UNIVERSITY OF ZAMBIA SCHOOL OF HEALTH SCIENCES/ BIOMEDICAL SCIENCES

TRANSFUSION TRANSMISSIBLE INFECTIONS (TTI's)

(1) HIV

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Introduction

Mandatory Tests:

In Zambia blood is tested for the following disease markers:

1. HIV
2. Hepatitis B
3. Hepatitis C
4. Syphilis

In this lecture; HIV will be considered.

Overview

HIV

- Historical background
- Introduction

  -Epidemiology

  -Classification & distribution

  -Window period

  -Structure of the HIV virion

  -Transmission
- **Laboratory Diagnosis**

Historical Background

- HIV/AIDS first found in ;
- Homosexual men in USA, injecting drug users
- Later found in heterosexuals and bisexuals who had contact with individuals in first two groups
- Later found in hemophiliacs & other transfused patients
- Finally in infants born to infected mothers
- Isolated in 1983
- WHO response: global security of blood stocks
- In Zambia; testing started in 1986.

Introduction…

- HIV is a virus that causes AIDS
- HIV destroys part of the body's immune system resulting in acquired Immune deficiency syndrome (AIDS)
- AIDS is a complex condition which can manifest in so many ways. commonest opportunistic conditions are:

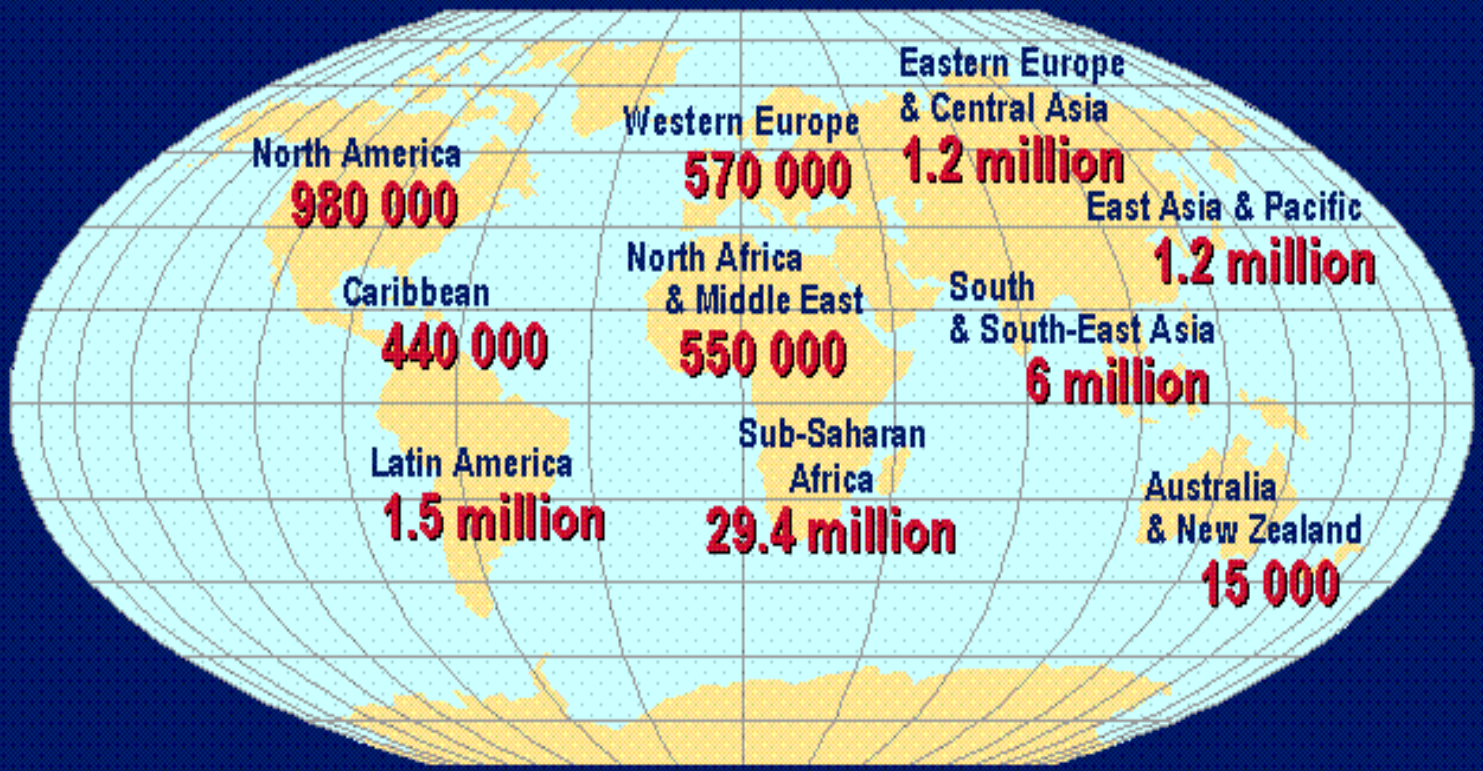
Introduction…

- Bacterial Infections e.g
  -Pneumonia
  -Mycobacterium tuberculosis
- Viral Infections e.g
  -meningitis
  -cytomegalovirus
- Cancers e.g
  -KS
  -Non-Hodgkins lymphoma
- Chronic diarrhoea etc
- Fungi e.g Candida albicans, pneumocystis karini

Introduction....

