STUDY OF HIV 1 & 2 SEROPOSITIVITY IN PATIENTS AND HEALTHY SUBJECTS IN G.G. HOSPITAL AT JAMNAGAR

by
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Under guidance of
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CERTIFICATE

THIS IS TO CERTIFY THAT

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UNDER MY CONSTANT GUIDANCE

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INTRODUCTION

AIDS the chilling acronym for acquired Immune Deficiency Syndrome evokes a response of familiarity to day. Seventeen years ago, this was not so. In the summer of 1981 the Centre for Disease Control (CDC) in the united state reported five previously healthy homosexual men in Los Angles suffering from an unusual type of pneumonia caused by pneumocystis carinii. CDC also reported 26 previously healthy homosexual men in New York and Los Angles, who had developed a rare form of skin cancer called Kaposis Sarcoma. This reports signaled the arrival of a mysterious acquired disorder of the human immune system, which disable the body's defenses. This was the beginning of one of the biggest public health problems of this century.[1]

HIV seems likely to have originated in central Africa, where serum samples from as early as the 1950s have been found to be seropositive.[2] Although HIV appears to have been present in Africa longer than in the united states; the rapidly rising incidence of cases in Africa also suggest a new epidemic. Subsequently the syndrome was found in Haitian immigrants on the east coast of the USA. Earlier in Europe three Norwegian cases reported. This HIV infection was present several years before AIDS was recognized, clinical manifestations present as early as 1966 and serological confirmation was in 1971. These patients are the first proven case of AIDS in Europe. The daughter seems to be the earliest recorded case of paediatric AIDS.[3]

AIDS in Africa, sometimes called slim disease, was usually characterized by opportunistic outcomes such as disseminated tuberculosis and chronic diarrhoea. Because the outcomes of AIDS were often different in different geographical areas of different population group.[4] In the absence of any identifiable cause, the syndrome was labelled as Gay Related Immunodeficiency Disease (GRID)[5].

The present term, acquired immunodeficiency syndrome (AIDS) came couple of year time later, when similar cases were identified among the blood transfusion recipients, intravenous drug addicts and heterosexual. The year of 1983 which saw the emergence of the modern Sherlock Homes Dr. Lue Montagnier, Dr. Robert Gallow and other scientists, who solved the mystery by sheer hard work and intelligence to describe the act and the actors. The heroes of the story were those who died and consented to autopsy.

The term AIDS was officially adopted in 1982.[6] The causative retrovirus, called Lymphodenopathy associated virus (LAV), was identified first in 1983 by Montagnier and colleagues in Paris. Simultaneously Gallo and colleagues reported isolation from patients of a virus, which they called Human T-cell Lymphotropic virus type-III. (HTLV-III) Investigations conformed the identity of LAV and HTLV III by international agreement this virus is referred to now as the Human Immunodeficiency Virus type-1 (HIV-1). The second AIDS virus HIV-2, was isolated in 1985 from West African patients by Montagnier and his collegues[7]. The most characteristic lesion of AIDS is a progressive loss of T lymphocytes. In most infected individuals this progresses to pathological levels and disease develops within 6 to 8 years.

In the mid 80s when the HIV epidemic was making fast inroade in USA, Africa and Western Europe we, in India, reacted timely to detect whether this virus has reach our country also. In 1985 the Indian Council of Medical Research (ICMR) established a tasked force and intuted HIV screening of the presumed risk groups to detect the presence of this virus in India. Since that time we have come a long way having established an extensive surveillance network in the whole country[8].

The prevalence of HIV infection in India was first detected in February 1986 among a small group of prostitutes at Madras. First AIDS case was reported from Bombay at May 1986[9]. First HIV-2 reported from Bombay, Vishakapatnam and Madras at January. 1991[10]. The infection is transmitted in majority of cases through sexual contact, blood and blood product, contaminated needles and from infected

mother to the newborn. Through the surveillance network we have observed that the situation is highly variable in the different states of India, being determined by the socio-economic factors as well as the magnitude and pattern of distribution of high risk behaviour practices in all state excluding state of Maharashtra and Manipur. The highest rate of infection have been observed among injecting drug users in Imphal (Manipur). The second highest rate of HIV infection was found in female sex workers in Bombay (Maharashtra). Maharashtra has reported highest number of cases in the country.

Though HIV infection has been reported from all the state and union territories 5 states and union territories have not reported a single case of full blown AIDS. They are Arunachal Pradesh, Andaman and Nicobar, Island, Dadra and Nagar Haveli, Lakshadweep and Tripura[11].

Table - I

Calendar of events related to HIV / AIDS in India[10].

1985 Oct.	HIV Serology testing introduce at CMC Vellore and NIV, Pune.
1986 Feb.	1st case of HIV ab +ve CSW in Madras.
1986 March	HIV ab +ve CSW detected in interior towns of Tamil Nadu (Madurai and Vellore)
1986 April	ICMR Initiates National AIDS surveillance programme with at least one testing station in every state.
1986 May	1st reported case of patients with AIDS in Bombay (Acquired through unscreened blood in USA)
1986 Dec.	1st seropositive man detected at STD clinic in Vellore (TN)
1987 July	1st seropositive blood donor detected in Vellore.
1987 Oct.	1st seropositive infant detected in Pune.
1990 Jan.	HIV ab detected among IVD's in Manipur.

1991 Jan. HIV-2 reported from Bombay, Madras and Vishakapatnam. (It is clear that HIV will continue to be a global problem well into the twenty first century).

UNAIDS and NACO estimate about 5 million people are living with HIV/AIDS infection and 62785 cumulative AIDS cases reported in Jan. 2004. NACO reported 3596 AIDS cases in Gujarat at Jan. 2004[12].

AIDS has emerged as one of the most grasome and dreadful disease of mankind which has evoked wide spread alarm not only in the medical profession but also almost the general public. In light of all that we know about this diabolical virus, currently there is no vaccine so India needs to act vigorously to dramatically enhance awareness.

In the mean time we will have to bring out the best in each one of us by way of compassion, understanding and love for those of are afflicted and already affected. India can do no less.

So let us stop AIDS before it is too late !!

AIMS AND OBJECTIVES

To dertermine the incidence of HIV infection in patients and healthy blood donors.

- To dertemine the incidence of HIV infections in symptomatic patients of tuberculosis.
- To determine the incidence of HIV infections in sexually transmitted disease patients.
- To determine the incidence of HIV infection multi transfused thallassaemic children.
- To determine the incidence of HIV infection in pregnant women (antenatal mother).
- To determine the incidence of HIV infection in blood donors.

REVIEW OF LITERATURE

HISTORY :

ORIGIN OF AIDS :

Acquired Immune Deficiency Syndrome (AIDS) was first described as a new and distinct clinical entity in 1981[15]. The first cases were recognized because of unusual clustering of diseases such as Kaposis Sarcoma and Pneumocystis Pneumonia in young homosexual men. Although such syndromes were occasionally observed in different well defined subgroup of the population, such as older men of Mediterranean origin in the case of Kaposi's Sarcoma and severity impaired cancer patients in the case of pneumocystis pneumonia, the occurrence of these diseases in previously healthy young people was unprecedented.

Within a brief period of time AIDS cases were also reported in other populations, such as intravenous drug abusers and hemophiliacs[14]. In the case of hemophiliacs, this would be associated with clotting factor preparations, which were prepared from the pooled blood of a huge number of donors. In the case of intravenous drug abusers, increased exposure to foreign tissue antigens might occur when recipients used dirty needles contaminated with small amounts of blood from previous users. Even independent of clinical AIDS, asymptomatic hemophiliacs and intravenous drug abusers were often found to have inverted T-lymphocyte helper to suppressor ratios, as did AIDS patients and a proportion of asymptomatic promiscuous homosexual men.

Soon after the first cases of blood transfusion associated AIDS were suspected. These were suspected because some individuals with clinical AIDS were found to lack any of the characteristics of the previously defined risk group homosexuality, hemophilia or intravenous drug abuse but were found to have a history of receiving blood transfusion within the preceding 3 to 5 years.

Although still dismissed by many, it seemed increasingly logical that an infectious etiology for AIDS had to be considered. Subsequently, several studies were initiated to determine seroprevalence rates for exposure to numerous microorganisms, especially viruses, and to compare exposure to given agents in AIDS patients and controls. High on the list of candidate viruses was cytomegalovirus, because it was already associated with less severe immunosuppression in kidney transplant patients; Epstein Bar virus, presumably because it was a lymphotropic virus, and hepatitis B, because it was already known to occur at elevated rates in both homosexual men and recipients of blood or blood products. Yet, since AIDS was a new disease, it was difficult to imagine how it might be caused by a viral agent that was not itself new. If a virus such as hepatitis B, Epstein Barr, or cytomegalovirus were to be etiologically involved, it would presumably have to be a newly mutated or recombined genetic variant.

At that time, Gallo and his colleagues and Montagnier and his colleagues, postulated that a variant T-lymphotropic retrovirus (HTLV) might be etiologic agent of AIDS. Among the most compelling reasons for considering such a retrovirus was that the human T lymphotropic retrovirus discovered as a cause of adult T-cell leukemia by Gallo and colleagues was the only human virus known to infect T-helper lymphocytes, the cells that became impaired or eliminated in individuals with AIDS. Along with this cell tropism, HTLV was known to be transmitted by all the appropriate routes; sexual contact, with transmission apparently more efficient from males, transmission by blood; and transmission from mothers to newborn children[15].

Several approaches were taken to determine if a virus related to HTLV might be associated with AIDS, and enticing but inconclusive results were initially obtained. Soon after, proof that the disease was linked to a T-lymphotropic retrovirus was obtained by Gallo and his colleagues. Further characterization of the agent, now termed human immunodeficiency virus type-1 (HIV-1) revealed that it was only distantly related to HTLV but was the same as the isolate detect earlier by Montagnier and his colleagues.

ORIGINS OF HUMAN RETROVIRUSES :

HTLV-1, the first human retrovirus identified, was known to be present at elevated rates in regions such as southwestern Japan, the Caribbean Basin, Northern South Africa and Europe. The theory that this virus originated in Africa was initially suggested by Gallo, who cited early reports of Africans in Southwestern Japan[16] Miyoshi and his colleagues then identified a virus related to HTLV-1 in Asian monkeys. This virus, designated simian T-cell leukemia virus (STLV), was later found in African monkeys and apes and was associated with lymphoproliferative diseases in captive macaques.

Seroepidemiologic studies in Old World primates from both Asia and Africa revealed that more 30 species of monkeys and apes had widespread infection with an STLV. However, on further molecular characterization, it was recognized that the STLV viruses from Japanese macaques and related Asian species of monkeys were not as closely related to HTLV-1 as were STLVs isolated from African primates such as chimpanzees and African green monkeys. All isolates of HTLV-1, whether from Japanese, Caribbean, or African people, were highly related to African strains of STLV but not as highly related to Asian strains of STLV. This suggested that HTLV-1 evolved from a subgroup of STLVs present in Africa but not in Asia. It also suggested that the STLV/HTLV-1 family of retroviruses was present in numerous species of Old World monkeys for some time before it was introduced to humans from an African species of monkey or ape.

HTLV-II, the second human retrovirus detected, was only about 40% to 50% related to HTLV-1 at the genomic level, HTLV-II was also found at high prevalence in selected human populations of New World origin. The closest HTLV-II related virus of subhuman primates was in a New World species, the South African spider monkey.

Whereas substantial genetic variation is seen among different isolates of HIV-1, particularly in the envelope gene, the same degree of variation is not seen for HTLV-1. Presumably, the rate of genetic drift seen in retroviruses is related to their

rate of replication. Although HIV-1 can replicate to high titers and be detected as free virus in serum or plasma, HTLV-1 cannot. Because HTLV-1 is apparently transmitted only in a cell-associated manner.

ORIGIN OF HIV-1 :

After HIV-1 was recognized as the probable cause of AIDS, it soon became apparent that this virus was new to populations in the Western hemisphere. This raised the question of whether HIV-1 was also new in Old World human populations or whether it had recently been introduced from another species. If HIV-1 had been present in some human populations in Africa to the point of evolutionary equilibration (as had HTLV-1), it probably would have been limited to isolated tribes of people, and selection for host immunity as well as selection of an virulent virus would have occurred. Such isolation seemed essential for evolutionary equilibration to have occurred, because, in both the United States and Haiti, blacks were just as likely as whites to develop clinical AIDS after exposure to HIV-1.

The possibility that HIV-1 or a related virus was present in human populations in central Africa at the same time or even before AIDS was diagnosed in the United States seemed even more probable after what was apparently the same syndrome was reported in Africans who sought treatment in Europe. Subsequently, it was recognized that HIV-1 infection and clinical AIDS were rapidly spreading in Central Africa.

Serum samples collected from Africans at earlier periods were also examined for the presence of antibodies reactive with HIV-1. In some cases, the examination of stored samples suggested elevated rates of infection in Africa during the period 1965 to 1975. Subsequently, it was revealed that most of those surveys were conducted with first stage tests that were imperfect, and the reactors were mostly false positive cases, caused either by contamination of the HIV antigen or by "Sticky sera" containing antibodies that reacted non specifically because of repeated freezing and thawing and maintenance under poor conditions.

While examining sera taken from African patients in the period 1955 to 1965, one antibody positive sample was found that was clearly specific[17]. When tested by radioimmunoprecipitation, this sample was found to contain high titers of antibodies that were reactive with virtually all the major antigens of HIV-1 detectable by this technique; gp^{160}, gp^{120}, gp^{55}, gp^{41}, p^{27}, p^{24}, and p^{17}. However this sample represented only a rare positive reactor in a high risk group of individuals exposed to general infections and AIDS like illness in a region that subsequently had high rates of infection with HIV-1. Positive results were obtained from fever than 1% of sick individuals tested from Kinshasa, Zaire, which is now classified as a region of moderate to high prevalence. This suggests that the virus was only rarely present at that time in places that would now be considered within the AIDS belt of Africa. It was speculated that HIV-1 or a virus very similar to it had moved to the cities of this region of Africa before the mid 1950s, either by introduction from subhuman primates or by migration of a few resistant carriers from a previously isolated tribe or tribes. Population redistribution was occurring at that time, with movement of previously isolated people into the newly expanding cities. Still it seems unlikely that HIV-1 would have been present, as such, for many generations in isolated tribal regions. If this were so, we would expect to find Africans who show greater resistance to infection and disease development, owing to genetic evolution of the human species. However, it prospective studies conducted to date, exposed Africans appear to develop clinical AIDS and other signs and symptoms of HIV disease as rapidly as individuals in the United State of Europe. Furthermore, as has been mentioned, the degree of genomic variation seen in African isolates of HIV-1 is greater than that seen in isolates from Europe or the United States.

A virus that could be a progenitor of HIV-1 has been isolated from a chimpanzee in central Africa. This finding, combined with the knowledge that all HIV-1 viruses tested appear to be a virulent when inoculated into chimpanzees, is also compatible with a subhuman primate origin for HIV-1. Some African isolates of

HIV-1 appear to be as close to the chimpanzee isolate as to other prototype strains of HIV-1.

ORIGIN OF HIV-2

Because a relative of HIV-1 (SIV) had been found in wild African monkeys but was only about 50% related to HIV-1 at the genomic level, it seemed logical that viruses more highly related to SIV may also be present in human populations. Serum samples from West African prostitutes were examined to determine whether they had antibodies that were more highly cross-reactive with SIV than with HIV-1. West Africa was chosen because, at that time, it was largely free of HIV-1 and clinical AIDS, and female prostitutes were selected because they represent a group at high risk for amplification of prevalence rates for infection with sexually transmitted viruses.

Through Western blot techniques, it became clear that a significant proportion of Senegalese prostitutes had antibodies that were highly reactive with all the major antigens of SIV detected by this technique. These included the gag-encoded p24, the pol-encoded p64/53 and p34, and the env-encoded transmembrane protein p34. Yet, when the same SIV antigens were reacted by Western blotting with sera from HIV-1 infected individuals of either European or central African origin with classic disease manifestations little or no reaction was seen with the envelope antigens. Because the transmembrane protein of SIV is usually smaller than the comparable protein of HIV-1, this is manifested as the loss of reactivity where it might be expected at gp41 and the acquisition of reactivity with gp32, the carboxy1-terminus peptide of the env gene of SIV. The class of reactivity seen with serum samples from West African prostitutes was virtually indistinguishable from that seen with serum samples from African monkeys or captive rhesus. Similar results were also obtained by radioimmunoprecipitation, except that this procedure readily detects the gp^{120} amino-terminus env glycoprotein that is often missed by Western blotting. In this case, serum samples from the West African prostitutes

reacted very well with the gp^{120} and gp^{160} of SIV but reacted only infrequently and weakly with the gp^{120} and gp^{160} of HIV-1.

