tests 2
(Antigen antibody interactions)
Lab 3

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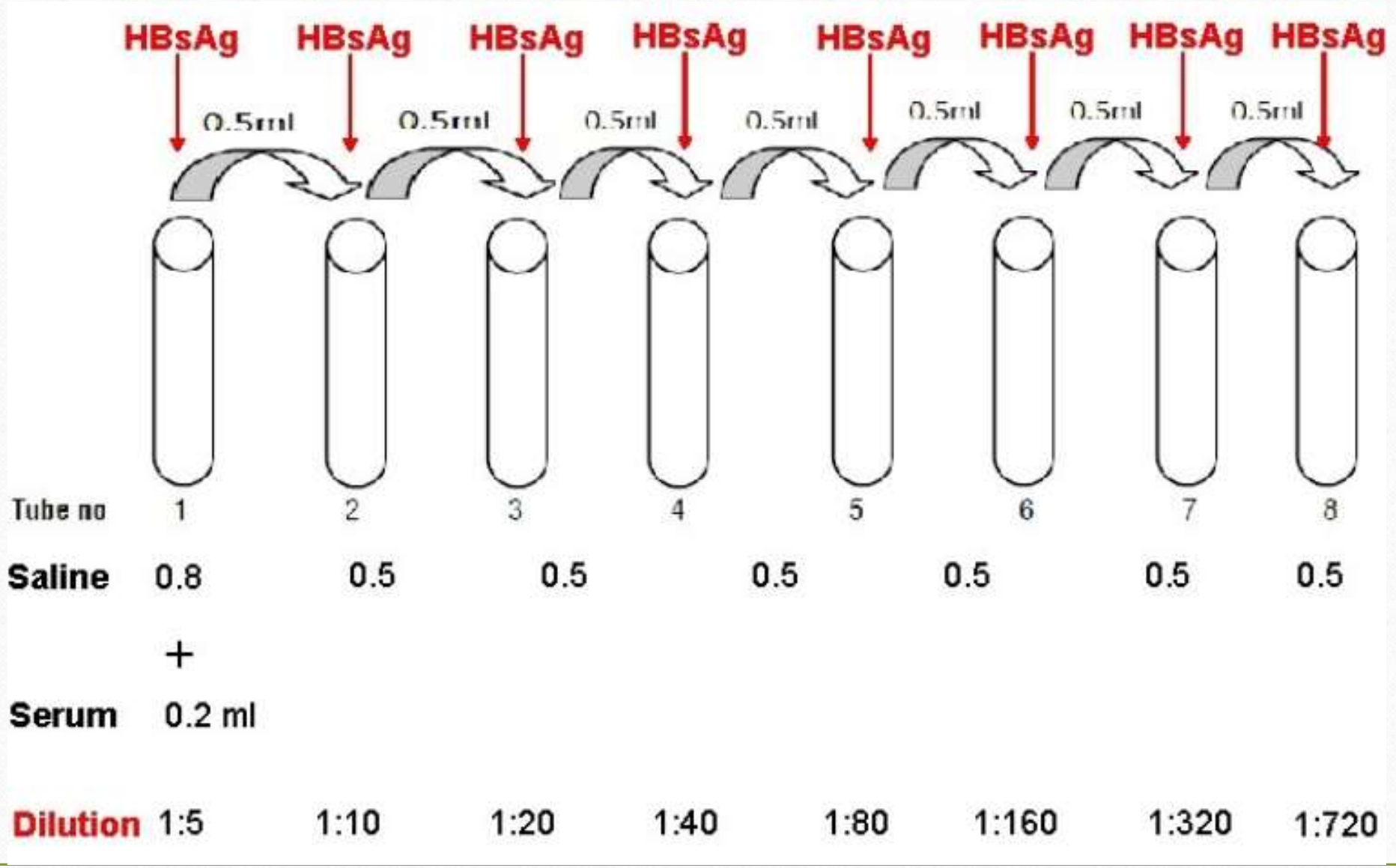
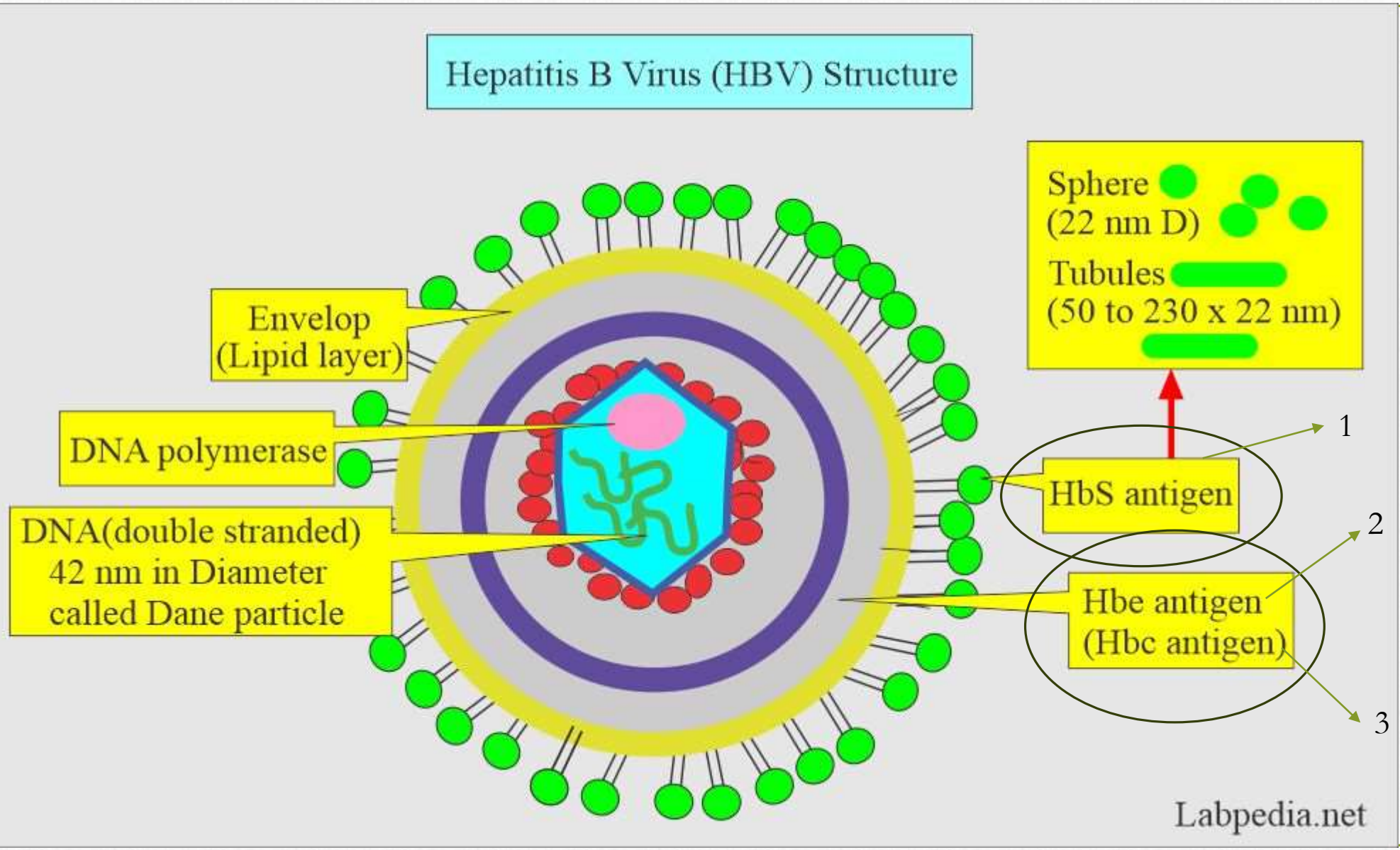
Examples of kits