- There is no cure for HIV infection
- Antiretroviral drugs are available.
- ARVs keep viral particles to minimal levels in many ways including; blocking entry points on susceptible cells, disturbing transcription/replication process and inhibition of enzyme activity e.g protease inhibitors

Adults and children estimated to be living with HIV/AIDS as of end 2002



Total: 42 million

UNAIDS

00002-E-4 – 1 December 2002

World Health Organization

Epidemiology

- HIV is a serious global problem. more than fifty million people are estimated to be living with HIV/AIDS world wide.
- Sub saharan africa worst affected with 60 % of all global cases of HIV.
- Two types of HIV: HIV-1 & 2.
  other several variants exist
- In Zambia it is estimated that 14% of sexually active adults are living with HIV/AIDS.

HIV Classification and Distribution (env subtype)

HIV type	Group	Subtype	Distribution
HIV-1	***M*** (Major)	A (A1,A2)	Middle Africa
		B	South/North America, Europe, Asia, Oceania
		C	***South/East Africa, India, Brazil***
		D	Middle Africa
		F(F1, F2)	Middle Africa, South America, Rumania
		G	Middle Africa, Taiwan, Russia
		H	Middle Africa
		J	West Africa
		K	
	O (Outlier)	O	West Africa, France
	N (New)		
HIV-2		A-G	West Africa

Questions

End of part one (HIV)

Window period

POST HIV INFECTION (PHI)

- The natural history of HIV disease is generally classified into the following stages:

  -Viral transmission.

  -Primary HIV infection (with or without symptoms).

  -Seroconversion (evidenced by the detectability of HIV-specific antibodies).

  -Asymptomatic chronic infection.

  -Symptomatic HIV infection.

  -AIDS (defined as the occurrence of an indicator condition, such as an opportunistic infection, or a CD4 T-cell count less than 200 cells/mm3).

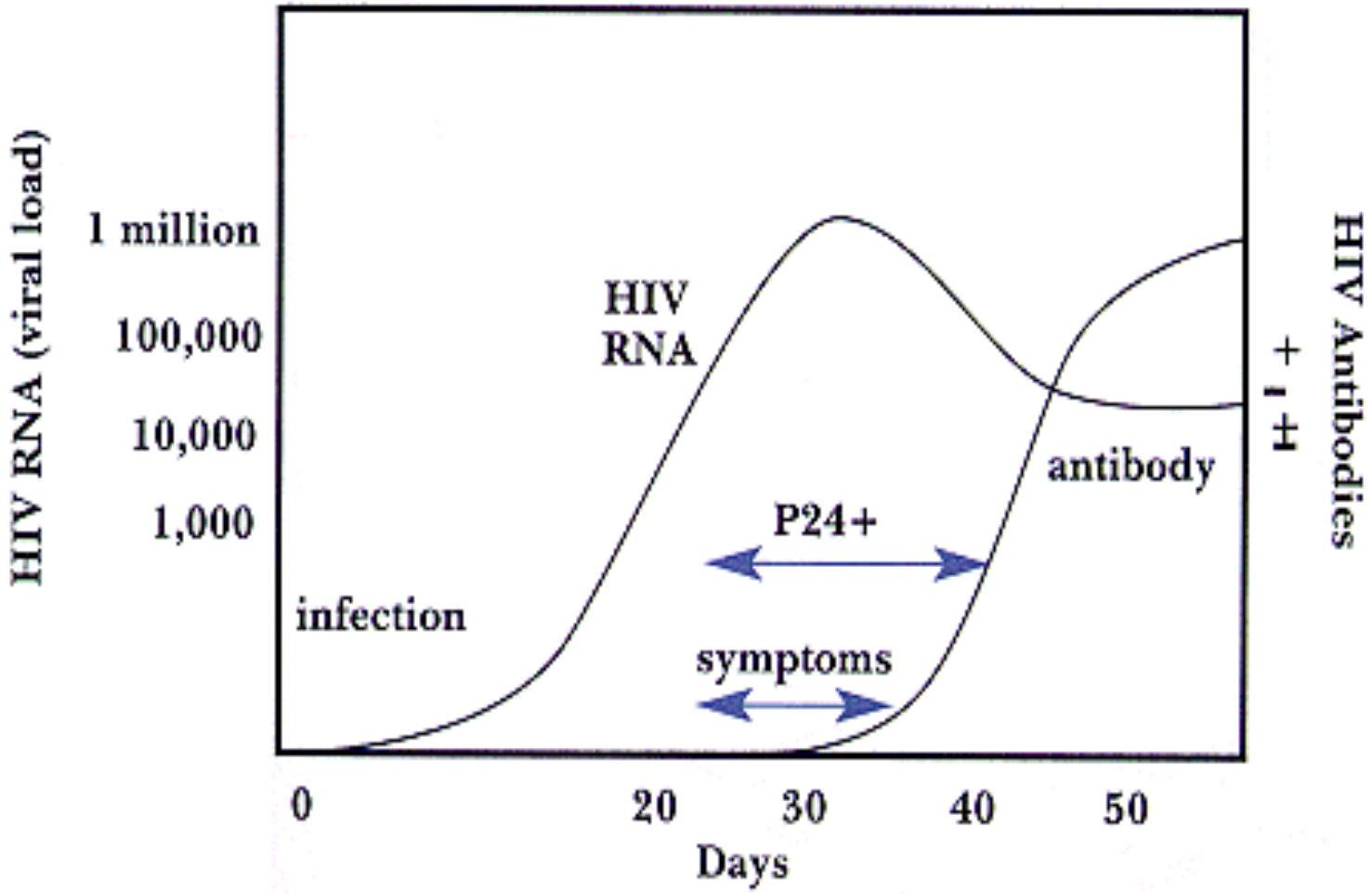
  -Advanced HIV infection (characterized by a CD4 T-cell count less than 50 cells/mm3).