With evidence that a virus more closely related to SIV than to HIV-1 was present in Senegalese prostitutes, more extensive studies were undertaken to determine whether the SIV-related virus was more widely distributed in Africa in general, particularly in West Africa. The screening of more than 2000 high-risk individuals from central Africa, including many persons with AIDS and other sexually transmitted diseases, revealed no evidence that the virus, termed HIV-2, was present in the same regions in which HIV-1 was rampant. However, pockets of infection with HIV-2 were detected in Mozambique and in Angola; although distant from West Africa, these regions are often on the same trade routes as Guinea Bissau and Cape Verde, West African countries whose rates of infection are among the highest. Within Senegal, prevalence rates for HIV-2 were substantially higher in the southern region of Casamance , which borders Guinea Bissau, than in the northern region.

Infection with HIV-2 was also substantially higher in female commercial sex workers than in other population groups, indicating that this virus is also sexually transmitted. However, HIV-2 is transmitted less efficiently than HIV-1, both perinatally and sexually. Analysis of circulating lymphocytes by polymerase chain reaction techniques reveals that most HIV-2-infected carriers or fewer copies of proviral genomes per infected cell, or both. As with the infrequently pathogenic HTLV-I, but not with the highly virulent HIV-1, the age-specific prevalence for HIV-2 increase with increasing age. This presumably happens because relatively few HIV-2 - infected people are lost from the population because of death.

The observations that HIV-2-infecteed carriers have lower loads of virus and slower rates of sexual transmission between people are compatible with the observations that HIV-2s appear to evolve and deviate less rapidly than HIV-1s. This does not appear to be caused by any reduction in the error rate of the HIV-2 reverse transcriptase. The slower rates of HIV-2 replication and spread also help

explain why the HIV-2 epidemic is largely restricted to West Africa. Further, ven within West Africa some HIV-2s are essentially indistinguishable from the SIVs that occur naturally in a particular monkey species living in the same area.

The lower rates of replication for HIV-2 may also help explain why this virus appears less virulent than HIV-1 within individual hosts. Case reports and cross-sectional studies reveal that some HIV-2 infected people develop clinical AIDS[18]. However, even early studies in HIV-2 endemic areas suggested that disease rates were much lower than expected, based on HIV-1 associated disease with proportional prevalence rates. One possible explanation for this dichotomy was that HIV-2 entered the human population more recently that HIV-1. This possibility was largely dismissed after it was recognized that HIV-2 rates had stabilized in West Africa. It seems clear that HIV-2 had stabilized in some regions of West Africa before HIV-1 had even moved in. Also, analyses of West African sera stored from earlier time periods have shown that HIV-1 has been present in the region for an extended period.

Recently, a natural history study was completed that followed disease development for seroconverting commercial sex workers infected with either HIV-2 or HIV-1 in the same cohort. HIV-2 infected individuals resisted clinical AIDS development much better than did HIV-1 infected women. HIV-2 infected individuals also had stable T4 helper cell numbers for prolonged periods and did not experience the high rates of skin test energy seen with HIV-1infected patients. It is too soon to say whether the slower pace of disease development with HIV-2 is associated with complete long-standing resistance to AIDS or whether most HIV-2 infected people eventually succumb to disease. A small fraction of HIV-1 infected persons, perhaps 2% to 5%, remain healthy with stable T4 cell numbers for 10 to 15 years. This small fraction appear to have a natural history that is similar to that of most HIV-2 infected individuals. However, for both HIV-2 and HIV-1 it is also too soon to conclude that all strains and clades in different geographic regions show the

same patterns of virulence characterized by HIV-1 in the West and HIV-2 in Senegal.

MOLECULAR BIOLOGY :

The causative agent of acquired immunodeficiency syndrome (AIDS), human immunodeficiency virus type-1 (HIV-1), is a retrovirus of the lentivirus subfamily. Although a lentivirus was one of the first known viral agents[19], the study of lentiviruses intensified after the discovery of HIV-1 and its similarity to other lentiviruses.

The lentiviruses are exogenous, nononcogenic retroviruses causing persistent (i.e. chronic / active) infections; the diseases have long incubation periods. These viruses usually infected cells of the immune system, such as macrophages and T-cells, and have cytopathic effects in permissive cells; such as syncytia and cell death. Lentiviral infections are not cleared by the immune system, and their damage accumulates over many years. This important characteristic is reflected in the name of the subfamily; lenti means slow. Lentiviruses have a larger RNA genome (approximately 10 kilobases (kb), which encodes additional proteins. They produce a large and heavily glycosylated envelope protein (Env) and, in the case of HIVs, a magnesium ion (Mg^{2+}) dependent reverse transcriptase. They encode essential regulatory and accessory genes that allow regulation of their own expression in the infected cell. Unlike other retroviruses, lentiviruses can infected non-dividing cells.

Many lentiviruses affect the immune system, causing acquired immunodeficiency. The ovine and caprine lentiviruses primarily infect monocytes, and the viral DNA is integrated into the cellular DNA. The provirus remains silent unit the monocyte matures into a macrophage. Equine infectious anaemia virus also infects macrophages, but the feline simian, and human immunodeficiency viruses primarily infect T lymphocytes. Replication of the lentiviruses usually is toxic to the cell and leads to cell dysfunction and death. The structure and replication properties

of the lentiviruses may be the reason that the immune system is unable to eliminate the infection.

GENOME AND VIRION STRUCTURE :

Retroviruses are enveloped, positive strand RNA viruses that rely on a unique enzyme reverse transcriptae, to convert their RNA genome into a DNA "Provirus" which is integrated into the cellular genome. The viral envelope is a lipid bilayer that is produced by the cellular plasma membrane and contains the protruding viral Env. glycoprotein. The core viral partical is composed of the P24 capsid (CA) protein and contains the viral RNA and enzymes.

The genomic organization of HIV-1 is shown in HIV is 120 nm icosahedral, enveloped RNA virus. HIV comprises of an outer envelope consisting of a lipid bilayer with uniformly arranged 72 spikes or knobs of gp^{120} and gp^{41}. Glycoprotein gp^{120} protrudes out on the surface of the virus and gp^{41} is embedded in the lipid matrix. Inside is the protein core surrounding two copies of RNA. Core also contains viral enzymes reverse transcriptase, integrase and protease, all essential for viral replication and maturation. Proteins p^{7} and p^{9} are bound to the RNA and are believed to be involved in regulation of gene expression[20] (Fig. 1)

All retroviruses have in common the three coding regions gag pol and env, which encode the capsid proteins (Gag), the viral enzymes necessary for replication (Pol), and the external glycoprotein (Env) that protrudes out of the lipid viral envelope and is responsible for the infectivity of the viral particle by means of attachment to specific cellular respectors. The viral enzymes encoded by pol are reverse transcriptase, integrase, and protease.

Retroviruses have one promoter and one polyadenylation site within the long terminal repeats (LTRs) and express one primary transcript.

The additional proteins expressed by HIV-1 are part of the viral particle (i.e. Vif. Vpr, Vpx) regulate directly viral gene expression (i.e. Tat, Rev), or interact with the cellular machinery to promote virus propagation (i.e. Vpu, Nef). The additional

proteins increase the complexity of the organization and expression of HIV and the other lentivitruses.

Table - II

Human immunodeficiency virus and simian immunodeficiency virus proteins

Name	Size	Function	Localization
Gag MA	P^{17}	Membrane anchoring, Env interaction, nuclear transport of viral core (myristylated protein)	Virion
Gag CA	P^{24}	Core capsid	Virion
Gag NC	P^{7}	Nucleocapsid, binds RNA	Virion
	P^{6}	Binds Vpr	Virion
Protease	P^{15}	Gag-Pol cleavage and maturation	Virion
Reverse transcriptase, RNase H	P^{66} P^{51} (heterodimeter)	Reverse transcription, RNase Hactivity	Virion
Integrase		DNA provirus integratior	Viron
Env	gp^{120}/gp^{41}	External viral glycoproteins bind to CD4 receptor	Plasma membrane, virion envelope
Tat	P^{16}/P^{14}	External viral glycoproteins bind to CD4 receptor	Primarily in nucleolus / nucleus
Rev	P^{19}	RNA transport, stability and factor (phosphoprotein)	Primarily in nucleolus/nucleus use shutting between nucleolus and cytoplasm
Vif	P^{23}	Promotes virion maturation and infectivity	Cytoplasm (cytosol, membranes) virion
Vpr	P^{10-15}	Promotes nuclear localization of preintegration complex, inhibits cell division, arrests infected cells at G_2/M	Virion, nucleus (nuclear membrane?)
Vpu	P^{16}	Promotes extracellular release of viral particles, degrades CD4 in the endoplasmic reticulum (phosphoprotein); only in HIV-1 and SIV cpz	Integral membrane protein
Nef	P^{27}/P^{25}	CD4 downregulation (myristylated protein)	Plasma membrane, cytoplasm (virion?)
Vpx	P^{12-16}	Virion protein, vpr homologue (not in HIV-1, only in HIV-2 and SIV)	Virion (nucleus?)
Tev	P^{28}	Tripartite Tat-Env-Rev protein (also named Tnv)	Primarily in nucleous / nucleus

STRUCTURAL PROTEINS :

Gag Proteins :

The Gag precursor Pr 55 forms the core viral particle and interacts with other viral and cellular components, including RNA. Pol and Env proteins, and the plasma membrane, to facilitate their incorporation into the budding viral particle. Pr 55 is a self associating protein capable of assembling into virion like particles without the requirement of any other viral factors. In addition to Gag precursor, a Gag Pol fusion protein (Pr $160^{gap\text{-}pol}$) is produced by ribosomal frameshifting. The self associating properties of the Gag portion also direct the incorporation of some molecules of the Gag Pol fusion protein into the forming particle.

During or shortly after assembly, the protease is activated, and the Gag precursor is cleaved into four proteins (i.e. matrix (P 17 mA), Capsid (P. 24cA), nucleocapsid (P7 NC) and (p6 gag) and two small peptides (i.e. P1 gag and P2 gag.) The proteolytic cleavage products form the core of the mature virus. Pr $160^{gag\text{-}pol}$ polyprotein is also cleaved by protease into the Gag components and several active enzymes; protease, reverse transcriptase and integrase.

Proteolytic cleavage by cellular and viral enzymes, myristylation, and phosphorylation of the Gag Precursor are essential step in the viral life cycle. After translation of the Gag precursor, the initiator Met residue is removed and a myristoyl group is attached to the amino group of Gyl 2 by cellular enzymes. The P^{17MA} & P^{24CA} proteins are posphorylated by cellular kinases. The P^{7NC} protein binds two zinc ions to form the two zinc fingers of the NC domain, which are necessary protein RNA interactions.

Gag interacts with additional cellular factors, which are essential for formation of infectious virions. A region between residues 178 and 300 in the CA domain of the Gag precursor has been shown to bind cyclophilin A, a peptidyl prolyl cis-trans isomerase thought to play a role in immune regulation. This protein has been found in purified HIV-1 virions. Viral particles formed by $Pr^{55\ gag}$, in contrast to particles formed by the Gag polyproteins of other retroviruses, contain significant

amount of cyclophilin A. Mutation of the cyclophilin A binding domain results in noninfectious viral particles. Cyclosporine and cyclosporine analogs disrupt the binding of cyclopuilin A to Gag and inhibit HIV-1 replication. In contrast, a cyclosporine analog was shown to be inactive against SIV mac, which does not incorporate cyclopuilin A. Moreover a cyclosporine analog (SDZ NIM 811) that is completely devoid of immunosuppressive capacity was found to have potent and selective anti HIV-1 activity. SDZ NIM 811 may interfere with Gag cyclophiln A interaction and affect the infectivity of the virions and early events of viral nuclear transport and integration[21].

The arrangement of the Gag-pol transcription and translation unit is essential for packaging the viral enzymes encoded by Pol. The pol encoded products are expressed as a Gag Pol fusion protein after a 1 ribosomal frame shifting, which happens at a frequency of approximately 5%. A stemloop structure 3' of the HIV-1 shift site is important for wild type levels of fromshifting in Vivo. In vitro studies did not show the importance of the 3 stemloop structure for frameshifting. The resulting fusion protein, the Gag Pol precursor ($Pr^{160gag\text{-}pol}$), contains the Gag proteins required for incorporation into the virion.

The Capsid :

The structure of the nucleocapsid of retroviruses is not well understood. When virions are carefully treated with mild detergent, two rather fragile structures are formed, depending on virus and conditions. The nucleoprotein contains the virus RNA, the NC protein, and a fraction of the RT activity, The core contains, in addition, the CA protein. Electron microscopy of whole or fractionated virions has not been particularly revealing of the fine structure; the particles are somewhat pleomorphic and do not reveal obvious overall symmetries, although local icosahedrally symetric regions can be seen. Immature virions containing unprocessed Gag proteins seem to have a much better defined structure[22]. Most of the structural features observed are atributable to gag proteins. Indeed, almost normal looking virions can be formed in the absence of pol and env proteins as well

as of genome RNA. The major gag proteins are most readily studied as the cleavage product found in virions; and they act in two different forms at different times.

During virion assembly, they are unified as a precursor protein; it is however, the cleavage product that enter the cell to initiate the infection cycle. Thus, domains filling important roles in assembly may not be neatly distributed among the cleavage products. As a consequence of the mode of assembly, it can be expected thatall gag proteins will be present in equal numbers in the virion. This expectation seems to be borne out, and it has been estimated that there are about 2000 to 4000 copies of each cleavage product per virion.

Matrix Domain (MA Protein) :

Are necessary for targeting the Gag precursor to the cell membrane resulting in the anchoring of Env. to the viral particle. The mature MA protein remains associated with the inner side of the lipid envelope.

Several domains of the MA protein in which single amino acid substitutions dramatically reduce the efficiency of virion particle production were identified by mutagenesis. Mutagenesis results also suggest the existence of a specific functional integration between the MA protein and the gp^{41}cytoplasmic tail; virion incorporation of envelope glycoproteins with long, but not short, cytoplasmic tails was blocked by specific, single amino acid substitutions in the MA protein[23].

Capsid Domain (CA protein)

In the CA domain of HIV-1, amino acids 240 through 430 are essential for self associaton[24]. The CA domain of all retroviruses contains a major homology region (MHR) that is required for efficient viral replication and particle production. Deletions within the MHR or deletions C-terminal to this region blocked viral replication and significantly reduced the ability to form viral particles.

Nucleocapsid Domain (NC Protein)

The Gag precursor binds to and is responsible for the packaging of the viral genomic RNA. The NC domain of the Gag precursor is directly involved in this nucleic acid binding. Rich in basic residues (i.e. Lys and Arg). the NC domain

contains two copies of a zinc-binding sequence [Cys $(x)_2$) cys$(x)_4$ His $(x)_4$ Cys] referred to as a retroviral CCHC zink finger. The basic residues confer a nucleic acid binding property to the domain and the CHC zink fingers contribute to the specificity for the viral RNA. During viral assembly, the NC domain of the Gag precursor bind to the encapsidation site (ie Psi site) near the 5' end of the genomic RNA.

Disruption of either of the two zink fingers in the NC domain resulted in noninfectious particles with reduced levels packaged viral genome[25]. Deletion the zink fingers also eliminated virus particle formation. Gag sequences up to and including the first zink finger domain are necessary and sufficient for virion formation. Mutants of the NC protein that still packaged RNA but had alterations coition the zink finger structures, reduced infectivity.

The NC protein also catalyzes the formation of the genomic RNA dimer found in virion particles. HIV-1 encoding a defective protease incorporated RNA with a different dimer structure. This indicates that dimer formation is probably catulyzed by the mature NC protein and not by the Gag precursor.

P^{6gag} protein:

The only established function of $P^{6\ gag}$ is the interaction with Vpr, resulting in virion incorporation of this accessory protein. The $P^{6\ gag}$ protein is necessary and sufficient for this incorporation. The role of Vpr is not known exactly, but it appears to block cells in the G_2 or S phase of the cell cycle[26].

P^1 and P^2 Peptides :

The role of the P^1 peptide is unknown. The P^2 sequences were shown to modulate the rates of Gag processing. The proteolytic processing sites of the Gag precursor are cleaved in a sequential manner by viral protease. The exact hierarchy of protedytic processing may be important for capsid formation and viral infectivity. The presence of a C-terminal P^2 tail on processing intermediates slows cleavage at the upstream CA/P^2 site. Deletion of the P^2 domain of Gag resulted in released

virions that were less infectious despite the presence of the processed final product of Gag[27].

The Envelope :

As with most enveloped viruses, the retroviral envelope is derived by budding from the cell membrane and compries preexisting lipids and other membrane components modified by the insertion of viral proteins in place of normal cellular components. All retroviruses have a similar complement of envelope proteins. The three dimensional structure of a retroviral env protein has yet to be determined.

