Ministry of higher Education and Scientific Research Southern Technical University

Institute of Medical Technology /AMARA

Virology

Viruses

Students of Second Class Medical Laboratory

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M. Sc. Biology

Definition of viruses

- Are infectious agents that are too small to be seen with a light microscope.
- A cellular (absence of nucleus, organelles, cytoplasm, plasma membrane).
- No ATP generating metabolism
- Do not undergo binary fission
- Sensitive to interferon

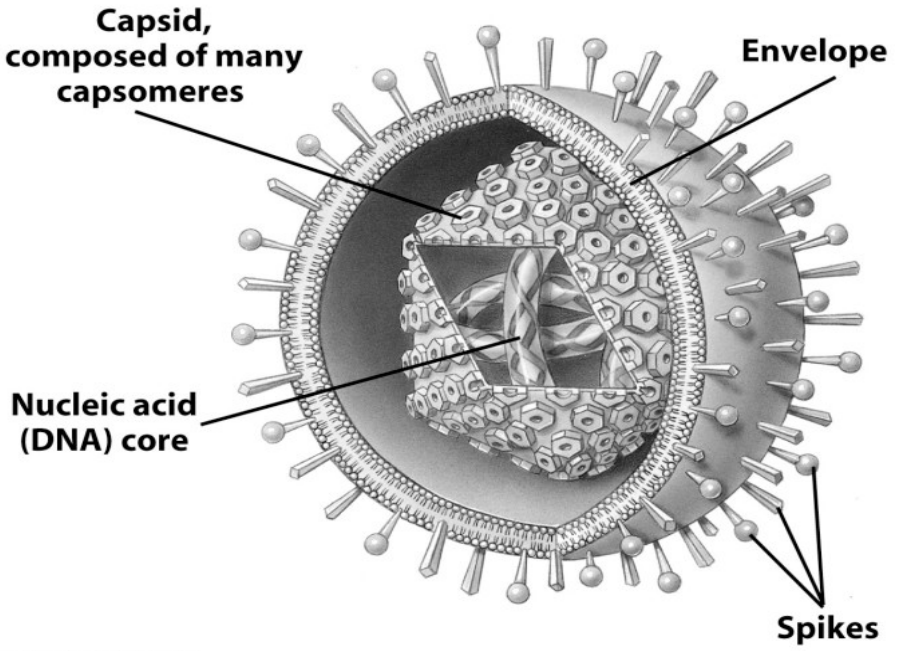

Figure 10-1 Microbiology, 6/e
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General Characteristics of Vir

Obligatory intracellular parasit

- Contain DNA or RNA
- Contain a protein coat
- Some are enclosed by an envelope
- Some viruses have spikes
- Most viruses infect only specific types of cells in one host
- **Host range** is determined by specific host attachment sites and cellular factors

Viruses Lab Microscopy

?What is a m e

- Is an optical instrument consisting of a combination of lenses which magnifies the image of the object seen through it.
- It is used for the morphological study of a very small organisms which are not visible by naked eye.

scope=to view**Micro**= small

Types of microscopes

- Simple
- Compound
- Electron

Incubator

- Is a device used to grow and maintain microbiological cultures.

- The incubator maintains optimal **temperature**, **humidity** and other conditions such as the **carbon dioxide** (CO_2) and **oxygen** content of the atmosphere inside.

Incubator

Autoclave

- An **autoclave** is a pressure chamber used to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C for around 15–20 minutes depending on the size of the load and the contents.

- Used to sterilize culture media, discard, and other equipments.

Autocla ve

Oven

- .device used in sterilization
- .oven uses dry heat to sterilize
- used to sterilize items that might belt (e.g.,damaged by moist heat .powders, oils) glasswares,

HOT AIR OVEN

Close View

Open View

Laboratory refrigerator

is used for a wide variety of purposes such as:

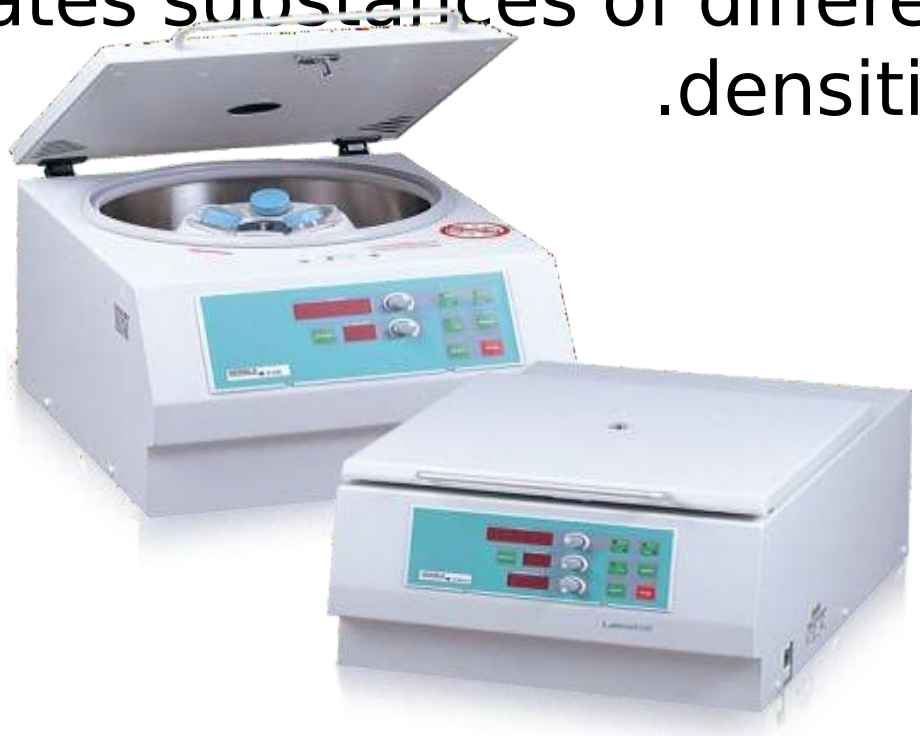
- maintenance and storage of stock culture between subculturing periods.
- storage of sterile media to prevent dehydration.
- also used as repository for thermolable solutions, antibiotics and serums.

Lab. refrigerator

Centrifuge

- is an apparatus that rotates at high speed

and separates substances of different densities.

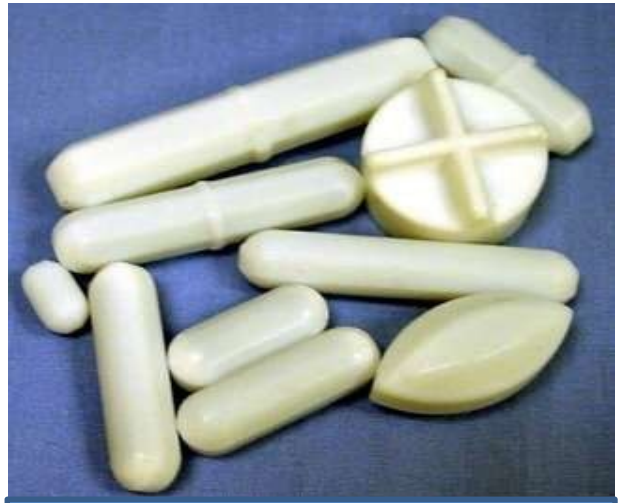


Balance

- used to measure an object's mass to a very high degree of precision.

hot plate / stir plate

- used to heat and stir substances



Magnetic stirring bars

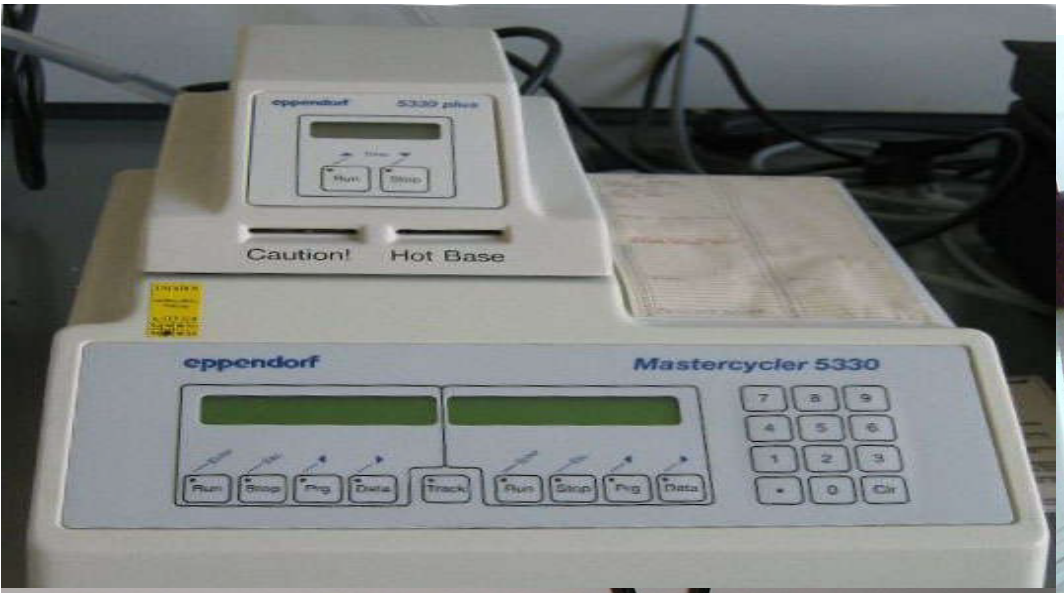
Water bath

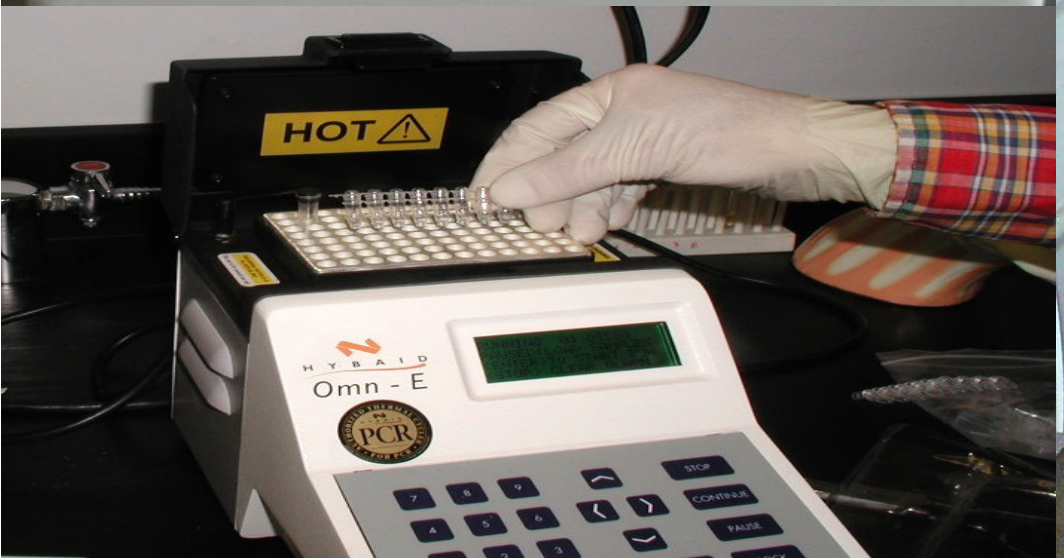
- is a device that maintains water at a constant temperature.
- It is used in the microbiological laboratory for incubations.

Biological Safety Cabinets

- is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with pathogens

NEW AUTOMATED PCR





OLD PCR

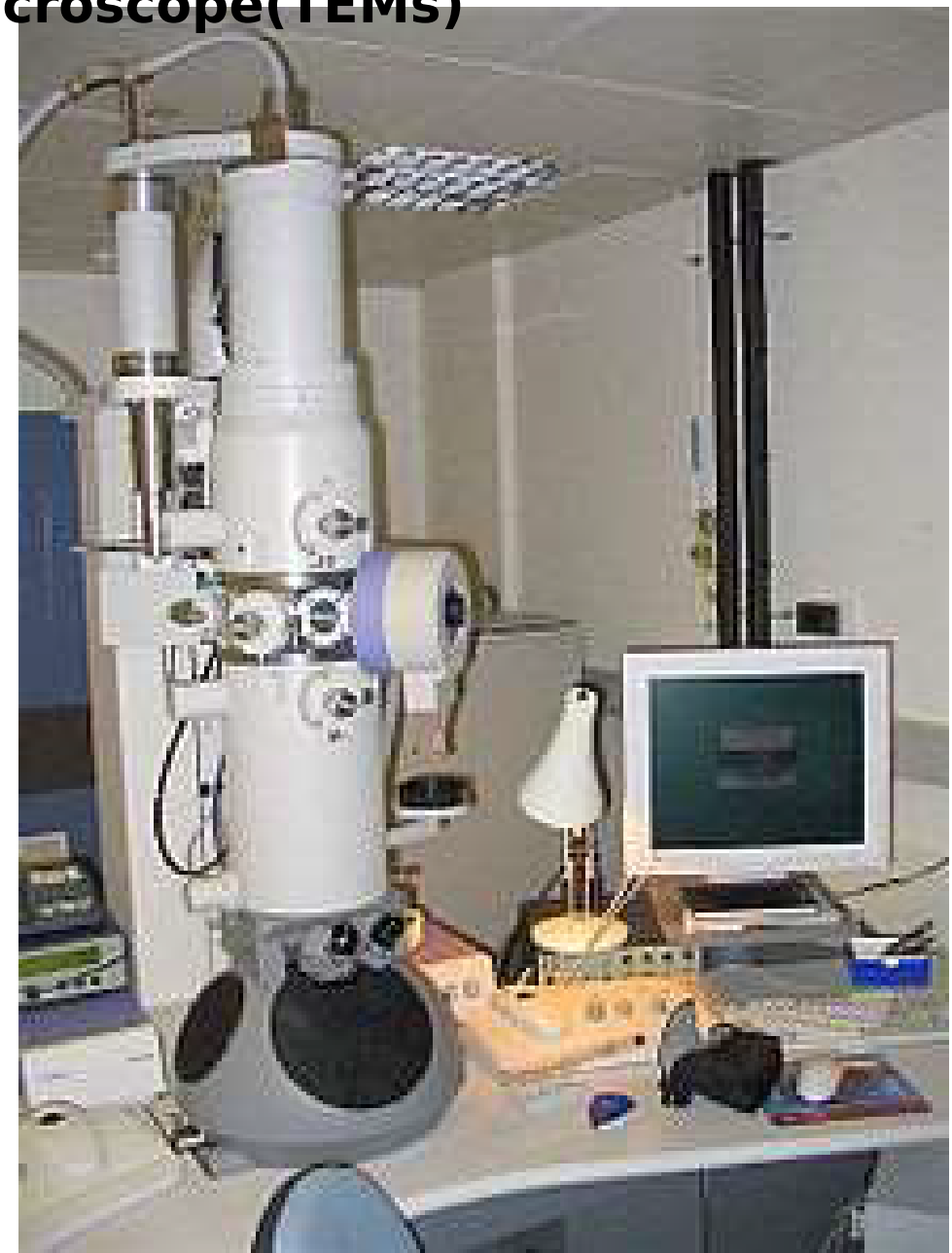
Micro plate readers

ELISA READER

Transmission Electron Microscope(TEMs)

