Antigen antibody reaction-II

TYPES

- PRECIPITATION
- AGGLUTINATION

  Complement Fixation Test
- Neutralisation test

  Radio immuno assay
- ELISA
- Immunochromatography
- Chemiluminescence immuno assay
- Immunofluorescence
- Western blot assay

IMMUNOFLUORESCENCE

WHAT IS FLUORESCENCE ?

Ability to absorb rays of short wavelength and convert to visible light of higher wavelength

Fluorescent dyes conjugated to abs to identify ag in tissues

Commonly used dye- FITC

Direct Immunofluorescence

Identification of bacteria, virus or other ags with fluorescent dye labeled with the specific antiserum

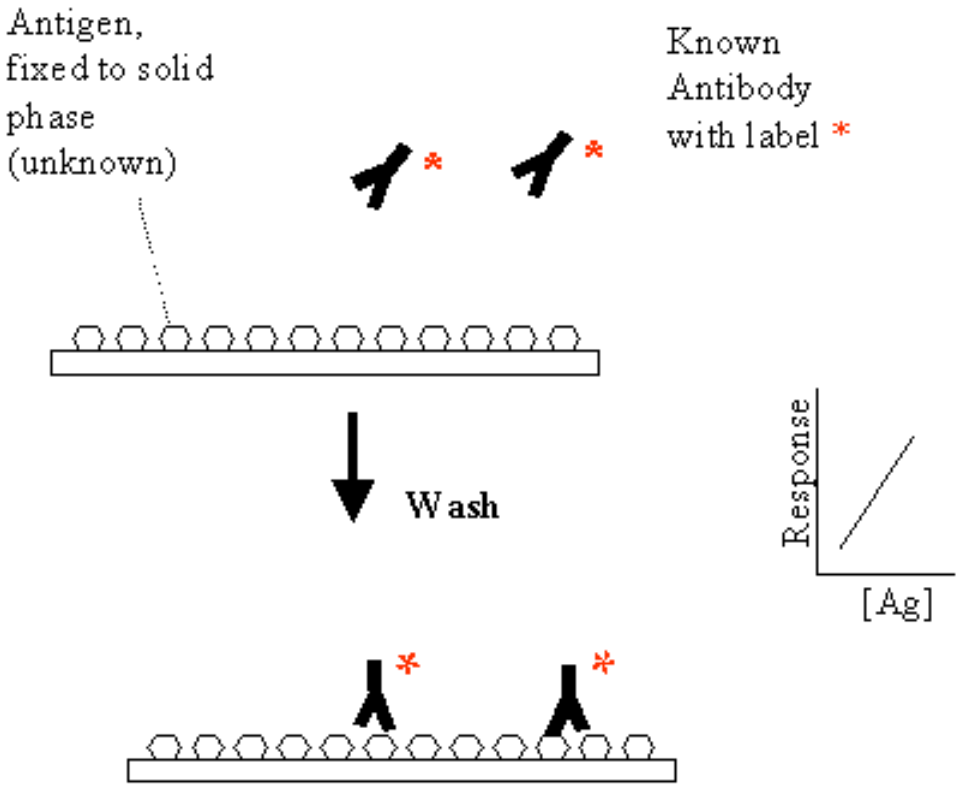
- Used to detect rabies virus ag in brain smears
- 1.sample containing the cells carrying the surface antigens smeared on a slide
- 2.Ab specific to antigen tagged with fluorescent dye is added
- 3.Washed to remove unbound abs and viewed under IF microscope

Disadvantage – separate fluorescent conjugates have to be prepared against each ag

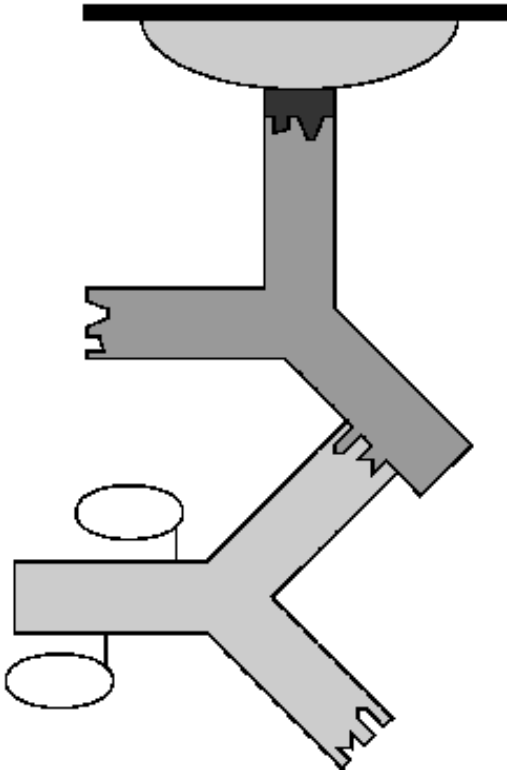
Indirect IFT:

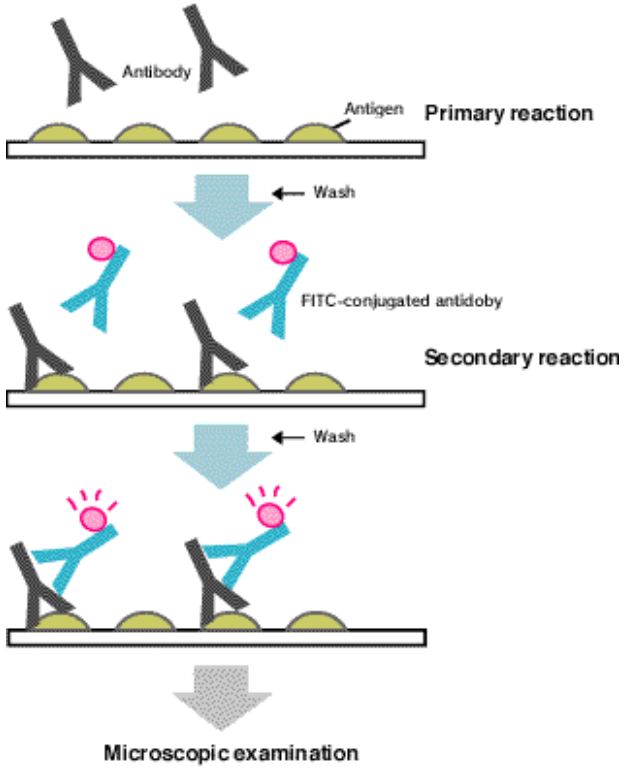
- Slides smeared with cells containing known Ag is taken
- Test serum with specific Ab added
- Washed – secondary ab (antihuman ab) tagged with IF dye is added
- Washed and viewed under IFM

Direct Immunofluorescence



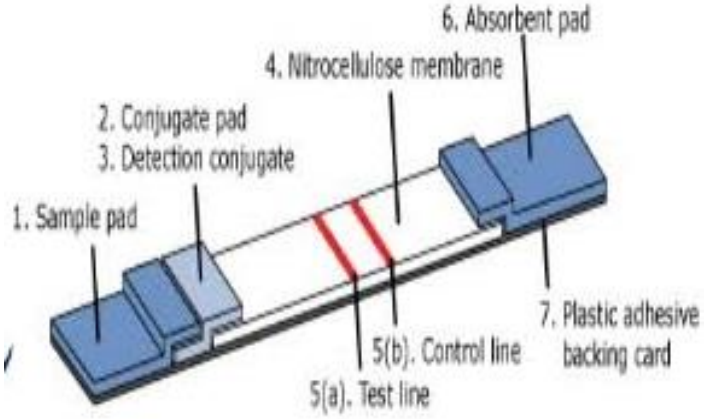
Indirect immunofluoresence





immunochromatography

- Lateral flow test- rapid card test
- Principle-
- Consists of nitrocellulose paper , coated with 2 abs-
- Ab Specific to Ag as test line and antihuman Ig as control line



Specific antibody is labelled with colloidal gold

- Sample pad
- Conjugate pad
- Test and control lines
- PROCEDURE-
- Test serum containing the antigen is added to the sample well
- It reacts with the Ab labelled with gold and forms complex
- This moves by capillary action along the NCP laterally and binds to the monoclonal ab in the test line to form a colored band; free colloidal gold labelled ab moves further and binds to HIG and form control band

ICT

- Advantage-
- ag and ab detected
- Economical, easy to perform,
- Rapid test
- Disadvantage – less sensitive than ELISA
- At a time one sample can be tested

Indirect ELISA

1. Antigen/sample is added to plate.
2. Blocking buffer is added to block remaining protein-binding sites.
3. Next a suitable ***primary antibody*** is added.
4. A suitable ***secondary antibody – HRPO conjugate*** is then added which recognizes and binds to the primary antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and is converted by HRPO to detectable form.

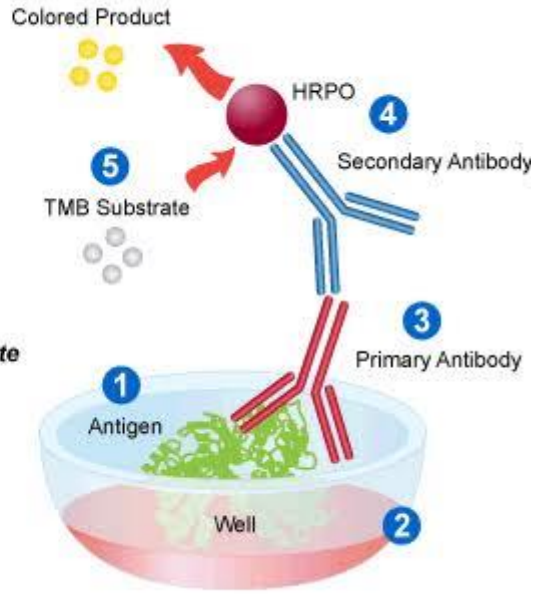


Diagram 1: *Illustration of Indirect ELISA method.*

- Enzyme Linked Immunosorbent Assay

"Serological test which detects either antibodies or antigens in the serum, which utilises an enzyme to label the antibody and react with the substrate chromogen producing color change"