Env. Protein :

The HIV-1 Env. precursor gp^{160} is cleaved intracellularly into gp^{120} and gp^{41} by a cellular protease. The surface glycoprotein gp^{120} binds CD4 in the initial step of virus-cell interaction. This binding leads to conformational changes and membrane fusion assisted by the N terminus of gp^{41}. The part of Env. interacting with CD4 has been identified within four noncontiguous regions of gp^{120}. The overall structure of gp^{120} is important for virus receptor interaction and virus entry. Env. is a heavily glycosylated protein. N linked glycans are necessary for the creation, but not the maintenance of a bioactive conformation, and drug induced alteration of the glycosylation pattern can impair virus infectivity[28].

Different isolates of HIV-1 very in their cell tropism, that is the range of cell types in which they are able to establish a productive infection. The env. glycoprotein gp^{120} is responsible for the difference in cell tropism in many cases. The region of Env. involved in the determination of cell tropism includes sequences that encode the V3 loop of gp^{120}. Control of cell tropsim by a part of Env. including the V3 loop may be general phenomenon that applies to many different HIV-1 isolates.

Interactions between gp^{120} and CD4 are responsible for the entry of HIV-1 into host cells in most cases. HIV-1 into host cells in most cases. HIV-1 replication is commonly followed by the disappearance or down modulation of cell - surface

CD4. This process potentially reders cell nonsus ceptible to subsequent infection by HIV-1 and other viruses that use CD4 as a receptor. Disappearance of CD4 from the cell surface is mediated by several different viral proteins that act at various stages throguh the course of the viral life cycle.

Virion Enzymes :

The product of the pro and Pol regions constitute the enzymatic activities found in retrovirus virion. Their mode of synthesis, as occasional extension products of gag, results in their presence in much smaller numbers, less than 100 compared with thousands of copies per virion.

Protease (PR) :

HIV-1 protease in an aspartic protease containing the characteristic active centre of such enzymes (i.e. Asp Thr Gly). Protease is only active as a dimer, which is formed after Gag and Gag Pol multimerization, and contains two identical subunits of approximately 10 Kb In contrast cellular aspartic proteases (eg. Pepsinogen) are monomers containing two Asp Thr Gly domains that form the active centre. Premature activation of protease does not occur before capsid formation at the plasma membrane, because the amount of Pr160 gag pol is limited. Several mutations prevented protease activation in the context of a Gag protease polyprotein, perhaps by preventing polyprotein dimerization. The appropriate activation of protease is essential for virus maturation, but excess protease activity is deterimental to the virus[29].

Inhabitation of HIV protease results in noninfectious particles and is an attractive target for therapy designed to block the progression of HIV infection. Elucidation of the tertiary structure of HIV-1 protease has aided the rational design and development of protease inhibitors. Inhibitors of HIV protease from a variety of chemical classes have been synthesized and antiviral activity has been demonstrated in lymphocytes and cell of the monocytemacrophage lineage. Several of these inhibitors have shown promise in clinical trials. Saquinavir (Invirase, Hoffman La Roche) was the first protease inhibitor in clinical trials, and other protease inhibitors

are under clinical evaluation, including MK-639 (Merck), ABT-538 (Abbot Laboratories), KNI-272 (Kyoto/national cancer institute), U 96988 and U 103107 (Upjohn) and AG 1343 (Agouron).

Reverse Transcriptase / Rebonuclease (RT) :

Active reverse transcriptase (RT) is a heterodimer produced by protecolytic processing of $Pr^{160gag\text{-}pol}$ by the viral protease. The gag pol precursor first produces P^{66}, which presumably dimerizes. The P^{66} subunits in the homodimer are loosely associated, and the dimer has low enzymatic activity. During viral infection, the P^{66} homodimer is further cleared at the C-terminal part of one P^{66} subunit to give a tightly associated P^{66}-P^{51} heterodimer, which has full enzymatic activity.

Enzymatic Activities :

Reverse transcriptase possesses three enzymatic activities : RNA dependent DNA polymarase, DNA dependent DNA polymurase and ribonuclease H (RNase H). The polymerase activity of reverse transcriptase lise within the N terminal portion P^{66} and the RNase H activity lies within the C terminal portion of P^{66}. A 'tether' region lies between the two domains. Rt also binds specifically to t RNA lys, which is preferentially encapsidated and subsequantly hybridized to the PBS. When isolated from HIV virions, the t RNA is annealed to the RNA at the PBS.

The three dimensional structure of HIV reverse transcriptase reveals an unprecedented degree of asymmetry within the heterodimer[30]. The P^{66}. submit has a large cleft similar to that of other polymarases, while the P^{51} subunit has a very different structure.

Rt uses RNA primers with 3' OH ends to initiate DNA synthesis. The RNase H activity is responsible for the cleavage of the RNA strand in the RNA-DNA hybrid. The synthesis of the minus strand starts at the PBS by the tRNA primer, while the synthesis of the plus strand starts at the PPT, next to the U3 region.

The fidelity of the DNA polymerases is largely attributable to a two step nucleotide binding michanism. In the first step binding contacts are initially made between the template and the incoming nucleotide triphosphates. In the second step,

a change in protein conformation occurs, which leads to rapid incorporation of the nucleotide triphosphates into the growing polymer. The fidelity of polymerization due to these mechanisms approaches one errors in 10^5 or 10^6 nt. The proofreading function of the DNA polymerases increases the fidelity by another 10^3 to 10^4, resulting in the extraordinary overall fidelity approaching one error in 10^{10} nt. Right does not have a 3' exonuclease proofreading activity, which results in high error rates. During one replication cycle, the viral genetic information is copied twice, first by the reverse transcriptase to proviral DNA and second by the RNA polymerase II to RNA. The combined error results in about one error in 10^4 nt. which means that every genomic RNA molecule of approximately 10^4 nucleotides contains one misincorporation.

Inhibitors of RT :

RT has been the first and most successful target for anti HIV-1 intervention[31]. Nucleoside analogs were found to be present inhibitors of rt, resulting in widespread clinical utility. The nucleoside analogs that have been approved for clinical use or are at various stages of clinical development include AZT (Zidovudine, Retrovir) ddi (didanosine, Videx), ddC (zalcitabine, Hirid), d4T (Stavudine, Zerit), and 3TC (lamivudine). These nucleoside analogs act after phosphorylation to nucleotide triphosphates to inhibit elopngation at the active site of rt.

The major problems with these drugs are their toxicity, caused in part by inhibition of cellular polymerases, and the rapid development of resistance to the drugs by HIV-1. Resistant viruses can be detected soon after treatment. Multiple reverse transcriptase mutations have been shown to confer resistance to AZT, including mutations at amino acids 41, 67, 70, 215 and 219. Accumulation of more than one mutation leads to higher resistance. Treatment with different nucleoside analogs may result in different mutations conferring resistance. Many, mutations have been observed in vitro and in vivo. Ongoing clinical trails are testing

combinations, with the hope that different inhibitors may prevent or delay the development of resistance.

Nonnucleoside analogs, such as nevirapine, TIBO, and L 697661, that inhibit reverse transcriptas also have been developed, these inhibitors are specific for HIV1.

Integrase (IN) :

Integrase is the viral protein responsible for the integration of the proviral DNA into the host nuclear DNA. Integrase is a 31 Kd protein produced from the C-tgerminal part of Pol after processing of Pr 160 gag pol Integrase possesses the 3' processing and DNA strand transfer activities that are required to integrate HIV-1 DNA into a host chromosome. It cleaves the ends of the liner viral RNA, leaving 3' recessed termini that may slow self ligation and favour integratgion of the linear two LTR DNA. Integrase also cleaves the cellular DNA randomly and joins the viral DNA to the host's chromosome. These activities have been reconstituted and studied extensively in vitro.

Biochemical analysis of HIV-1 integrase with purified protein and synthetic DNA substrates has revealed extensive information regarding the mechanisms of action of the enzyme and identified critical residues and functional domains. In vitro, a stable complex containing only purified HIV integrase and a model viral DNA substrate processively executes the 3' trimming and DNA joining step in the integratgion reaction. Purified integrse was also shown to promote the same action in reverse, a process called disintegration. Integrase is an important component of the preintegration complex. Association of integrase, but not reverse transcriptase or MA proteins, with viral DNA was stable in the presence of detergents. The domains involved in the integration of integrase with the viral DNA substrate have also been studied[32].

Integration is an obligate step in productive HIV-1 infection of activated peripheral blood mononucler cells (PBMCs) and primary human macrophages. Mutations introduced into infectious DNA clones of HIV-1 affect virus replication at a variety of steps. Mutations that altered virion morphology, levels of particle

associated integrase and reverse transcriptase, and viral DNA synthesis have been identified. Although none of the replication defective mutants were able to intgrate into the host genome, a subset of them with alterations in the catalytic triad were capable of Tat-mediated transactivation of an indicator gene linked to the viral LTR promoter. Although unintegrated viral DNA can serve as a template for Tat expression in infected indicator cells, this level of expression is insufficient to support a spreading viral infection in CD4 positive lymphocytes. Integration of the HIV-1 provirus is essential not only for productive infection of T cells, but also for virus passage in cultured peripheral blood lymphocytes and macrophages.

REGULATORY PROTEINS :

The two essential regulatory proteins of HIV-1 are Tat and Rev. These are the two gene products for which there is direct proof of involvement in different steps of gene expressions transcription for Tat and posttranscriptional regulation for Rev. Because the other nonstructural gene products do not appear to be directly involved in gene expression control, they were named accessory to differentiate them from Tat and Rev. which act on gene expression. Tat and Rev appear to recognize specifically a limited number of functional group in the major groove of an RNA double helix distored by virtue of unpaired or non watson crick paired nucleotides.

Tat :

Tat is an essential regulatory protein for HIV-1 replication[33]. The 16 kd, two exon Tat protein is encoded from two separate exons of multiply spliced mRNAs. Whereas a one exon, 14 kd form of Tat (Tat-1) is produced by singly spliced mRNAs. Tat varies in size among the different isolates (86 to 101 amino acids); turncated (58 to 72 amino acid) forms encoded by the first exon are also functional. Tat accumulates in the nucleolus and is responsible for the transcriptional activation of HIV expression. It is the first charactrized eukaryotic transcription factor that binds to a nascent leader RNA and affect the HIV enhancer promoter.

All HIV-1 mRNAs contain a region at the 5' end named TAR, which has been mapped to nt + 1 through 44. This sequence is necessary for transactivation of viral gene expression by Tat. TAR is predicted to form a stable stem loop structure, with a bulge in the stem. Result of mutational analysis indicate that the bulge and the stem are necessary for transactivation Tat binds directly to the bulge region. of the TAR element. The nucleotide sequence of the bulge, UCU, is critical for Tat binding. Specific sequences are also required for Tat binding in the double stranded region that flanks the bulge. These interactions are necessary but not sufficient for Tat function; cellular factors have also been shown to bind to TAR or to Tat and some cooperate with Tat in promotor activation. Factors produced by the human chromosome 12 contribute to the binding of Tat to TAR. The HIV promoter is significantly less activated in the presence of Tat.

All RNA binding Tat proteins contain an 'activation domain' and an arginine rich basic domain which are necessary for activation of transcription, specific binding to TAR RNA, and nuclear localization.

Viruses defective for Tat do not replicate, except in some in vitro cell lines after cytokine stimulation. 41 irradiation can also activate expression of tat-integrated proviruses. Tat defective proviruses could easily be detected in patient tissues. Transitions from tat + to tat - and back may be a mechanism for tetancy in human tissues. Tat has been found in the medium of infected cells, suggesting that it may have an extracellular role. Tat has also been implicated as one of the cofactors in Kaposis sarcoma induction.

Tat is a potent and essential transactivation element acting through RNA binding. Its role in the virus life cycle is complex and has the potential to interact with multiple cellular components. It has been used as a reliable marker for HIV-1 infection, and as a target for development of antiviral strategies.

Rev :

Rev is an essential regulatory viral protein that accumulates in the nucleolus[34] and shuttles between the nucleolus and the cytoplasm. Rev is a small,

positively charged protein of approximately 116 amino acids. The abnormal mobility of Rev on denaturing gels (19 Kd) probably results from the structure of the protein. Rev. is phosphorylated on ser residues. Mutagenesis studies indicated that phosphorylation is not essential for Rev. function in vitro.

In the absence of Rv. most of the viral mRNAs are multiply spliced, the production of the structural protein is very low, and no virions are formed.

ACCESSORY PROTEINS :

The accessory genes (i.e. Vif, VPr, Vpu and nef) and proteins of HIV-1 were so named because they were dispensable for virus replication in many cultured cells in Vitro, although their conservation in all virus isolates demonstrates that they are essential during the virus life cycle in the host. Most of the accessory proteins appears to enter the virion, and they interact with viral and cellular components to increase viral replication in the host. Two of the accessory genes, vif and Vpr, have recognizable homologs in nonprimate lentiviruses.

Vif :

The viral infectivity factor Vif (previously named sor or Q) has been shown to influence the infectivity but not the production of virus particles. The vif ORF is located after the Pol ORF and overlaps with the 3' part of pol. Homologs of vif exist in all lentiviruses except equine infectious anaemia virus. The vif gene encodes a 23 Kd protein, which is immunogenic in infected individuals. Vif is expressed from partialy spliced mRNAs; its expression is activated by Rev[35]. Vif is a cytoplasmic protein, existing in a soluble cytosolic form and a membrane associated form. The latter form of Vif is a peripheral membrane protein that is tighty associated with the cytoplasmic side of cellular membranes. This localization and other observations suggest that vif is incorporated in the virion.

Vif is required for HIV-1 replication in the CD4 positive T cell lines CME and H9 and in peripheral blood T lymphocytes.

The mechanism of function of Vif is not clear several studies agree with the conclusion that Vif affects late events in the viral life cycle, which result in lower

infectivity. Vif may affect virus particle maturation. It was shown that nonhomogeneous packing of the core takes place in most vif virions production in CME and Jurkat cells. In the absence of Vif, the cone shaped virus core contained dense material in its broad end, but in contrast to Vif+ virions, the material inside its narrow end appeared transparent. Notably, vif virions recovered from restrictive cells, but not from permissive cells, were abnormal in terms of morphology and viral protein content.

Vif is required for proper assembly of the virion and for efficient Env. mediated infection of target cells. Failure to infect target cells results from a defect in the formation of the viral particle in PBMCs or nonpermissive cell lines. In a single round of infection vif virus is approximately 25 (from CEM x 174 cells) to 100 (from H9 cells) times less infectious than wild type virus.

The observation that vif virions do not produce cDNA after infection led to an alternative proposal, suggesting that vif stimulates efficient nucleocapsid. internalization or activation of reverse transcription after infection. The levels of viral DNA were examined by RNA PCR during infection by Vif + and vif - viruses of MT2 and H9 cells, in which Vif is required for HIV-1 replication. Inefficient viral DNA synthesis correlated with restricted replication of the vif virus. Instead, vif virions were severely impaired in their ability to complete the synthesis of viral DNA after they were internalized in the target cell. The impaired reverse transcription may be the result of defects in virus maturation in the nonpermissive producer cell.

The C terminus of Vif is required for the stable association of Vif with membranes and is essential for Vif function, suggesting that this association is likely to be important for its biologic activity.

Vpr :

Vpr (Viral Protein R) is a 96 amino acid, 14 Kd protein that is incorporated into the virion. It interacts with the P^{6} part of the Pr^{55} precursor[36]. Vpr detected in the cell is localized to the nucleus. Proposed functions for Vpr include the nuclear

import of preintegration complexes, cell growth arrest, transactivation of cellular genes and induction of cellular differentiation.

Most retroviral integration requires cell division. In contrast, HIV-1 and the other lenmtiviruses can inegrate their genomes in nondividing cells by transporting the preintegration complex into the nucleus. Vpr is one of the two identified nucleophilic componants that promote nuclear localization of viral nucleic acids in nondividing cells.

Serval observations raise the possibility that some aspects of HIV-1 induced pathologies are caused by a disturbance of cells by Vpr. Although Vpr has no effect on the initial cytopathic effect of HIV-1, viruses that contain an intact Vpr gene are unable to establish a chronic infection of T cells, whereas viruses with a mutated Vpr gene can readily establish such longterm cultures. Expression of Vpr alone affects the progression of cells in the cell cycle, arresting the cells in Gz / M phase. These results suggest hat HIV-1 uses Vpr to modulate chronic infection of T cells. Vpr can directly inhibit cell proliferation and induce cells differentiation of the human rehabdomyosarcoma cell line TE671 These results may be related to the ability of Vpr to block cell division, and link this proein with cellular proliferation pathways possibly relevant to the control of HIV-1 replication.