The electron beam passes through .the specimen

ᴧᴧ Electrons are deflected as they pass through the material and the pattern is converted into an image



Stereoscope

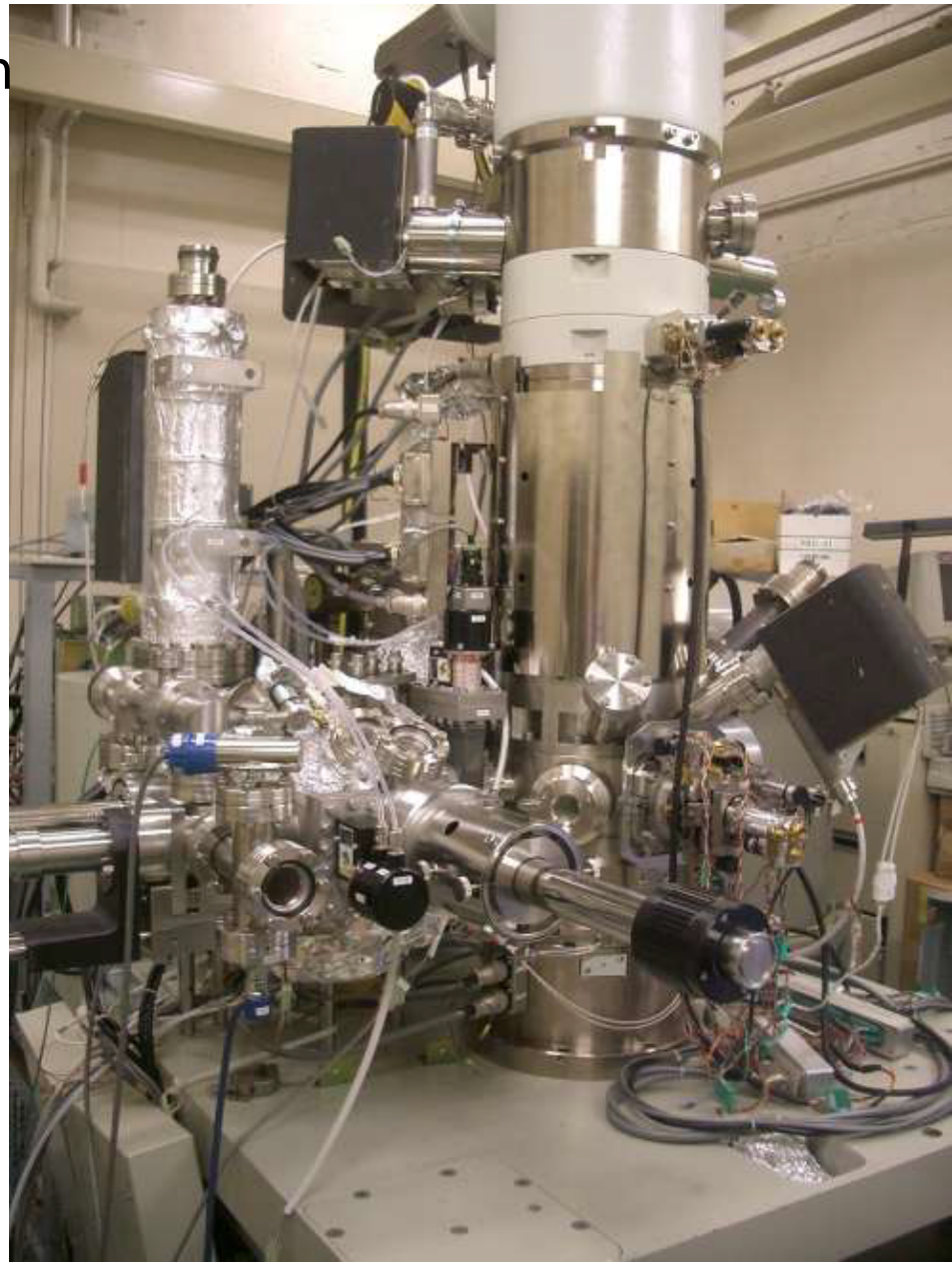
ssection or surgery requires 3D vision

Insects

Scanning Electron Microscope (SEMs)

SEM record the electrons that are reflected off surface of a specimen the

- Thin specimens are not needed and 3-D images are produced



ight microscopes

Analytical Balance

Reaction Plates

Reaction plates *(or multi-well plates)* are used when we want to perform many small scale reactions at one .time

Cultivation and identification of viruses

?□ What are viruses

Viruses are infectious ,intracellular,obligate,parasites

:-□ Methods to cultivate animal viruses

Embryonated Chick egg) 1

Live animals)2

Tissue culture)3

:□ The primary purposes of viral cultivation are

to isolate and identify viruses in clinical specimens. 1

to prepare viruses for vaccines. 2

,to do detailed research on viral structure. 3

.multiplication cycles, genetics, and effects on host cells

Regardless of the type of virus, the•

parasite diverts the host cell's resources

.for viral production

:The host cell provides•

- **Nucleotides for nucleic acid production**
- **Enzymes**

- **ATP**

Embryonated chick egg:-

Good Pasteur.1
Burnel-Modified.2
:-Animal eggs eg.3
CHICKEN,DUCK and TURKEY
4.5to12 days old eggs

Tissues
Choriallantonicm embrane
Amniotic cavity,

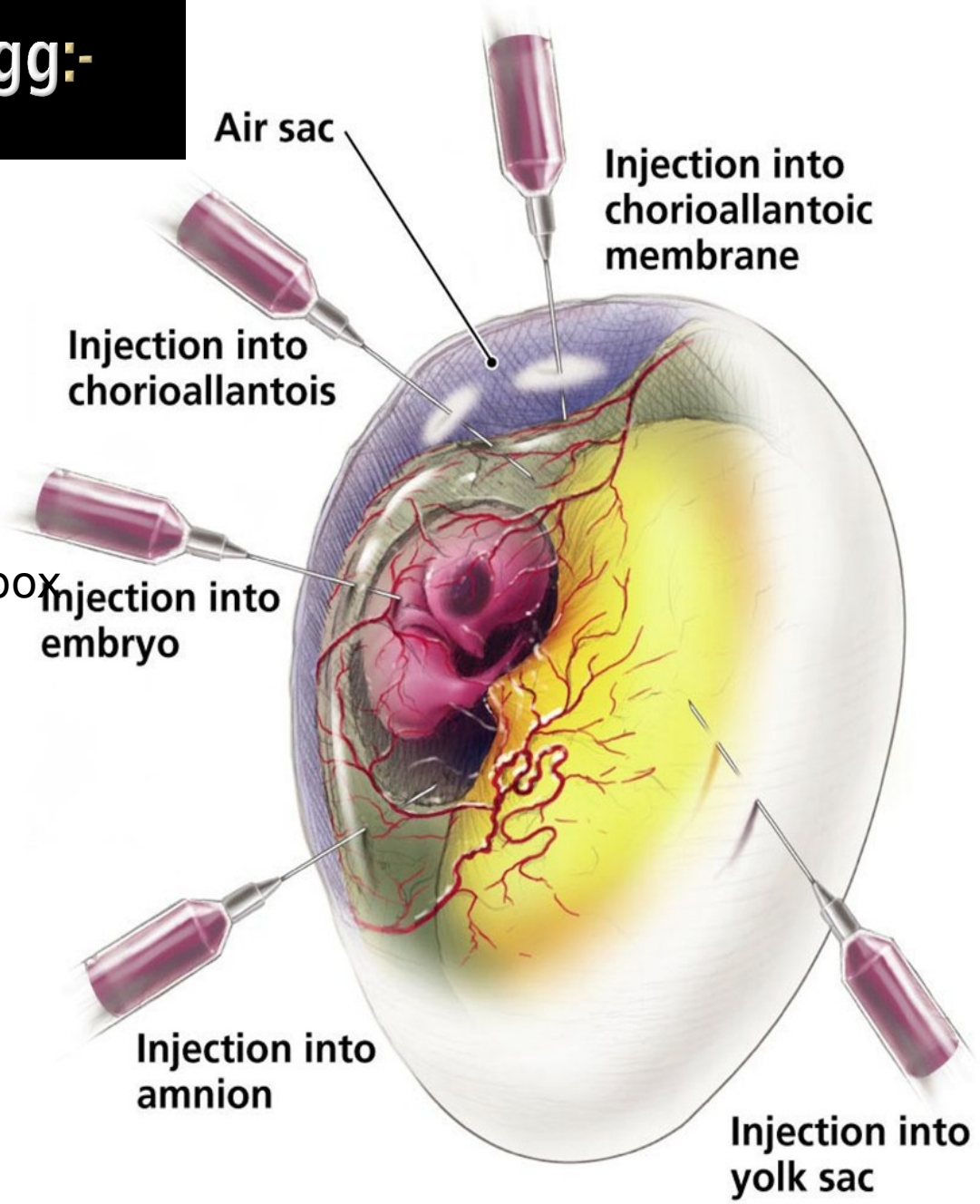
Viruses)cultivated(
Herpes,Small pox virus,Rous Sarcoma virus
Mumps virus
Influenza,
Yellow fever,Rabies

Yolk Sac

Embryo

Influenza

Mumps ,



PROCEDURE :

1. Candling

2.Drilled the hole

3.Inject the suspension with syringe

4.Hole sealed with Paraffin wax

5.Eggs incubate at 36C for 2to3 days

Viral culture in eggs: Some viruses, ,such as influenza viruses are grown in embryonated chicken eggs .

ELISA
Enzyme Linked( )ImmunoSorbent Assay

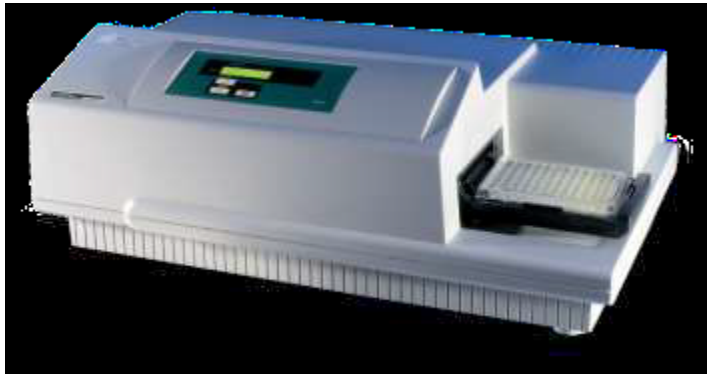
:Principle of ELISA

- **Based on Basic Immunology Response**

:- Lock and Key Concept
:Antigen (key) 2) Antibody (lock)) 1
Key fits into the lock–

- Enzyme conjugate substrates Bound to a secondary antibody that binds with the .antibody-antigen complex

ELISA kits are commercially available, which can
be conveniently used for laboratory .purpose



ANTIGEN (Ag)

- Any molecule that induces production of antibodies when introduced in the body of an animal is called .antigen

OR

- any “thing”, foreign to the immune .system. e.g bacteria, viruses, (or their parts), pollen, .etc
- Protein molecule
- Carbohydrate molecule
- Microorganisms
- Allergens
- Viruses Etc

SYMBOL FOR ANTIGEN

ANTIBODY (Ab)

- Antibody: proteins produced by the immune system which help defend against antigens

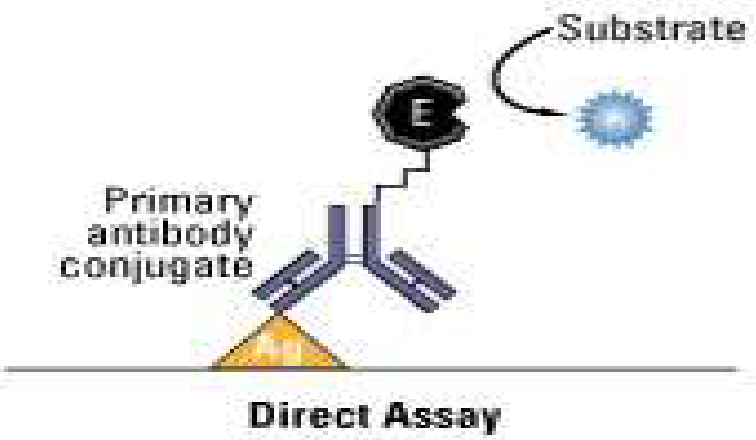
SYMBOL FOR ANTIBODY

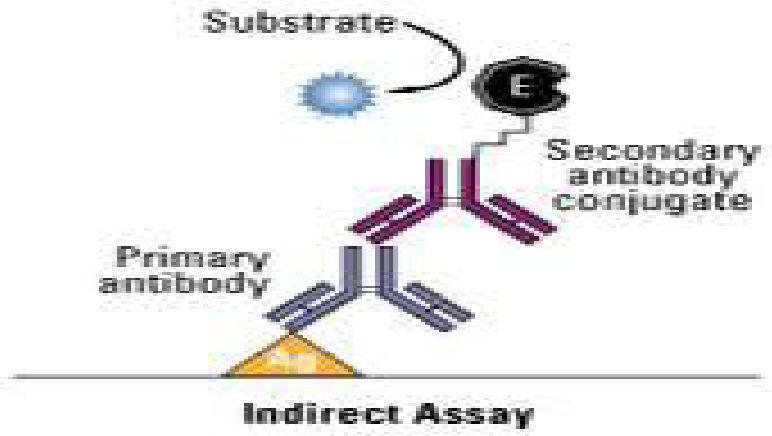
TYPES OF ELISA

Direct ELISA. 1
Indirect ELISA. 2
Sandwich ELISA. 3
Competitive ELISA. 4

DIRECT ELISA.1

- It is used in the detection of antigen .in the given biological sample
- Microtiter wells are initially coated with antigen to be detected which is followed by an antibody linked to an enzyme conjugate. This follows the addition of substrate which produces colour detected using ELISA .detector





INDIRECT ELISA. 2

- It is used for detection of an .antibody in the given sample
- Microtiter wells are initially coated with antigen specific for antibody to be detected, followed by the addition of sample. Enzyme conjugated Secondary Antibody is added followed by the substrate which forms a coloured reaction .product