PRINCIPLE- Antibodies in the serum binds to the antigen coated in the microtitre plate, the enzyme which is labelled to the antibody reacts with the substrate activating the chromogen to produce a color detected by spectrophotometry

MICROTITRE PLATE

Enzyme linked immunosorbent assay (ELISA)

- Ag-Ab complex- enzyme+ substrate -→ activates the chromogen → color change→ detected by spectrophotometry

- **Enzymes – horse radish peroxidase & alkaline phosphatase**
- **Substrates , o-phenyl diamine dihydrochloride & p-nitro phenyl phosphate** TetraMethyl Benzidine

- REQUIREMENTS
- MICROTITRE PLATE
- ELISA WASHER
- ELISA READER
- TEST SERUM
- CONTROL REAGENTS
- DILUENT
- ENZYME CONJUGATE
- SUBSTRATE
- STOP SOLUTION
- MICROPIPPETS

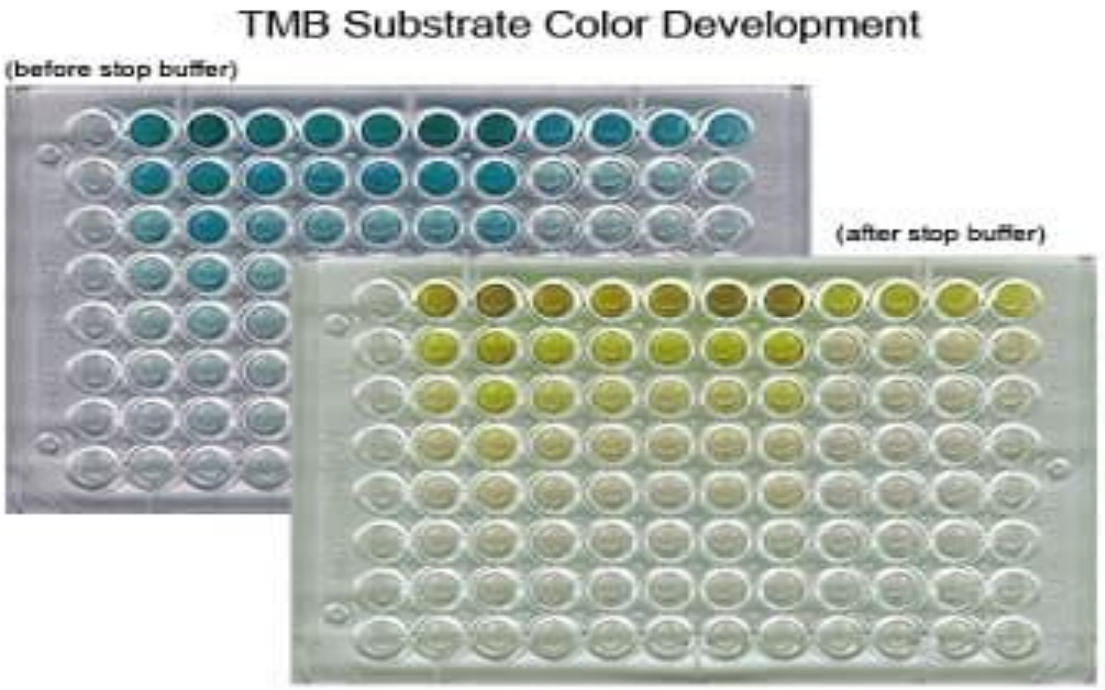
- PROCEDURE
- Step-1: Take Solid phase of the wells of microtitre plate precoated with antigen
- Step-2: Negative control sample to the 1st and 2nd wells and positive control to 3rd well
- Step-3: Test sera (containing antibody specific to coated antigen) added to the wells from 4th well onwards- incubation at 37C
- Step-4: after washing 5times enzyme labelled secondary Ab (antihumanIg)is added incubation
- Step-5: washing 5times add substrate and keep in room temp. at darkness for 30 mts
- Step-6: add stop solution and take reading by ELISA reader

ELISA WASHER

ELISA READER

INTERPRETATIONOF RESULT

Find out the OD value for each sample and Calculate tha cut-off value



1.DIRECT ELISA

- Detection of Ag
- Microtitre plate not coated with ag or ab
- Test serum is added to the well- ag binds to the well by passive adsorption
- After washing ab labelled with enzyme is added
- After washing chromogen is added and color change is noted