- **The course and time frame of the infection is illustrated** . The course of PHI is limited to a few weeks or months, whereas the entire course of HIV infection can span many years.

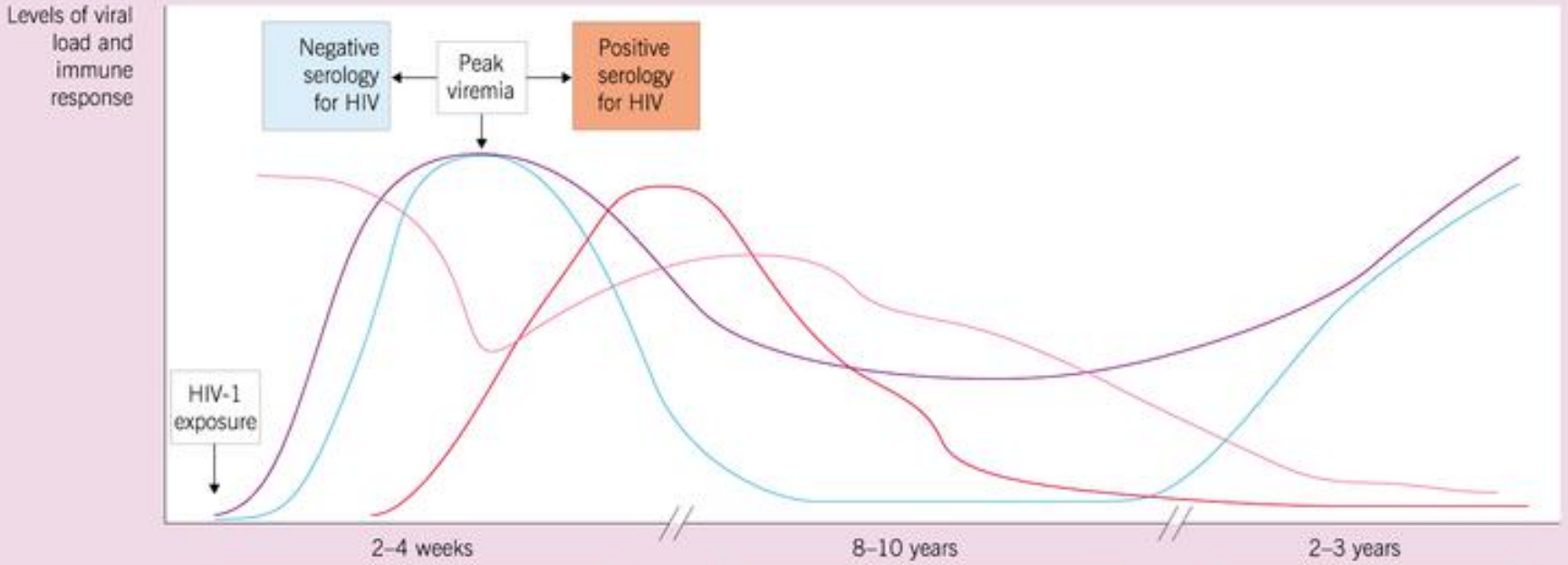
Window period (cntd)

- Specifically, PHI is the period *after* infection with HIV but *before* the development of detectable antibodies. It is a period of active HIV replication and transient immune suppression. Viremia rises rapidly in the plasma, often reaching levels in excess of 1 million copies per milliliter, with widespread dissemination into lymphoid organs throughout the body. While some patients have no clinical symptoms during PHI, it has been estimated that 50% to 90% of patients are symptomatic with an illness resembling infectious mononucleosis or influenza and that a large percentage of these patients present for care at health care facilities.
- This symptomatic manifestation of infection is generally referred to as "acute retroviral syndrome" (ARS). Some of the most prevalent symptoms associated with ARS are listed in Table.

FIGURE 1. Basic Course of Primary HIV Infection (PHI)



KINETICS OF VIRAL LOAD AND IMMUNE RESPONSE DURING THE PHASES OF HIV-1 INFECTION



Acute clinical syndrome	
Symptoms	**% of patients**
Fever	80–90
Fatigue	70–90
Rash	40–80
Headache	32–70
Lymphadenopathy	40–70
Pharyngitis	50–70
Arthralgia	5–70
Myalgia	50–70
Night sweats	50
Gastrointestinal symptoms	30–60
Aseptic meningitis	24
Oral or genital ulcers	5–20