SANDWICH ELISA.3

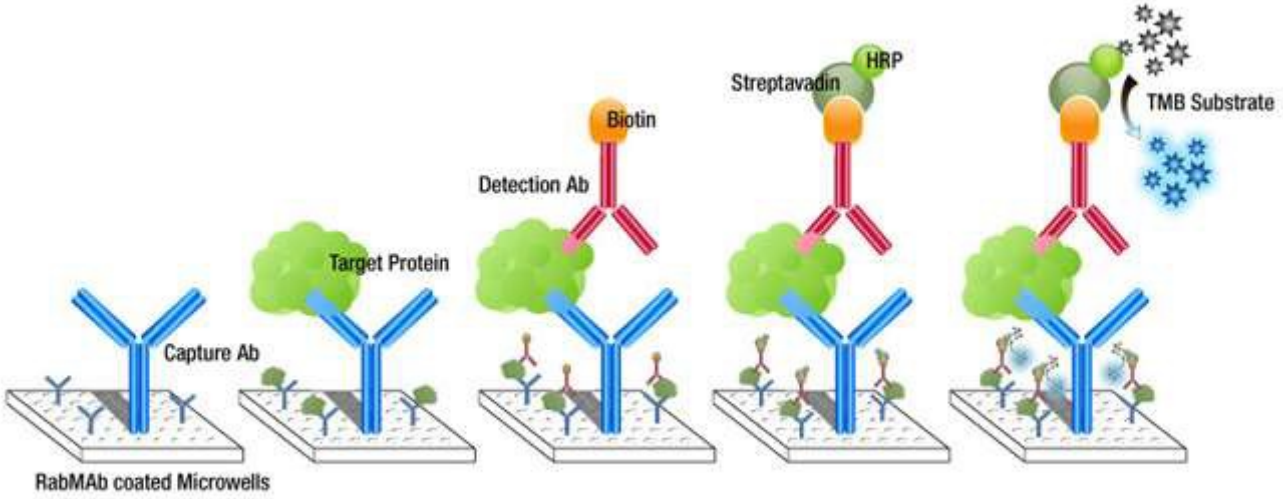
It is used for detecting an antigen in the-1 .given sample

Microtiter wells are initially coated with monoclonal antibodies(called capture antibody) raised against antigen to be .detected, followed by addition of sample

Any trace of antigen is detected by adding primary antibody (a MAb),followed by enzyme conjugated secondary Ab and a chromogenic substrate; or by directly .adding an enzyme conjugated primary Ab

Specimen sample for ELISA

Serum HIV
CSF Neurocysticercosis
Sputum Brucellosis
Urine Legionnaire's disease
Stool Giardiasis

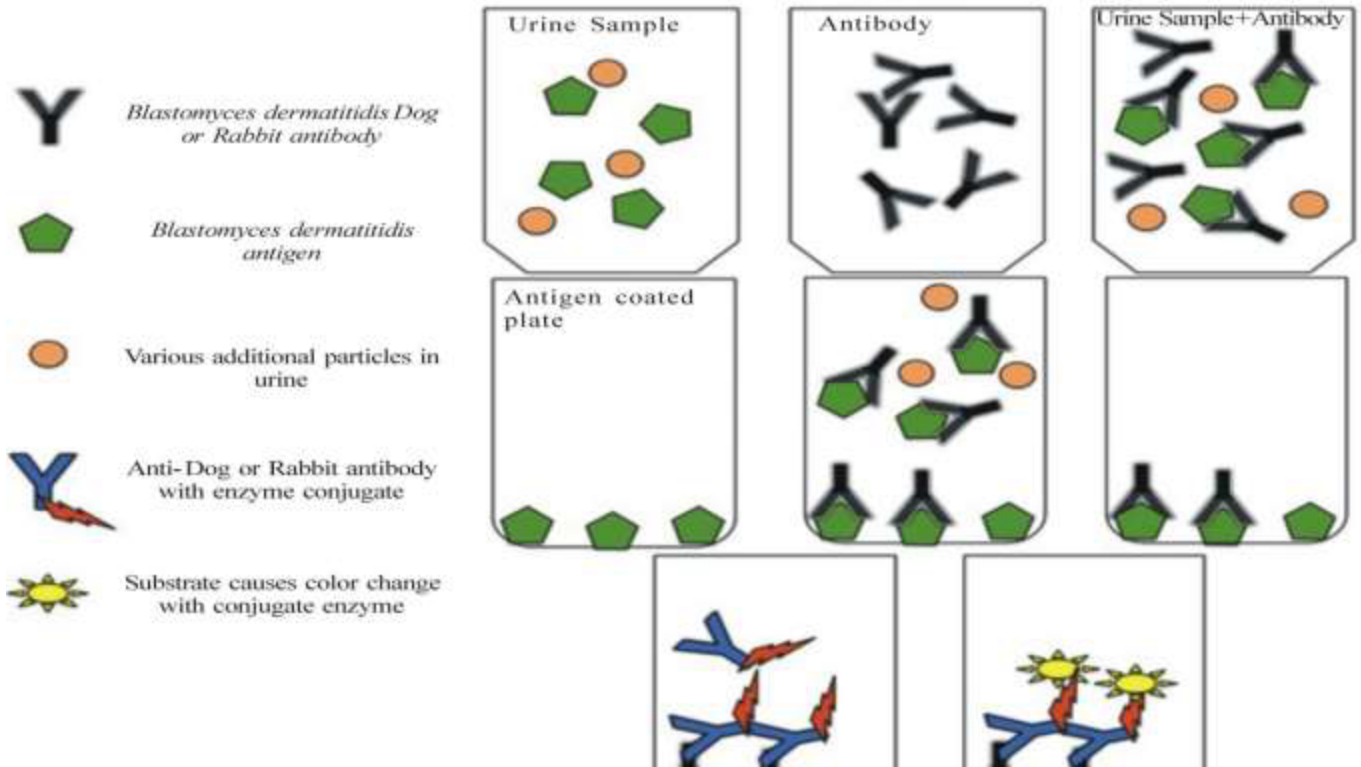


SANDWICH ELISA

COMPETITIVE ELISA.4

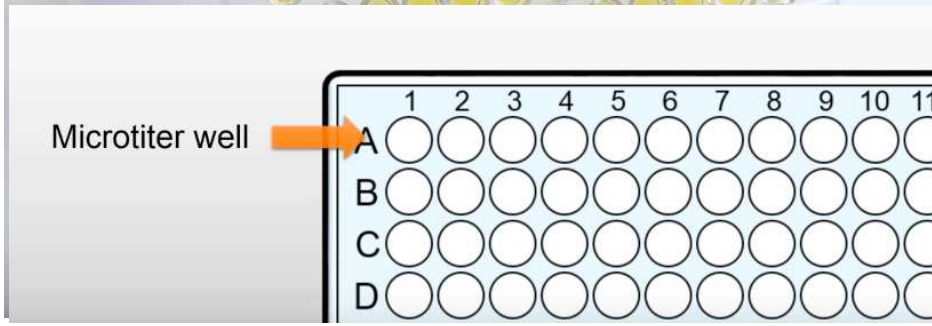
- This variation of ELISA is used to quantitatively estimate the amount of antigen in the given .sample
- Ag and Ab are initially incubated so that they form Ag-Ab complex. This mixture is then added to microtiter wells coated with synthetic analogue of antigen to be ,detected any free antibody binds to these antigens

This complex is estimated by enzyme conjugated secondary antibody by chromogenic detection .More the amount of antigen in the sample, lesser is the antibody .available to bind to microtiter wells



:Equipments

:Washing Device) 3



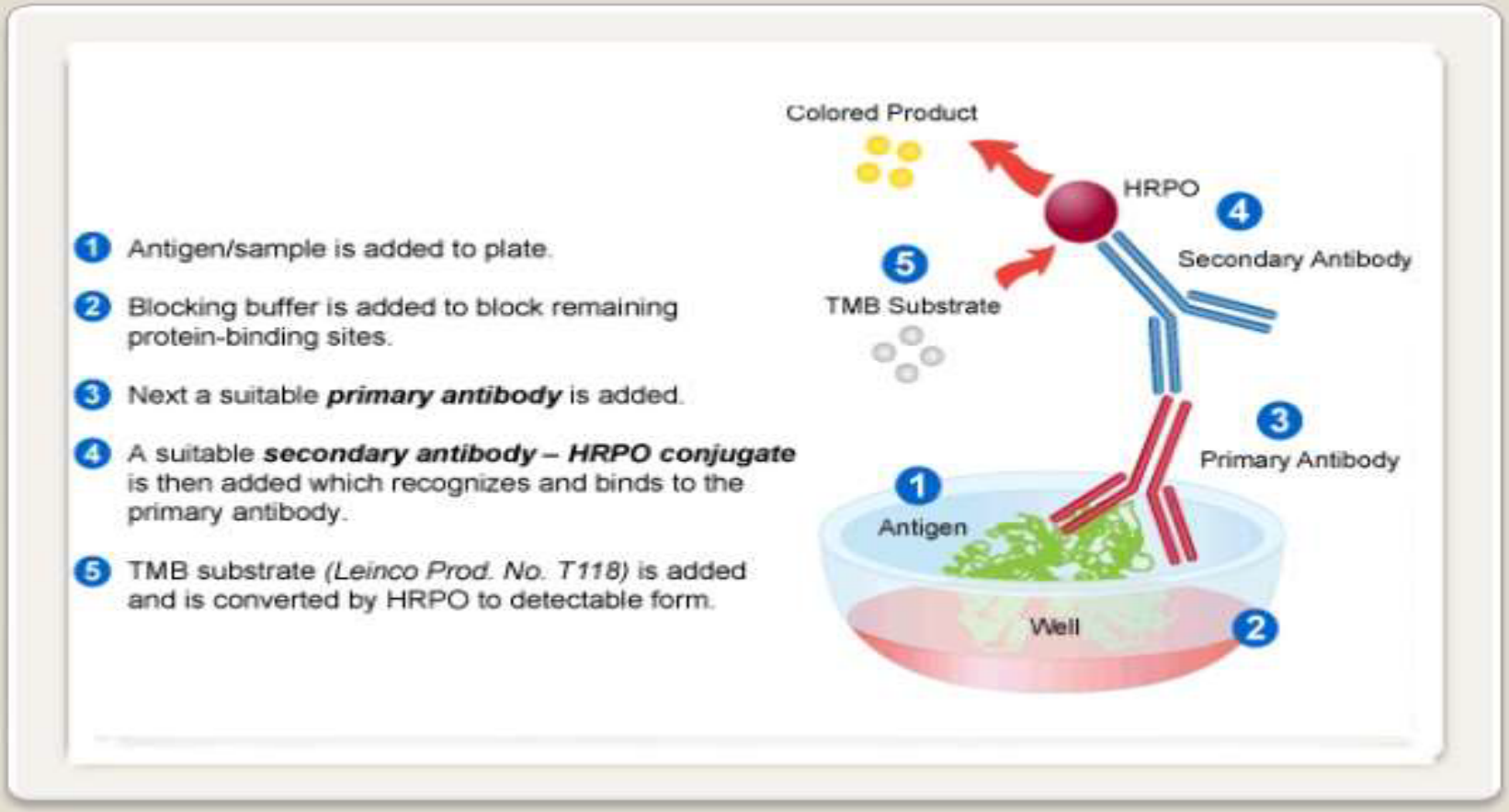
:Microplate washer) 4

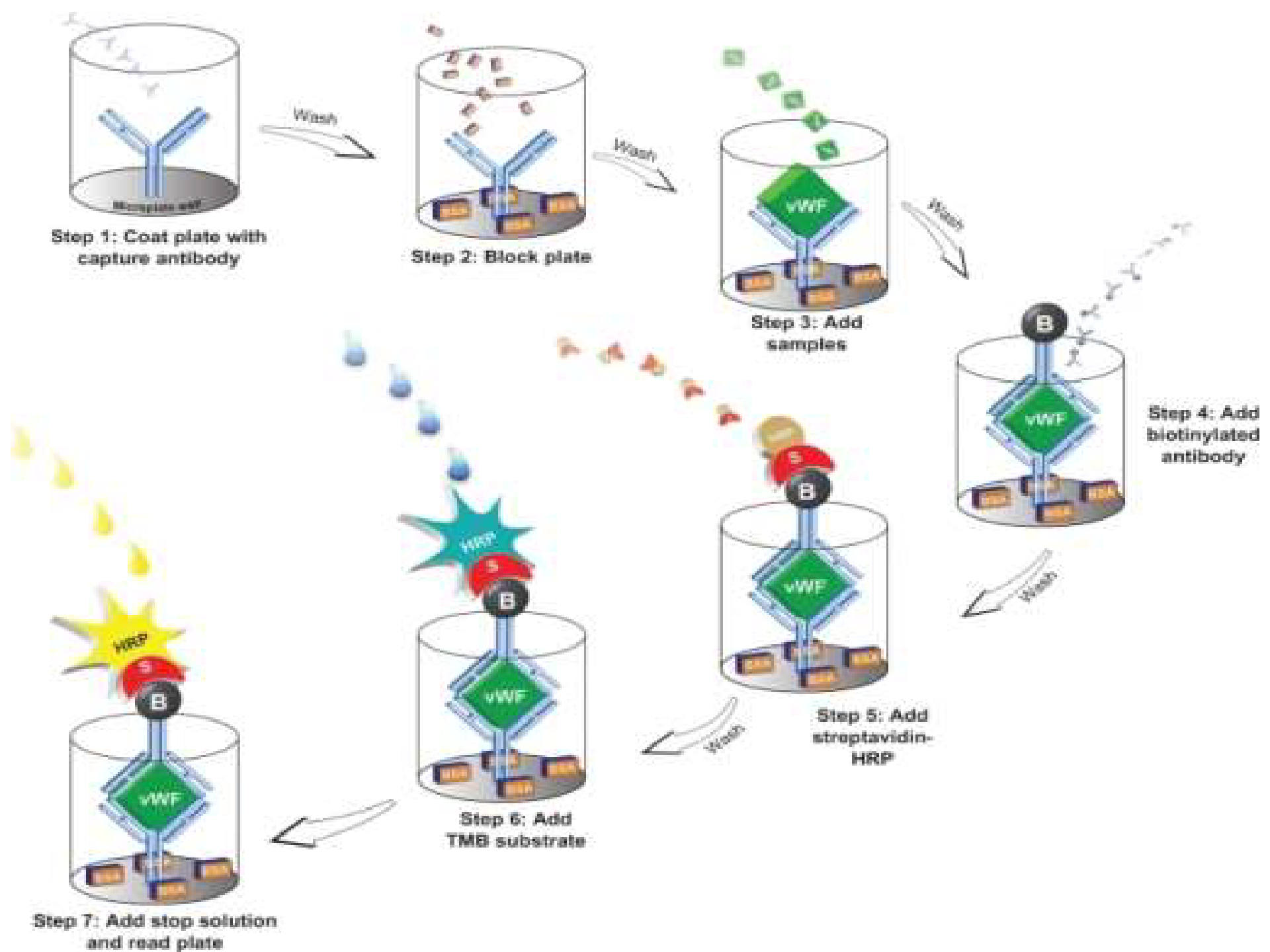
:Multipipette)