Users Manual
Brucella Antibody
ELISA
An ELISA testkit to detect antibodies against polysaccharide epitopes of Brucella melitensis in serum and milk samples


CODE
J79
PACK
25T
RHEUMATOID FACTOR
LATEX TEST KIT (R.A. TEST KIT)
Contents
Reagent 1 : RF Antigen (Gamma Globulin)
Reagent 2 : Positive Control
Reagent 3 : Negative Control
Accessories : Disposable Plastic Dropper, Disposable Applicator Sticks, Rubber Teat, Glass Slides
Shake well before use
CE
ISO 9001 & 13485
Certified Company
Mfg. Lic. No. G-639
BEACON
DIAGNOSTICS PVT. LTD.

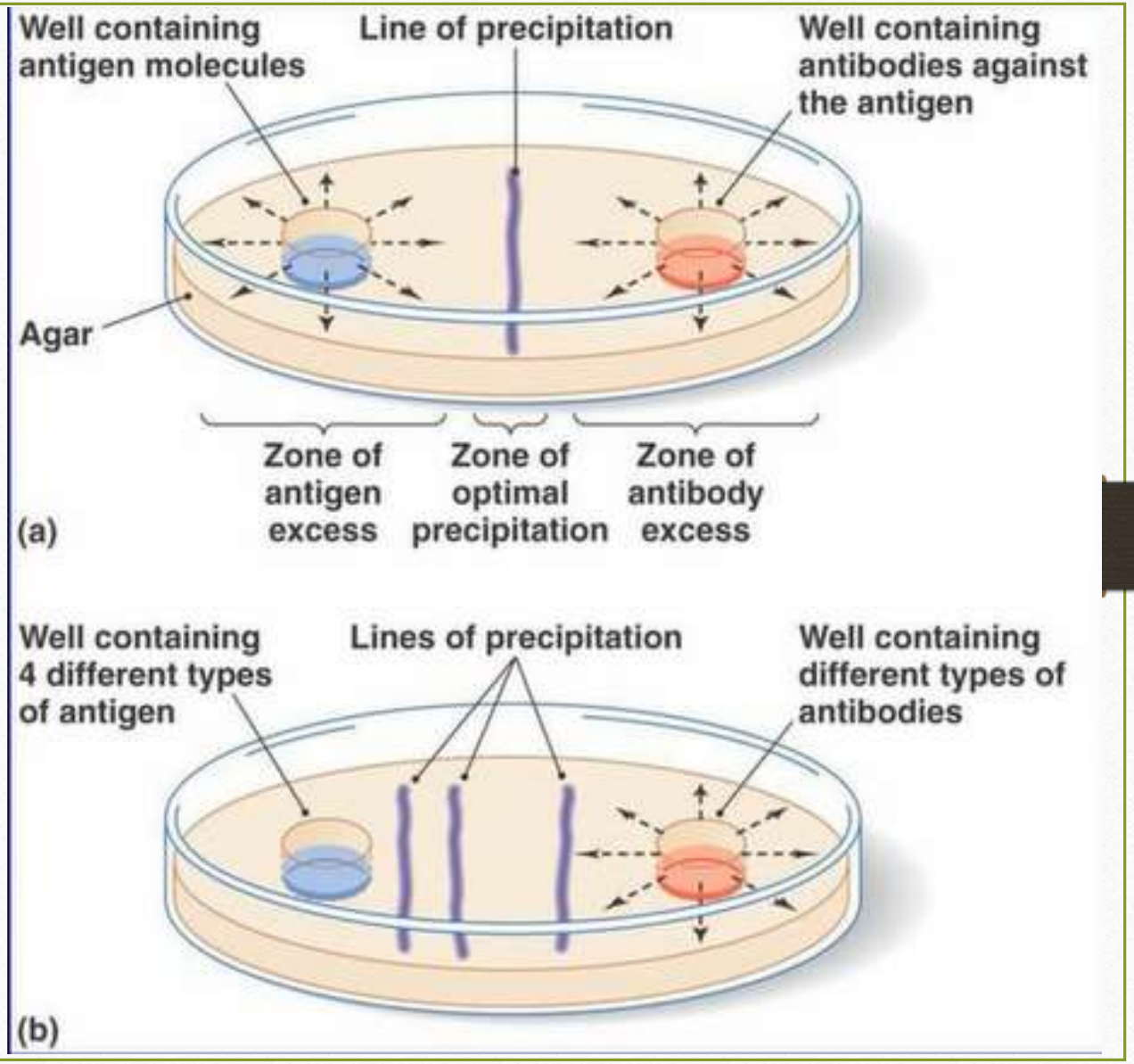
Blood grouping kit

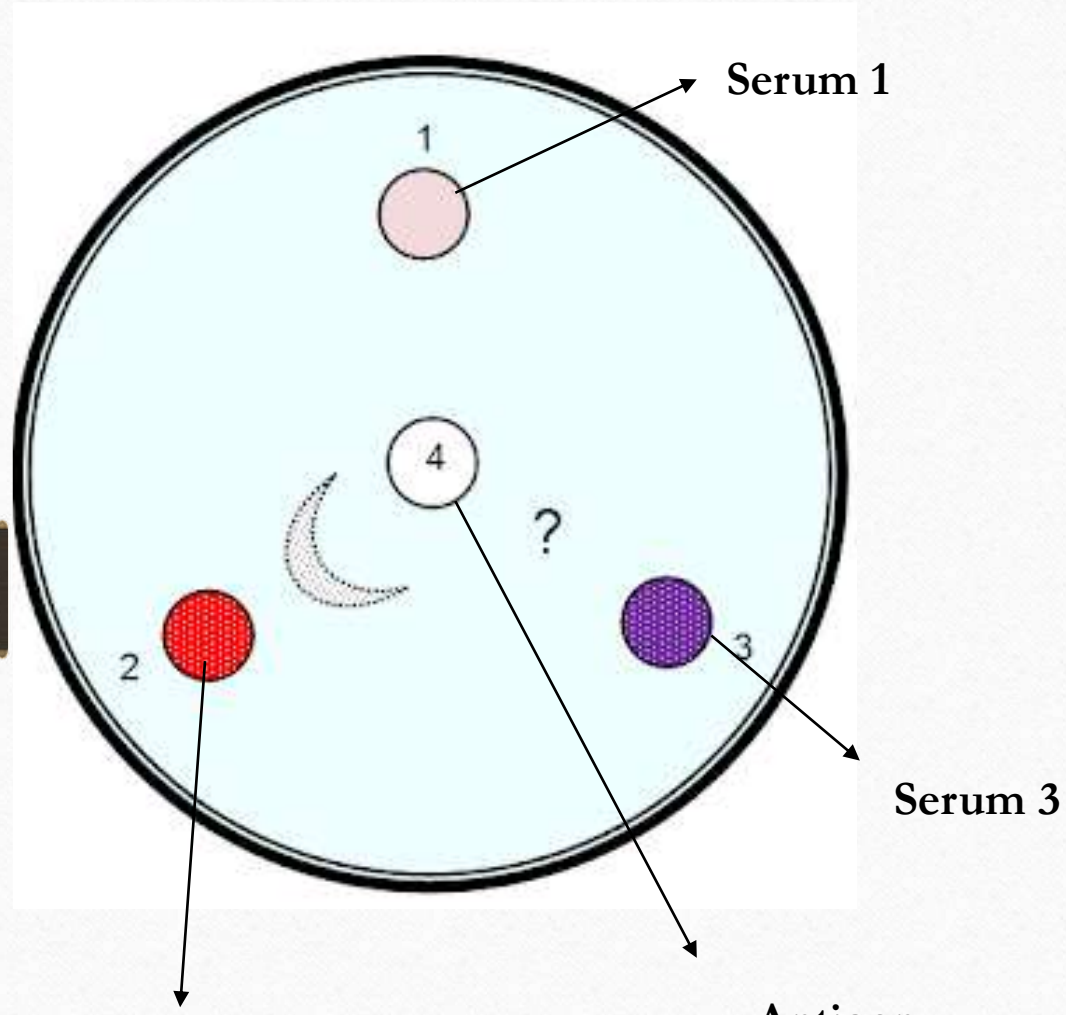

HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
HBsAg
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
0.5ml
Tube no 1 2 3 4 5 6 7 8
Saline 0.8 0.5 0.5 0.5 0.5 0.5 0.5
+
Serum 0.2 ml
Dilution 1:5 1:10 1:20 1:40 1:80 1:160 1:320 1:720

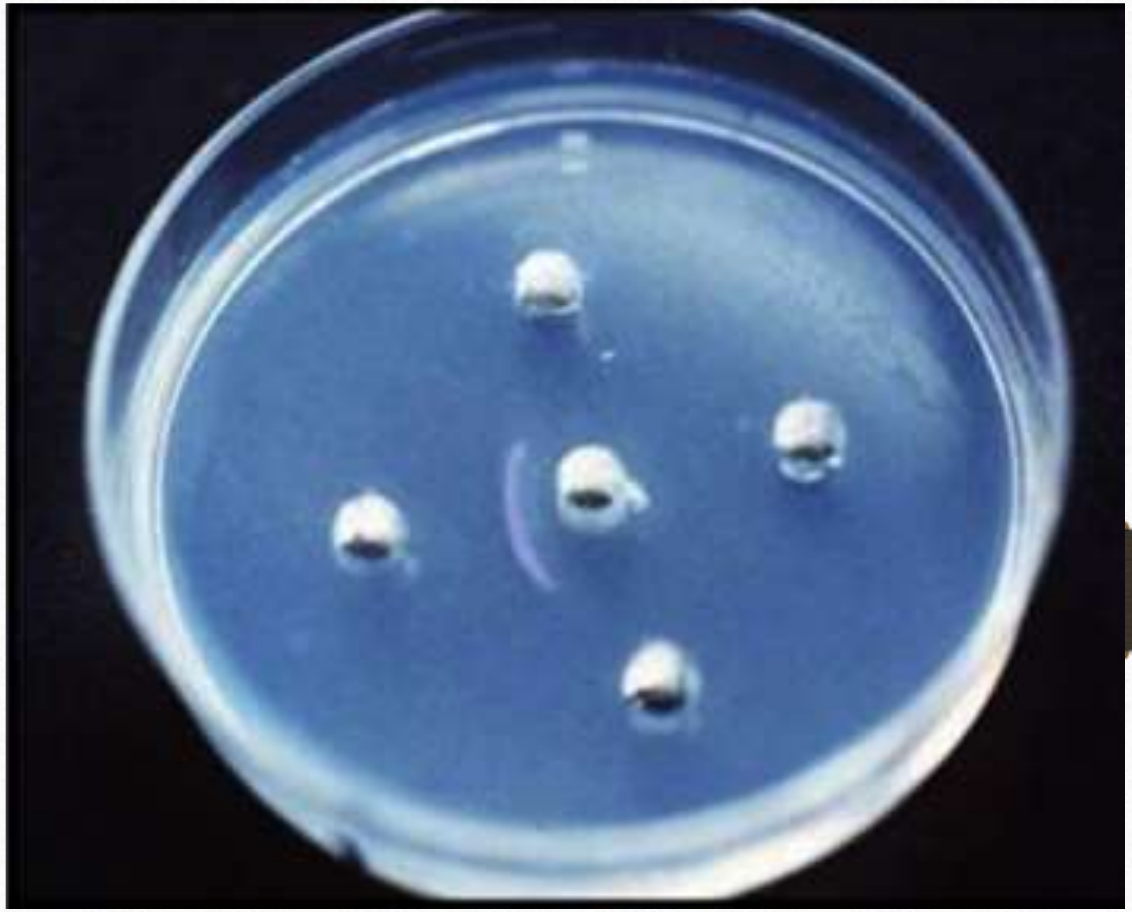

Hepatitis B Virus (HBV) Structure
Sphere
(22 nm D)
Tubules
(50 to 230 x 22 nm)
Envelop
(Lipid layer)
DNA polymerase
DNA(double stranded)
42 nm in Diameter
called Dane particle
HbS antigen
Hbe antigen
(Hbc antigen)
1
2
3
Labpedia.net

Ouchterlony test

- The Ouchterlony Test is used for qualitative analysis, not for quantitative measurement.