2. Indirect ELISA

- ab detection
- Wells coated with ag
- Sera to be tested added to this
- If ab present in specimen it binds to coated ag
- To detect the combination AHG conjugated with enzyme added .
- Substrate added, if positive acted on by the enzyme to give a color change
- Incubation & washing done at every step

3.Sandwich ELISA

- Detects Ag
- Well coated with capture antibody
- Serum having Ag is added – it gets attached to Ab
- After washing enzyme labelled primary specific detector Ab is added
- After washing substrate chromogen added and color developed

4. Competitive ELISA

- Ag in the serum competes with another Ag coated in the well to bind to the Primary Ab
- Primary Ab is incubated in a solution with the serum containing the test Ag
- This mixture is added to the well coated with the same type of Ag
- The unbound free Ab binds to the coated Ag
- If more Ag in the serum less free Ab to bind with the coated Ag

Competitive ELISA contd …….

After washing enzyme labelled secondary Ab added

- **Substrate is added and color developes**
- **The intensity of colour inversely proportional to the amount of Ag in the test serum**

Applications of Immunoassays [RIA & ELISA]

- Analysis of hormones, vitamins, metabolites, diagnostic markers
  - Eg. ACTH, FSH, T3, T4, Glucagon, Insulin, Testosterone, vitamin B12, prostaglandins, glucocorticoids,
- Therapeutic drug monitoring:
  - Barbiturates, morphine, digoxin,
- Diagnostic procedures for detecting infection
  - HIV, Hepatitis A, B etc

Uses of ELISA

- Used to detect ags & abs
- E.g : 1. Detection of HIV abs in serum

  2.detection of mycobacterial abs in T.B

  3. detection of Rotavirus in feces

  4. detection of Hepatitis markers in serum

  5. detection of enterotoxin of E-coli in feces

Chemiluminescence assay

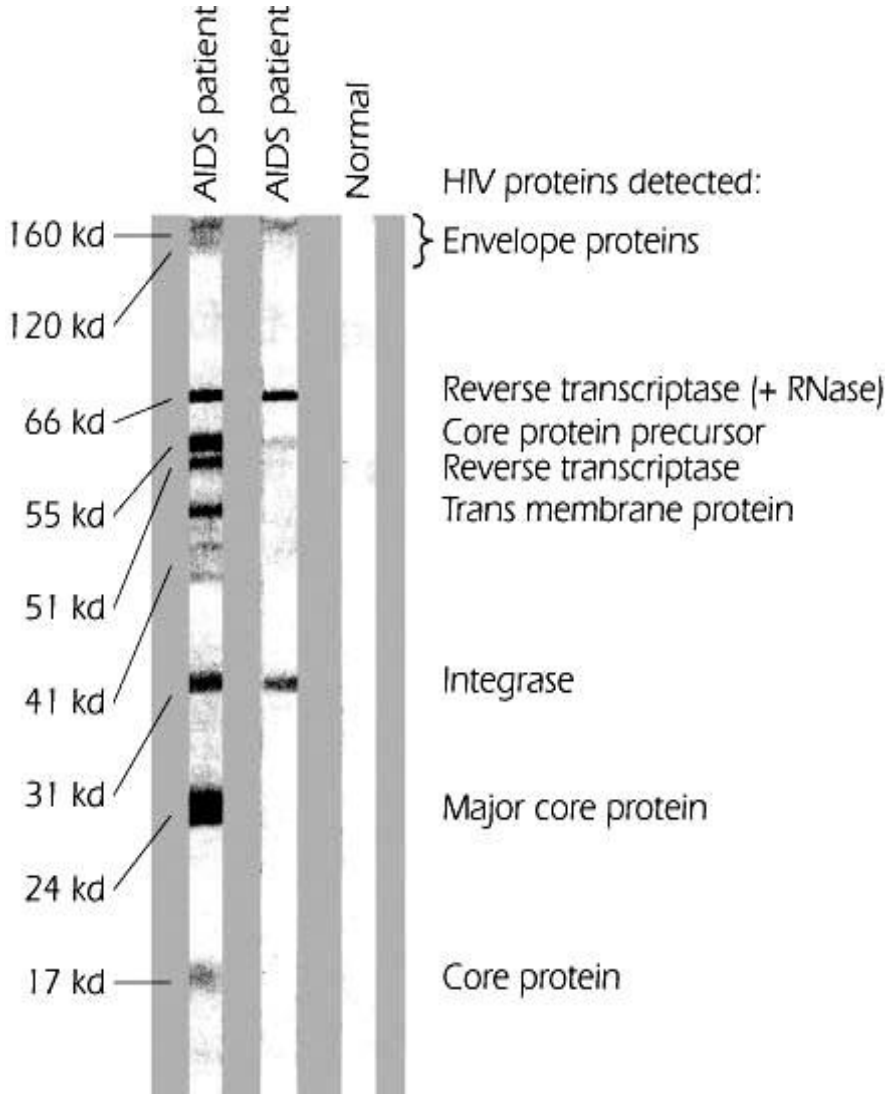
- Chemical reaction in which energy is emitted in the form of light
- These compounds used to provide signal during ag-ab reaction
- The emitted light can be measured & the concentration of analyte calculated
- Fully automated, more sensitive than ELISA