:Reagents Used

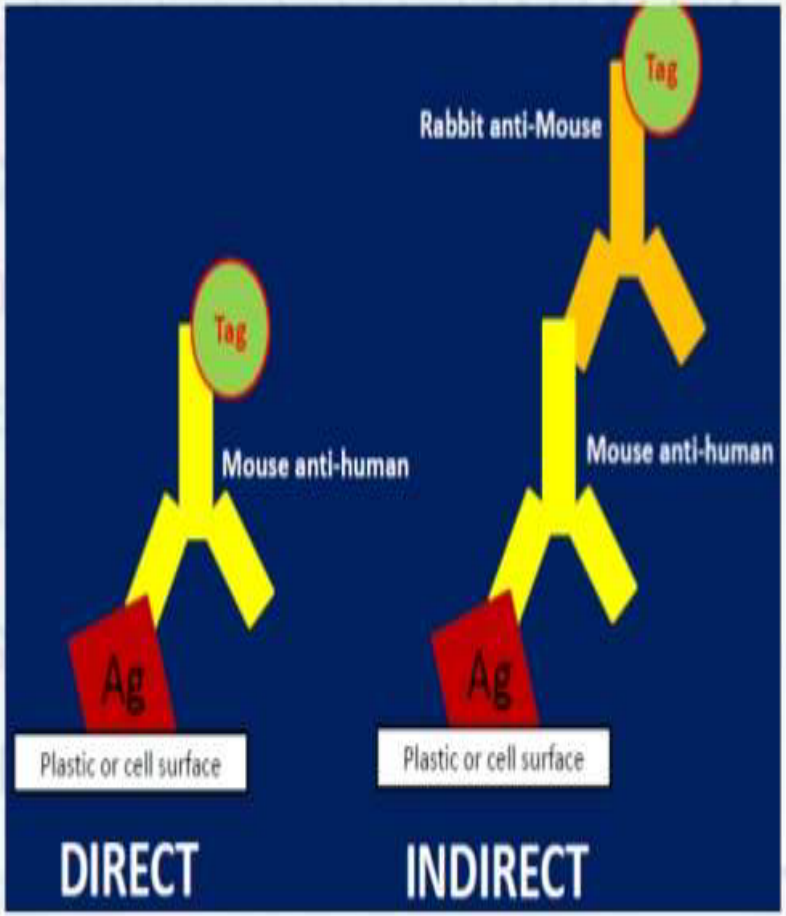
Coating Buffer 0.01 M Phosphate Buffer
M NaCl (PBS) 0.15+
Diluting/Washing Buffer 0.01 M Phosphate Buffer
M NaCl + 0.1% Tween 20 0.50+
Blocki
Albu
)BSA(

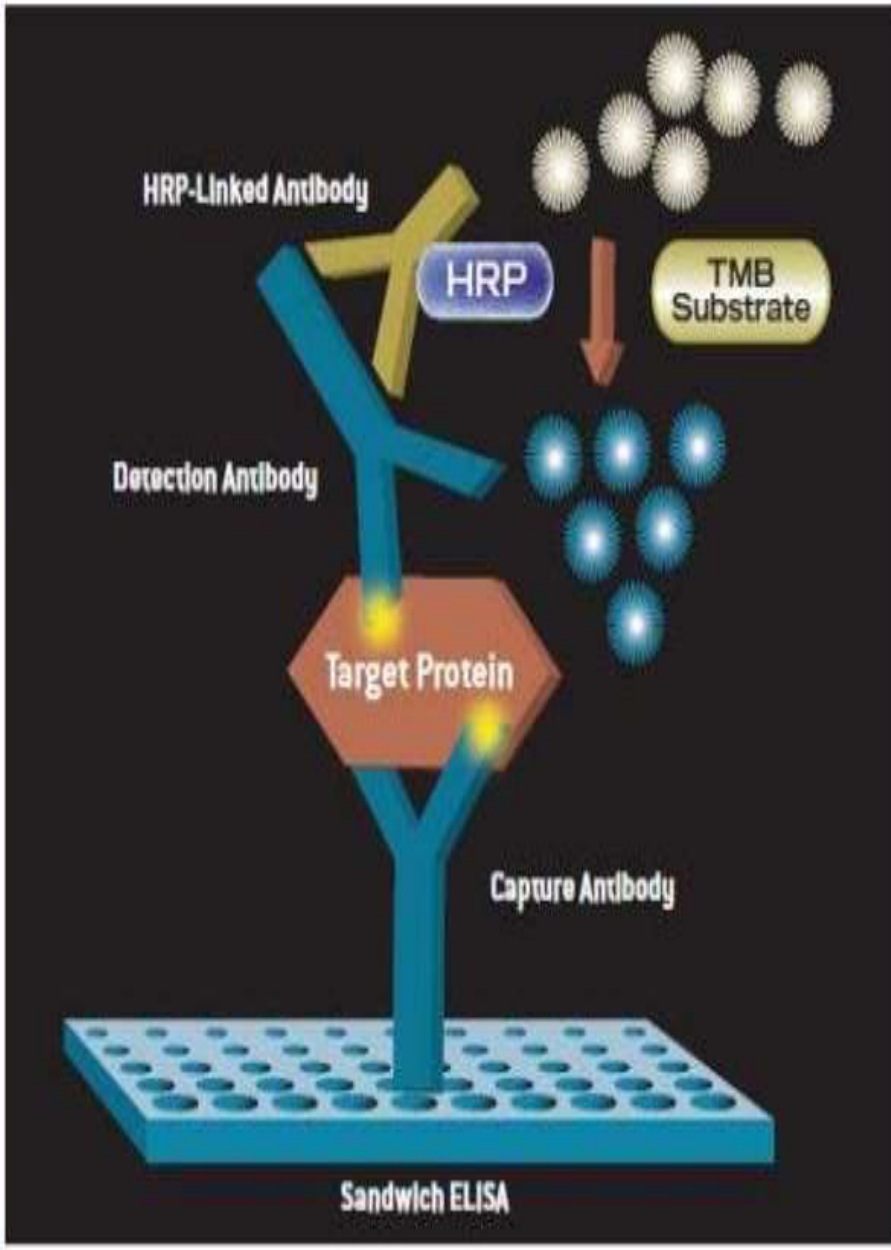
Enzyme Horse-redish peroxidase)HRPO(
Chromogenic Substrate Trimethyl benzidine)TMB(
Stop Solution 0.5 M H_2SO_4

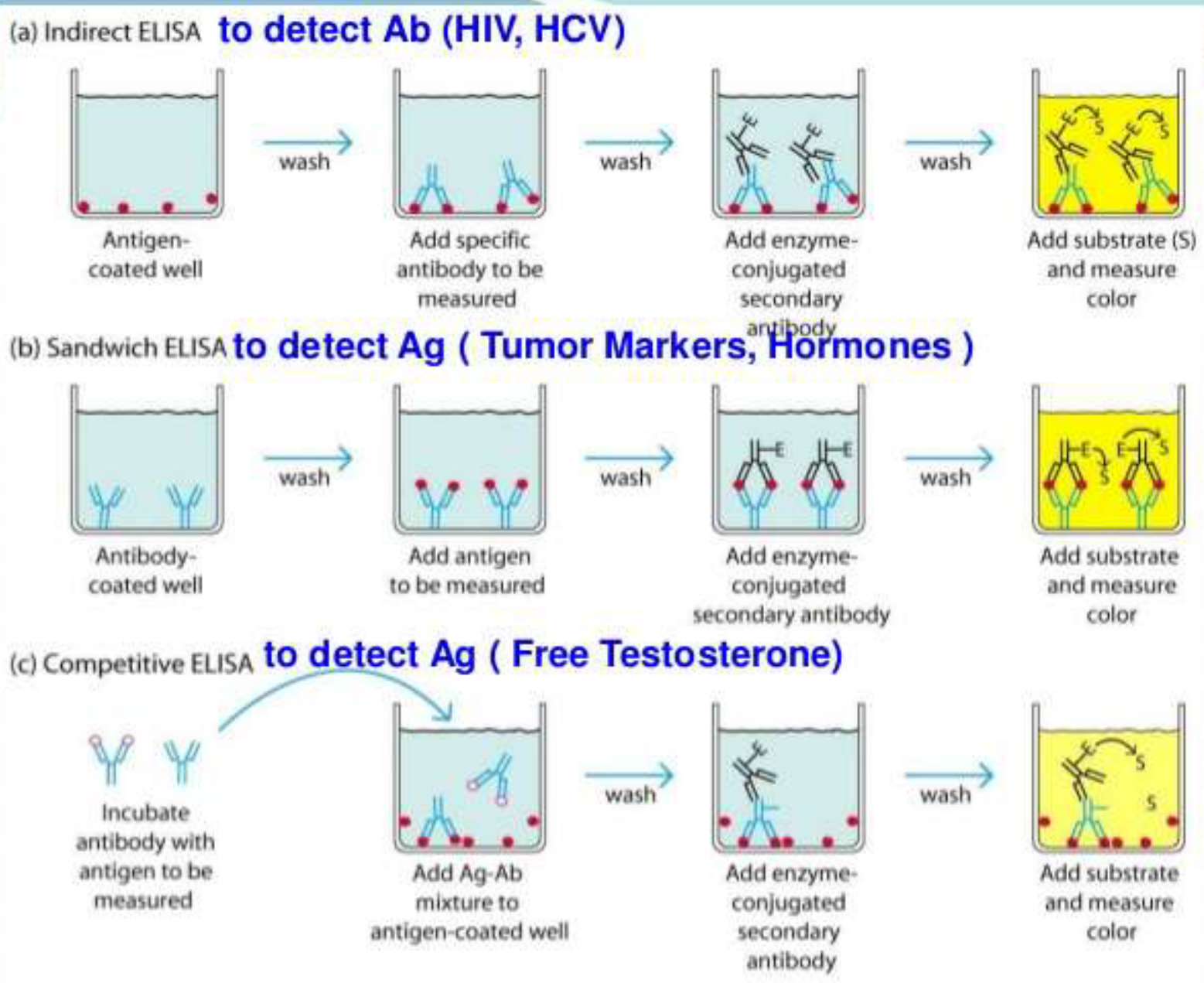



Step 1: Coat plate with capture antibody
Wash
Step 2: Block plate
Wash
vWF
Step 3: Add samples
Wash
B
vWF
Step 4: Add biotinylated antibody
Wash
S
B
vWF
Step 5: Add streptavidin-HRP
Wash
HRP
S
B
vWF
Step 6: Add TMB substrate
HRP
S
B
vWF
Step 7: Add stop solution and read plate