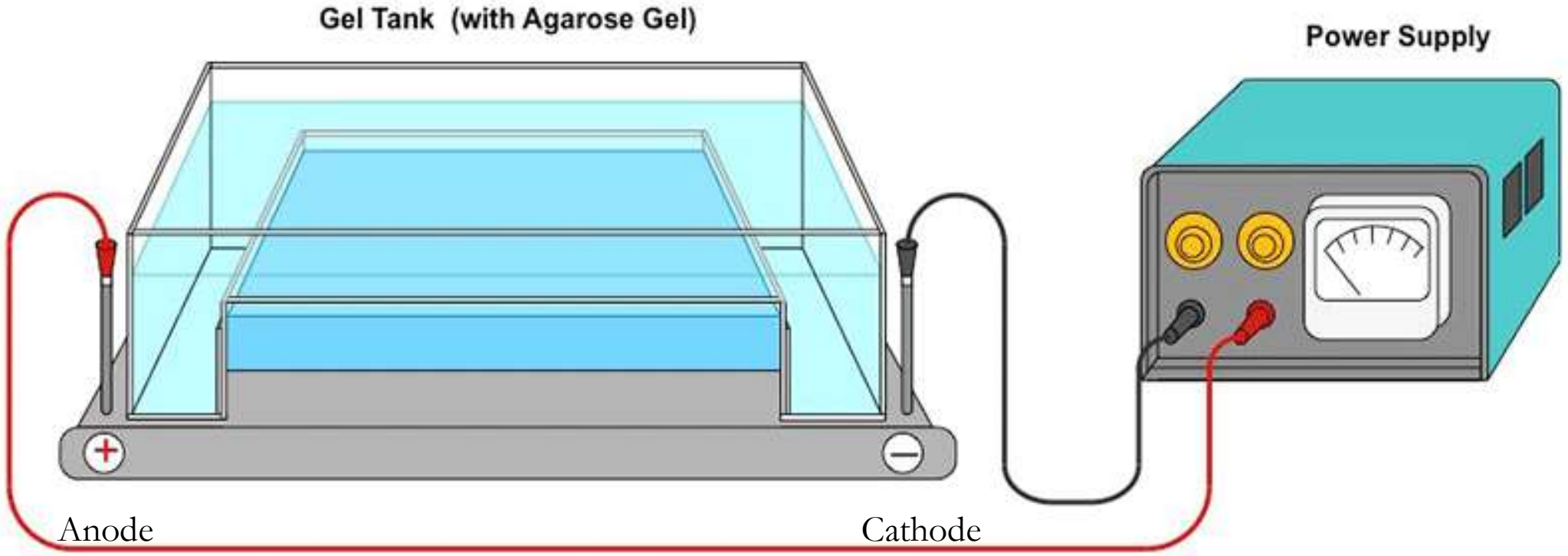

Serum 1
1
4
?
2
3
Serum 3
Antigen
Serum 2



Immunoelectrophoresis

- When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size.
- Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration, and diffusion is allowed to occur.
- Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with their antibody.

Immunoelectrophoresis



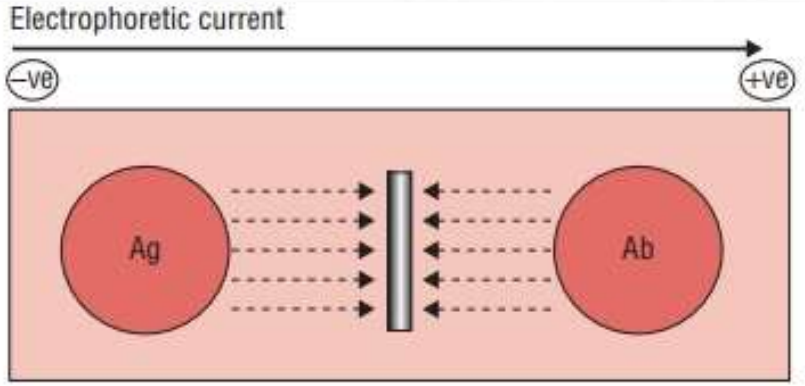


FIG. 14-6. Counter-current immunoelectrophoresis.