Phase of clinical latency

Late appearance of constitutional symptoms

Overt AIDS

Severe constitutional symptoms

Opportunistic infections

Neoplasms

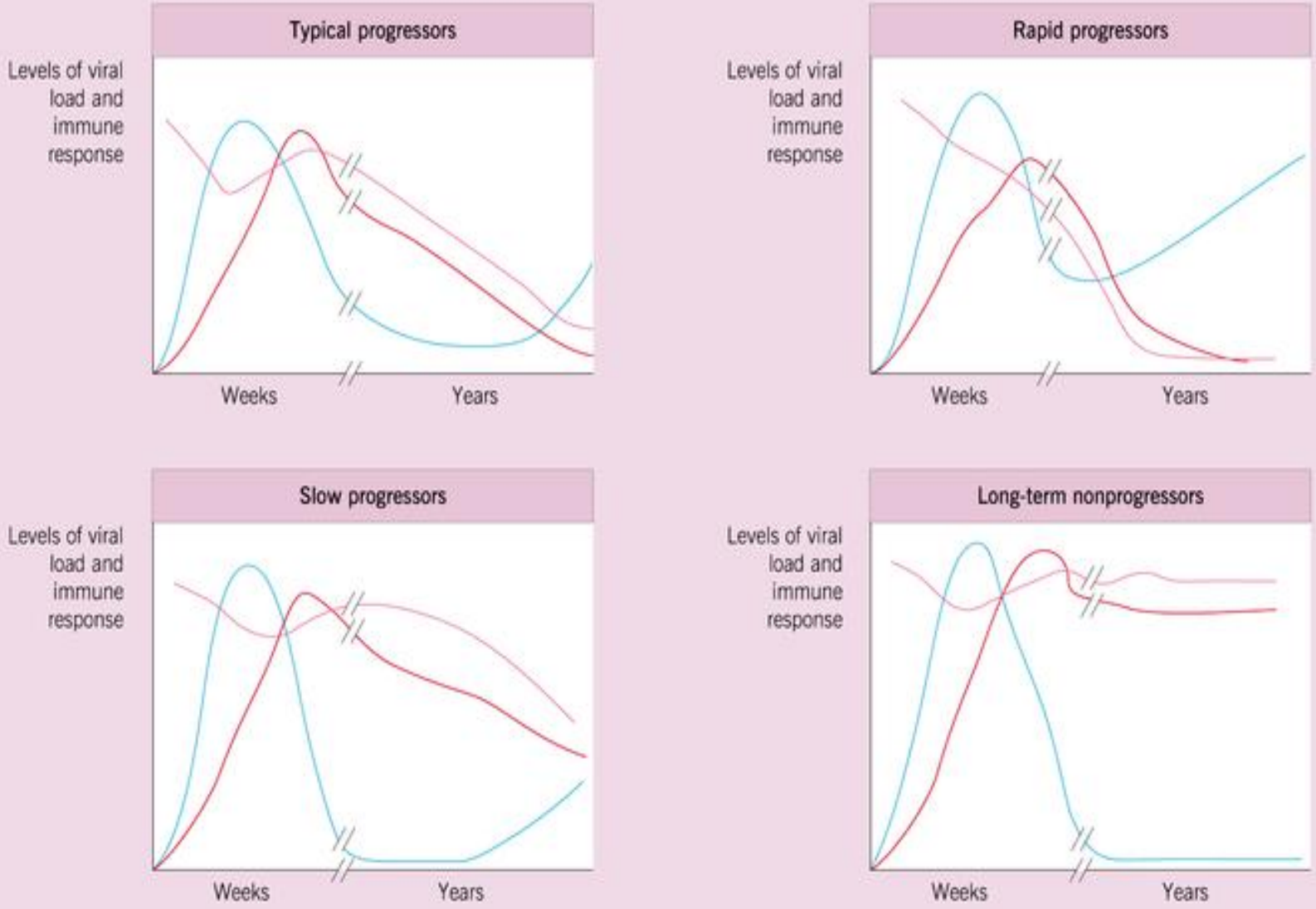
Plasma viremia

Viral load in lymphoid tissue

Immune response (HIV-1-specific cytotoxic lymphocytes)