Direct and Indirect ELISA

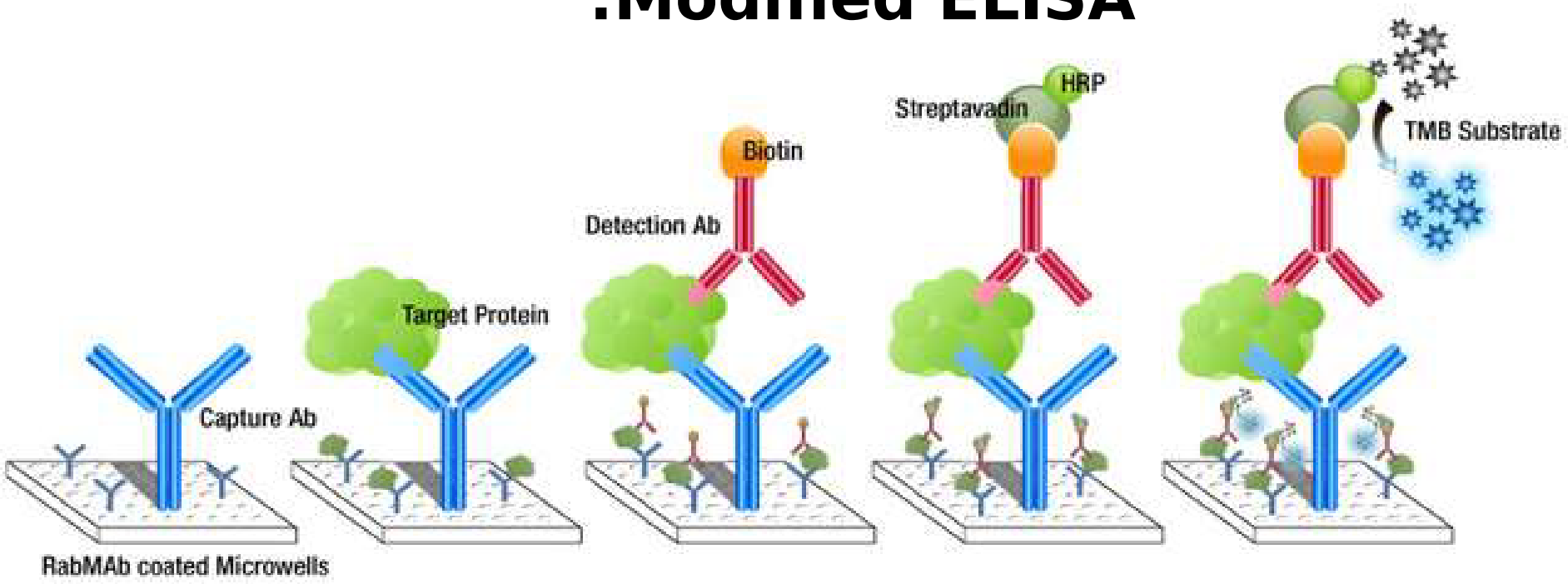




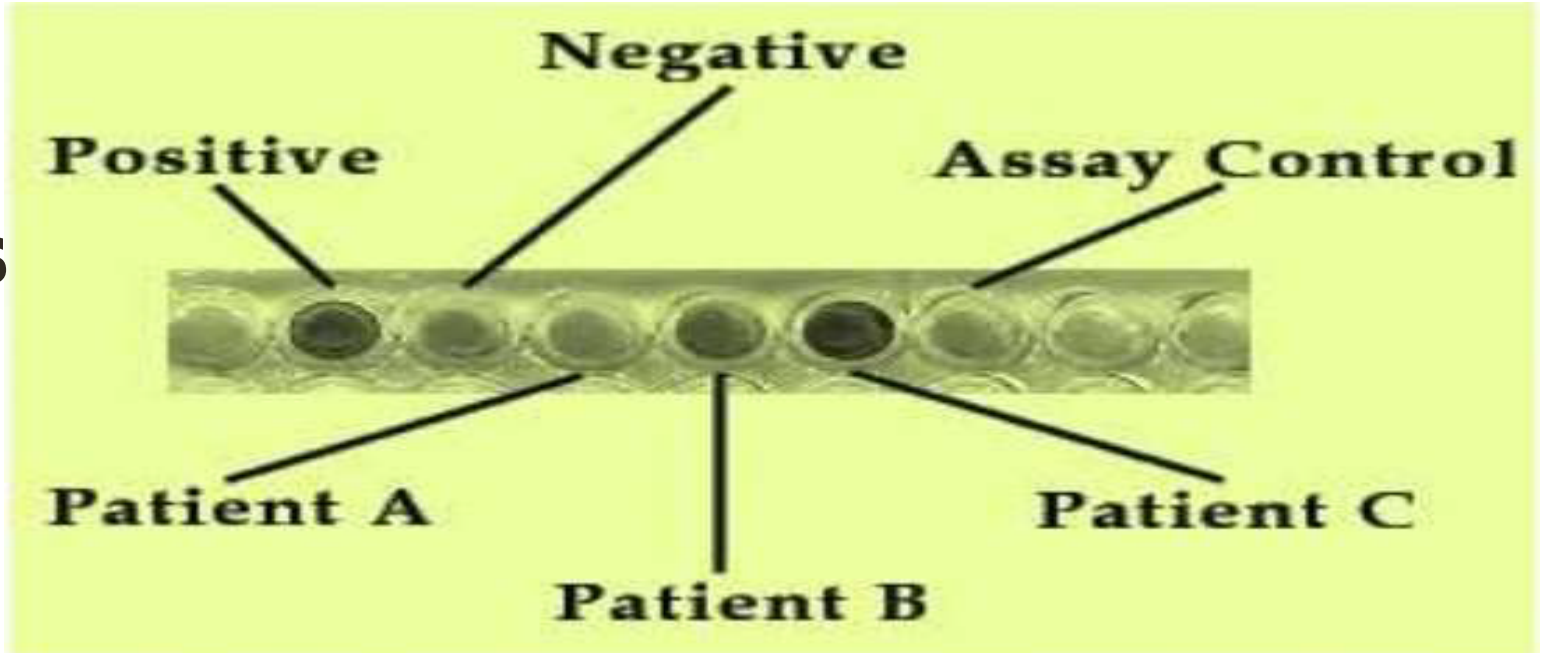


Comparison between Indirect Sandwich & Competitive ELISA

:Modified ELISA



:Results



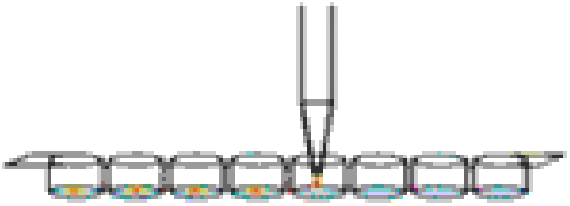
APPLICATIONS OF ELISA

Hormones 7- Vaccine Quality- 1 Control
Proteins 8- FOR GMO (Genetically- 2 modified
)organism
,Infectious Agent (Viral, Bacterial- 3
)Parasitic, Fungal
For Rapid Test- 9
Drug Markers 10- IgG, IgM, IgA- 4
Tumor Markers 11- In New Born- 5 Screening
Serum Proteins 12- In Clinical Research- 6

What Are The ?Reagents And What Function Do They ?Perform

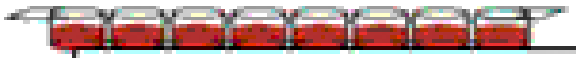
Antigen: **Elisa plate coated with the A60 .antigen-antibodies complexes**
Primary antibody: **Human Serum IgG, , &IgA IgM**
Secondary antibody: **Peroxidase-labelled antihuman IgA, IgG, or IgM antibodies that bind .to the antibobdy complexes**
' –Enzyme substrate: **3,3',5,5 tetramethylbenzidine (TMB) – a colorless solution that when oxidized by HRP turns .blue**
Stop Solution: **Sulphuric Acid 0.5N (H_2So_4)**

ASSAY PROCEDURE

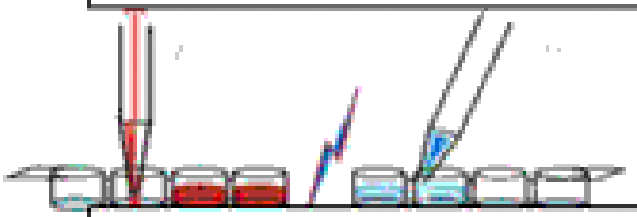


1. Dispense diluted sample

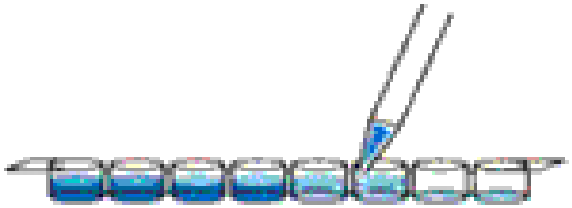
2. Add 100 ul of enzyme conjugate



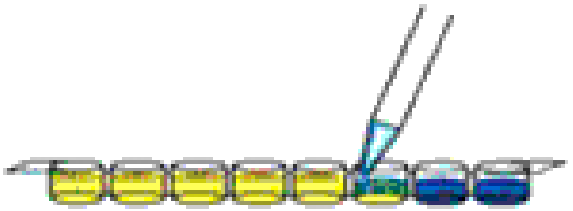
3. Incubate for 1 hour at room temperature



4. Wash plates 6 times with deionized water



5. Add TMB colour developer substrate and incubate for 30 min



6. Add dilute acid stop solution and read at 450nm

Polymerase Chain Reaction (PCR)

?What is PCR

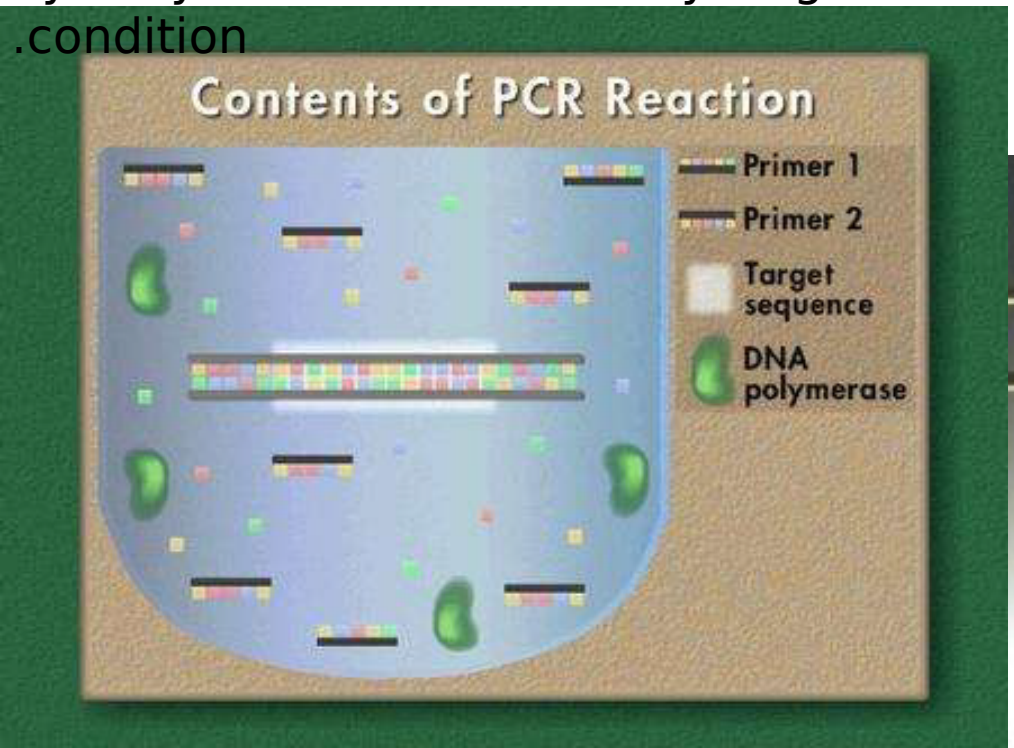
PCR is a technique that takes• specific
sequence of DNA of small amount and
amplifies it to be used for further .testing

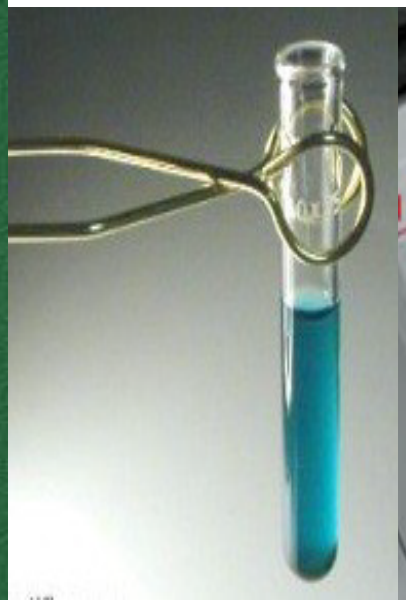
Purpose

To amplify a lot of double-stranded• DNA molecules
in vitro technique•
with same (identical) size) fragments(and sequence
by enzymatic method and cycling
.condition

Chemical Components

- DNA Template
- Primers
- Taq polymerase
- Deoxynucleoside triphosphates(dNTPs)
- Buffer solution
- Divalent cations(eg.Mg_2





Principle

To amplify a lot of doublestranded DNA molecules with same) fragments(size and) identical(sequence by enzymatic method and cycling .condition

Steps of PCR

1. Denaturation of ds DNA template.
2. Annealing of primer s . . s
3. Extension of ds DNA molecules.

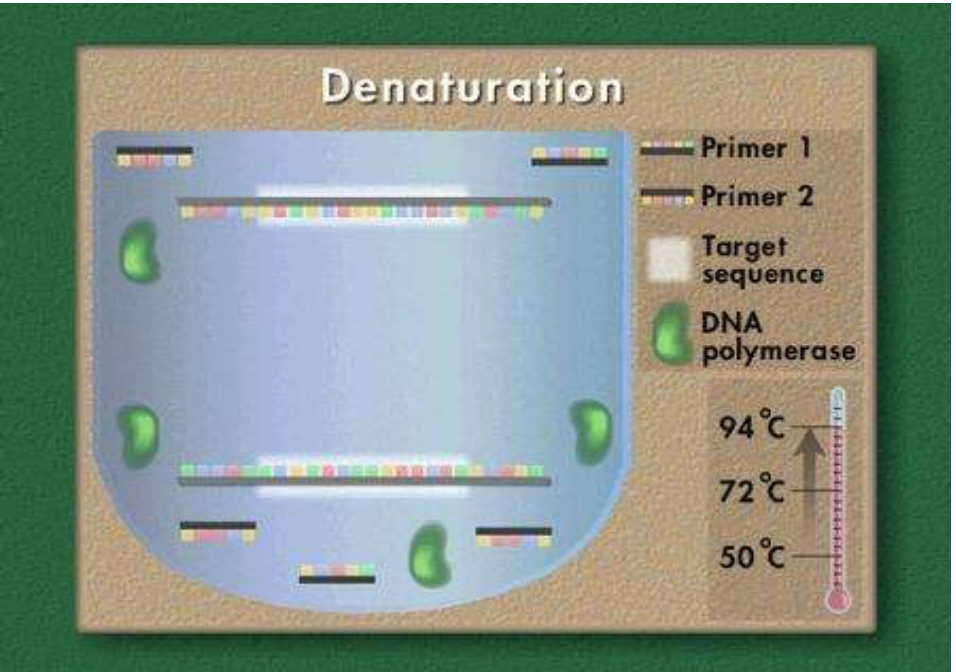
Denaturation. 1

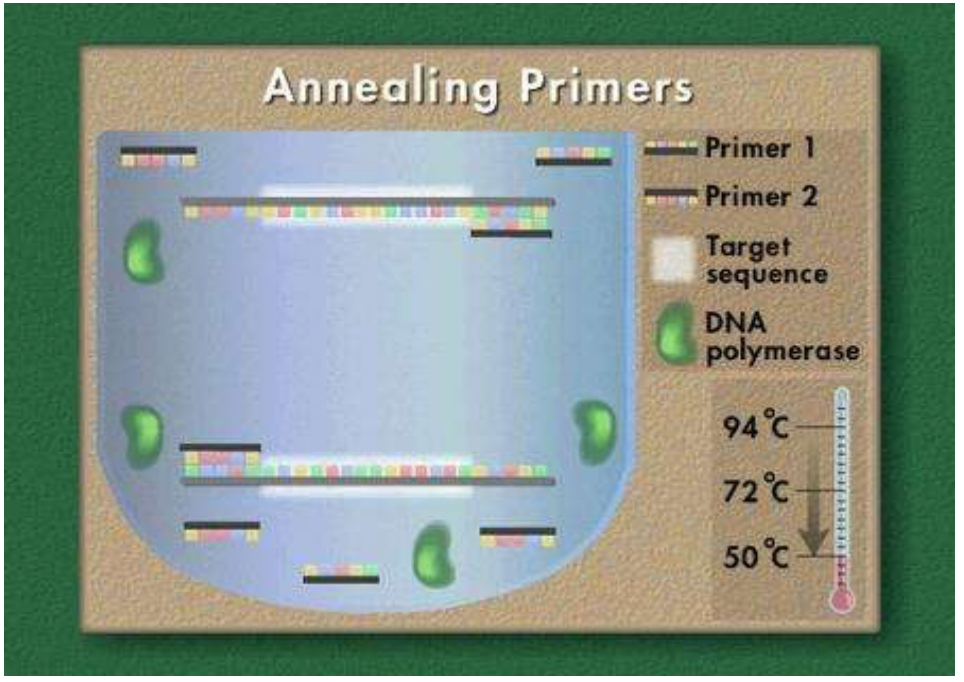
C so that the ds DNA is 90-98° denatured into single strands