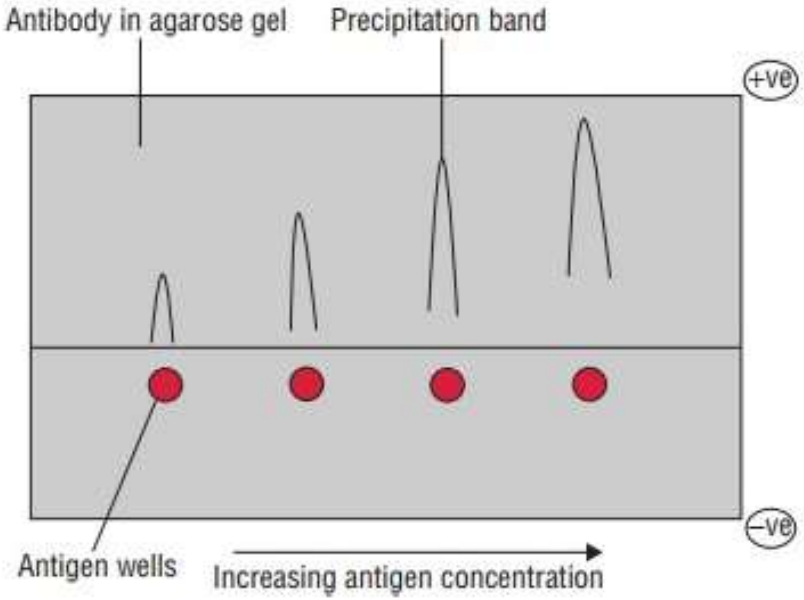
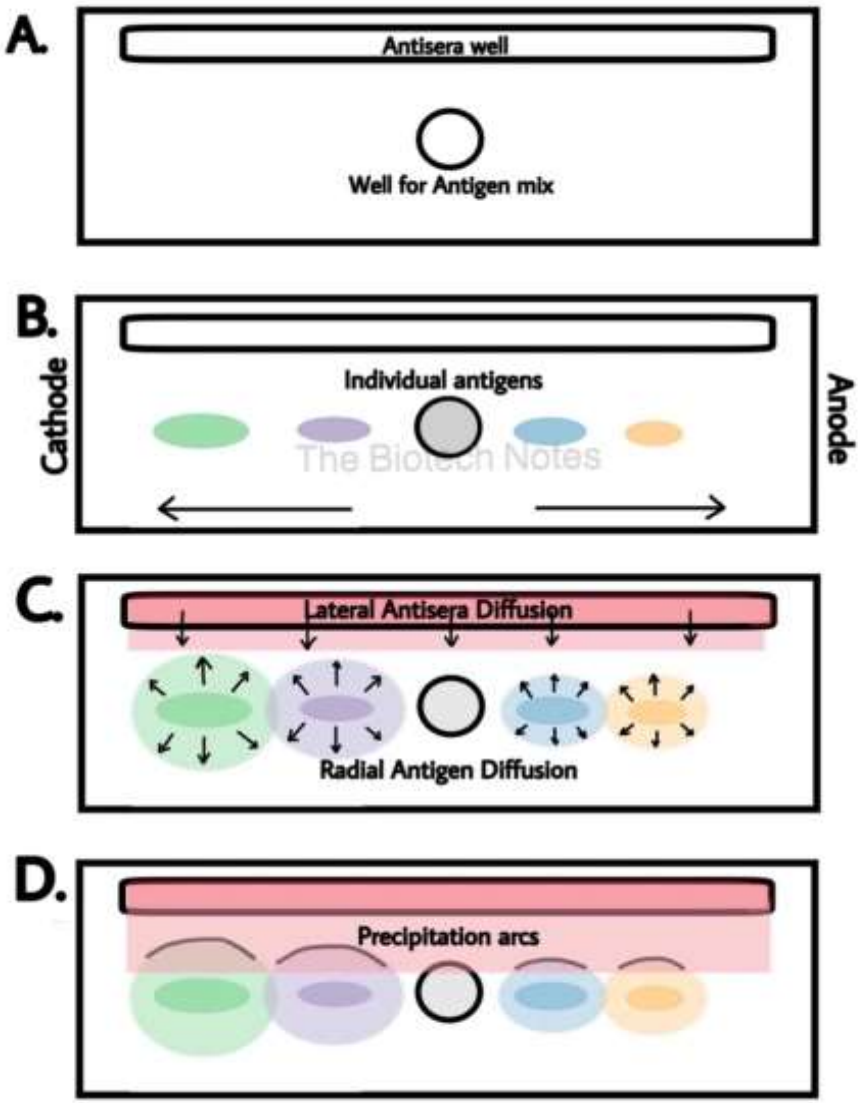


FIG. 14-7. Rocket electrophoresis.



Neutralization Tests

- Used to assess the effectiveness of neutralizing antibodies against specific pathogens or toxins.
- These tests applications in the fields of virology, immunology, and vaccine development.
- The primary purpose of neutralization tests is to determine if a given antibody or serum can neutralize the infectivity or toxicity of a particular pathogen or toxin.

These tests are broadly of two types:

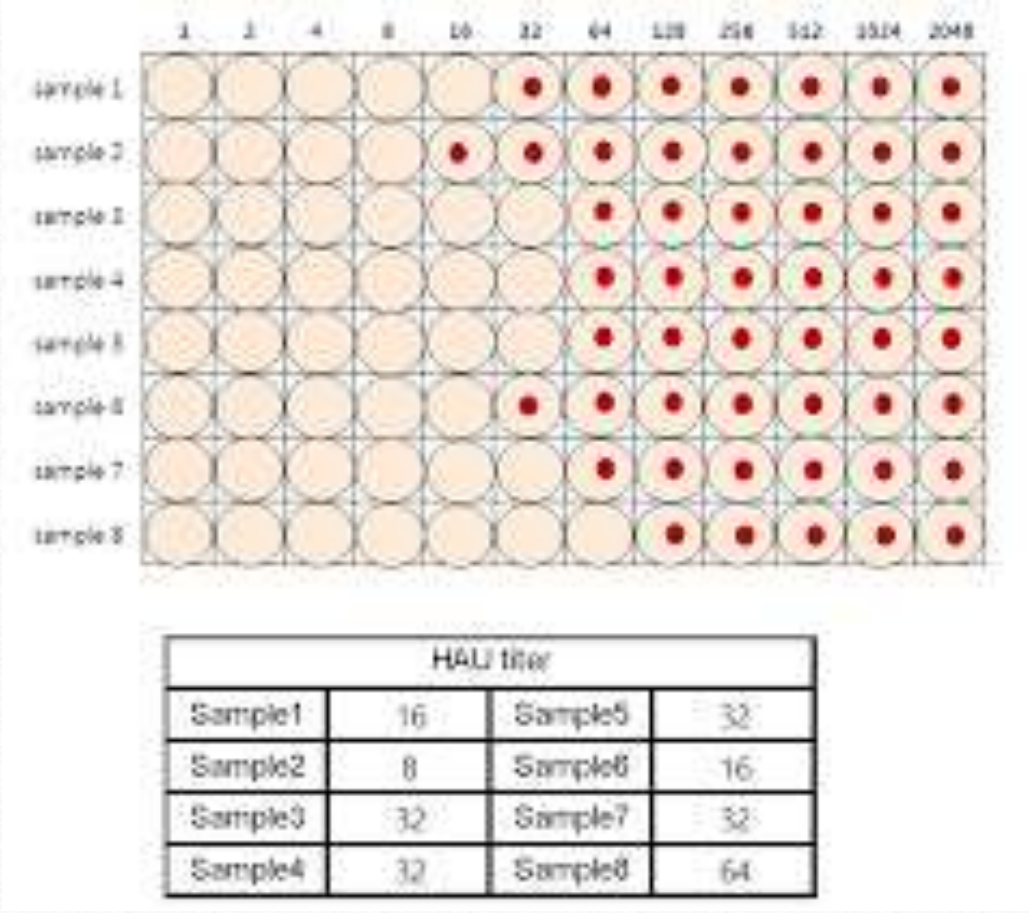
(a) Virus neutralization tests.

(b) Toxin neutralization tests.

Virus neutralization tests

- Neutralization of viruses by their specific antibodies are called virus neutralization tests. Inoculation of viruses in cell cultures, eggs, and animals results in the replication and growth of viruses. When virus-specific neutralizing antibodies are injected into these systems, replication and growth of viruses is inhibited.
- **Viral hemagglutination inhibition** test is an example of virus neutralization test frequently used in the diagnosis of viral infections, such as influenza, mumps, and measles. The test involves mixing the virus with serum or antibodies and observing whether the virus is able to infect host cells. If the antibodies can neutralize the virus, there will be no infection, and the test result is positive for neutralization.