Immunoblotting

- Abs can detect proteins (ags) in mixtures
- The proteins electrophoretically separated in a gel
- Transferred to nitrocellulose paper
- These paper strips are reacted with test sera & subsequently with enzyme conjugated anti-human globulin
- Substrate added,color produced if ab in test sera has reacted with the separated proteins on strip

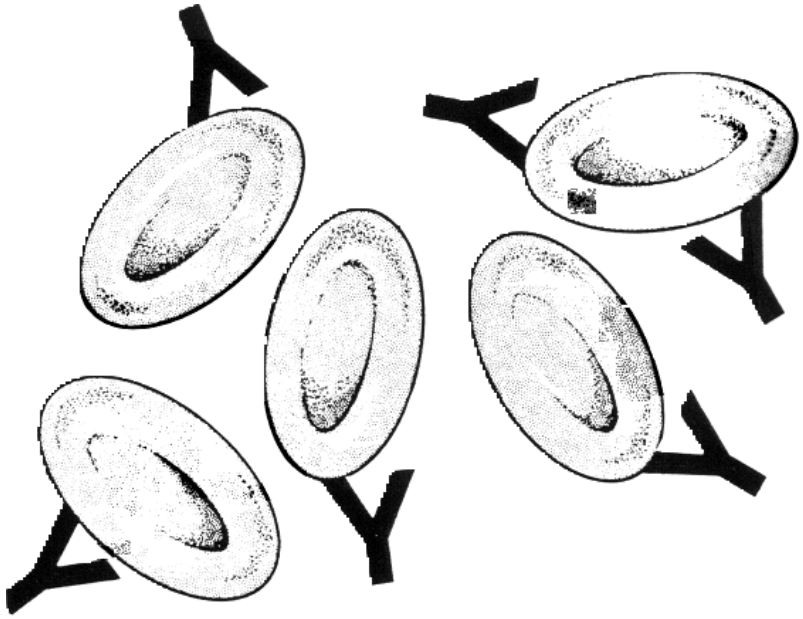
- **Used to detect ELISA positive HIV serum – known as Western Blot test**
- **May be applied to analyse DNA or RNA**
- **DNA transferred to gel-Southern Blot test**
- **RNA – Northern Blot test**

Western Blotting



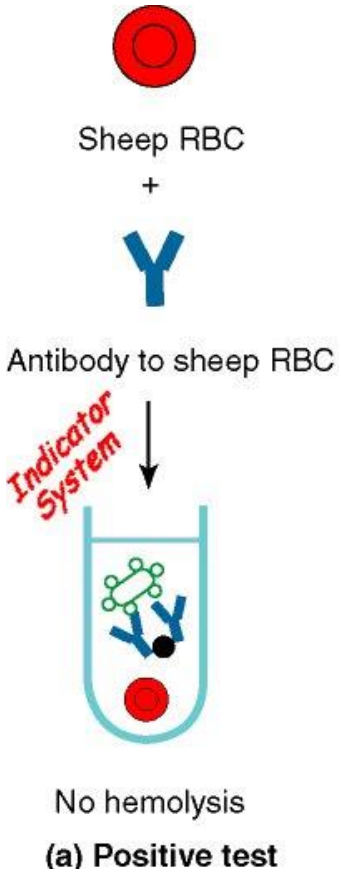
THANK YOU…..

Complement fixation tests



- C’ is absorbed during the combination of ag with their abs
- In the presence of appropriate abs C’ lyses bacteria, immobilises motile organisms, promotes phagocytosis & immune adherence
- Very versatile & sensitive test
- Detects 0.4 µg of ab & 0.1 µg of ag
- Consists of 2 steps
- 5 reagents – ag, ab, C’, sheep erythrocytes & amboceptor

CFT contd……..

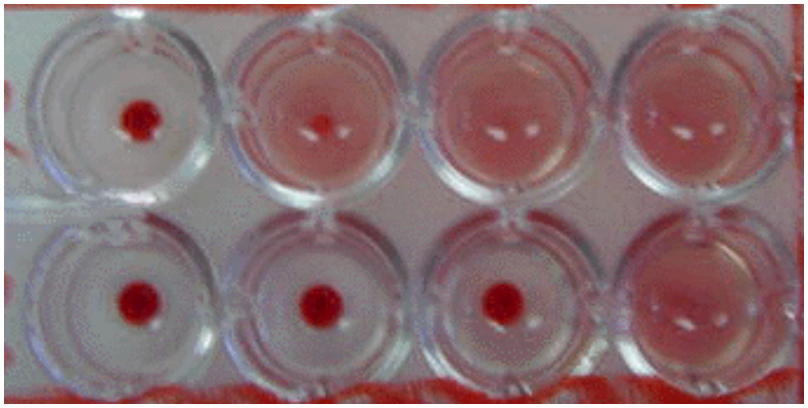


- C' in the antiserum inactivated by heating at 56ºc
- The source of test C' is G.pig serum
- Classical example – Wassermann reaction