$CD4^+$ T lymphocyte count

CHANGES IN VIRAL LOAD, CD4⁺ T LYMPHOCYTES AND IMMUNE RESPONSE IN THE DIFFERENT NATURAL COURSES OF HIV-1 INFECTION

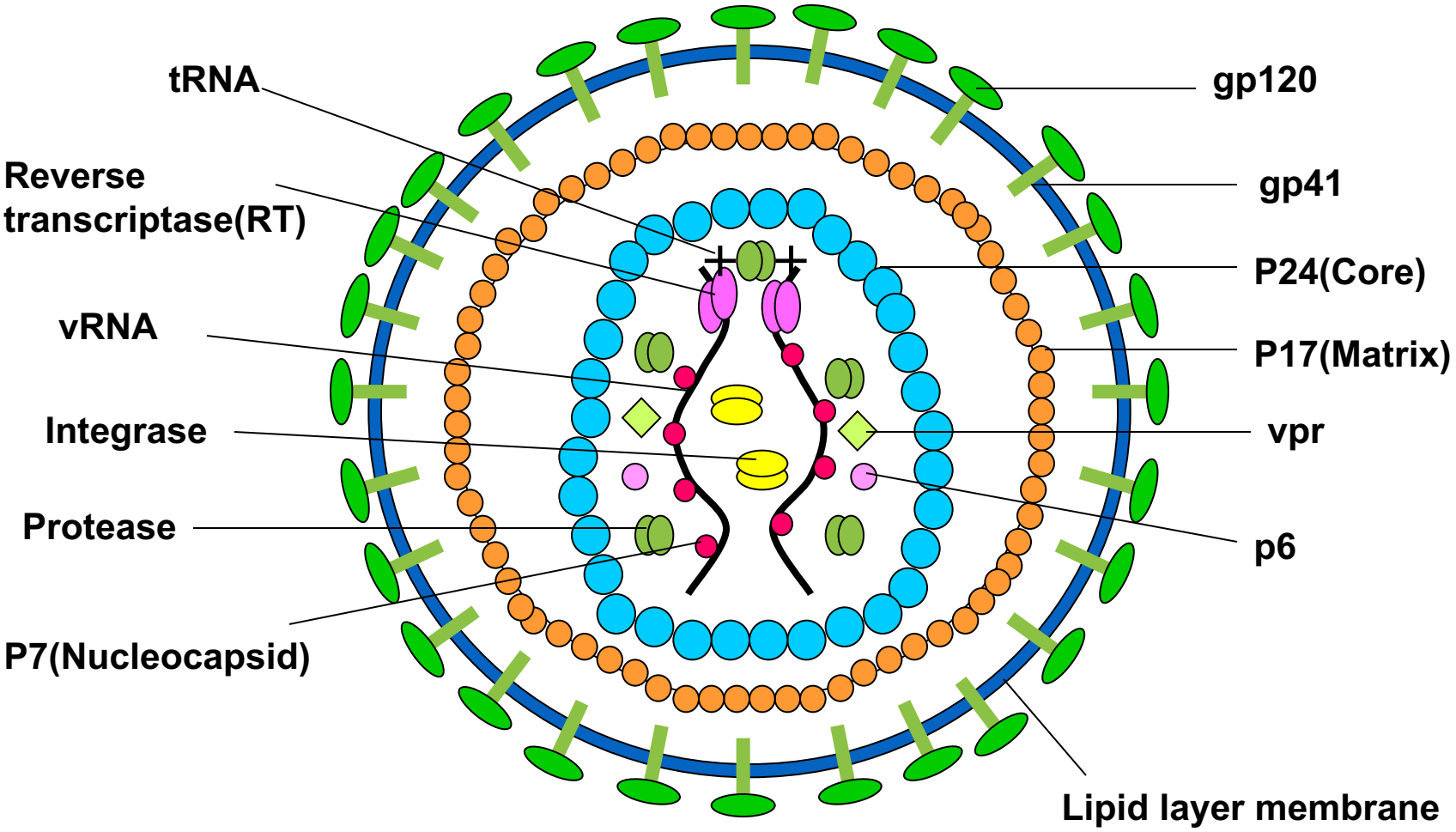


HIV VIROLOGY

Mature virion

- HIV has a Diameter of 100-120 nm
- It has a spherical morphology with 80 spikes on its surface
- It has a dense cone shaped core surrounded by a bilayered Phospholipid membrane.
- The genome consists of dimer of two identical RNA molecules

Structure of the HIV Virion



Human Immunodeficiency Virus
(Transmission).

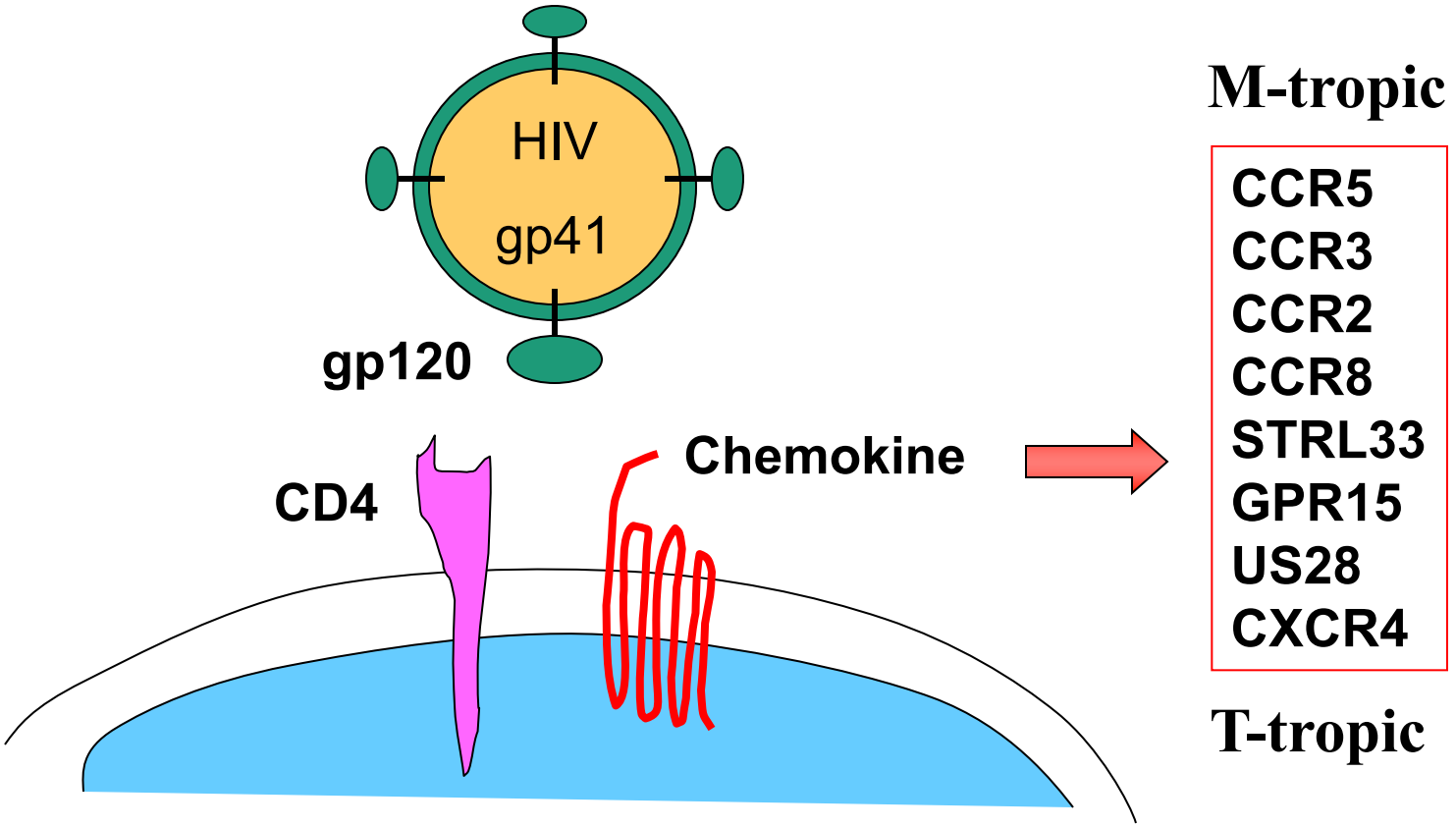
- **BLOOD – PARENTERAL TRANSMISSION.**
- Blood transfusion, contaminated needles and surgical instruments. high risk groups include health workers, iv drug users.