Viral hemagglutination inhibition



HAU titer			
Sample1	16	Sample5	32
Sample2	8	Sample6	16
Sample3	32	Sample7	32
Sample4	32	Sample8	64

Toxin neutralization tests

- Toxin neutralization tests are laboratory assays used to assess the ability of antibodies or other agents to neutralize the toxic effects of specific toxins, often produced by bacteria or other microorganisms. These tests are important for diagnosing and treating diseases caused by toxins.

Examples of neutralization tests include:

- ***In vivo***— Schick test to demonstrate immunity against diphtheria, and

-*********Clostridium welchii* **(*Clostridium perfringens*)**toxin neutralization test in guinea pig or mice.

-***Clostridium botulinum*** toxin botulism.

- ***In vitro***— (a) Anti-streptolysin O test. a blood test used to measure the level of anti-streptolysin O antibodies in the bloodstream. This toxin produced by ***Streptococcus pyogenes***

(b) Nagler reaction used for rapid detection of *Clostridium perfringens*, to identify the presence of lecithinase that hydrolyzes lecithin, a component of cell membranes.

*** *Clostridium welchii* is an outdated term for a bacterium.

Immunofluorescence

- The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules.
- They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope.
- This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test

2. Indirect immunofluorescence test

Direct immunofluorescence test is widely used for detection of bacteria, parasites, viruses, fungi, or other antigens in CSF (Cerebrospinal Fluid), blood, stool, urine, tissues, and other specimens.

useful in the diagnosis of suspected autoimmune disease, connective tissue diseases and vasculitis

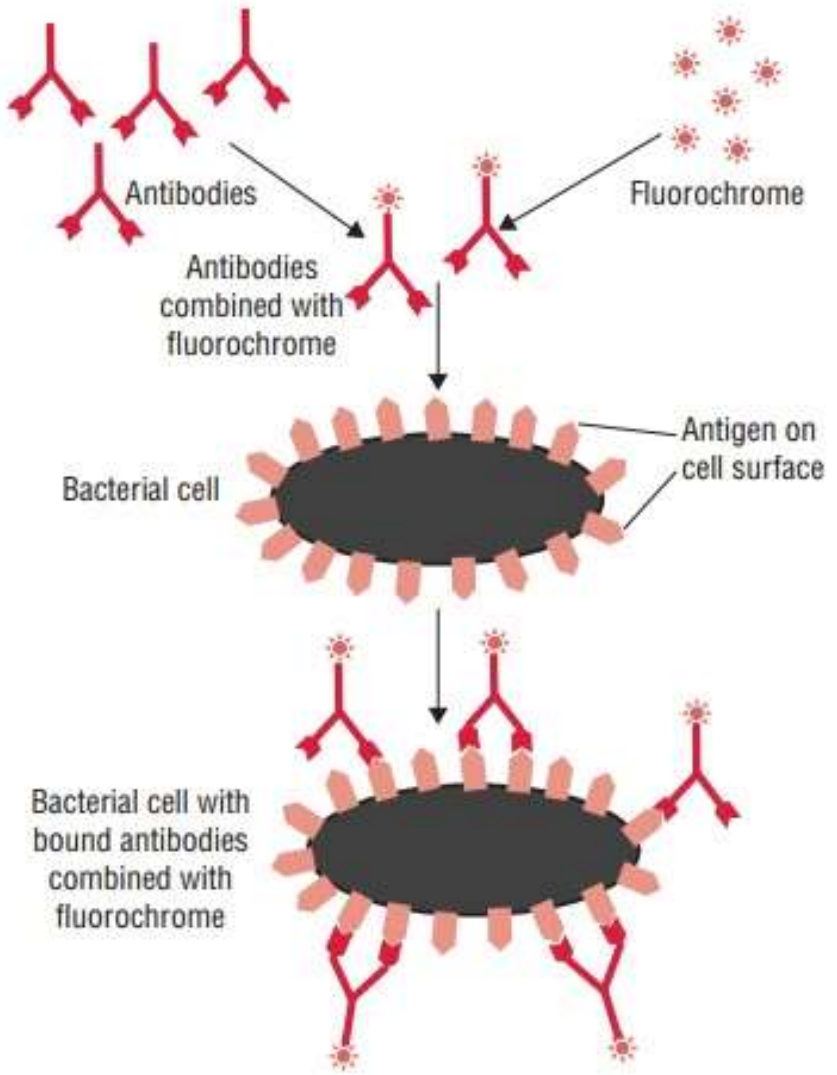
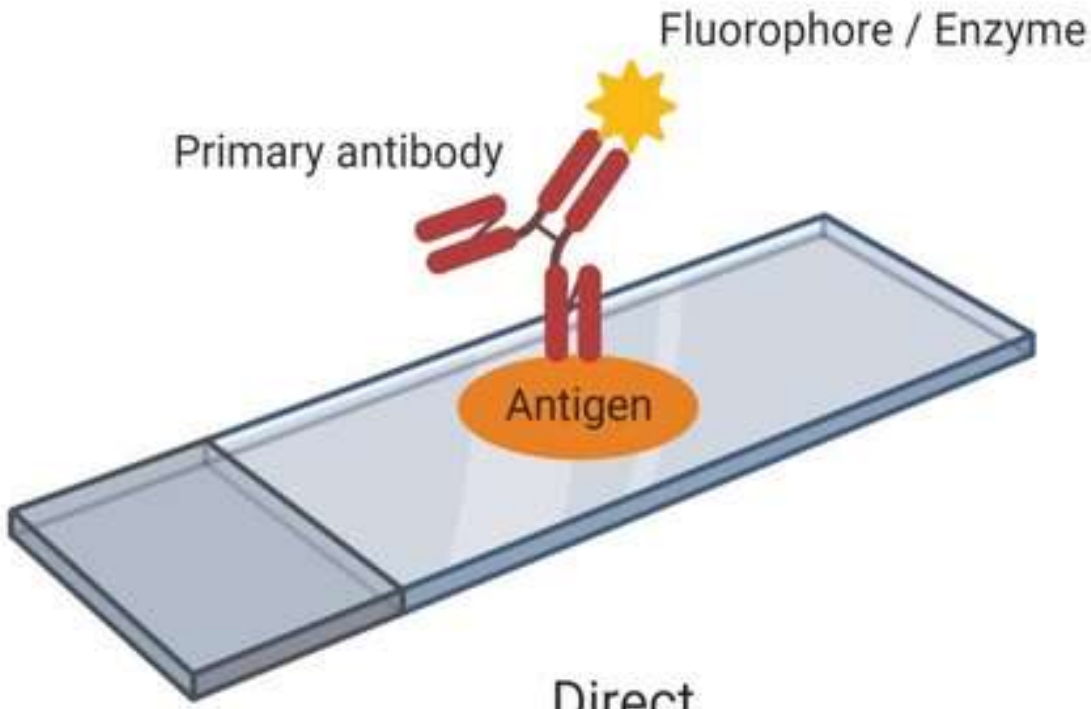


FIG. 14-13. Direct fluorescent antibody test.