Duration of this step is 1-2 mins

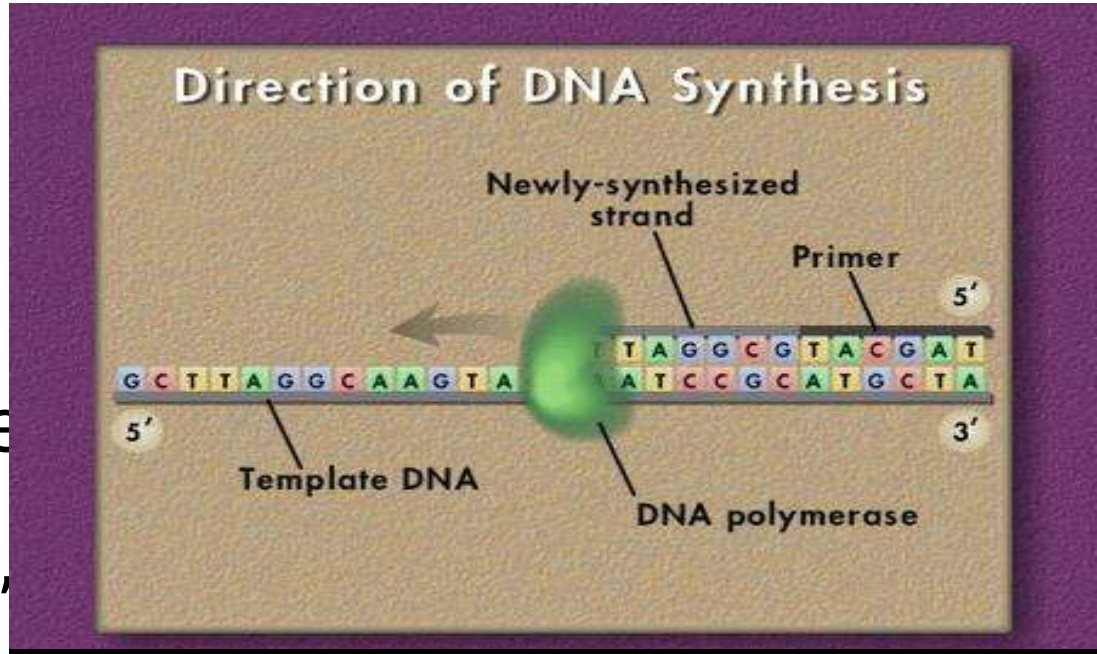
Annealing-2

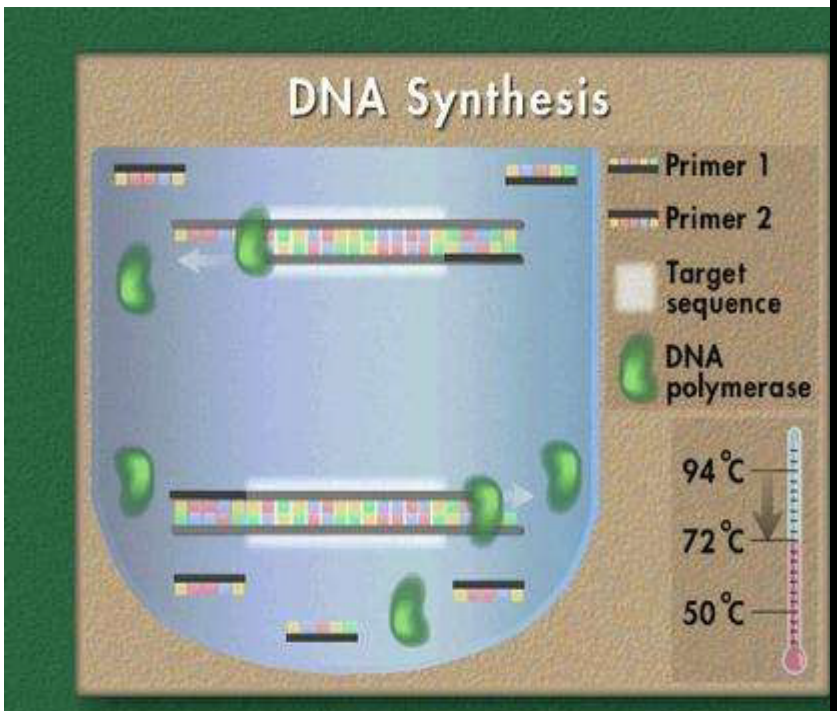
- Temperature: ~55-68 oC
- Primers bind to their complementarysequences

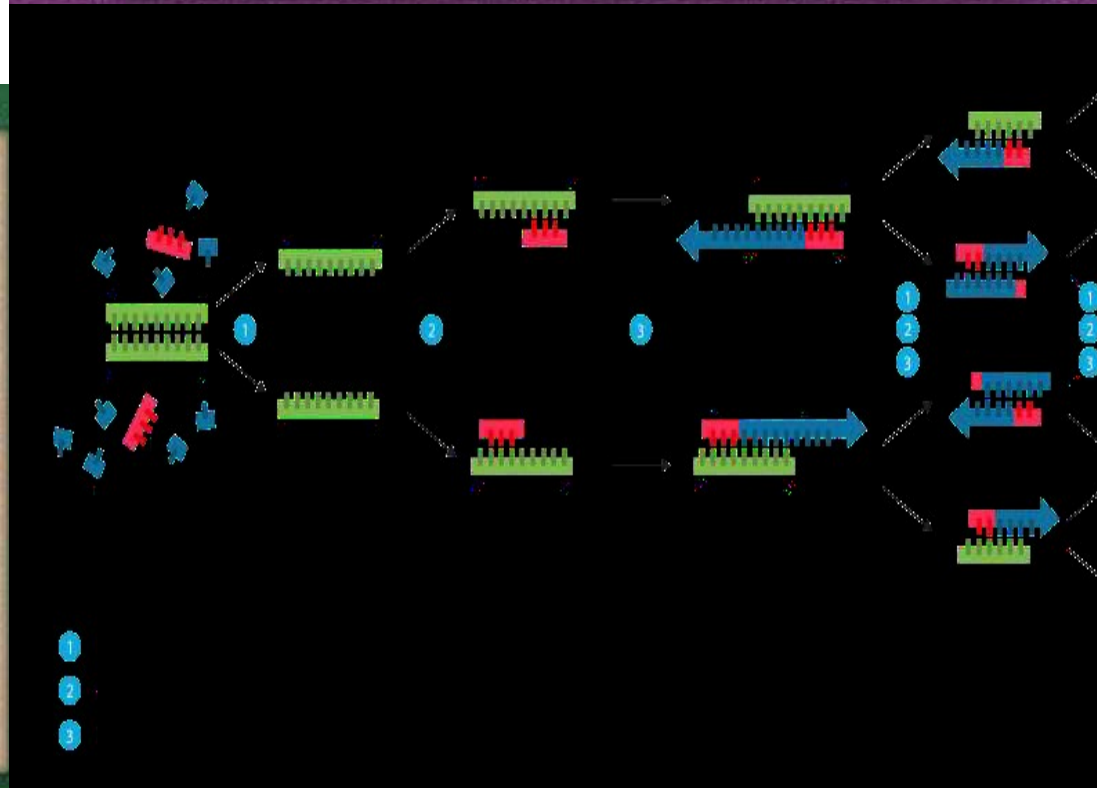




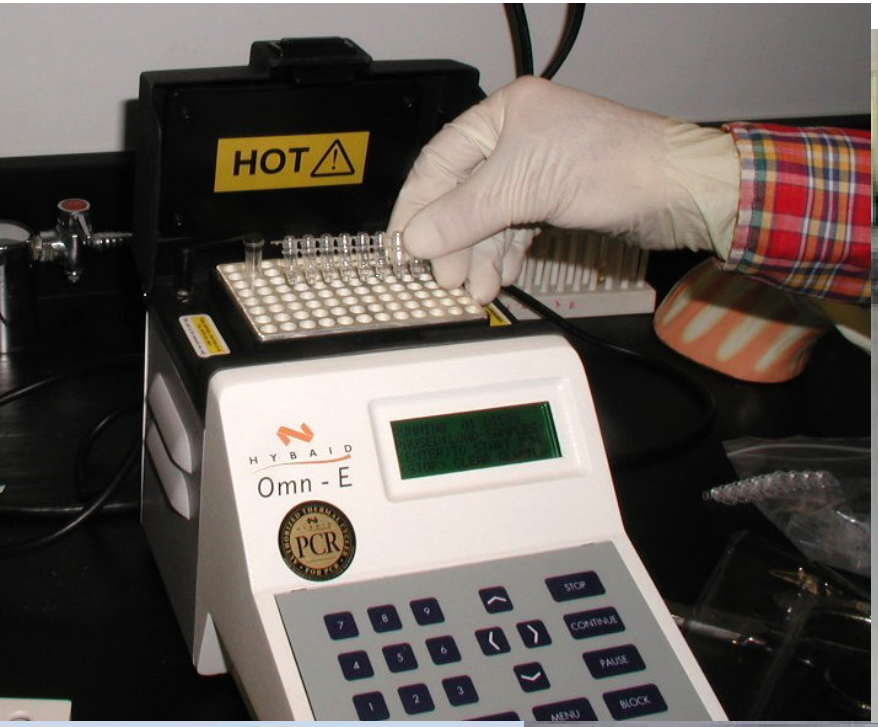
Extension-3
Temperature: ~72C•
Time: 0.5-3min•
DNA polymerase bind
to the annealed prime
and
extends DNA at the 3’
end of the chain

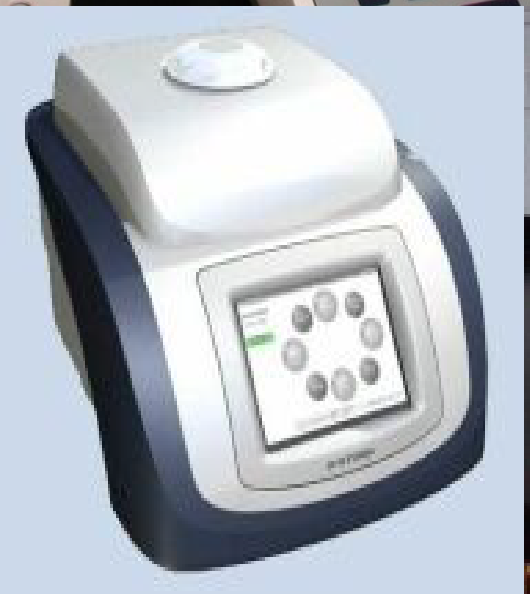






Thermo cyclers





Applications of PCR

Molecular Identification

Engineering

Molecular Archaeology

Molecular Epidemiology

DNA fingerprinting

Molecular Ecology

Classification of organisms

Genotyping

Pre-natal diagnosis

Mutation screening

Drug discovery

Genetic matching

Detection of pathogens

Sequencing

Bioinformatics

Genomic Cloning

Human Genome Project

Genetic Engineering

Site-directed mutagenesis

Gene Expression Studies

Differences

TEM Transmission Electron Microscope	SEM Scanning Electron Microscope
Based on transmitted electrons	Based on scattered electrons
Electrons are directly pointed toward the sample and the parts through which electrons are passed are illuminated in the image.	The scattered electrons produced the image of the sample after the microscope collects and counts the scattered electrons.
TEM seeks to see what is inside or beyond the surface	SEM focuses on the sample's surface and its composition
TEM shows the sample as a whole.	SEM also shows the sample bit by bit
TEM delivers a two dimensional picture.	SEM also provides a three dimensional image
TEM has up to a 50 million magnification level.	SEM only offers 2 million as a maximum level of magnification.
The resolution of TEM is 0.5 Å.	SEM has resolution of about 0.4 nm.
TEM The image is produced by the microscope via fluorescent screens.	In SEM, The image of the sample is projected onto the CRT or television-like screen.

DNA Extraction

DNA extraction is a procedure used to isolate DNA from the nucleus of cells•

Purpose of DNA Extraction•

To obtain DNA in a relatively purified form which can be used for further investigations, i.e. PCR, sequencing, etc

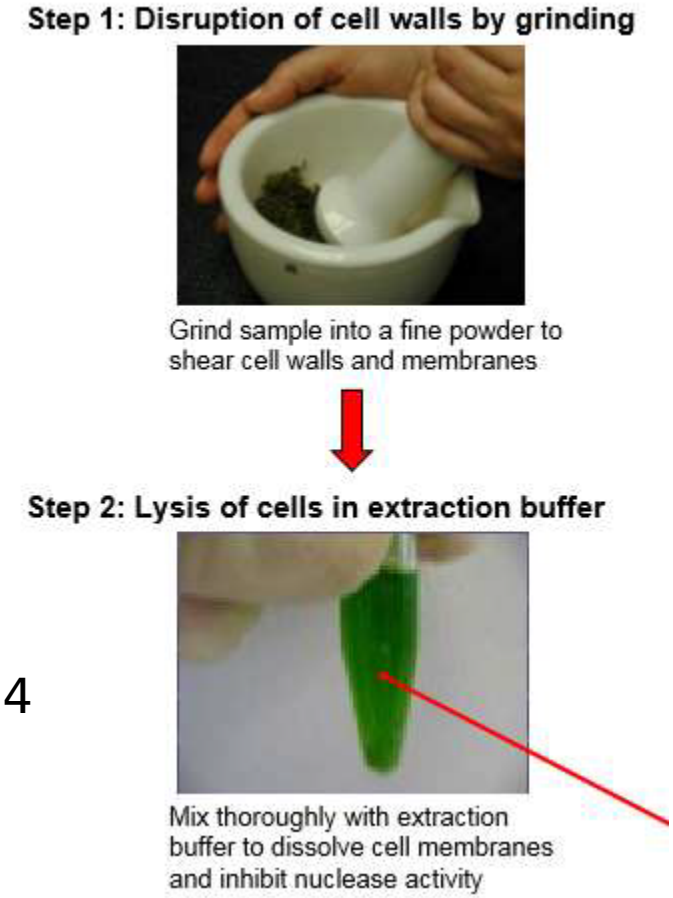
Basic steps for DNA extraction

Breaking the cells open, commonly referred. 1 to as cell disruption or cell lysis, to expose the DNA within. This is commonly achieved by grinding, sonicating or treating the sample with lysis buffer.