First step : inactivated patients serum incubated at 37ºc for 1 hr with the Wassermann ag & a fixed amount of G.pig C'

- If syphilitic ab present the C' will be utilised
- No C' will be left behind

CFT CONTD……



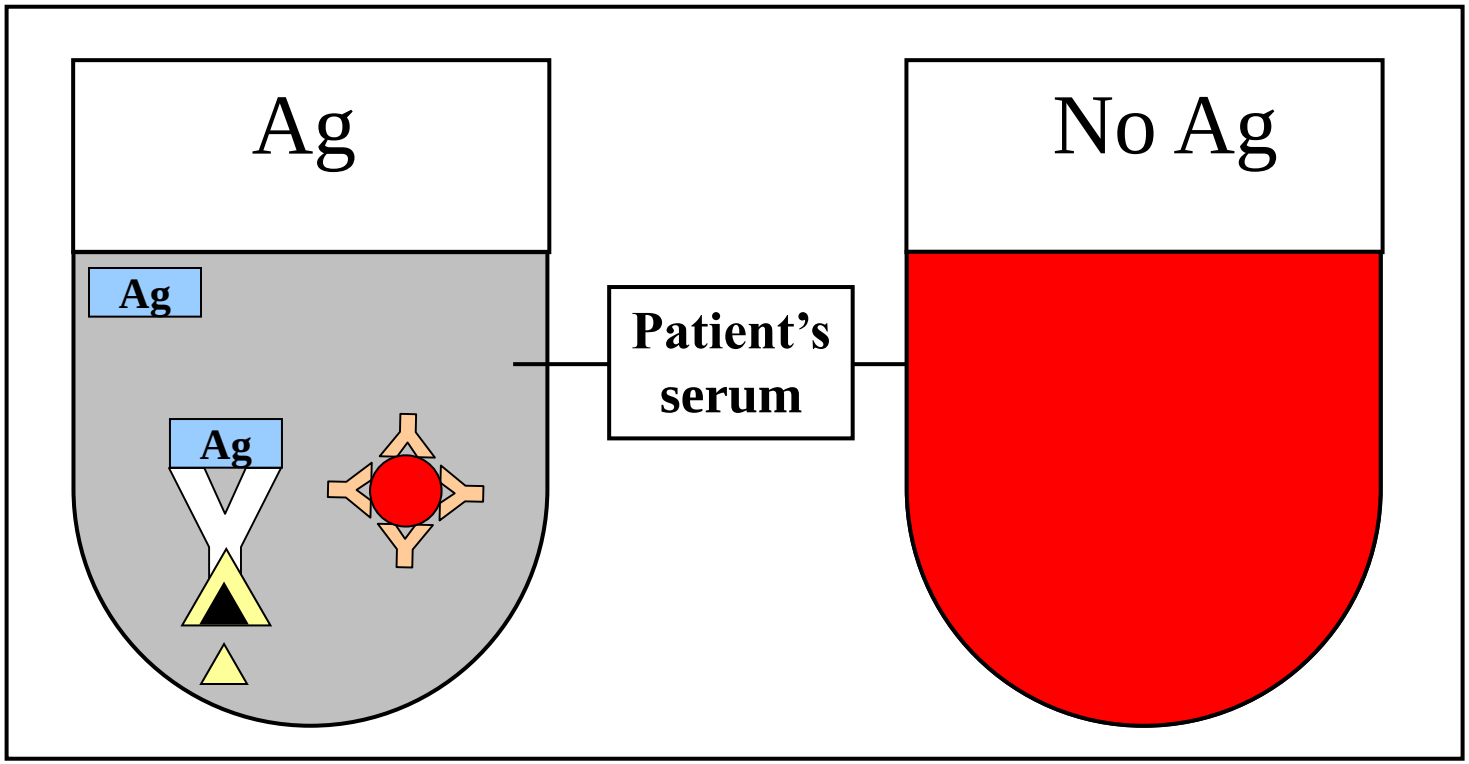
Second step : adding sensitised cells (sheep RBC coated with its ab)

- Incubation at 37ºc for 30 mtes
- Lysis of erythrocytes – test negative
- No lysis test positive

Complement Fixation

- Ag mixed with test serum to be assayed for Ab

– Standard amount of complement is added
– Erythrocytes coated with Abs is added
– Amount of erythrocyte lysis is determined



Neutralisation tests

Virus neutralisation tests:

- **neutralisation of bacteriophages by plaque inhibition test by specific antiphage serum**
- **Neutralisation of animal viruses - demonstrated in animals, eggs & tissue culture**

Toxin neutralisation test: **tested in vivo or vitro**

- **in animals injection of toxin & antitoxin mixtures. Estimation of the least amount of antitoxin that prevents death or disease of animal.E.g Shick test**
- **In vitro – inhibition of some demonstrable toxic effect**

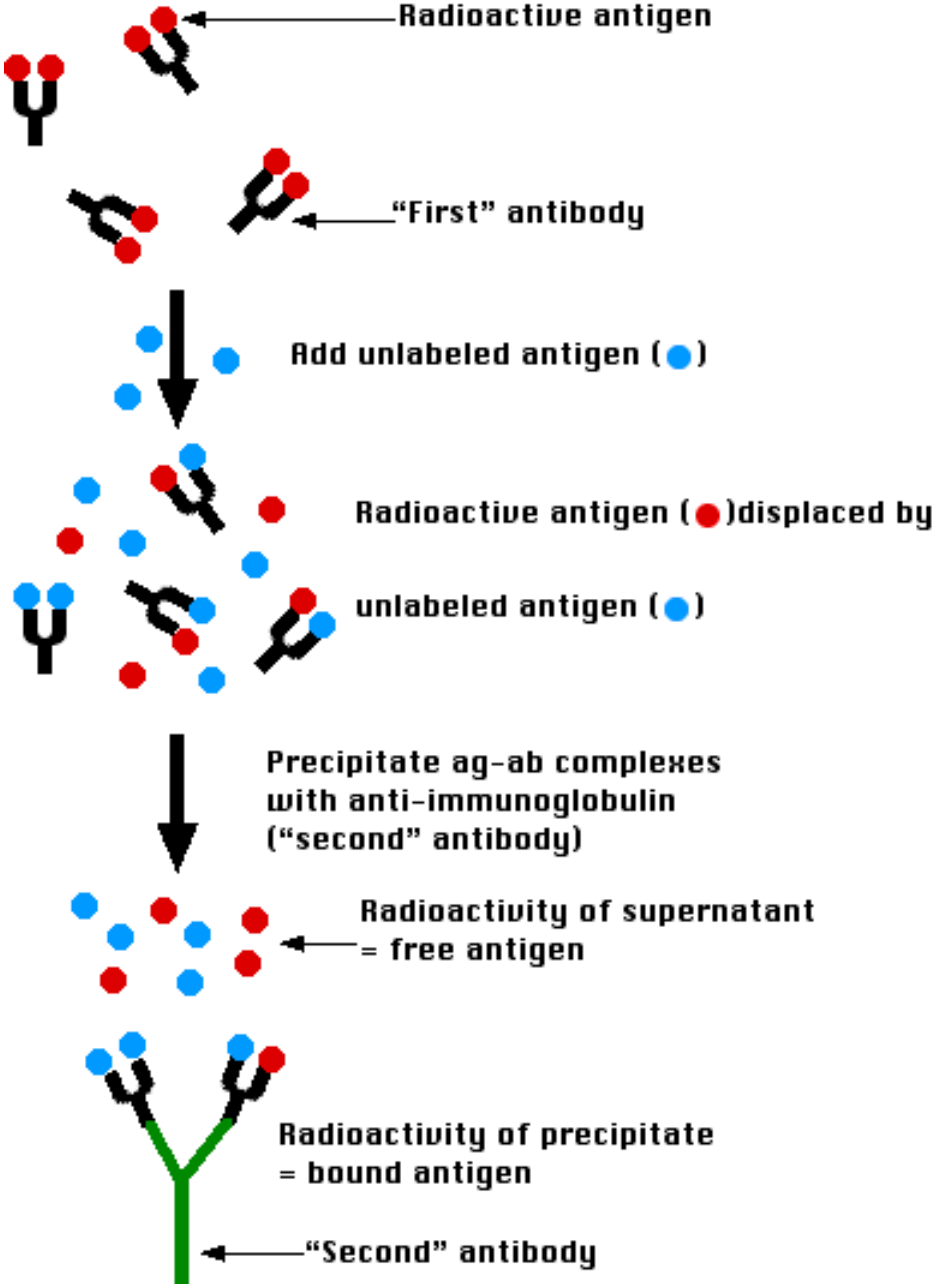
E.g ASO test

Opsonisation

- **Opsonic index is measured by incubating fresh citrated blood with the bacterial suspension at 37 º c for 15 mtes & estimating the average number of phagocytosed bacteria per PML from stained blood films**

Radioimmunoassay

- Based on competition for a fixed amounts of specific ab between a known radio labeled ag & unknown unlabeled (test) ag
- This competition is determined by the level of test ag present in the reacting system
- After ag-ab reaction the ag is separated into the free & bound fractions & their radioactive counts measured
- The concentration of the test (unlabeled) ag is calculated from the ratio of the bound & total ag labels using a reference curve
- Useful in the quantitation of hormones,tumor markers,drugs,IgE & viral ags
- Can detect ags up to picogram quantities
- Disadvantage -hazard