The C terminal third of the Vpr protein caused cell growth arrest and structural defects in yeast cells, indicated by osmotic sensitivity and gross cell enlargment. The Vpr molecular clones were viable and did not have any obvious defects when growth in T cells, but their growth was severely delayed in macrophages. Loss of Vpr reduced viral antigen production in macrophages by up to 1000 fold but only marginally affected replication in lymphocytes. The block to infection in monocytes was identified at a step in the viral life cycle after entry and reverse transcription, but before or at the time of proviral transcription. Infection of mononuclear phagocytes with virions that had been loaded with Vpr molecules in the producer cells by transcomplementation still showed a Vpr phenotype, suggesting a role for Vpr produced in newly infected cells in addition to its

presumed function in the virion. In support of this theory, Vpr was found to be produced by multiply spliced (Rev. independent) and by singly spliced (Rev. dependent) mRNAs, suggesting that Vpr is present early after infection.

Vpr contains an amphipathic alpha helical domain in the N terminus (residues 17 through 34) that is highly conserved among Vpr sequences of different isolates. Acidic and hydrophobic residues and the helial structure in this region are critical for the stability of Vpr and its efficient incorporation into virions. Mutation of the highly conserved acidic residues in the N terminal domain (residues 17 through 34) disrupted the alpha helical structure and eliminated virion incorporation.

Vpu :

Vpu (Viral Protein U) is unique to HIV-1 and SIV, a close relative of HIV-1. Vpu is a 16 kd, has two different biologic functions. degradation of CD4 in the endoplasmic reticulum and enhancement of virion release from the plasma membrane of HIV-1 infected cells.

CD4 Degradation :

Intracellular complexing between Env. and CD4 results in trapping of both molecules in the endoplasmic reticulum. The presences of Vpu releases Env. from these complexes. In several systems, Vpu was shown to act on CD4 in the absence of Env. or any other viral protein. Degradation of CD4 by Vpu requires the two proteins to be present in the same membrane compartment. Immunoprecipitation experiments showed that Vpu specifically binds to he cytoplasmic tail of CD4[37]. The ability of CD8 / CD4 chimeric molecules and various CD4 mutunts to form complexes with Vpu correlates with their sensitivity to degradation, and amino acid residues in the CD4 cytoplasmic tail that are important for degradation are also necessary for Vpu binding.

Virus Release :

In addition to CD4 degradation, Vpu appears to have another function, it enhances the release of virions from the infected cells. This function of Vpu may be related to the multimerization of Vpu and potential ion channel forming capacity. A

structural homology of Vpu with the influenza M2 membrane protein has been observed. M2 forms ion channels and may modulate the pH of intracellular compartments. This activity of Vpu is not restricted to HIV-1. It was reported that Vpu enhances the release of capasids produced by the Gag genes of widly divergent retroviruses in CD4 negative cells.

Nef :

Nef (previously named 3' ORF) is an approximately 27 kd myristylated protein produced by an ORF located at the 3' and of the primate lentiviruses. Other forms of Nef are knonw, including nonmyristylated variants. Nef is predominantly cytoplasmic and associated with the plasma membrane through the myristly residue linked to the conserved second amino acid (Gly.) Nef has also been identified in the nucleus and found associated with the cytoskeleton in some experiments. Its association with the virion is suspected but not proven. Nef is not preferentially accumulated in the virion, but its membrane localization may result in virion incorporation.

Initially thought to be a negative factor, nef was found to be important for viral replication in vivo. A nef + HIV-1 molecular clone induced severe depletion of human thymocytes in immunodeficient (Scid) mice containing human lymphoid tissues (scid-hu) within 6 weeks of infection, but a nef-HIV-1 did not[38]. Thus HIV-1 nef is required for efficient viral replication and pathogenicity in vivo.

Down regulation of surface CD4 :

A well established function of Nef is the downregulation of CD4, the primary viral receptor. Nef acts by inducing CD4 downregulation is strongly enhanced by the association of Nef with cell membranes through myristylation.

This function of Nef appears to be redundant with the function of Vpu, which degrades CD4 in the endoplasmic reticulum. For both proteins, membrane association is essential for CD4 degradation involves binding to CD4. Binding has been demonstrated for Vpu; however, no direct evidence exists for an association between Nef and CD_4 and attempts to demonstrate CD4-Nef complexes have failed.

Whatever the mechanism, this function of Nef results in rapid CD4 downregulation and possible prevention of superinfection. In concert with the function of Vpu, these effects may result in higher levels of intact Env. protein in the capsid and increased virus production.

CD4 down regulation is a property of nef alleles found in many primary HIV-1 isolates. It was proposed that CD4 down regulation is a conserved function of Nef, selected in vivo during human HIV-1 infection.

VIROLOGIC AND BIOLOGIC FEATURES OF HIV-2 :

Both HIV-1 and HIV-2 are human lentiviruses with a number of similar virologic properties. Both viruses have a target cell tropism for CD4 + T lymphocytes and a propensity for establishing latent infections. Like other retroviruses, the HIVs are single positive strand RNA viruses with particles approximately 100 nm in diameter. Virions have a characteristic dense, cylindrical, protein core that encases the genomic RNA molecules and viral enzymes (reverse transcriptase, integrase, and protease). This ultrastructurl morphology of HIV-2 can be distinguished from HIV-1 and other animal lentiviruses; it is also distinct from type C retroviruses. Distinct for HIV-2 is the prominant protruding envelope projections and the electron lucent paranucleoid space, which is an open ring around the triangular core, compared to HIV-1 where the lucent space extends from the core to the envelope binding of the envelope of HIV to the CD4 molecule is only a first step in viral entry, with subsequent fusion events that appear to require both viral envelope and other cellular factors[39]. HIV-2 isolates have demonstrated tropsim for cells bearing the CD4 marker, similar to both HIV-1 and SIV. Blocking studies with recombinant soluble CD4 have verified that SIV and HIV-2, like HIV-1, bind via their external envelope glycoprotein, gp^{120}, to a common cellular receptor, the differentiation antigen CD4. It is of interest that HIV-2 has a lower binding affinity for the CD4 receptor than does HIV-1.

Once inside the cells, the processes of reverse transcription and integration occur. The HIV replication cycle is unique, with a complex array of various viral encoded factors that regulate both positively and negatively on the level of virus expression. This culminates in the final steps of virus assembly, maturation, and release, similar to other retroviruses. HIV replication is closely linked to the death of the CD4 + cell. This cytopathicity is a hallmark of HIV infection and has been studied extensively both in vitro and in vivo. In vitro studies of HIV-2 isolates by a number of laboratories have described differences in cytopathicity of HIV-2 as compared with HIV-1. In comparison with HIV-1, HIV-2 isolates demonstrate decreased cell killing, less syncytial cell formation, reduced virus replication, and difference in interaction with CD4 in some cases related to the clinical stage of the HIV-2 infected individual.

The antigenic relatedness of both SIV and HIV-2 to the prototype HIV-1 virus prompted both the discovery and further classification of these related viruses. Subsequent genetic analysis confirmed the relationship indicated by antigenic studies, demonstrating that HIV-2 was more closely related to SIV than to HIV-1 (approximately 40% nucleotide homology). Similar to HIV-1, restriction site polymorphism and sequence data indicate variability among HIV-2 strains. As more sequence data have become available from various HIV-2 and SIV strains, it has also become apparent that no branching order of divergence can be specified and that these virus types may in fact share a common ancestor. Sequence variability of structural genes such as env and gag has been similar between HIV-2 and HIV-1, with greater variability in the env gene. Interestingly, regulatory gene sequences are more variable among HIV-2 isolates, compared with HIV-1.

Like all retroviruses, HIV-2 encodes gag, pol and env structural gene products, flanked by two long terminal repeat sequences (LTRs). The overall genomic organizations of HIV-1 and HIV-2/SIV are similar except for the presence of the unique gene termed VPx found in both HIV-2 and SIV and the Vpu gene unique to HIV-1. The gag precursor polyprotein of HIV-1is translated from the full

length genomic RNA to a 57 KD protein that is myristylated and cleaved at five cleavage sites to yield six products, P^{17}-P^{27}-P^{2}-P^{1}-P^{6}. The major core protein P^{27} is slightly larger in HIV-2 than in HIV-1, whereas the myristylated matrix protein P^{17} is of similar size. After a gag-pol polyproptein precursor is formed, the complete Pol precursor is cleaved and forms a protein of approximately 180KD. This product is in turn cleaved to form the P64 (reverse transcriptase / RNase H), P36 (endonuclease / integrae), and P11 (Protease) of HIV-2. The major viral structural proteins are readily distinguished on immunoblats and regularly recognized by HIV-2 serum samples. The gag and pol genes are well conserved among both HIVs and SIVs, the high degree of conservation in the structural genes accounts for the cross reactivity of HIV-2 sera on HIV-1 gag and pol antigens, as seen in some serologic assays.

In HIV-2 the envelope polyprotein precursor is a heavily glycosylated protein which is elevated to form the mature (group 105-120) and the transmembrane (group 32-40) envelope proteins. Like HIV-1, genetic variation between HIV-2 isolates has been demonstrated, with the most marked variation in the envelope gene. The envelope gene demonstrates genetic variation from isolate to isolate with some plymorphism exhibited at a protein level. Sequence analysis of several HIV-2 strains has demonstrated a stop codon in the middle of the open reading frame encoding the transmembrane protein; this finding explains the smaller transmembrane protein size that is seen with certain HIV-2 isolates.

Functionally important sites on the external glycoprotein appear conserved between HIV-1 and HIV-2. This includes the CD4 binding domain, precursor processing, fusion, principal neutralizing domain of V3 and at least some cytotoxic T-cell epitopes, unlike HIV-1, antibody binding activity appears to concentrate in the middle protein of the Group 105 envelope molecule, as opposed to the HIV-1 antibody response, which is directed more broadly across the group^{120} molecule. Another important immunogenic domain of the envelope of HIV-2 is found in the amino terminus of the transmembrane protein, which has been used to advantage for a variety of peptide and recombinant based type specific diagnostic assays.

Like other complex retroviruses, HIV-2 possesses a number of accessory genes with variable homology to HIV-1 genes, including tat, rev, nef, vif and vpr. Both SIV and HIV-2 possess a Vpx gene which is absent in HIV-1 and they both lack the Vpu gene, the unique accessory gene of HIV-1. Matational studies have shown that Vpx is not required for virus replication, yet may fucilitate infection in fresh lymphocytes and monocytes. Thus, the Vpx gene product apears to be necessary for efficient viral replication under certain circumstances. Although the general genomic structure of HIV-2 and HIV-1 are similar, the reading frames differ. The reading frames for HIV-2 gag, pol and vif are similar to HIV-1, but the reminder of the open reading frames are different from that of HIV-1. This frame shift occurs at the site of Vpx, the unique gene of HIV-2.

HIV-2 encodes the vif gene which, by analogy to HIV-1, is likely to be involved in the infectivity in certain cell types. The nef gene, originally considered on the basis of tissue culture experiments to encode a negative regulatory element, is now known to encode a protein which actually increase SIV and presumbly HIV-2 replication in Vivo. The product of the Vpr gene appears to be a virion associated structural protein which has been shown to cause a modest increase in HIV-1 replication to be critical for macrophage infectin. Recent data suggest that Vpr may function as a regulatgor of cellular permissiveness to HIV-1 replication. Serum Vpr from HIV-1 infected patients activated virus expression in latently infected cell lines and in resting peripheral blood mononuclear cells, thus indicating that the Vpr gene product may control HIV latency in vivo. The distant genetic relatedness of Vpr to Vpx has suggested that Vpx in HIV-2 results from an ancient duplication of the Vpr gene. The relevance of this observation to the function of these genes in HIV-2 is still not known.

The tat protein of HIV-2, like that of HIV-1, transactivates to stimulate viral production and is assumed to be essential for viral replication. The tat proteins are unsual viral transactivators in that their action is mediated through a site or sites

downstream of the RNA initiation site. It is generally believed that the primary role of tat is to permit elongatgion of properly initiated viral transcripts. Whereas the RNA target for HIV-1 tat has only one stem loop structure, the HIV-2 tat target sequence has two. It appears that the distal site may be employed only when the proximal site can not be used. Functional evidence of tat activity has been shown for HIV-2 and SIV similar and cross reactive with HIV-1 tat.

Another regulatory gene of HIV is the rev gene, which exhibits a complex machanism of action and is required for viral replication. Rev. acts through its RNA target in the env gene. The rev responsive element (RRE), to fucilitate the transport of HIV structural gene transcripts to the cytoplasm. This is accomplished by decreasing the transport of unspliced (structural gene) viral RNA from the nucleus and by regulating RNA splicing. Interaction of Rev. with the RRE RNA, either alone or with additional cellular factors, presumably mediates the export of these RNAs from the nucleus to the cytoplasm. In the absence of Rev, the viral proteins encoded by these mRNAs are not expressed because their respective mRNAs are trapped in the nucleus. While less well, studied, HIV-2 rev appears to function similarly to HIV-1 rev, the HIV-2 rev is phosphorylated, albeit toamuch lesser extent, and is localized to the nucleus with marked accumulatation in the nucleolus. While HIV-1 rev can function with both the HIV-1 and HIV-2 RREs, HIV-2 rev function only with its own RRE.

In general, regulation of viral gene expression in HIV-2 seems to resemble that observed in HIV-1. However, several differences have been described thatmay play a role in the differential pathogenicity of these virus for example; sequence of HIV-1 and HIV-2 have demonstrated differences in the LTR structure. Whereas HIV-1 has two NFkB enhancer binding sites, only one can be identified for HIV2. Regulation and response to T-cell activation via the viral. LTR also appear to be distinct in HIV-2. Specific and unique lements in the HIV2 LTR may regulate HIV-

2 gene expression independently of the T-cell activation signals. Mutational studies of the unique sites in the HIV-2 LTR responsible for inducible enhancer function demonstrate that this function is more readily disrupted in HIV-2 compared with HIV-1.

The invitro behaviour of HIV-1 viruses have been used as an indicator of pathogenicity. In contrast to most HIV-1s, HIV-2 isolates demonstrate decreased cell killing less syncitial cell formation, reduced virus replication and difference in interaction with CD4, frequently related to the clinical stage of the HIV-2 is more difficult to isolate from asymptomatic people than is HIV-1, but when isolated, it appears to show the "slow low" pattern. Similarly, the "rapid high" isolates were more likely to be from HIV-2 infected people with advanced stage disease. Evaluation of intrapatient variation in the V3 region of HIV-2 in asymptomatic and symptomatic individuals followed over time has shown less variation appears to be a distinct feature of HIV-2 infection that may result from decreased viral burden and also may contribute to lower rate of transmission and disease development.

LIFE CYCLE :

The human immianodeficiency virus (HIV) shares features common to all retroviruses. First, retroviruses are so named because of their ability to route genetic information from RNA to DNA. This is accomplished by a unique enzyme, reverse transcriptase (RT), which is encoded by a gene within the retroviral genome. This gene (Pol) and two others, gag and env, are the major coding regions for the structural proteins needed for retroviruses to maintain and complete a life cycle. Although the seven major genera of ritroviridae are usually differentiated by the pathogenic processes they initiate in their host, all use the products of their gag, pol and env genes (Table III) to proceed through a single cycle of replication.

Table - III

HIV-1 structural and regulatory proteins

Gene	*Protein (kd) and structure of function*
gag	Group-specific antigen. Encodes structural core matrix (17-kd), capsid (24-kd) and nucleocapsid (7-kd) proteins.
pol	Polymerase enzyme activity. Encodes the protease (10-kd), reverse transcriptase/RNase H (66-kd/51-kd dimmer, and integrase (32-kd) enzymes.
env	Envelope glycoproteins. Encodes transmembrane (41-kd) and external (120-kd) glycoproteins.
tat	Transactivator of transcription. Encodes a one-exon (14-kd) or two-exon (16-kd) protein that increases the overall level of steady-state HIV-1 RNA.
rev	Transactivator of structural gene expression. Encodes a 19-kd protein required for expression of HIV-1 unspliced and single spliced mRNAs in the cytoplasm.

The binding of an extracellular infectious virion to a susceptible cell begins the afferent protein of the HIV retroviral life cycle. (Fig.2) Binding occurs between the surface gp^{120} Env. protein of the virus and the cellular CD4 molecule. This reaction permits the transmembrane gp^{41} Env. molecule to fase the viral lipid envelope with the cell membrane. Cytoplasmic penetration by virus core results, liberating the viral genomic RNA as a nucleoprotein complex. The RNA is than rapidly converted by RT into a copy of double stranded DNA and transported to the nucleus. Here, in association with viral integrase, the viral DNA is integrated by cleavage steps into the host chromosomal DNA. This begins the efferent portion of the life cycle. The proviral DNA now functions as a mammalian gene and can replicate synchronously with host chromosomal genes. Expression of the viral genes begins when the proviral DNA serves as a template for DNA dependent RNA

polymerase activity. Such activity results in production of viral messenger RNA (mRNA), a procedure that can be divided into early and later steps. Early steps involve production of viral regulatory factors that enhance and stabilize the late stage mRNAs responsible for structural function. These structural proteins assemble around viral genomic RNA at the plasma membrane. The final step consists of proteolytic cleavage of Gag precursor molecules, which occurs as the particle buds free from the cell, incorporating the Env. protein and portion of the plasma membrane into its outer envelope coat.