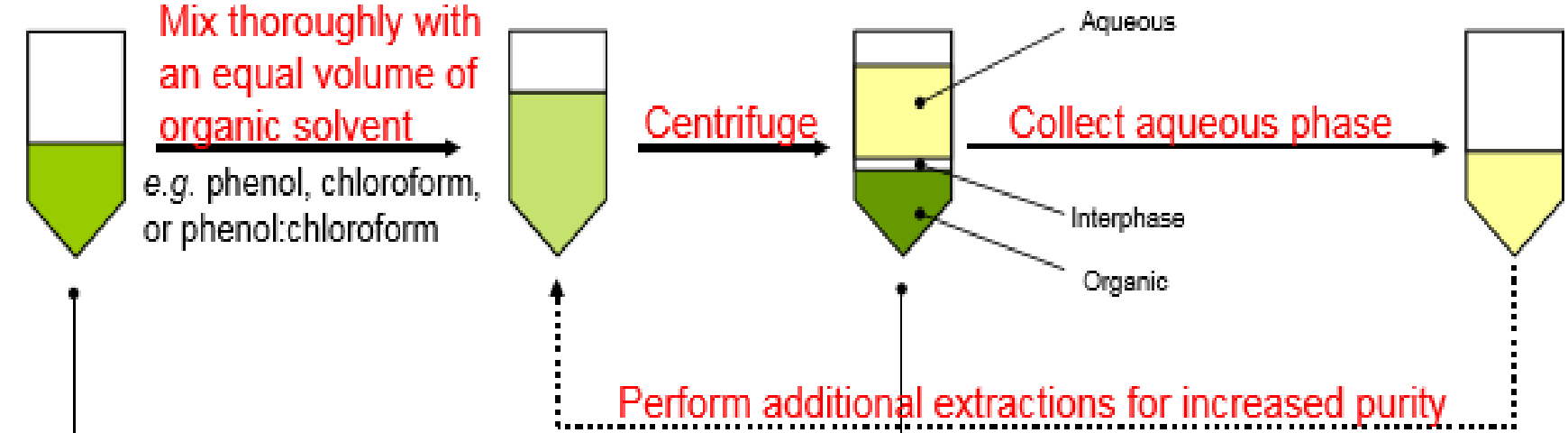
- detergent

Removing membrane lipids by adding a. 2 Removing proteins by adding a protease. 3 (optional but).almost always done

Precipitating the DNA with an alcohol — usually. 4 ice-cold ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-

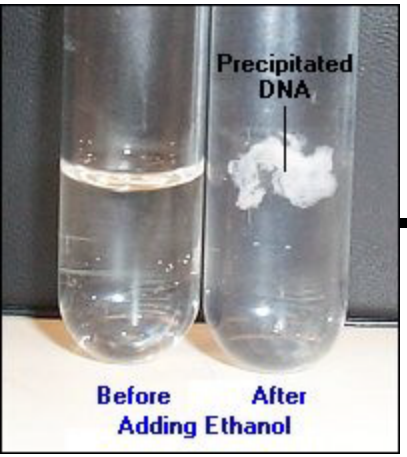


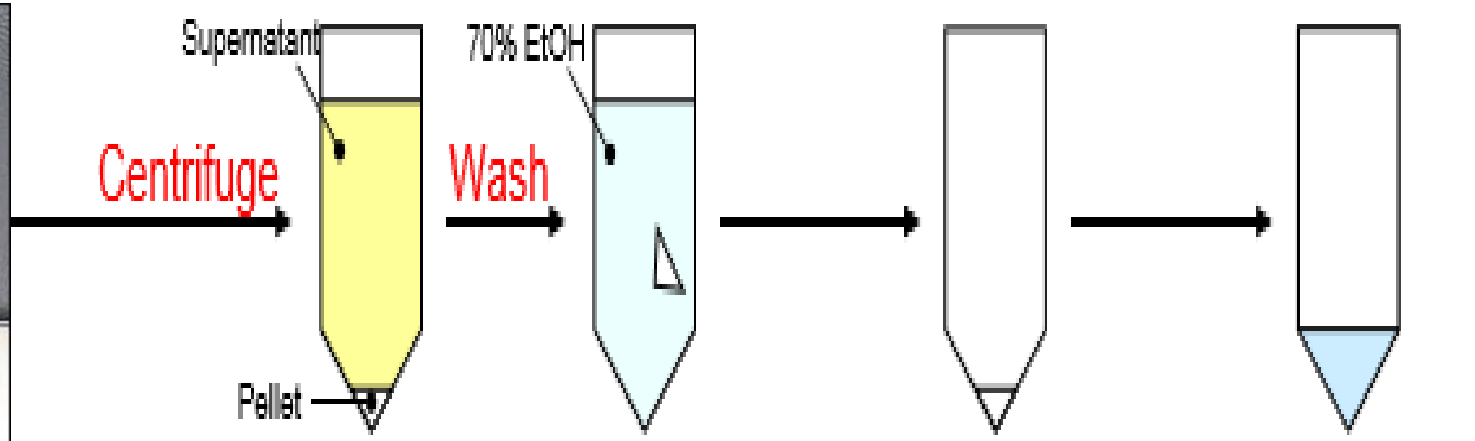
Step 3: Organic extraction



Crude lysate containing nucleic acids and other cell constituents

The **aqueous phase** contains water-soluble molecules, including nucleic acids. Proteins and lipids become trapped in the **organic phase**, and are thus separated away. Insoluble plant debris become trapped in the **interphase** between the two layers





Dissolve pellet (H_2O, TE, etc.)

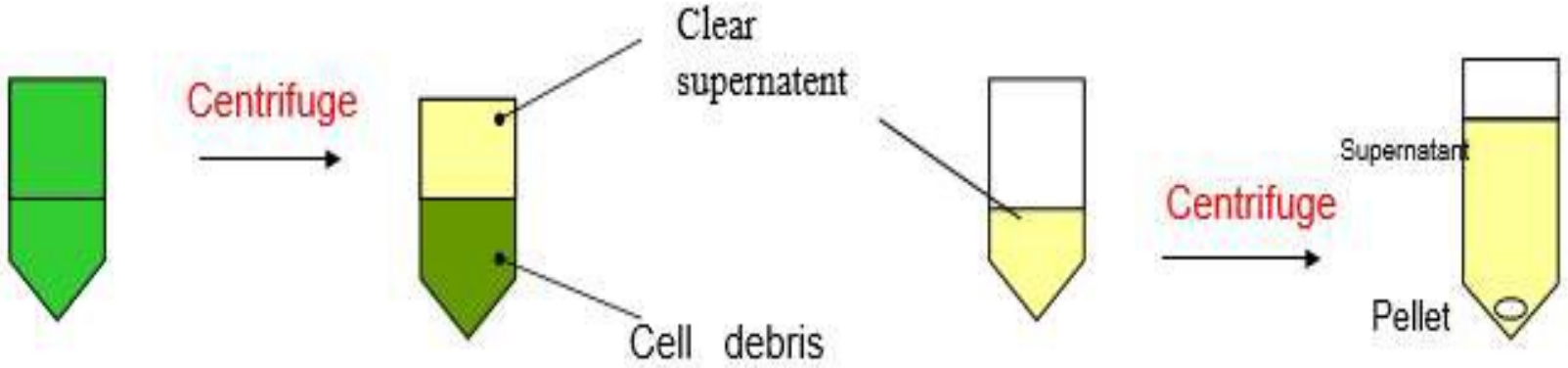
Add alcohol and salt to precipitate nucleic acids from the aqueous fraction

- Pellet down nucleic acids.
- Wash pellet with 70% ethanol to remove residual salts and other contaminants.
- Discard ethanol and allow pellet to dry.

Non-Phenol Chloroform based extraction of DNA

What are the essential components of a DNA extraction ?Procedure

Maximize DNA recovery. 1
Remove inhibitors. 2
Remove or inhibit nucleases. 3
Maximize the quality of DNA. 4

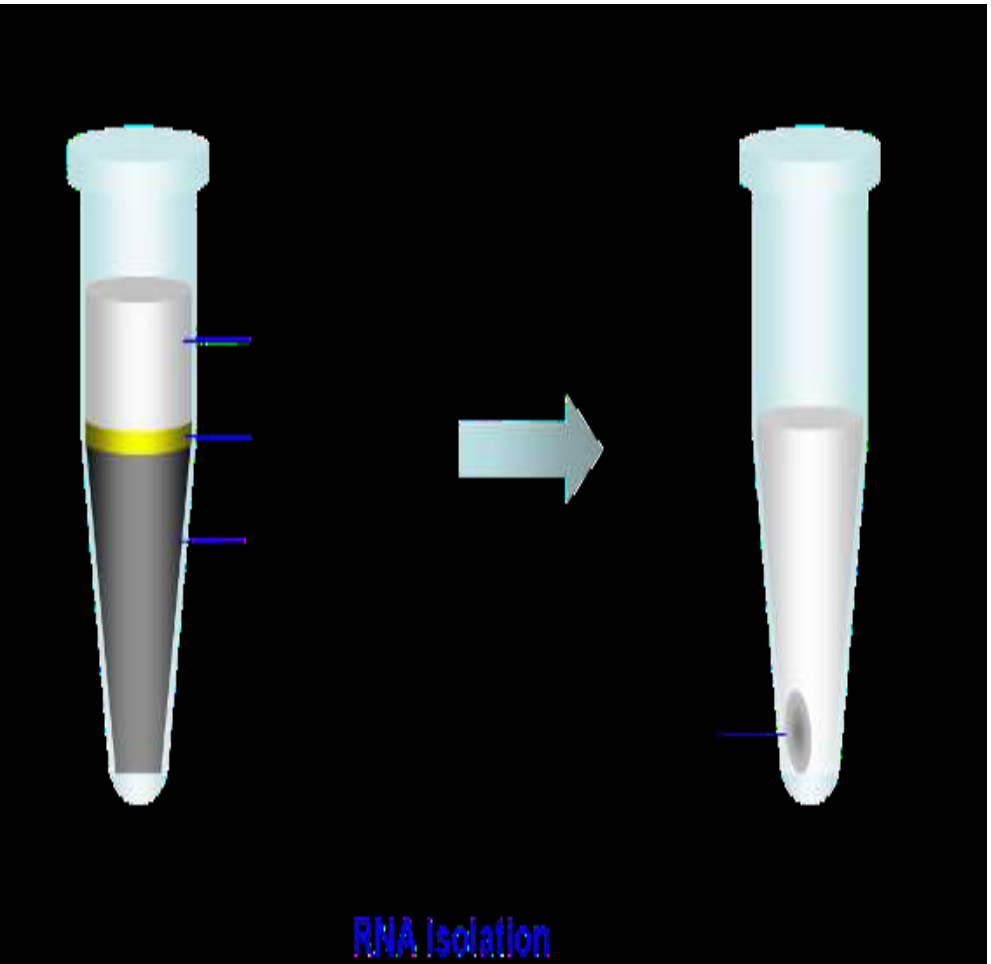


Add isoprpanol /alcohol and salt to precipitate nucleic acids from the aqueous fraction

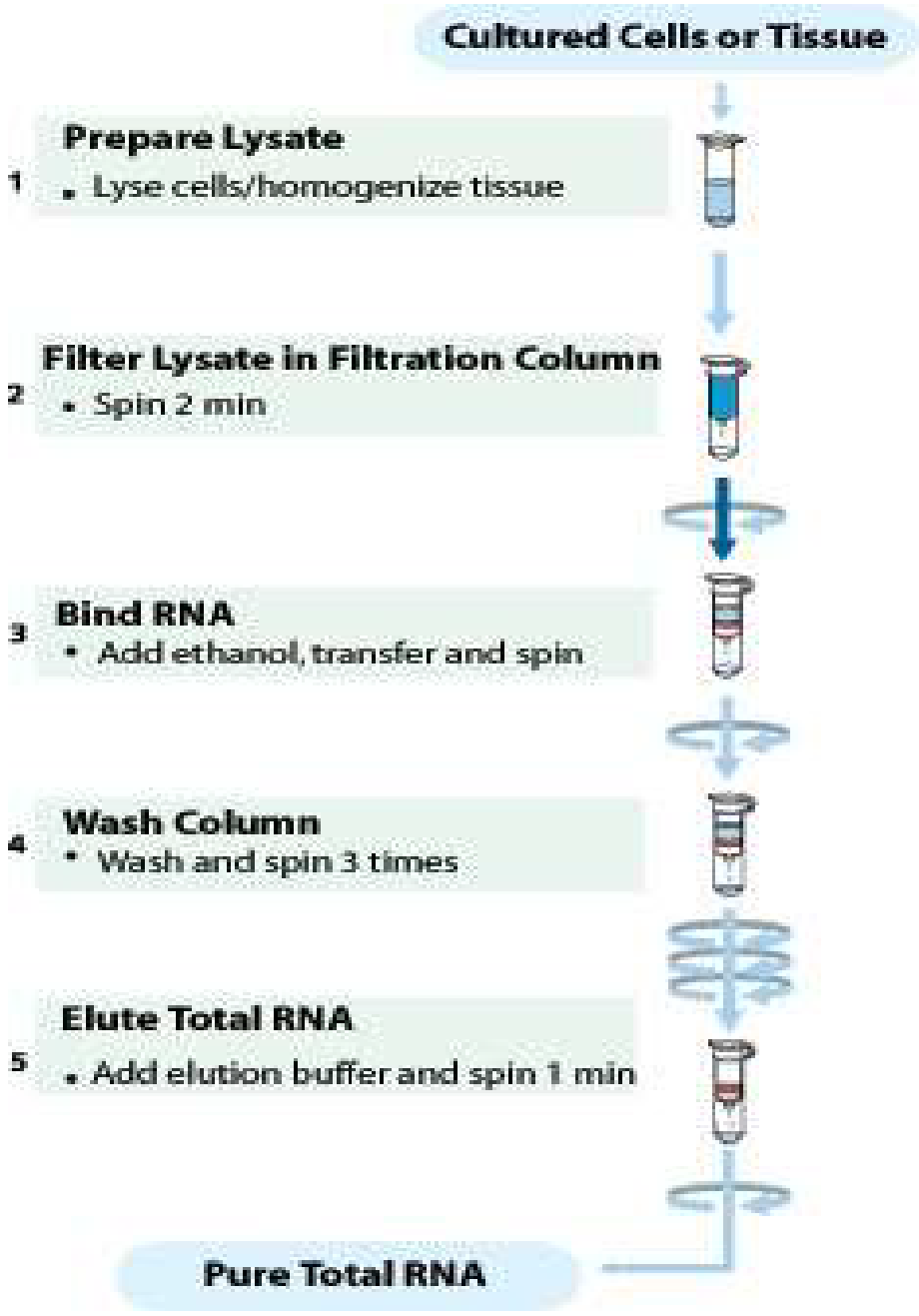
MORE TECHNIQUES (RNA ISOLATION)

- Organic Extraction Methods
- Filter-based RNA isolation
- Magnetic Particle Methods
- Direct Lysis Methods
- RNA extraction in liquid nitrogen