Human Immunodeficiency Virus
(Transmission).

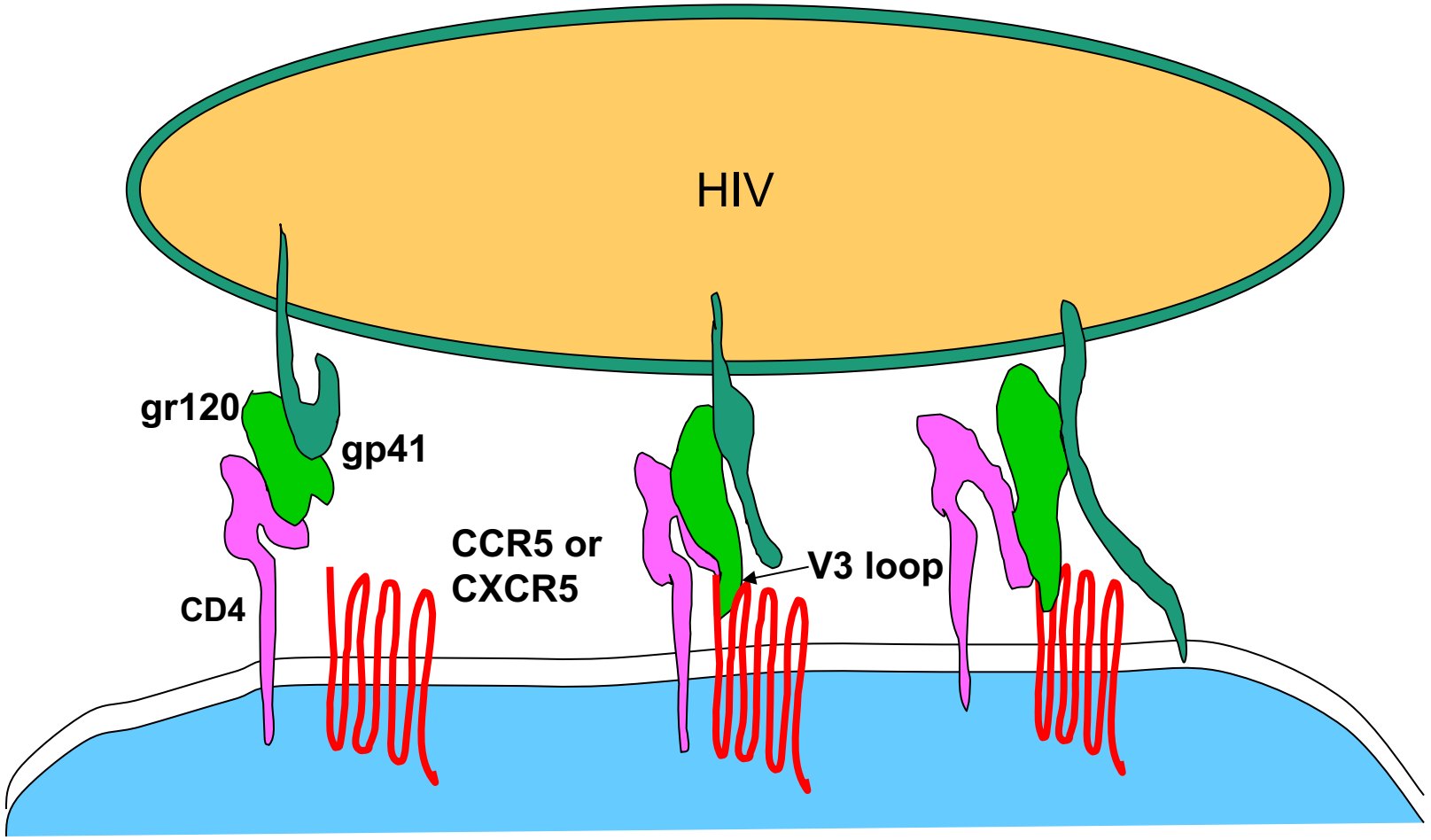
- **Sex – sexual transmission.**
  - Hetero and Homosexual. risk increases with more partners, other std esp. ulcerative, risky sexual practices.

- **Birth - perinatal transmission (MTCT).**
  - Transplacental especially if mother has advanced disease.
  - through contact with genital fluids during labour.
  - through breast feeding.