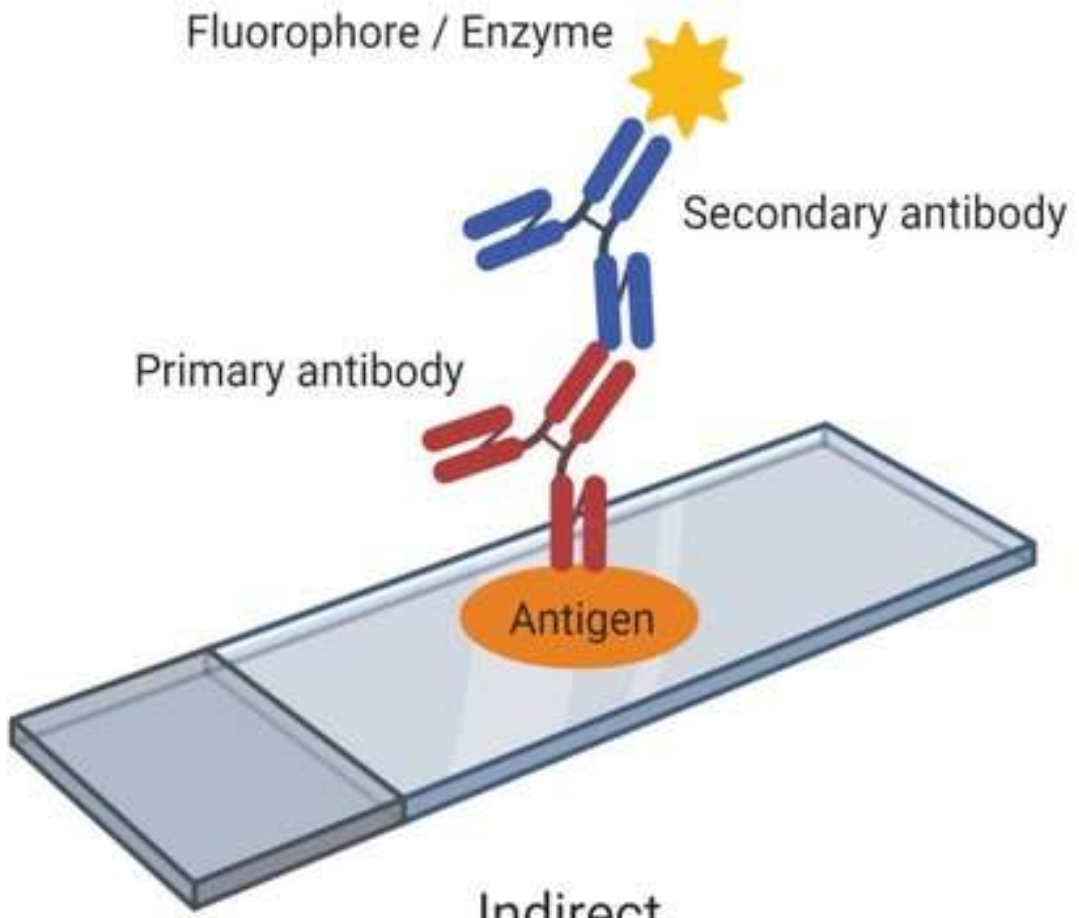
Indirect immunofluorescence

Is a two-stage process.

- **First stage**, a known antigen is fixed on a slide. Then the patient's serum to be tested is applied to the slide, followed by careful washing. If the patient's serum contains antibody against the antigen, it will combine with antigen on the slide.
- **Second stage**, the combination of antibody with antigen can be detected by addition of a fluorescent dye-labeled antibody (Secondary Ab) to human IgG, which is examined by a fluorescence microscope.



Direct
immunofluorescence assay



Indirect
immunofluorescence assay

NK

NK cells

- are a type of lymphocyte critical to the innate immune system. are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes.
- 10 % of mononuclear cells in blood and spleen and rare in lymphoid organs

NK

- Act very early against **viruses and intracellular microbes and tumor cells** or altered expression of surface MHC 1 molecule until T cells become activated.
- There activity increase by IFN alpha and beta (secreted by virally infected cells).
- activated cells secrete IFN gamma

NK receptors

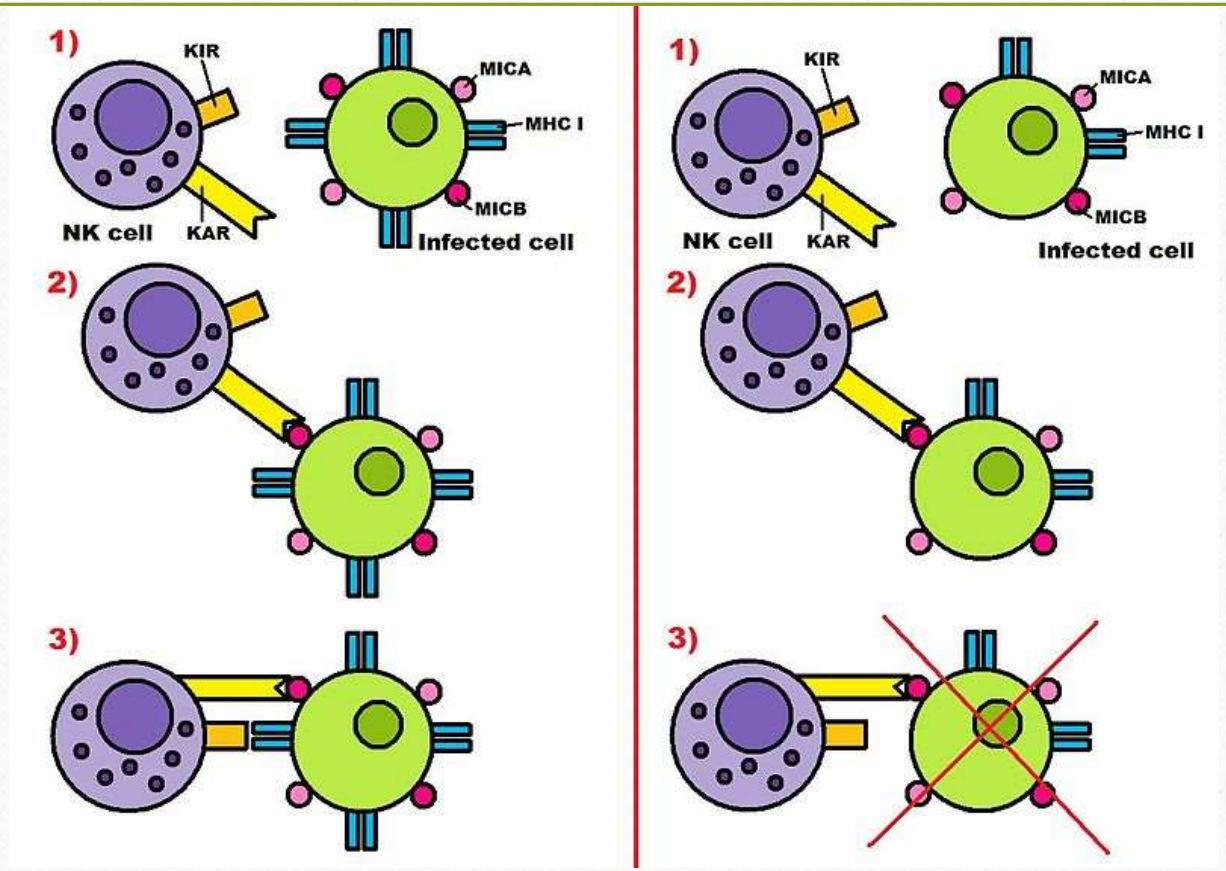
1. Killer inhibition receptors (KIR) of NK cells (most important), used to detect the presence of MHC 1 protein on host cells any binding means inhibition of killing