Binding and Entry :

One of the most fortuitous findings in AIDS research was that the molecule used to identify the cell type (CD4), which had been observed to be depleted during disease progression, was also the major receptor for HIV-1[40]. CD4 is part of the immunoglobulin /G super family; it is expressed on the surface of helper T lymphocytes and participates in normal class II major histocompatibility complex (MHC) recognition in association with T cell responsiveness to foreign antigens. The mature virion of HIV-1 makes primary contact with CD4 through a constellation of surface gp^{120} envelope glycoprotein spikes consisting of variable (V1 through V5) and conserved (C1 through C5) domains. This glycoproteins are noncovalently linked to a transmembrane gp^{41} glycoprotein. Critical binding may occur between the C3 and C4 domains of gp^{120} and the N terminal extracellular domain of the CD4 molecule during primary attachment. Although the V3 loop of gp^{120} does not bind to CD4, its role in overall virus binding and entry may be important. Certainly, syncytium induction, neutralization, and T-cell versus macrophage tropsim are dependent on the integrity of this region of gp^{120} Other cell surface molecules, such as MHC class-II HLA-DR, $\beta 2^{-}$ microglobulin, and CD4, which are 'trapped' in the virion coat during budding, have also been considered as possible accessory ligands in virus binding.

After gp^{120} CD4 binding, a number of events take place that position the virion for entry. Conformational changes may occur after binding that uncover domains of the gp^{41} transmembrane molecule. It is theorized that exposure of the N - terminus of gp^{41}, which contains a stretch of hydrophobic amino acids, allows the host cellular membrane to fuse with the lipid envelope coat of the virion. Cleavage of the gp^{120} V3 region by proteases (possibly as a second receptor) has been postulated and may facilitate exposure of the fusogenic gp^{41} domain.

In general, enveloped viruses enter a cell by either receptor mediated, PH dependent endocytosis or PH independent membrane fusion. Most data support a membrane fusion mechanism for HIV-1, and this process has been observed directly by electron microscopy. After insertion of the hydrophobic gp^{41} N-terminal domain into the lipid bilayer of the cell membrane, the viral membrane fuses with the cellular membrane. At this point, accessory cellular factors (receptors) may facilitate entry.

After fusion of viral and cellular membranes, the internal virion core is released into the cytoplasm as a ribonucleoprotein complex, allowing for reverse transcription of the genomic RNA. Not only are the Gag proteins important for assembly and release of HIV, but their association with the two plus strands of the HIV genome are critical for reverse transcription and nuclear import of the preintegration complexes. The P^{17} matrix protein has been shown to contain nuclear localization signals that are responsible for transport of the nucleoprotein complex to the nucleus. The Vpr and Nef accessory proteins may facilitate this process. In addition, the matrix protein may be responsible for transport of the preintegration complex in the absence cell proliferation.

Reverse transcription, Nuclear import and Integration of viral DNA :

Subsequent to internalization and uncoating of the viral core, the HIV-1 nucleoprotein complex in the infected cell cytoplasm consists of Gag and Pol. proteins and genomic viral RNA. The pol protein containing the RT and

ribonuclease H enzymatic activities (RT/RNase H), is a 66^{-} and 51-kd heterodimer that use the single stranded genomic viral RNA to synthesize a double stranded linear proviral DNA. The reverse transcription process also generates the long terminal repeat (LTR) region on both the 5' and 3' ends of the proviral DNA that are characteristic of retroviruses and necessary for integration of the proviral DNA into the cellular chromosomal DNA.

Reverse Transcription :

The infecting HIV-1 virion carries the RT / RNase H enzyme and a cellular tRNA bound to the genomic RNA at the primer binding site for negative strand DNA synthesis, the PBS (-). HIV-1 and other lentiviruses use $tRNA^{lys}$ as a primer for the initiation of RNA based, DNA synthesis. The 18 nucleotides of the 3' and of $tRNA^{lys}$ bind to a perfect complementary region of the BPS(-) that is located immediately 3' to the U5 region in the genomic viral RNA. The RT enzyme initiates synthesis of the negative strand proviral DNA at the PBS (-) by copying the R U5 region at the 5' and of the genomic RNA. The RNase H activity degrades the R region tRNA in the newly synthesized RNA-DNA hybrid. The RNase H degradation of the 5' R region induces the newly synthesized negative strand DNA (R-V5) + $tRNA^{lys}$ to rehybridize to the intact 3' R region of the same genomic RNA or to the other copy of genomic RNA (intermolecular jump). Negatgive strand DNA synthesis is then continued through the env. pol and gag regions, including the PBS(-). RNase H degrades the RNA in the DNA-RNA hybrid except for a 16 base, polypurine tract immediately 5' to the U3 region. This polypurine tract, termed PBS (+), is used as the primer for initiation of positive strand DNA synthesis. Positive strand DNA synthesis begins by replication of the 3' LTR and stops in the adjacent $tRNA^{lys}$ primer. After RNase H removes the RNA primers in the PBS(+) and PBS (-), a final intramolecular jump occurs wherein the PBS(-) region in the newly synthesized positive strand DNA hybridizes with the complementary PBS(-) in the negative strand DNA. The RT enzyme completes the synthesis of both strands of

DNA; the negative strand is completed with synthesis of 5' LTR, and the positive strand is completed with the synthesis of all the protein coding regions and the 3' LTR.

The reverse transcription process is the point in the replication cycle that generates the rapid genomic variability characteristic of HIV-1. Some of the genetic heterogencity may result from possible intermolecular strand switching of the RT / RNase H enzyme during synthesis of the negative strand proviral DNA. Moreover, the RT enzyme is highly, error prone, in part because of the absence of 3'-5' exonuclease activity, which is required for the replacement of misincorporated bases. In vitro assays have reported an error rate of 1 per 6000 nucleotides and an in vivo mutation rate of 1 base per HIV-1 genome[41]. This high error rate in replication has afforded the virus an intrinsic capacity to avoid immune surveillance, to quickly develop resistance to antiviral therapy, and to generate the diverse strtain of HIV-1 world wide that have impeded vaccine development.

Nuclear transport of the preintegration complex :

The product of reverse transcription, the double stranded linear viral DNA, remains associated with the nucleoprotein complex, aptly termed the prientegration complex at this point in the virus life cycle. In addition to the double stranded viral DNA, the preintegration complex contains the viral Gag matrix, Pol integrase, and Pol RT proteins that were present in the infecting virions. The Gag matrix protein contains at least two nuclear localization signals that may be pivotal in targeting the nucleoprotein complex to the nucleus. Recent data has indicated that the HIV accessory protein, Vpr, may be important for nuclear import of the preintegration complex.

HIV-1 can infect non dividing monocytes and macrophages in addition to activated CD4 + lymphocytes, the preintegration complex has to traverse an intact nuclear envelope for access to the host chromosomal DNA. Nuclear import of the preintegration complex is a two phase process consisting of the initial prenuclear

pore transport, which is independent of high energy cofactors, and transport across the nuclear pore, which is dependent on high energy cofactor because of their relatively low metabolic state, and they are presumbly unable to transport the preintegration complex across the nuclear pore.

Proviral DNA Integration :

Once inside the nucleus, the linear double stranded viral DNA associated with the preintegration complex is capable of integrating into the chromosomal DNA. Chrgomosomal integration of the viral DNA requires the pol gene encoded 32 Kd integrase enzyme contained in the preintegration complex. HIV-1 integrase removes the two nugcleotides from the 3' and of both viral DNA strands, producing receassed 3' termini. Integrase also generates the integration site by cutting the chromosomal DNA in a way that produces a 5' five nucleotide overhang on each of the newly formed chromosomal ends. Cellular DNA repair enzymes complete the integration process by ligating the recessed 3' ends of viral DNA with the protuding 5' ends of genomic DNA and filling the resulting five nucleotide gap in the genomic DNA. The completed integration process results in a provirus minus two base pairs at the ends of both LTRs and an identical five base pair sequence immediately upstream and downstream of the provirus. There is conflicting evidence on the necessity of an integrated provirus for viral replication. Infectious HIV-1 proviral DNA clones with integrase mutation support viral gene expression in transient DNA transfection assays, but these same integrase defective DNA clones do not allow the spread of an HIV-1 infection through out a cell culture.

Regulation of viral transcription :

HIV-1 RNA transcription initiates in the 5' LTR at the begining of the R region and terminates in the 3' LTR at the end of the R region. The combind effects of extracellular signals and cellular and viral factors control the amount and type of viral RNA transcripts produced in infected cells. The cellular HIV-1 steady state RNA levels range widely, from essentially a latent state (undetecteble viral

transcription) to a highly activated state of traqnscription that produces enormous, levels of HIV RNA.

Tat Transactivation :

In addition to the upstream cis-acting elements just described, a major control element of HIV transcription is the trans acting response element (TAR), which is located downstream of the transcription initiation site. The TAR region (+1 to +80) is the target for the viral transactivation of transactivation protein, Tat. Tat transactivation induces a dramatic overall increase in steady state HIV RNA and is required for the high level of virus production observed in human T cell lines and activated human peripheral blood lymphocytes in culture. Although interest has been generated as the unique features of the HIV-1 tat TAR activation mechanism are discovered, three other lentiviruses, HIV-2, S1V and equine infectious anaemia virus, also encode a Tat protein TAR mechanism that is very similar to the HIV-1 transactivation system.

Post transcriptional control of HIV RNA expression :

The Tat induced increase in HIV transcription results in an abundant pool of nascent viral RNA in the nucleus that must be processed and transported to the cytoplasm for viral protein synthesis to occur. In the eukaryotic nucleus, the RNA splicing mechanism for splice competent cellular transcripts is usualy rapid and complete[42]. In an HIV infected cell, both unspliced and spliced mRNAs must be co ordinately produced from the same HIV proviral DNA. The discovery of the HIV-1 rev protein coding sequence has led to the identification of a second HIV-1 encoded transactivation mechanism that is required for the cytoplasmic expression of intron containing, unspliced and single spliced viral mRNAs. Rev activity has also been confirmed in at least eight other retroviruses, including HIV-2.

The HIV-1 Rev protein is a 19 kd phosphoprotein encoded by two exons. In contrast to the situation with tat, both rev exons are required for function; one of two oligomerization domains spans the exon 1 and exon 2 splice junction, and the

nuclear localization and activation domains are located in exon 2. The oligomerization domains induce Rev protein multimers that are proposed to be the active form of the protein. The nuclear localization domain of Rev includes an arginine rich RNA binding domain, similar to Tat, that binds to a cis-acting Rev response elements (RRE) in viral RNA. The RRE RNA is a 234 nucleotide region located in the env gene that is predicted to form a stem loop secondry structure. The RRE is contained in all viral mRNAS that encode the unspliced gag pol and single spliced vif, vpr and vpu env genes. Transcripts that encode tat, rev. and nef are multispliced mRNAs that do not contain the RRE RNA. The activation domain is a leucine rich region that appears to mediate protein protein interactions and is not involved with RRE bindng. Rev protein that contains mutations in the activation domain binds RRE RNA but is functionally incative.

The exact mechanism of Rev. function is not determined, the final result of Rev. activity is the cytoplasmic (Transport of viral mRNA) containing the structural gag pol for the production of infectious virions. Moreover the absence of Rev. activity has been proposed to play a role in HIV latency.

Translational control of HIV protein synthesis :

Most cellular mRNA are monocistronic they encode only one protein that can be translated in a relatively uninterupted process. Many of the HIV mRNAs are multicistronic, with open reading frames that either partially overlap or are arranged in tandem. Ribosomal frame shifting and initiation site scan through are two control mechanisms during viral protein synthesis that allow the translation of more than one protein species from a multicistronic mRNA. In addition to production of different viral proteins from one species of mRNA, appropriate stoichiometric amounts of these proteins must be maintained to ensure efficient virion assembly.

Expression of Gag and Pol proteins from the same unspliced mRNA occurs by ribosomal frameshifting, Gag and Pol genes are located in different reading frames, which overlap in their 3’ and 5’ regions, respectively.

In most translations of the Gag Pol mRNA, the Gag protein is synthesized to completion and released from the ribosome. However, as the ribosome traverses the 3' and of the gag open reading frame, a – 1 frame shift can occur that puts the ribosome in the pol open reding frame. This results in a Gag Pol fusion protein that is subsequently cleaved, by the viral encoded protease, into the mature Gag and Pol proteins. Pol translation is totally dependent on the – 1 frame shift because the pol gene does not have a functional AUG start site. The 1 – frame shift is dependent on a specific heptamer sequence at the actual frame shift side and a predicted RNA stem loop structure directly downstream of the heptamer*[6]. The presence of the RNA stem loop is predicted to cause a pause of the ribosome at the heptamer sequence and thereby allow the 1 – frame shift in a limited number of translations.

Many of the spliced mRNAs of HIV-1 are multicistronic and are therefore capable of translating more than one protein. The flanking sequences surrounding AUG start codons determine how efficient a particular start site is in initiating translation. If the most 5' start site in a multicistronic mRNA is suboptimal, the ribosome may continue past this site and begin translation at a more efficient start site further downstream. This suboptimal initiation site scan through mechanism occurs readily in the translation of HIV-1 Env. protein. All of the Env. encoding mRNAs contain the Vpu start site upstream of the start site, however, allows a majority of the ribosomes to scan through and find the strong Env. start site Because all HIV-1 regulatory genes (-) and accessory genes are encoded in multicistronic HIV-1 mRNAs, the relative differences in the strengths of their respective translation start sites suggest that translational control of these protein is important in the virus life cycle.

Virion Assembly :

Before the virion can be released from the cell, the structural viral proteins must coordinately assemble. HIV has been observed by electron microscopy to assemble both at the plasma membrane and within intracytoplasmic vacuoles. The

actual mechanism responsible for this bidirection trafficking is unknown. Nonetheless, as the major structural precursor proteins (55kd Gag, 180 kd Gag Pol and gp^{160} Env) begin to accumulate, a number of steps are required for completion of the life cycle. first, the Gag (P^{65}) polyprotein oligomerizes, at which point the matrix (MA) domain of P^{55} (P^{17} processed matrix protein) is responsible for transport of the entire gag protein to the plasma membrane. As the polyprotein Gag complex aproaches the cellular membrane and begin forming the inner framework of the virion the nucleocapsid (NC) domain of p^{55} permits two copies of HIV genomic RNA to assemble around the "cys His" box, which is an array of cysteines similar to a zinc finger motif. Discrimination among multiple viral and nonviral mRNAs and the full length genomic RNA for Gag encapsidation lies in a cis derected sequence, designated psi. This site is located at the 5' region of the HIV genom and is not present in spliced viral mRNA transcripts. To complete the encapsidation process, it is thought that the specific tRNA lys primer (necessary for priming negative strand synthesis by RT) associates with the Pol domain in the larger Gag Pol 180 kd polyprotein and is included in the infectious virion. The capsid protein (CA) domain of Gag is essential for the morphogenesis of the mature, cone shaped core.

Virus Budding and Release :

As a final step in the HIV life cycle, the infectious virus matures after if has been released from the plasma membrane. This occurs as a coordinated process by which the viral protease (located between the Gag and Pol domains) cleaves the Gag Pol protein in an ordered fashion. The conserved P^2 domain of Gag is required to regulate this sequential proteolytic processing. The protease begins its processing by cleaving the P^{55} Gag protein at the N-terminus of the P^{15} NC protein and than at the $P24^{43}$ CA. P7 and P6 are the last proteins processed. The Pol polyprotein (RT and IN) remains inactive.

As the Gag and Gag Pol proteins begin to encapsidate the HIV genomic RNA, the plasma membrane also begins to form a lipid bilayer around the viral core.

Precursor gp^{160} Env. undergoes glycosylation and oligomerization in the endoplasmic reticulum. From this point, it is transported to the Golgi complex, where cellular enzymes process the glycoprotein to surface gp^{120} (Su) and transmembrane gp^{41} (TM). Here, they associate noncovalently and are transported to the cell surface. Critical to the final budding step is myristolation of the N terminal glycine of the P17 MA protein, This event culminates as the final process in condensing the inner core shell and linking it with the glycoprotein incorporated lipid membrane.

Cell Activation :

The early events (afferent protein) of the viral life cycle are known to occur in the absence of activation. Howeve, for efferent functions (integration, transcription, and translation) to efficiently take place exogenous T cell stimulation is required. Several activation mechanisms are possible and, influence the viral life cycle. Probably the one that has the greatest positive effect on viral expression is activation through the T3-T1 specific antigen receptor on CD4 + lymphocytes. Similar activation can be achieved through mitogens and superantigens. Other exogenous factors (eg. cytokines, physiological stimuli) also have been shown to have dramatic effects on HIV expression process. Some investigators have predicted that repid progression in HIV disease.