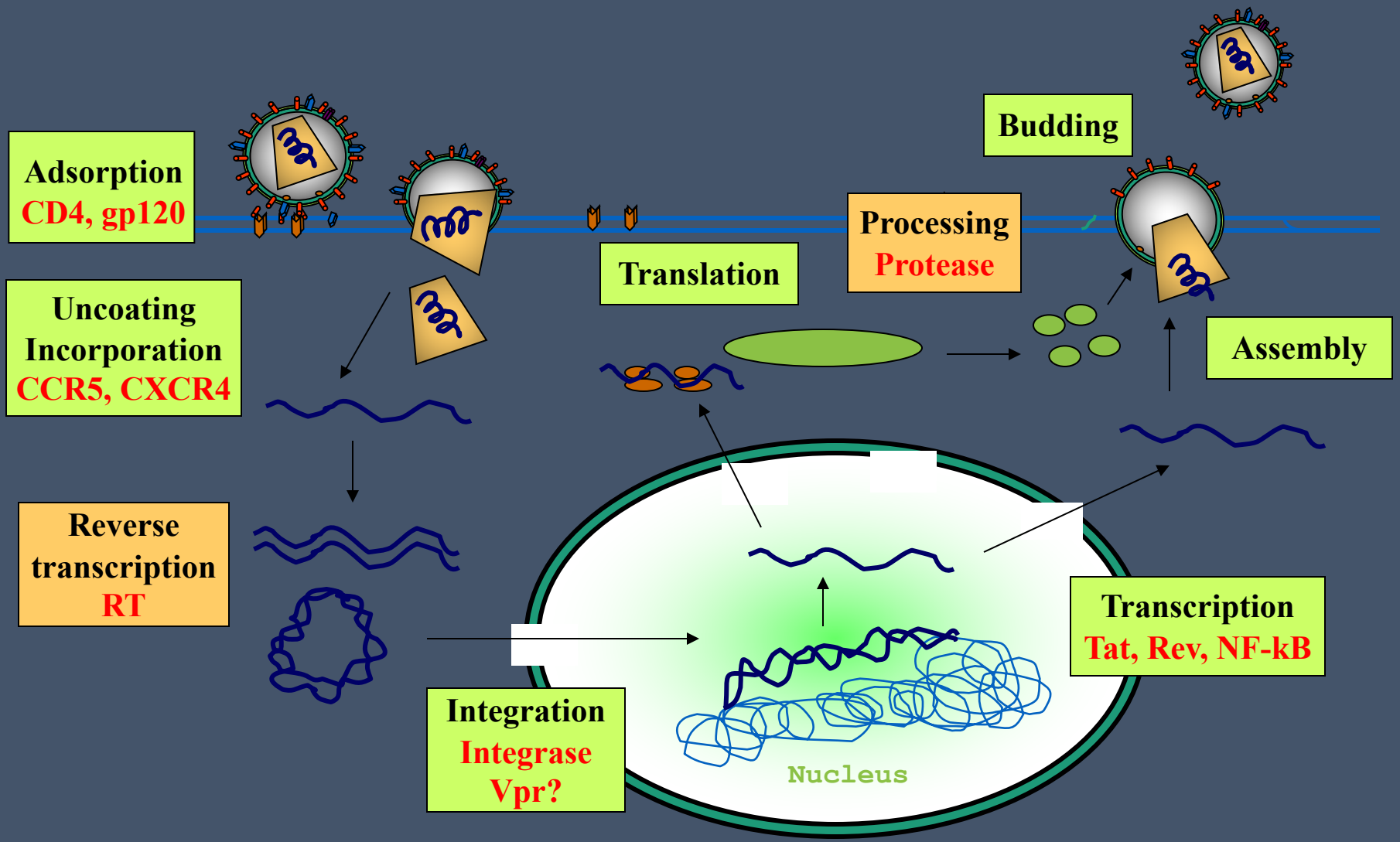
Chemokine Receptor



Interaction HIV env (Gp120 & gp41) with CD4 and Co-receptor



HIV Replication and Targets for Anti HIV Drugs



Human Immunodeficiency Virus

- **How does HIV cause AIDS?**
  - HIV multiplies in white blood cells important in preventing infection.
  - progressive destruction of these cells occurs.
  - disease develops when rate of white blood cell loss increases.
  - the body's ability to fight germs is damaged to a point where opportunistic infections and cancers develop e.g. skin cancer.

Laboratory Screening/Diagnosis

- Laboratory Strategies:

1. **Screening** for e.g epidemiological surveys, Blood safety
2. **Diagnosis** for disease in a patient

HIV 1 &2 SCREENING

HIV detection Methods:

- Based on :

- Identification of various markers

Markers include :

1. Indirect or Serologic ;

- Specific Antibodies against HIV

2. Direct ;

-Whole Viral particles, circulating antigens, or viral nucleic acids in biological samples

ANTIBODY SCREENING ASSAYS

- Three main kinds of primary screening assays to detect anti-HIV antibodies

  1. EIA (ELISA)
  2. Particle agglutination assays
  3. Specialised rapid assays

ELISA

INDIRECT

- **PRINCIPLE;**

  -Patient` s serum is added to the solid phase containing the antigen and is incubated for a specific period and at a particular temperature.

  -specific antibody if present in serum binds to the viral antigen

  -At end of incubation, wells are washed to remove excess serum

TESTING

Semi-Automated Elisa Testing

TESTING

Automated TTI Testing

INDIRECT ELISAs CONTND

- Conjugate is added to the dried well.

  -Conjugate solution contains an anti-human immunoglobulin antibody (IgG) which has been chemically linked to an enzyme (peroxidase or phosphatase)

  - Enzyme is an oxidizing agent.

INDIRECT ELISA (PRINCIPLE CONTD)

- Wells are washed to remove excess unbound conjugate

  -Substrate solution is added and incubated at specified tempt and period

  -Substrate solution contains a chemical called chromogen

  - The oxidizing agent will act on substrate

  resulting in color change

  -Wells not containing bound antibodies will remain colorless

INDIRECT ELISA (PRINCIPLE CONTND)

- Diluted acid is added to inactivate the enzyme thus stopping the reaction and in some cases even intensify color
- Optical density (OD) values are read and results determined after calculating the cut off.