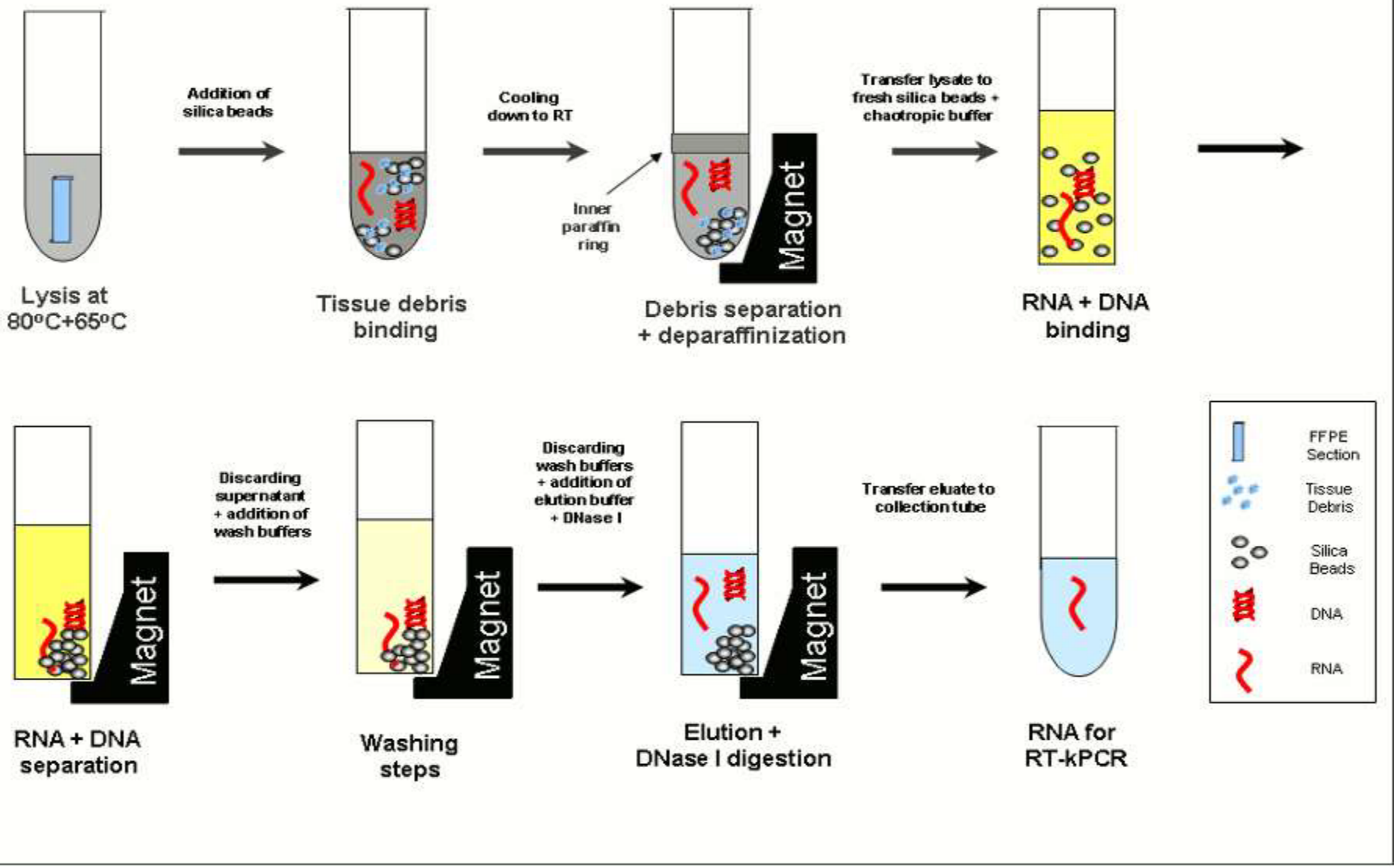
Organic Extraction Methods



Filter-based RNA isolation

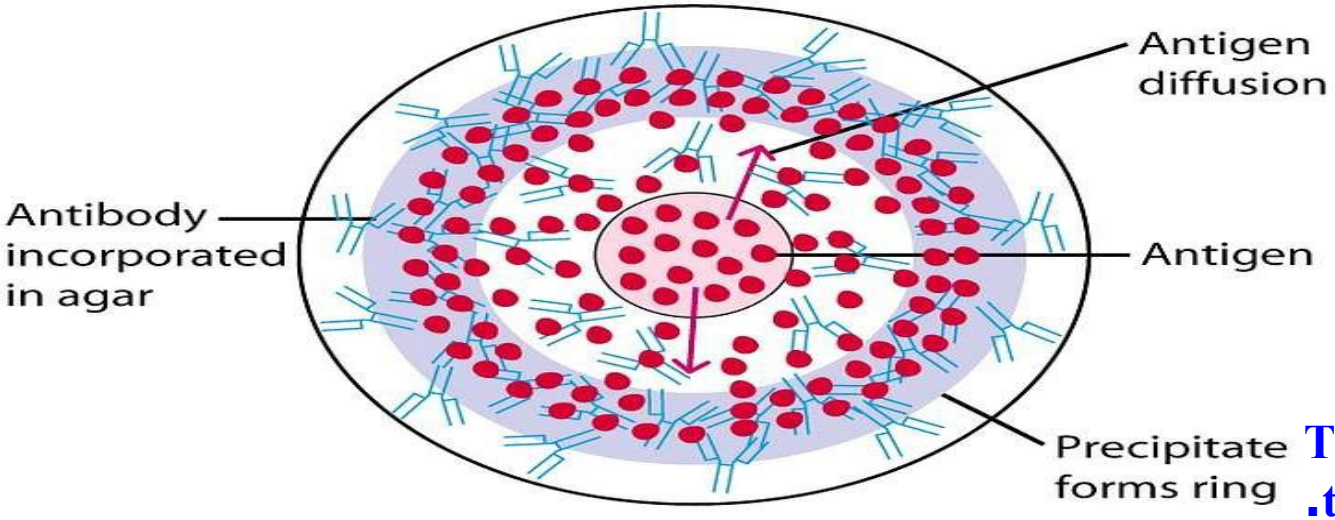


Magnetic Particle Methods



Precipitation Reactions

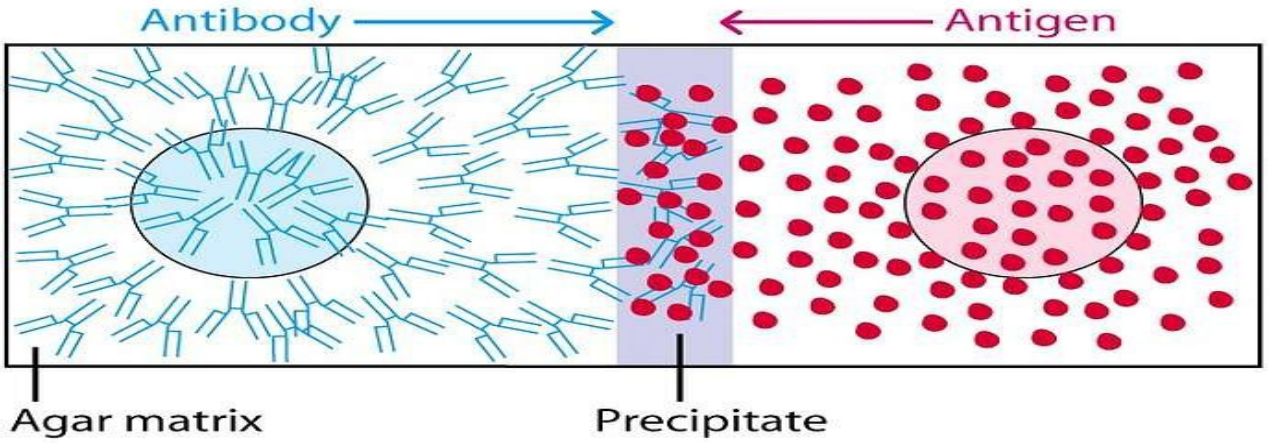
RADIAL IMMUNODIFFUSION



The area is proportional to .the conc. of Ag

The region of equivalence

DOUBLE IMMUNODIFFUSION

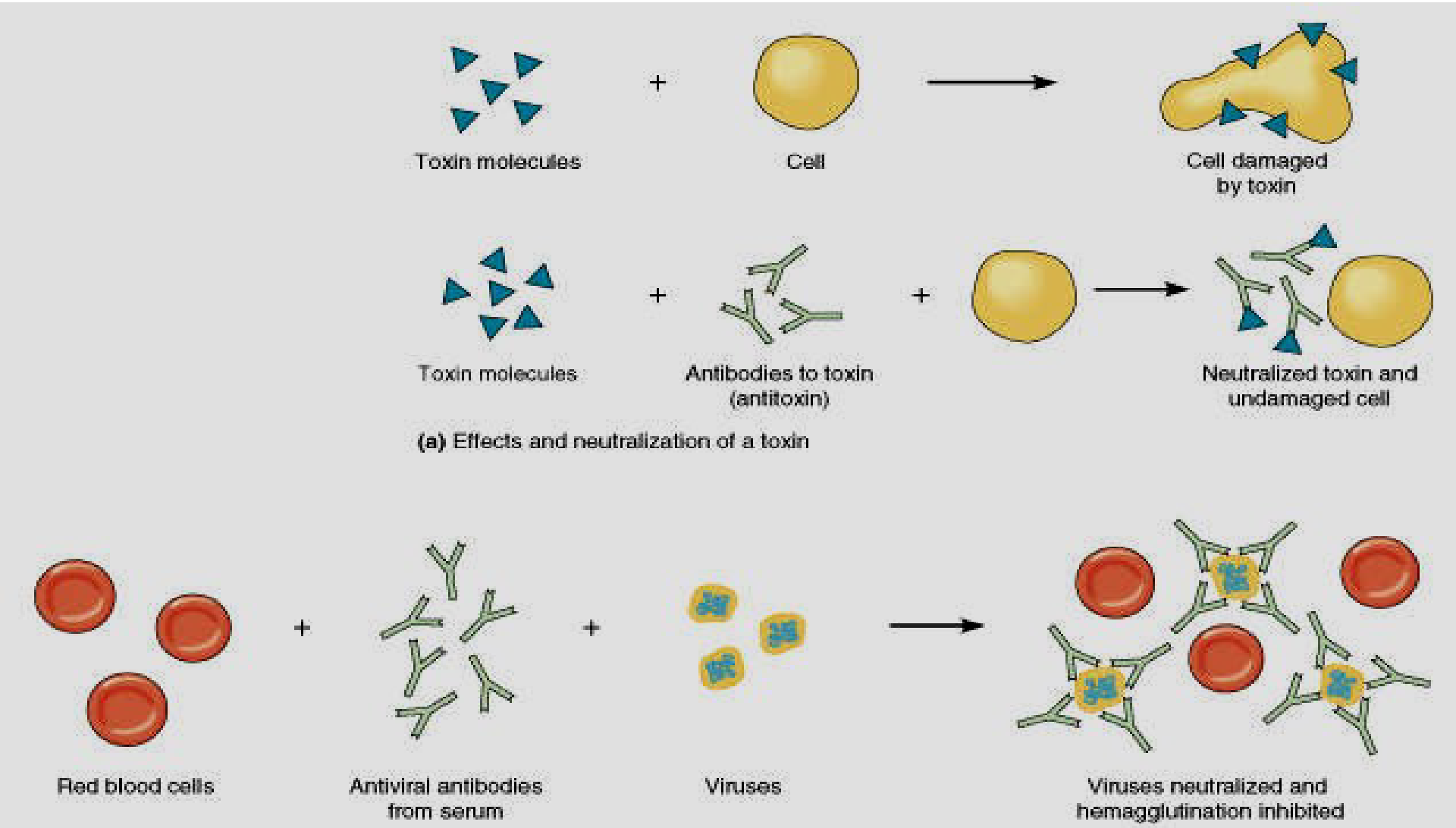


.Diagrammatic representation of radial & double immunodiffusion

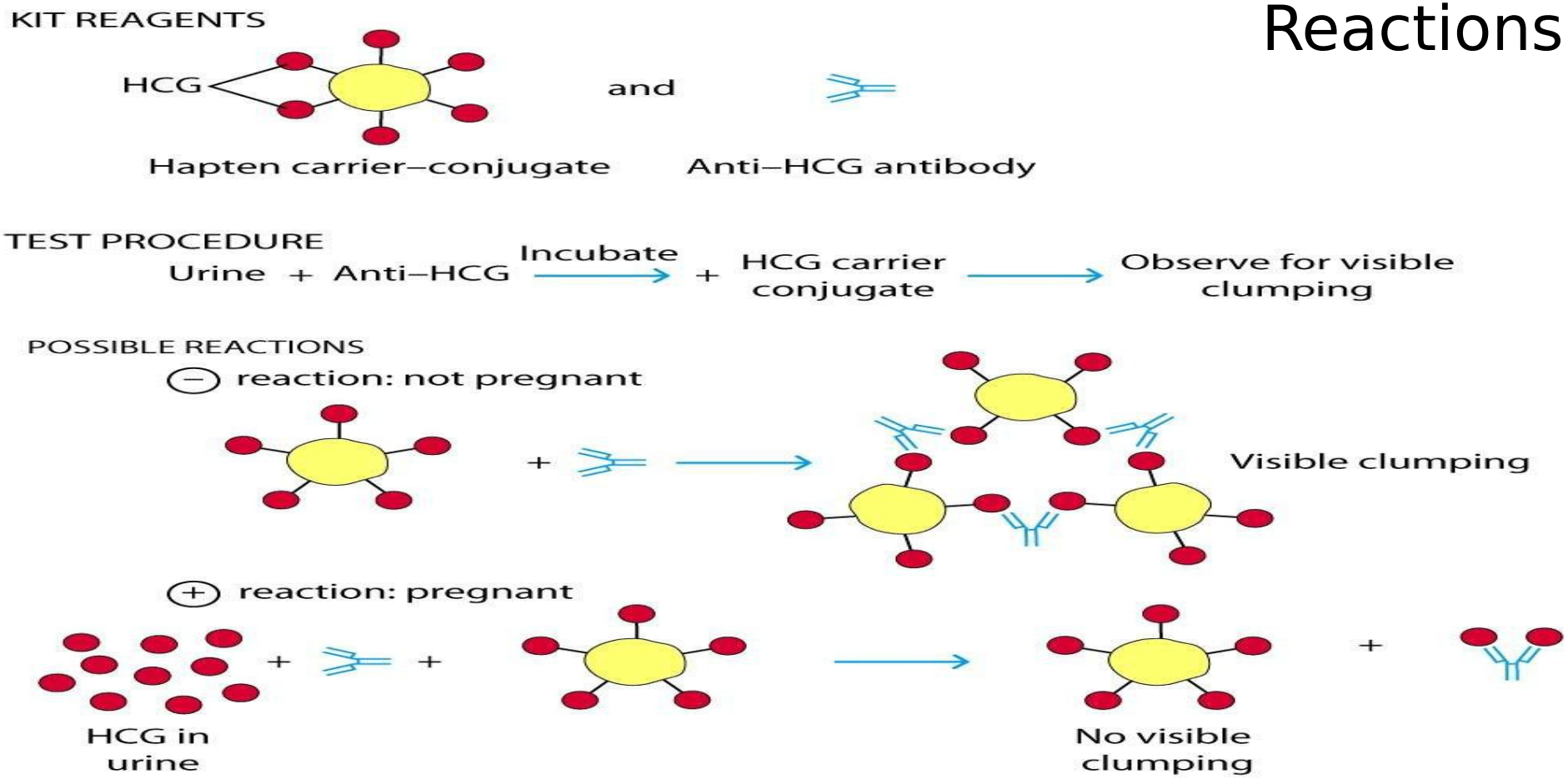
;Precipitation reactions in gels yield visible precipitin lines:

.no visible precipitate forms in regions of Ab or Ag excess

NEUTRALIZATION test



Agglutination inhibition Reactions



The original home pregnancy test kit employed hapten inhibition (agglutination
inhibition) to determine the presence or absence of human chorionic gonadotropin
The kits currently on the market use ELISA-based) >>> HCG(.assays
Also used to determine the use of illegal drugs & immunity (Ab) to

Latex agglutination tests