T cell lines have been developed to better understand these activation process and have been extremely useful in synchronizing stages of the viral life cycle so that coordinate expression of the life cycle can be analyzed. Second messenger signalling by Ca^{2+} and protein kinases is required for linking the exogenous stimulation to the viral life cycle dependent events. Viral transcription is one of the best studied mechanisms that is dependent on cellular activation. Intracellular second messenger pathways leading to NF-kB activation and other factors interacting with the viral LTR appear to be only a small segment of the control over the life cycle that T cell activation can impart. Many believe that control of the viral life cycle through its

dependence on cellular functions offers some of the greatest hope for therapeutic intervention[44].

Role of Latency in the life cycle :

Much attention has been focused on the afferent and efferent portions of the life cycle, primarily because of findings that throughout the course of HIV infection there is no microbiological latent period associated with the clinical latent perioid. However, there are microbiologically latent infected cells. HIV is replicating with virion turnover from the time of infection until the time of death of the patient. Nevertheless, high ratios of viral DNA to viral RNA and latent expressing cell lines teach us that not every infected cell is programmed to immediately produce virus. It is probably premature to discard latency as not contributing to the pathogenic process, as some have indicated. In vitro studies have recently implicated Vpr and possibly Nef in the control of transcriptional expression of HIV through cellular activation pathways. Many factors could be responsible for controlling expression and inhibiting the efferent phase of virus replication.

Cell Death :

Because HIV buds from the plasma membrane there appears to be no selective advantage for, the death of the virus producing cell. However, direct cytotoxicity by replicating virus has been demonstrated most of the direct killing effects have been attributed to the env gene products (gp^{120}, gp^{160}) and their affinity for CD4 positive cells. Recently the C terminus of gp^{41} has been demonstrated to have a potent toxic effect on cells independent CD4[45]. Syncytium or multinucleated giant cell form resulting from fusion of HIV infected and uninfected cells, has been shown to contribute to cell loss in culture. However, this process is not usually found in tissue of infected patients and may only be an artifact of in vitro virus propagation. Apoptosis or programmed cell death has been suggested to explain the loss of CD4+ cells seen in HIV infection. Apoptosis differs from necrosis because the latter takes place faster, causing loss of plasma membrane integrity but no

chromatin condensation. However, indirect effects of HIV infection resulting in the apoptotic process are thought to account for the majority of CD4 loss in vivo. Such indirect effects include gp^{120}. CD4 crosslinking followed by cell activation, autoantibodies, and cellular immune destruction. Recently, the HIV regulatory protein, Tat, has been implicated with the APO-1/Fas receptor (cD95) as a strong inducer of apoptosis. This area of investigation continues to be intensely pursued to better understand CD4 depletion in AIDS and the role that products from the viral life cycle play in host pathogenesis.

IMMUNOPATHOGENESIS OF HIV INFECTION :

The immunopathogenesis of HIV infection is extremely complex. A variety of virologic and immunologic mechanisms contribute to the progressive deterioration of immune function and to progression of HIV disease to the acquired immunodeficiency syndrome (AIDS). Becaue of the multifactorial and multiphasic nature of HIV infection, these mechanisms may vary according to the different phases of infection. Among the multiple patghogenic mechanisms that have been proposed, four are critical for the establishment and propagation of HIV infection over time and for the progration of HIV disease : (1) Lack of elimination of HIV after primary infection (2) persistant virus replication in lymphoid organs throughout the course of HIV infection. (3) Chronic stimulation of the immune system, which may cause inappropriate immune activation and progressive exhaustion of the immune response; and (4) destruction of lymphoid tissue, which results in severe impairment of the ability to maintain over time an effective HIV specific immune response and to generate immune respones against new pathogenes. Identification of these pathogenic mechanisms has resulted from recent advance in the delineation of the virologic and immunologic events associated with primary infection, and of the anatomic compartments that serve as reservoirs for HIV and the sites at which virus replication primarily occurs. These observations represent a fundamental advance in our understanding of the pathogenesis ofHIV infection. Recent studies on the

kinetics of virus turnover have supported previous findings that virus replication is continuous in all stages of HIV infection and have provided a precise determination of the number of virions produced every day. In the same studies, a mathematic model has been used to calculate the potential numberof CD4+ T lymphocytes that are depleted and replenished every day, and it has been proposed that there is a very high tournover of CD4 + T cell (a mean of 1.8×10^9 cells per day).

The clinical course of HIV infection has been well studied. Although progression to AIDS occurs in most HIV infected individuals, in a small percentage, HIV disease does not progress for an extended period of time. This latter finding, together with the observations that HIV specific immune responses may be detected in individuals who are multiple exposed to HIV despite the fact that they are seronegative, supports the concept that HIV infection can be effectively controlled or even prevented by the immune response.

The course of HIV infection :

On the basis of the duration of HIV infection and the kinetics of virologic and immunologic events observed throughout HIV disease, three dominant patterns of evolution of HIV disease have been described :(1) 80% to 90% of HIV infected persons are "typical progressors" and experience a course of HIV disease with a median survival time of aproximately 10 years (2) 5% to 10% of HIV infected persons are "rapid progressor" and experience an unusually rapid (3 to 4 years) course of HIV disease. (3) About 5% of HIV infected person do not experience disease progression for an extended period of time (at least 7 years) and are termed "long term nonprogressors" (LTNP)[46].

Typical Progressors :

The typical course of HIV infection includes three phases : primary infection, clinical latency and clinically apparent disease. The diagnosis of primary HIV infection is made in only a minor percentage of cases : Difficulties reside in the variable severity and nonspecificity of the clinical syndrome which is characterized by mononucleosis or flue like symptoms such as fever, lethargy sore throat, malaise,

macupapular rash, lymphadenopathy arthralgias, myalgias, headaches, retroorbital pain, photophobia and rarely meningitis. Hospitalization is required in only a minority (10% to 15%) of individuals, and this phase therefore usually goes unnoticed. Furthermore, during the initial period of primary infection the laboratory blood test that is widely used for the diagnosis of HIV infection (i.e. detection of HIV antibody specific for various viral proteins) may be negative. On the basis of the clinical history, however, it is thought that an acute clinical syndrome of variable severity may occuring a relatively large proportiong (50% to 70%) of HIV infected individuals, and although HIV specific antibodies may not be detected, the initial period of primary infection is characterized by high levels of virus in the circulation (i.e. viremia). Determination of plasma viremia or p^{24} antigenemia represents the only valid laboratory approach for the diagnosis of primary infection. Appearance of symptoms during acute primaryg infection usually occurs within 3 to 6 weeks of infection, together with the high levels of viremia. In most patients, both resolution of symptoms and downregulation of viremia occur within 9 to 12 weeks after the onset of symptims : both of these events are associated witgh the appearance of HIV specific immune responses.

The phase of primary infection is followed by the long, clinically latent period of HIVg infection. In typical proressors, this phase of infection may last for year (median, 8 to 10 years) The observations that active replication of HIV is continuous and occurs primarily in the lymphoid organs throughout the entire course of infection, together with the development of highly sensitive and quantitative polymerase chain reaction techniques for the determination of viremia have clearly demonstrated that HIV disease is active and progressive even during this prolonged asymptomatic phase. These findings have helped to explain the discrepancy between the absence of clinical signs of active disease and the proressive decline of CD4+ T lymphocytes that invariably accompanies the clinically latent period.

Progression to AIDS and clinically apparent disease occurs within 8 to 10 years in typical progressors. The progression to AIDS results from the continuous

replication of virus in the lymphoid tissue, which is associated with progressive destruction of this tissue and severe impairment of immune function. This advanced phase of infection is characterized by severe and persistent constitutional signs and symptoms or by opportunistic infections or neoplasms, or both. In those persons who develop generalized lymphadenopathy or the AIDS defining conditions of Kaposi's sarcma or neurologic disease clinical disease may be apparent before the progression to advanced stage disease.

Rapid Progressors :

In a minor percentage (5% to 10%) of HIV infected persons, rapid progression to AIDS occurs wthin 2 to 3 years after seroconversion. Immune responses are usually defective in these rapid progressors. Levels of antibodies against HIV proteins and neutralizing antibody are low to absent. Although it is unclear whether HIV specific cytotoxicity is defective in rapid progressors, it has been shown that the CD8+ T cell mediated suppression of HIV replication (i.e. mediated by a soluble factor) is severely impaired. A series of immunologic abnormalities typical of HIV infected individuals in late stage disease are usually observed in rapid progressors, including high percentages of activated CD8+ T cells expressing CD38 and HLA-DR and elevated serum levels of β_2 microglobulin, neopterin, soluble CD8, and soluble interleukin 2 (IL-2) receptors. The levels of viral load are usually very high in rapid progressors. In particular, it has been reported that rapid progressors have elevated levels of unspliced HIV mRNA compared with typical progressors and that HIV isolates from rapid progressors are more homogeneous that those from typical progressors and LTNP2.

Long Term Nonprogressors :

Now that considerable experience has accumulated in prospective studies of HIV infected individuals, it has become clear that a small percentage of infected persons (5%) do not experience clinical progressoion of HIV infection and have stable CD4+ T cell counts for many years (7 or more years) despite lack of therapy. The criteria we have used for nonprogression include documented HIV infection for

more than 7 years, stable CD4+ T cell counts higher than 600 cell/μL, absence of symptoms, and no antiretroviral therapy. From an immunologic standpoint, immune functions are conserved in LTNP2, and both HIV specific humoral and cell mediated immune responses are very strong. In addition to normal and stable CD4+ T cell counts, the absolute number of CD8+ T lymphocytes is significantly and consistently higher in most LTNPs. In addition, quantitative differences in the distribution of certain surface markers on CD8+ T cells, which have been associated with either a favourable or a severe prognosis in HIV infection have been observed in LTNPs. Other immunologic parameters such as serum levels of β microglobulin, elevated levels of which have been associated with an unfavourable progresis in HIV infection are within the normal range in LTNPs furthermore in contrast to typical progressors. LTNPs exhibit preserved proliferative responses to mitogens (eg. phytohemagglutinin, pokeweed mitogen), alloantigens, and soluble antigens (eg. tetanus toxoid).

HIV specific cytotoxic T lymphocytes (CTLs) against various HIV proteins, including Env and Gag, can readily be detected in LTNPs, either in freshly isolated peripheral blood mononuclear cells or after in vitro stimulation. In contrast to typical progressors who experience a decline over time of HIV specific activity, this activity remains stable in LTNPs. Although these differences are clearly observed if LTNPs are compared with typical progressors who have been infected for a comparable period of time, no differences in HIV specific cytotoxicity may be observed between LTNPs and HIV infected individuals in early stage disease

Analysis of virologic and immunologic events associated with HIV infection in peripheral blood and lymphnodes :

Primary HIV infection :

The sequelae of virologic and immunologic events associated with primary HIV infection in peripheral blood and lymph nodes have only recently been delineated. The SIV model of acute infection has been an ideal experimental system to determine the initial anatomic site of virus localization and spread. For this

purpose, sequential lymphnode biopsies were performed in monkeys, and analysis of virus distribution demonstrated that virus may be detected in lymph nodes as early as 1 week after inoculation[47]. Significant changes in virus distribution occurred after primary infection and during the transition from the acute to the chronic phase of HIV infection. Virus that is detected in lymph nodes early after inoculation (i.e. day 7) is exclusively cell associated, and a peak in numbers of indiviudal virus expressing cells is detected at this time. Rapid changes in virus distribution occur during the 2 to 3 weeks after inoculation. The number of virus expressing cells is dramatically reducedby day 14; this event is probably the result of the emergence of virus specific immune responses. By 3 to 4 weeks after inoculation, virus particules traped in the follicular dendritic cell (FDC) network in the germinal centers represent the predominant form of virus detected in the lymph nodes. Definitive evidence that the initial replication and spread of virus in lymph node may account for systemic virus dissemination was obtained by comparing the kinetics of virus replication in lymphnodes with that in the circulation (i.e. p26 antigenemia). This analysis revealed that the peak in numbers of virus expressing cells in lymph nodes precedes the peak in p26 antigenemia. These phenomena are usually observed during the first 2 weeks of primary infection, and this early period during which intense virus spreading occurs, has been designated the stage of virus dissemination.

Subsequent to the stage of virus dissemination, the course of primary infection is characterized by a dramatic downregulation of viremia. During this latter period, levels of virus replication are significantly reduced in both peripheral blood and lymph nodes; in addition, in lymph nodes there is a switch in predominance from cell associated virus (i.e. virus expressing cells) to extracellulat virus trapped in the FDC network. In addition to the elimination of virus expressing cells by HIV specific CTLs the trapping of virus particles in the FDC network represents an important mechanism for the decrease of virus in the circulation.

The major decrease in virologic parameters observed during the first weeks of infections are predominantly limited to those that reflect active replication such as

number of virus expressing cells. viremia p26 in SIV and p24 in HIV, and titers of infectious virus changes in the number of HIV proviral DNA copies have not consistently been observed. During the acute phase of SIV or HIV infection in addition to the systemic dissemination of the virus. other crucial pathogenic events may significantly influence the propagation of the infection over time; these include the generation of a large pool of HIV latently infected cells (i.e. cells containig proviral DNA) and the large number of trapped virus particles that may represent a source of virus for de novo infection of target cells that reside in or migrate through the lymphoid organs.

With regard to HIV specific immune responses, robust cell mediated and humoral responses are redily detected during primary infection Usualy, virus specific CTLs can be detected within 2 to 4 weeks from the onset of symptoms, and their appearrance coincides with the dramatic decrease in viremia in both humans and monkeys. HIV specific CTLs play a major role in the initial downregulation of viremia by killing virus expressing cells that are responsible for the high level of virion production. In the SIV model of acute infection, it has been shown that the appearance of virus specific CTLs coincides with a dramatic decrease in the number of virus expressing cells in lymph nodes, HIV specific CTLs have been detected against both structural (e.g. Env, Gag Pol) and regulatory (e.g. Nef, Tat) viral proteins. Variable frequencies and specificities of HIV specific CTLs have been observed among different individuals. However, it is unclear whether these quantitative and qualitative differences in HIV specific cytotoxicity significantly influence the initial down regulation of viremia and the ability to control virus replication and spread over time. In addition to typical HIV specific cytotoxicity, CD8+ T cells may also potentially mediate suppression of virus replication by release of a soluble factor; this CD8+ T cell mediated suppressor activity has been detected during primary infection. Taken together these observations strongly support an important role for CD9+ T cells in the primary immune response to HIV infection.

With regard to the virus specific hummoral immune response neutralizing antibodies, which represent the protective component of this response, are usually detected after the phase of downregulation of viremia, when the transition from the acute to the chronic phase of infection has already occurred. Although these findings suggest that neutralizing antibodies may have little effect in the control of the initial spread of HIV, they do not rule out the involvement of nonneutralizing antibodies in the initial downregulation of viremia.

The Clinically Latent Period :

Transition from the acute to the chronic phase of HIV infection is marked by the downregulation of viremia and resolution of symptoms. All virologic parameters, including viremia, virus expression and titers of infectious virus in peripheral blood mononuclear cells as well as frequency of cells containing HIV DNA are usually very low in peripheral blood during this clinically latent period[48]. However, determinations of viral load in lymphoid tissue have demonstrated that the frequency of cells containing HIV DNA and the levels of virus expression in mononuclear cells are 1 to 3 logs higher in lymph nodes that in peripheral blood. These observations indicate that virus replication is continuous throughout the entire course of infection, including the asymptomatic period. This concept has been further supported by the detection of viremia during the clinically latent period with the use of a highly sensitive polymerase chain reaction assay and by recent studies that have shown that not only is viral replication continuous but the turnover of virus is very high (up to 10^9 virus particles every 1.5 to 2 days).

Another important pathogenic event associated with the early stage of infection is the trapping of virus particles in the FDC network of the lymph node germinal centers. The trapping of virus is related to the histopathologic changes associated with the early stage of HIV disease, when CD4+ T cell counts are higher than 500 cells/μL. The typical histopathologic abnormality of this stage of HIV infection is follicular hyperplasia which is a reflection of the state of immune

activation of the lymphoid tissue and of the ongoing immune response to HIV or other pathogenis. These histopathologic abnormalities, together with increasd cellular activation, which is critical for effective virus replication may contribute to the dichotomy of viral load between peripheral blood and lymph nodes by promoting trapping of virus particles in the FDC network and by creating environmental conditions (i.e. close cellular contact, high concentration of HIV, and abnormal retrafficking of lymphocytes) that favor the sequenstration of HIV in the tissue.