COMBINATION ASSAYS

- HIV Ag/Ab Combination Assay
- The combination assay is based on : microwells coated with a synthetic peptide representing an immunodominant region of HIV1(0),recombinant protein derived from the envelope proteins of HIV 1&2 and an HIV pol protein and monoclonal antibodies raised against p24 of HIV 1
- The conjugate is a mixture of the same Ag epitopes, and different monoclonal antibodies also raised against p24,all labelled with horseradish peroxidase
- Serum will be added and incubated

Combination Assay (CONTND`)

- Specific corresponding antibodies and

  Antigens will get bound
- After washing, Conjugate is added.
- Conjugate will be bound to the antibodies and antigens already bound to the wells
- Samples without antigens and antibodies will not cause conjugate to bind
- Unbound Conjugate will be washed away at washing stage
- Substrate will be added and acted upon by enzyme system on the conjugate
- Color will develop & this will be read by spectrophotometry

COMPETITIVE ELISAs

- PRINCIPLE:
- Antigen is bound to the surface of the well

-Serum is added and at the same time the conjugate is added. Both are incubated together

- Conjugate is an enzyme labelled anti-HIV.

- Conjugate competes with any natural anti-HIV antibody for the antigen binding sites.

- The Concentration of the conjugate is set low this advantages antibody in serum if present

COMPETITIVE ELISAS (contd)

- If concentration of antibody in serum is high, very little conjugate can bind to antigen
- Wells are washed
- Substrate is added
- Because binding sites are taken up by anti-HIV antibody from serum, conjugate with enzyme would have been washed off in previous step .
- No color or very little color develops since very little enzyme is present to cleave substrate

COMPETITIVE (CONTD)

- Conversely with sample that contains no antibody, more conjugate will bind to antigen on solid phase and subsequent addition of substrate will cause more color development. Therefore, amount of unknown antibody in sample is inversely proportional to amount of color produced

PARTICLE AGGLUTINATION ASSAYS

- PRINCIPLE:
- `Carrier  substances` are coated with antigens. Common carriers include Red cells, latex particles gelatin particles and micro beads
- HIV antigens are absorbed onto carriers and these antigen coated reagents come ready to use.
- A lattice network is formed between antigen coated particles and antibodies in serum. The reaction brings about clumping /agglutination of particles

TESTING
Rapid Testing

SPECIALIZED RAPID ASSAYS

- **PRINCIPLE:**
- HIV antigen is immobilized on either a porous or semi-porous membrane or strip. The antigen is in most cases a recombinant or synthetic peptide antigens. They may be presented in a dot blot or straight line

CONTD`

- Membrane is coated with antigen
- Sample is applied to solid phase
- Anti-HIV antibody will bind to immobilized antigen
- Incubation period is short 5-10 minutes
- The module is rinsed by adding rinse solution
- *Some assays do not require this step
- Conjugate is then added to module
- *Conjugate composition varies between assays e.g Enzyme conjugated anti-human IgG

CONTND

- Others use Protein A labelled with colloidal Gold as conjugate
- When added, aggregates of gold particles are seen

CONTND`

- Antigens will get bound
- After washing, Conjugate is added.
- Conjugate will be bound to the antibodies and antigens already bound to the wells
- Samples without antigens and antibodies will not cause conjugate to bind
- Unbound Conjugate will be washed away at washing stage
- Substrate will be added and acted upon by enzyme system on the conjugate
- Color will develop & this will be read by spectrophotometry

SOLID PHASES :(GENERAL)

- RECOMBINANT BASED ASSAYS
- Recombinant antigens are produced when a portion of HIV genome is inserted into biological vehicle e.g bacterial cells resulting in production of gene product
- ADVANTAGES :
  - Antigen of interest is propagated e.g If gene that codes for gp120 were inserted into the vehicle, the antigen gp120 only would be produced without any p24 etc
  - Property exploited in producing assays of high specificity.

SYNTHETIC PEPTIDES

- Amino Acid sequence of antigen is determined, antigens are made in Lab by constructing it from a pool of free amino acids
- ADVANTAGE:
    - Highly sensitive and specific

CONTND

- DISADVANTAGES:
- Sometimes contaminants arise from the vehicles' components leading to false positive results

CONFIRMATORY TESTS

- W.BLOT
- PCR
- NAT

SENSITIVITY AND SPECIFICITY OF ASSAYS

SENSITIVITY:

- Ability of an assay to pick up the weakest possible positive marker.
- Expressed as a %:

  Sensitivity= True pos/True pos +false neg

SPECIFICITY:

Ability of an assay not to pick up a false or non specific positive marker.

Expressed as a %

Specificity=True Neg/True neg+False pos

Test algorithm

- For Blood Transfusion purpose :

  Test algorithm:

  Repeat testing, in duplicate of initially reactive sample

  Interpretation: At least 2/3 tests should be reactive for sample to be considered Pos.

  -An initial negative result: Sample is Non reactive therefore Negative

Conclusion …1

Strategies for managing HIV/AIDS

- Prevention[1]: for those who are not infected
- Screening/Diagnosis[2]: for everyone e.g VCT
- Treatment[3]: for those infected
- Care and support[4]: for those already infected

HIV/AIDS impacts negatively on household & countries' economies, social structures & total wellbeing of human race

CONCLUSION…..2

- UNDERSTANDING of principles

  Is important. This provides basis for choice of kit depending on the purpose;
- Counseling, Screening, Diagnostic etc
- Test algorithms are guides

KEY Messages…1

- Laboratory Screening for HIV:

  Based on

- Detecting the Viral particles or products of the virus
- Detecting products of immunological response to virus in body; e.g antibodies
- Technology of testing:

  -ELISA Technology ; semi/fully automated platform

  Principles:

  -Indirect ELISA

  -Competitive ELISA

Key messages….2

- Rapid Testing

  -Particle agglutination

  -Specialised rapid tests e.g determine
- Sensitivity : High sensitivity
- Specificity : High specificity
- Test algorithm