2. Killer activation receptors (KAR) of NK cells detect alteration in host cells as cancers. recognize stress related molecules as MICA, MICB

The killing function happens with or without the following receptors

1.receptors for antibodies; and kill these antibody- coated cells, this is called antibody dependent cell mediated cyto-toxicity (ADCC)

2. Expression fas ligands that bind fas on target cells and activation of caspases, this is a way in killing activated T cell (activation induced cell death) (AICD)

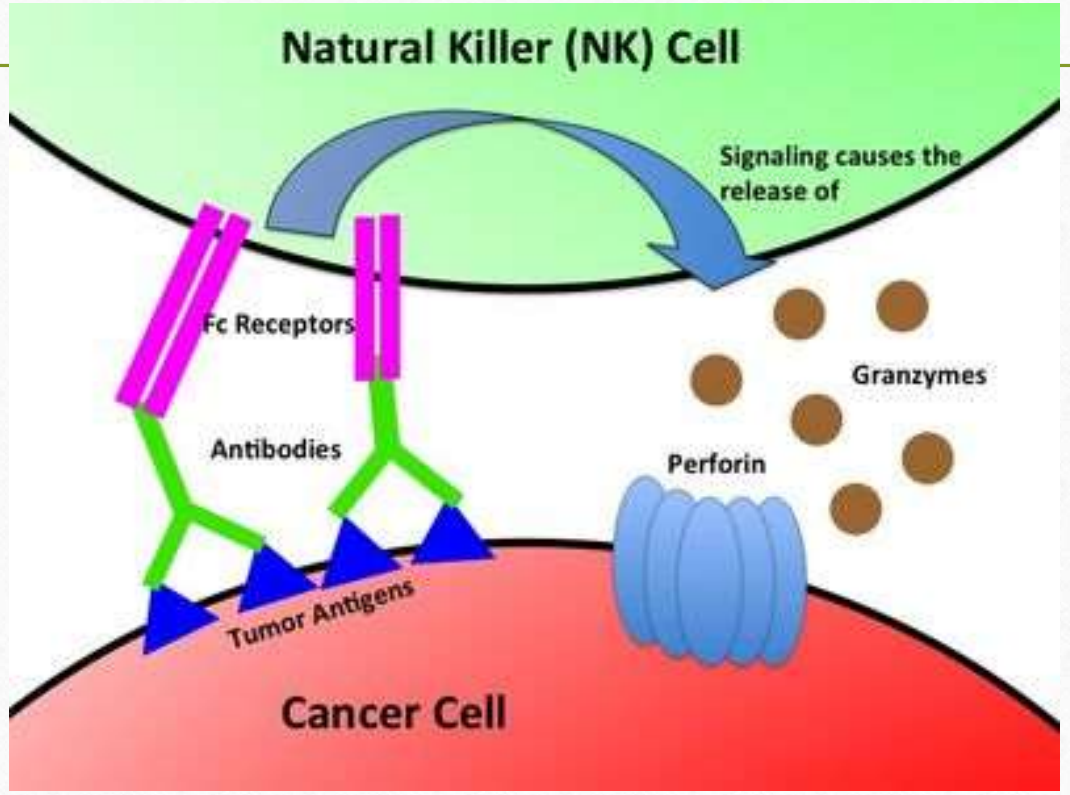


When a KAR binds to MICA and MICB molecules on the surface of an infected cell (or a tumor cell), a KIR examines the levels of MHC class I of this target cell. If the MHC class I levels are enough, killing of the cell doesn't proceed (left), but if they aren't, the killing signal proceeds and the cell is eliminated

NK ways of killing

- Effecter functions of NK cells
  - **Direct extracellular killing**
  1. by secretion Perforins; making pores then osmotic lysis. And Granzymes, enzymes enter through perforin pores and activate caspases leading to cell death
  2. -FAS-FASL binding
  - **Indirect killing** . increase macrophage phagocytosis and killing of microbe by secreting IFN gamma

Direct killing by NK cells



Name	Percent	Crosses placenta easily	Complement activator	Binds to Fc receptor on phagocytic cells
IgG1	66%	yes (1.47)†	second-highest	high affinity
IgG2	23%	no (0.8)†	third-highest	extremely low affinity
IgG3	7%	yes (1.17)†	highest	high affinity
IgG4	4%	yes (1.15)†	no	intermediate affinity

†: Quota cord/maternity concentrations blood. Based on data from a Japanese study on 228 mothers.

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	−	+++	−	−	−	−
Alternative pathway of complement activation	−	−	−	−	−	+	−	−	−
Placental transfer	+++	+	++	−/+	−	−	−	−	−
Binding to macrophages and other phagocytes	+	−	+	−/+	−	+	+	−	+
High-affinity binding to mast cells and basophils	−	−	−	−	−	−	−	−	+++
Reactivity with staphylococcal Protein A	+	+	−/+	+	−	−	−	−	−