With the progression of HIV disease from the early to the intermediate stage (i.e. CD4+ T cell counts between 200 and 500 cells/μL), levels of viral load in peripheral blood reflects in part a progressive deterioration of the immune system, which in turn results in defective control of virus replication. However, this increase is also the result of important histopathologic changes that occur in lymphoid tissue as disease progresses. Increasing proportions of lymphoid tissue show signs of disruption of tissue architecture, including abnormal location of germinal centers in the medulla, greater numbers of germinal centers undergoing follicular involution, and increased vascularity and fibrosis. The disruption of lymph node architecture leads to a progressive loss of the ability of lymphoid tissue to trap virus; this, together with the accelerated replication of virus as disease progresses, probably contributes to the increase in viremia observed in patients in the intermediate stage of disease.

Advanced Stages of HIV Infection :

From a virologic standpoint, the advanced stage of HIV infection (i.e. CD4+ T cell count <200 cells/μL) is characterized by a substantial increae in all virologic parameters in both peripheral blood and lymph nodes. In advanced stagedisease, the levels of viral load equilibrate between peripheral blood and lymphnodes. Lymphoid tissue has for the most part been destroyed and replaced by fibrotic tissue most of the virus is cell associated, and virus trapping is minimal or absent. The obvious

consequence of the destruction of lymphoid tissue is the severe impairment of immune function and profound immune suppression.

Induction of HIV expression :

Several factors have been proposed as being involved in the regulation of HIV expression and the potentiation and modulation of virus replication. These include endogenous viral regulatory proteins such Tat and Rev. cellular transcription factors acting at the level of the proviral long terminal repeat (LTR), the state of immune activation other pathogens and cytokines.

Both Tat and Rev are crucial for efficient virus replication[49]. Tat protein acts at both the transcriptional and postranscriptional levels; Rev protein plays an important role in the export of unspliced and incompletely spliced viral messages from the nucleus to the cytoplasm of the infected cell. Because these messages encode for structural proteins, optimal Rev function is essential for production of infectious virions.

A general state of immune activation is associated with all stages of HIV infection. The inability to eliminate HIV after primary infection and the continuous replication of virus thrughout the entire course of HIV disease represent the primary mechanisms responsible for the chronic state of immuneactivation. Both individual virus expressing cells and trapped virus in lymphoid tissue represent the source of antigen responsible for maintaining chronic stimulation of the immune system over time. Although a state of immune activation is necessary in order to maintain HIV specific immune responses, at the same time it may indirectly enhance virus replication, either by leading to the secretion of HIV inducing cytokines or by generating a large pool of activated target cells that efficiently support virus replication. HIV in vitro can infect both resting and activated CD4+ TG lymphocytes with equal efficiency; however it replicates only in activated T cells[50]. As in CD4+ T cells efficient virus replication in monocytes or macrophages depends on the state of cellular activation and differentiation.

Several pathogens, including cytomegalovirus, herpes simplex virus, hepatitis B virus, human herpesvirus 6, human T cell lymphotropic virus type I, and microbes such as Mycoplasma, have been shown to enhance HIV expression in several experimental in vitro systems. It is not certain whether these pathogenes mediate a similar effect in vivo.

Since the initial observation that certain cytokines induce HIV expression from a state of latent or chronic infection to that of active virus expression, the effects of several cytokines on virus expression and replication have been extensively investigated. Most cytokines induce HIV expression by mechanisms acting at both the transcriptional and postranscriptional levels : TNF-α, TNF-β and IL-1 induce HIV expression by activation of the transcription factor NF-kB. Although the regulatory effect of cytokines on HIV replication is limited to in vitro observations, the fact that several of these cytokines may be found at increased levels in plasma cerebrospinal fluid, and lymphoid tissue of HIV infected individuals suggests that they probably mediate similar effects in vivo. It is possible that cytokines play an important role in maintaining the constant levels of virus expression and replication particularly in lymphoid tissue.

Mechanisms of CD4+ T cell depletion and dysfunction :

CD4+ T lymphocytes are the primay targets of HIV infection[51]. The progressive depletion of cD4+ T lymphocyes is characteristic of every stage of HIV infection, and CD4+ T cell counts represent a valid surrogatge marker to monitor the progression of HIV disease. For these reasons, the potential mechanisms responsible for the depletion of CD4+ T lymphocytes have been studied extensively, and several mechanisms have been proposed. These can be divided into two groups : direct virologic mechanisms that result from an HIV mediated cytopathic effect, and indirect, nonvirologic mechanisms that include predominantly immunologic phenomena triggered during the course of HIV infection.

With regard to direct virologic mechanisms, HIV medited cytopathic effects may occur through either single cell killing or HIV induced syncytia formation.

However, that the evidence that HIV mediates direct killing of CD4+ T cells rests on the in vitro observation that cell death can be detected in cultures of cells that are acutely inoculated with virus even in absence of syncytia formation. It has been proposed that HIV may mediate single cell killing by both accumulation of unintegrated viral DNA and inhibition of cellular protein synthesis. The other primary mechanism of cell depletion observed after acute inoculation of virus in vitro in both peripheral blood and various CD4+ T cell lines is syncytia formation. This process involved multiple steps of cell membrane fusion between HIV expressing cells and uninfected CD4+ T cells : in the fusion process, one infected cell could potentially be responsible for the elimination of up several hundred unifected cells. Although the interaction of gp^{120} with the CD4 molecule represents a necessary step in this process, it has been demonstrated antigen (LFA-1) plays a fundamental role in the regulation of syncytia formation of peripheral blood CD4+ T lymphocytes. Experiments performed with a panel of antibodies directed againstg the α and β chains of LFA-1 molecules and the intracellular adhesion molecules-1, 2 and -3 (the natural ligands for the LFA-1 molecule) have provided evidence that the process of HIV mediated syncytia formation is regulated by the LFA-1/ICAM-1/-2/-3 pathway of cell to cell adhesion.

With regard to the induction of anergy in HIV infection, it has been shown that after binding of gp^{120} antibody complexes to the CD4 molecule. CD4+ T cells become refractory to further in vitro stimulation. Anti gp^{120} antibodies have been detected on CD4+ T lymphocytes in patients with AIDS suggesting that gp^{120} is bound to the surface of these cells by the CD4 molecule. In this context, it has been proposed that gp^{120} bound to CD4+ T cells may mediate a pathogenic role by being presented to other CD4+ T cells. This abnormal presentation of gp^{120} by other CD4+ T cells could induce energy in already activated CD4+ T cells and could generate CD4+ cytotoxic T cells from the pool of resting T cells.

Finally, HIV specific immune responses, including humoral and cell mediated immunity, play an important role in the control of HIV replication and

spread in vivo. These phenomena may contribute significantly to the elimination of HIV infected cells, including CD4+ T cells, macrophages, and FDCs, either by HIV specific CTLs or by antibody dependent cellular cytotoxicity. It has been hypothesized that HIV may cause immunosuppression and disease progression by virus specific cytotoxic T cell mediated immunopathologyg, Therefore, although in the initial phase of infection effective immune responses may be critical for the containment of virus. It is possible that during the chronic phase of infection these virus specific immune responses may have a pathogenic role.

IMMUNE RESPONSES TO HIV INFECTION :

The immune response to the human immunodeficiency virus (HIV) is determined by many complex factors. First, the extraordinary host virus interactions that lead to the pathogenesis of acquired immunodeficiency syndrome (AIDS) induce profound functional host immune defects, beginning soon after infection with HIV. Prominent forms of HIV induced immune dysfunction include defects in T and B cell responses to specific antigens, polyclonal hypergammaglobulinemia, enhanced autoantibody and immune complex formation, dysregulated cytokine production, decreased natural killer cell activity, and defective monocyte and dendritic cell function. At the time when the host immune system begins to mount an anti HIV immune response designed to neutralize free HIV and eliminate HIV infected cells, many of the cellular components of the immune responses are being adversely affected by HIV. Second, evidence suggests that the route of HIV infection, the amount of HIV in the inoculum, the pathogenic potential of a given HIV strain, and host genetic factors may modify the host response of HIV. Third, evidence suggest that some components of an immune response to HIV may enhance HIV infectivity or may be directly responsible for clinical manifestations of the disease. Fourth, the remarkable ability of HIV to mutate genome sequences and

change the primary amino acid sequence of HIV proteins effectively allows HIV to evade otherwise effective antiviral immune responses.

In individuals with advanced HIV disease, as many as 10^9 new HIV virions are produced each day and as many as 2 x 10^9 CD4 positive (CD4+) T cells turn over per day, with a half life of HIV and CD4+ T cells of approximately 2 days. Studies suggest that CD4+ T cell depletion in HIV infection result from the high replicative capacity of HIV and direct viral cell killing, although CD4+ cell loss could in part be caused by immune mediated cell killing or to T cell apoptosis, or both. The complexities of HIV host interactions create myriad obstacles to an effective immune response to HIV.

Antibody responses to HIV :

Studies have documented the immunologic and virologic events that occur during acute HIV infection[52]. Acute HIV infection frequently presents as an influenza or mononucleosis like syndrome, with fever, adenopaty, pharyngitis, rash, myalgias, and arthralgias as common symptoms. Abnormal liver function test results, hepatosplenomegaly, encephalopathy, and neuropathy occur less commonly. Between 5 and 10 days after HIV infection, serum p24 protein levels rise rapidly, serum infectious HIV levels rise, the numbers of circulating HIV infected CD4+ T cells rise, and the total number of circulating CD4+ T cells transiently decrease. Circulating infectious virus levels peak from 10 to 20 days after HIV infection and precipitously fall coincident with an increase in anti HIV cytotoxic T cell (CTL) activity and usually, resolution of initial clinical symptoms. However, the initial fall in plasma viremia may also reflect trapping of HIV in the spleen and lymph nodes.

HIV Neutralizing Antibodies :

Neutralizign (Anti HIV) antibodies inhibit the infectivity of free HIV or HIV infected cells and have been prosposed to be one component of a salutary or protective anti HIV immune response Human serum anti HIV antibodies are isolate

type specific or group specific. The epitopes to which most anti HIV antibodies bind have been located on envelope glycoproteins gp^{120} or gp^{41}.

Regions of HIV proteins to which relatively group specific HIV neutralizing antibodies bind are conformational epitopes around the CD4- binding site on gp^{120} or are carbohydrate in nature. After HIV infection, type specific neutralizing antibodies appear in serum, followed by broader reactive group specific anti HIV antibodies.

Serum levels of neutralizing antibody being to rise 2 to 4 weeks after primary HIV infection and peak during the asymptomatic phase of HIV infection. Most studies have demonstrated that anti HIV antibodies are present in the symptomatic stages of AIDS related complex and AIDS, although frequently at lower levels than in the asymptomatic stages of HIV infection.

Antibodies that promote antibody dependent cellular cytotoxicity of HIV infected cells :

Anti gp^{160} antibodies in the serum and cerebrospinal fluid of HIV infected persons bind to IgG Fc receptor (R) bearing natural killer (NK) cells by means of the antibody Fc region and sensitize IgG FcR positive cells to kill HIV gp^{160} expressing, or gp^{120} coated, target cells. Peripheral blood monocytes from AIDs patients can also mediate antibody dependent cellular cytotoxicity (ADCC) of HIV infected cells.

Although ADCC antibodies against p^{24} Gag proteins have been described, most studies have found serum anti HIV ADCC antibodies to react with HIV gp^{120} or gp^{41} envelope proteins. Anti HIV antibodies that mediate ADCC of $group^{120}$ or gp^{41} expressing target cells arise soon after infection with HIV, are predominantly of the IgG1 sub class, and are detected throughout all stages of HIV infection, although ADCC antibody levels decrease somewhat with the onset of AIDS.

Anti Gag Antibodies :

Anti p^{24} antibodies appear within the first 2 weeks of acute HIV infection. Rises in p^{24} antibody levels correlate well with the precipitous fall in infectious HIV antigenemia that occurs as the symptoms of acute HIV infection sub side.

Antibodies to HIV p^{24} Gag proteins rise to their highest levels during the asymptomatic seropositive stage and then fall to usually undetectable levels with the onset of AIDs. Antibodies against the p^{17} Gag protein of HIV have been reported to neutralize HIV and to cross react with the thymic hormone thymosin. α_1.

Antibodies to Other HIV Proteins :

Antibodies to HIV Rev, Nef, Tat, Vpu, Vpr and HIV protease proteins have been reported in various percentages of HIV patients. Antibodies to Nef proteins have been found in HIV infected persons who were otherwise HIV seronegative. In general, antibody levels to all of these HIV proteins decrease as HIV infection progresses to AIDS.

HIV Enhancing Antibodies :

HIV enchancing antibodies bind to epitopes of Env gp^{41}. The presence of HIV enahcning antibodies has been correlated with progression of HIV infection to AIDS. As new HIV variants emerge over time, new HIV variants are not neutralized by autologous sera, and in some cases, antibodies against newly emerged HIV variants may enhance HIV replication in vitro, although the significance in vivo of enhancing antibodies is controversial.

T-lymphocyte responses to HIV :

Cellular T lymphocyte responses are essential for the control of numerous viral infections. CD4+ helper T cell responses are required for induction of B cell antibody production and for induction of other T cell responses. In patients with HIV infection, anti HIV helper T cell responses, major histocompatibility complex (MHC) class I and MHC class II anti HIV CTLs, and non MHC restricted CD8 positive (CD8+) T cell and HIV activities have been identified.

Anti HIV major histocompatibility complex restricted cytotoxic T lymphocytes :

MHC class I restricted CTLs have been demonstrated against Gag, Env, Nef, and Pol HIV proteins. Remakably, anti HIV class I restricted CTLs in asymptomatic

HIV seropositive persons circulate in extraordinary frequency, on the order of 10 to 20 CTL precursors per 10^4 peripheral blood mononuclear cells.

In primary HIV infection, the initial fall in viremia correlates best with the appearance in peripheral blood of anti HIV MHC class I restricted CD8+ CTLs. CD8+ CTLs are also thought to be important in the immune response to HIV during the chronic phase of HIV infection for the elimination of productively infected cells and for control of the viral load. HIV specific CD8+ CTLs may also play a role in the immunopathogenesis of HIV infection by contributing to depletion of HIV infected antigen presenting cells or through tissue damage after the release from CTLs of certain cytokines (eg. tumor necrosis factor α, interferon β) during the process of cytolysis.

Cytotoxic T cells That Suppress HIV Replication by Secretion of Soluble Factors :

CD8+ T lymphocytes from HIV infected individuals have inhibited HIV replication in naturally infected CD4+ cells in vitro. This antiviral activity is not mediated by NK cells, is not HLA restricted, and depends on the number of CD8+ cells present. This type of anti HIV activity is not mediated though target cell killing but is mediated through CD8+_ cell secretion of undelineated soluble anti HIV factors.

Non-T-cell-mediated anti HIV cellular immune responses

Non T cell mediated immunity, as is mediated by NK and other FcR positive cells that directly kill virally infected cells or that mediate ADCC, is potentially important as an anti HIV immune response, because these forms of immunity can eliminate virally infected cells in a non MHC restricted fashion and do not require a memory T cell response for effector cell induction. Devising ways of augmenting or inducing NK responses against HIV infected allogeneic cells or against malignant cells that arise in the context of AIDS are important areas of research.

Although monocytes in AIDS patients have a chemotatic defect, monocytes from asymptomatic seropositive persons mediate ADCC against HIV coated target

cells and mediate monocyte tumoricidal activity in vitro a potential mechanism of immune response against Kaposi's sarcoma and other tumors that occur in AIDS.

Immune responses to HIV in children :

Most HIV infections in children occurs perinatally. Most infants born to HIV positive mothers passively acquire maternal anti HIV antibody in utero, which may persist for 15 to 18 months postnatally. In this situation, the polymerase chain reaction (PCR) assay for HIV proviral DNA sequences has been used to detect HIV infection infant with passively acquired Hiv antibody. In children younger than 12 months of age, AIDS frequently occurs in the presence of higher T cell levels (500 to 1000 cells / mm^3) than are seen in adults with AIDS. However, in older infants and children, anti HIV immune responses and progressive immune defects that develop over the course of HIV infection are similar to those seen in adults. The types of immune responses that may be protective in children born to mothers with HIV infection are unknown, but data sugest CTLs may be important[53].

Pathogenic versus salutary anti HIV immune responses :

Because of the observations that CD4+ T cells, human monocytes, macrophages, dendritic cells, and Langerhans' cells are all capable of infection by HIV in vivo, numerous investigators have suggested that one component of the pathogenesis of immune dysfunction in HIV might be immune mediated damage to HIV infected T cells and antigen presenting cells. Moreover, gp^{120} bound to the cell surface. The presence of enhancing antibody against HIV Eng gp^{41} has been associated with progressive HIV infection.