RIA

''PROGRAMMED BY KAZUSHI ARAKI''

oTESTOSTERONE 91-02

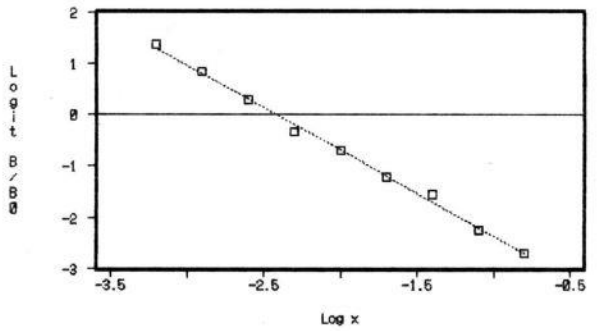
	No.	Dose/tube	B/B0 (%)
*	1	0.000625	79.8%
*	2	0.00125	69.7%
*	3	0.0025	57.2%
*	4	0.005	41.5%
*	5	0.01	32.9%
*	6	0.02	22.5%
*	7	0.04	17.3%
*	8	0.08	9.6%
*	9	0.16	6.3%
	10	0.32	2.8%
	11	0.64	2.1%

回帰分析の結果 (No.2)

Y 切片		-4.039076
Y 評価値の標準誤差		0.0824350
R２乗		0.9968677
標本数		9
自由度		7
X 係数	-1.6	
X 係数の標準誤差	0.03	

Y= -1.668641 X+ -4.039076

結合率= 28.276364 %



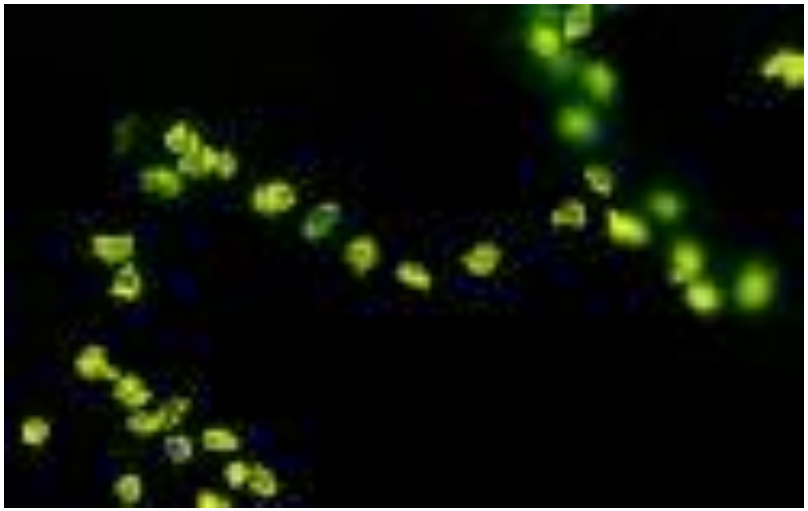
Advantages & Disadvantages of RIA

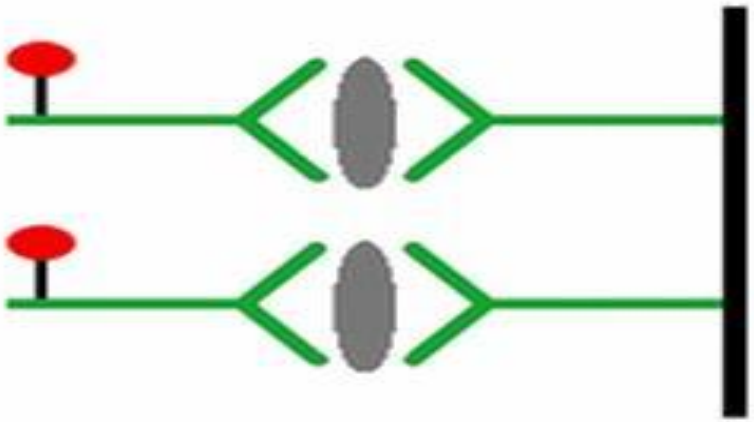
Advantages

- Highly specific
- High sensitivity

- **Disadvantages**
  - Radiation hazards: Uses radiolabelled reagents
  - Requires specially trained persons
  - Labs require special license to handle radioactive material
  - Requires special arrangements for
    - Requisition, storage of radioactive material
    - radioactive waste disposal.

Immuno contd ….





- For detection of ags by IF the sandwich technique is employed
- Ab, unlabeled ag ,which is then treated with fluorescent labeled ab
- Fluorescent dyes – fluorescein isothocynate (blue green) & lissamine rhodamine(orange red)
- Disadvantage – frequent occurrence of nonspecific fluorescence in tissues & other materials