It is difficult to determine if anti HIV immune responses are salutary, destructive, or both. There is reason to speculate that, at least in the case of anti HIV CTLs, salutary and destructive anti HIV immune responses occur. That HIV specific antibody responses and anti HIV CTL responses decrease in the wake of progression to AIDS suggests that these immune responses promote the asymptomatic HIV seropositive state. However, there is increasing evidence for the involvement of HIV specific CTLs in HIV induced pulmonary inflammatory disease, central nervous

system disease, and lymphadenitis. For example, high numbers of anti HIV CTLs have been isolated from the lung of HIV infected patients with lymphocytic alveolitis that are capable of killing HIV infected macrophages[54]. More over, the presence of antiHIV CTLs capable of killing a variety of typesof HIV infected antigen presenting cells in lymph nodes, bone marrow, and thymus support to the notion that, over time, anti HIV CTLs that originally keep HIV infection in check by killing virally infected cells, by continued killing of antigen presenting cells and other immune types could gradually promote progressive immune system dysfunction.

Anti HIV Immune Responses in Rapid Progressors and Non progressors to Acquired Immunodeficiency Syndrome.

Rapid progressors to AIDS have a profound decline in CD4+ T cell levels, usually within 2 to 3 years after primary HIV infection. Rapid progressors have lower levels of anti HIV antibodies and low or absent HIV neutralizing antibodies that neutralize autoloous HIV primary isolates grown in peripheral blood mononuclear cells. High levels of HIV enhancing antibodies have been reported in rapid progressors. Levy and associates found CD8+ noncyutolytic T cell responses that suppress HIV replication are decreased or absent in rapid progressors. Rinaldo and colleagues found low levels of memory CD8+ CTLs by precursor frequency analysis in rapid progressors compared with nonprogressors, although anti HIV CTL effector cell activity was present in fresh peripheral blood cells from rapid progressors that compared with CTL activity in nonprogressors Rapid progressors to AIDS have elevated number of CD8+, CD38+ and DR+ T cells, elevated levels of serum neopterin, and β_2 microglobulin levels that signify chronci immune system activation.

Nonprogressors to AIDS have strong peripheral blood CD8+ class I restricted anti HIV CTL level that do not fall over time, strong CD8+ non MHC restricted HIV supressor activity, and high levels of anti HIV antibodies. Increased HIV neutralizing antibodies have been reported in nonprogressors to AIDS.

Neutralizing antibodies may contribute along with cellular anti HIV responses to control of HIV in nonprogressors, although the specificity of such antibodies is unknown.

There appear to be quantitative and qualitative differences in anti HIV immune responses among nonprogressors and rapid progressors. It is unknown if nonprogressors only have higher levels of neutralizing antibodies and CD8+ CTL responses than rapid prgoressors or if nonprogressors have only salutary anti HIV immune responses while rapid progressors have a preponderance of pathologic immune responses to HIV that damage lymphoid tissue cells.

Immunologic Characteristics of HIV Exposed, Seronegative Individuals :

Studies of exposed and persons have suggested that rare individuals may be resistant to HIV or may have cleared the infection without making anti HIV antibodies. CD8+ CTLs have been found in seronegative sexual contacts of HIV infected patients and found in seronegative infants born to HIV infected mothers.

Bryson and coworkers reported an HIV infected neonate who initially had serum anti HIV cultures, but the infant eventually became HIV antibody and viral culture negative, having appearently cleared HIV infection. Clerici and colleagues to HIV envelope peptides in seronegative homosexual persons with multiple exposures to HIV through sexual contacts. Multiply exposed prostitutes in Africa have been reported who are HIV negative by serology and PCR for viral DNA and who have HIV specific CTLs.

ROUTES OF TRANSMISSION :

The appearance of AIDS in diverse populations implicated several routes of transmission : contaminated needles or injection equipment; clotting factor concentrates, blood, and selected other blood products; transplacental and intrapartum and breast-feeding; anal and vaginal sex.[55] (Fig. 3)

SEXUAL TRANSMISSION :

Worldwide, more than 90% of HIV infections are associated with heterosexual transmission. In the United States, cumulative 1981 through 1994

AIDS surveillance reports received through April 14, 1995 to the Centers for Disease Control and Prevention (CDC) indicate that 60% of adult cases of AIDS are linked to either male-to-male sexual (53%) or heterosexual (7.3%) exposure categories, with an additional 6.6% reporting both male-to-male sexual and IDU activity[56].

Infectiousness :

The risk factors and cofactors associated with HIV transmission through sexual contact are often amenable to intervention. Virus load may be the key unifying feature of transmission risk. Because the dominant HIV binding affinity is for immunologic cells expressing the CD4 molecule, any physical or physiologic facilitator of this contact may be expected to increase transmission risk. The complex associations that may exist between sexual risk factors and the difficulties in measuring these factors make it challenging to identify consistently the same significant factors in all populations at all times.

A second, more prolonged period of higher infectiousness occurs when the HIV infected person becomes increasingly immunosuppressed. This is likely to be a consequence of high viremia and increased free and cell-associated virus load in cervicovaginal secretions, rectal fluids, and ejaculate, consequent to the deterioration of the immunologic gauntlet and the structural disruptions of the lymph node architecture that traps circulating virus for presentation to lymph node-based humoral and cellular defenses.

Although it has been apparent that higher transmission rates have been associated with depleted CD4+ cells among HIV-infected sexual partners, causality is hard to establish because higher transmission rates in late stage disease may also reflect longer duration of exposure to an infected partner.

Further contributions to infectiousness are those that recruit more CD4+ cells into the genital tract of the infected individual. Sexually transmitted infections that result in frank genital ulcers may include chancroid, herpes, syphilis, and other diseases. Infections and conditions resulting in inflammation and exudation include

chlamydial infection, gonorrhea, trichomoniasis, bacterial vaginosis, and candidiasis. Recruitment of infectious lymphocytes, macrophages, or other cells into seminal or vaginal secretions may increase transmissibility, as occurs with sexually transmitted infections and their inflammatory urethral, prostatic, cervical, vaginal, and anal responses. Mucosal ulceration, inflammation, exudation, or trauma to protective epithelial surfaces, as occurs with infection of "dry sex," is likely to increase the efficiency of viral release and viral entry. STD patients commonly report other high-risk activities and high-risk partners.

Although hypothesized, it is unknown whether systemic coinfections in the HIV-infected person increase infectiousness. Coinfections such as cytomegalovirus, Estein-Barrvirus, human T-cell lymphotropic virus types Ior II, human herpesviruses type 6 or 7, Mycoplasma fermentans, and other may increase viral expression in vitro and could increase in vivo viremia and infectiousness.

Table - IV

Factors of interest in the sexual transmission of HIV

- Number of sexual partners
- Number of sexual exposures
- Likelihood sexual partner is infected
- Probability of transmission during sexual activity
- Nature of sexual contact; insertive most infectious versus receptive most susceptible.
- Noninjecting drug use (cocaine, alcohol, nitrites) through facilitation of high-risk sexual contact.
- Vaginal docuhes, astringents, abrasives, or trauma Anal doches or trauma.

The uncircumcised male may have a higher intraurethral and subprepucal load of potentially infectious cells than the uncircumcised man, explaining the data suggesting this as a risk factor for transmission to a sexual partner. It is plausible, but unknown, that a woman who has been circumcised with an infundibuletomy would be more infectious if infected because of the trauma to the unnatural postsurgical genital anatomy.

Host genetics may influence natural history and viral load. Some persons may be more infectious as a consequence of their greater genetic susceptibility to rapid progression.

Susceptibility :

Factors that probably facilitate transmission include those that increase contact with blood and infectious genital secretions during sexual acts and increase virus-cells contact with vulnerable cells in the mucosal epithelia of the rectum, cervix, vagina, or oral cavity. Increased contact time with infectious secretions that are kept warm and moist is likely to increase transmissibility, suggesting why male to female transmission rates are more efficient than female to male and why uncircumcised men may be at increased risk for heterosexual transmission from vaginal sex. Rates of female to male transmission in Thailand are much higher than noted in United State and European studies, suggesting at least one reason for the rapid expansion of the heterosexual epidemic in developing countries.

Nutritional status, particularly vitamin A deficiency, has emerged as a likely cofactor for perinatal transmission[57]. It will be important to assess whether sexual transmission occurs more easily in vitamin A deficient adults, because this introduces a potential intervention method for developing countries.

Sexually transmitted infections :

Although it is widely appreciated that STDs are associated with HIV risk, only recently has it been documented that a reduction in STDs can reduce HIV transmission. Ulcerative STDs disrupt the integrity of the epithelial mucosa and facilitate HIV contact with the lymphatic and circulatory systems, as well as recruting CD4+ lymphocytes and macrophages to the site of injury or infection. Genital ulcers have had the higher relative risk estimates, with a 5 fold to 10 fold excess risk demonstrated in cross sectional studies. Inflammatory and exudative STDs are less disruptive of the epithelial tissues, but the exudate may recruit large volumes of cervical or urethral discharge filled with susceptible cells, and inflammation may result in microulcerations and superficialization of capillaries,

facilitating contact with HIV with more easily infectable cells. Relative risk estimates for excess risk for HIV with nonulcerogenic STDs may be in the 2-fold to 5-fold range.

This may suggest that ulcerogenic STDs should be the target of intervention. However, inflammatory and exudative STDs such as chlamydial infection, gonorrhea, and trichomoniasis are far more common than ulcerogenic STDs such as herpes, syphilis, and chancroid. The risk within a such as herpes, syphilis, and chancroid. The risk within a given population attributable to STDs is greater, therefore, for inflammatory and exudative STDs than for ulcerogenic STDs, suggesting the strong need to target all STDs.

Risk and Age, Race of Ethnicity, and Gender :

Sociologic and behavioral characteristics, such as higher prevalence of IDU and poorer educational back grounds, are the roots of drug abuse frequencies and high sexual risk behaviors in some minority populations.

Creak house sex for drugs exchanges, most prevalent in eastern U.S. inner city and southeastern U.S. rural minority communities, is a powerful multiplier for HIV transmission, analogous to the bathhouse environments prevalent in San Francisco, New York City, Los Angeles, and elsewhere from 1977 to 1984. The bathhouse catering to homosexual and bisexual men, "swinging singles" clubs for heterosexuals, and crack house environments for drug addicts and their sexual partners are perfect for HIV transmission when a highly infectious individual is introduced into the arena of multiple, unprotected sexual encounters. The efficient multiplier is a classic feature of STDs with a "core" transmitter group, but rarely is there a more rapid spread than when the core transmitters combine expanded social networks with high frequency of infectious contacts.

Black women in Alabama have HIV and AIDS rates approximately 20 times that of white women, a frequency that may be related to higher rates of HIV in African American heterosexual men in Alabama and to higher efficiency of transmission because of the relative rarity of condom use and frequency of STDs.

Young and postmenopausal women may be at comparatively higher risk of HIV infection for a given number of exposures. Immature vaginal mucosa in adolescents, with large cervical ectopy zones may be more susceptible to trauma during sex and may be especially prone to infection with STDs because of the large transformation zone and exposed columnar epithelia. HIV transmission may be facilitated by sexual activity at times in which there is expected to be blood exposure, as during menses, rape, or first coital experience. Improved understanding of mucosa immunity is needed to appreciate the relative importance of physical and immunochemical barriers to transmission.

Anal sex may facilitate HIV transmission better than vaginal sex, as suggested by its independent risk association among heterosexuals. This would be biologically plausible because of the abundance of infectable cells in the distal large intestine. Similarly, oral sex is probably less infectious, although epidemiologic studies are weak in divining causal associations because of the congruence of oral sexual risk with anal or vaginal sexual activity. Animal studies may prove useful in assessing human risk through vaginal and viral challenges, microbicide screening, and the role of trauma as a facilitator of transmission.

Older persons are less likely to practice high risk behaviors, but those who do have high risk sexual behavior are less likely to take precautions. Hence, attention to risk beyond the adolescent and young adult populations is needed.

PARENTERAL TRANSMISSION:

INJECTION DRUG USERS :

Even before the first report associating AIDS and use of injectable drug in 1983[58], the epidemic among IDUs had spread worldwide.

IDU associated AIDS cases constitute the second largest risk exposure category in CDC surveillance statistics. In newly reported cases, IDU and heterosexual cases exceed male to male sex as the reported risk in many parts of the United States.

Thousands of infected women and men whose heterosexual partners were IDUs, children whose mothers were IDUs and children whose mothers were sex partners of IDUs may attribute their infections indirectly to the use of injectable drugs.

Secondary spread of HIV infection through heterosexual activity from the ever increasing reservoir of IDUs continues to rise. This is in dramatic contrast to the situation within the male homosexual and bisexual community, in which incident HIV infection has fallen since 1983 among older men, largely because of rapid and dramatic alterations in sexual behavior. Rates among young men and gay or bisexual men of minority background have not declined as notably.

Infectiousness :

Viral load, as with sexual exposure, may be the major factor predicting the infectiousness of an HIV infected IDU. Viral load is highest shortly after infection, before the host immune system has time to marshal its defenses, and late in the course of disease at the time of systemic immune collapse. Low CD4+ cell counts are though to be associated with increased infectiousness, although this is much harder to ascertain in needle sharing partners than among sexual couples. Behaviors related to drug use that increase cross contamination activities in the window period and late in disease would be expected to increase risk. However, given the parenteral nature of the inoculation, it is known that HIV can be transmitted at just about any time in the course of infection. An infected IDU in open member sharing groups for drug use, as is common in "shooting galleries", are expected to be an especially dangerous vector for wide spread transmission. Similarly, the non-IDU who is exchanging crack cocaine for sex can be in a highly efficient transmission environment in which IDUs are among the sexual clients and STD prevalence is substantial.

Susceptibility :

Needle sharing networks and the "human bridges" that connect them are critical in predicting the efficiency of transmission. Drug users tend to share in groups. Most dangerous is promiscuous sharing in a shooting gallery environment, where anonymous sharing occurs. Social sharing with a more defined needle sharing group is of intermediate risk.

Extension of the HIV epidemic among drug users in Asia will predictably increase drug related heterosexual transmission. Male drug users commonly have female sexual partners who do not use drugs. Heterosexual HIV transmission is more frequent among women than men, partly because of the greater numbers of infected heterosexual men who tend to be greater users of intravenous drugs. There is a greater likelihood of a woman encountering an infected male sexual partner in a locale with prevalent HIV and IDU than for a man to encounter an HIV infected woman. Moreover, sexual transmission appears to be more biologically efficient from male to female. Women who overcome addictions may continue to be at risk of HIV transmission through sexual encounters.

Female former IDUs whose sexual partners currently inject drugs display a higher HIV seroprevalence than do female IDUs whose sexual partners do not inject drugs. Most women who inject drugs are in their prime childbearing years. Heroin and cocaine are the drugs commonly associated with serpositivity among intravenous drug users.

Cocaine can effect a "high" by smoking (ie, frebasing), intranasal use, injection alone, or in combination with heroin (ie, speedballing). Studies suggest cocaine to be more strongly associated with HIV acquisition than heroin or other drugs, probably owing to the greater frequency of its use. Non-IDU crack use is HIV associated because of exchange of sex for drugs and other high-risk sexual activity.

High risk sexual practices and drug use are related behaviors. IDUs are less likely to incorporate safe sex behaviors than to use safer needle sharing practices.

HIV-Contaminated Instruments :

Use of HIV contaminated instruments used for puncture or injection in other non-traditional ways may transmit HIV. A 6 weeks period of acupuncture therapy was implicated in the seroconversion of a previously healthy 17 years old boy without any known risk factors. Two persons may have contracted HIV from tattoos administered in prison with unsterilized tattoo needles[59]. Adolescent brothers may have transmitted HIV through sharing shaving equipment contaminated with blood from cuts.

Even professionally administered needle sticks or injections may cause HIV infection if proper sterilization techniques are not used. The pressure to reuse equipment when funds for replenishment of medical supplies are short could explain the occurrence of some HIV without the classic risk factors. Epidemics in Romania and the former Soviet Union have been documented among hospitalized children with HIV seronegative mothers. Shortages of medical supplies have resulted in reuse of needles presumably contaminated with HIV.

In resource poor areas of Africa, Asia, and Latin America, the potential exists for transmission of HIV through unsanitary use of needles. Other areas of reported concern for parenteral transmission include tuberculosis screening, jet injections, medical waste disposal, neurologic pins, electroencephalographic testing, earlobe sampling by stylet, and prenatal diagnostic tests on seropositive mothers.

OCCUPATIONAL EXPOSURE :

Occupational exposure among health care workers has been the topic of recent review; deep punctures with large bore needles are the highest risk event[60]. Nonparenteral exposures such as splash exposures carry much smaller risks. Nurses are most commonly exposed. Zidovudine use has been associated with partial protection in an observational analysis performed by CDC, but it has not been demonstrated to protect all exposed health care workers.

Tuberculosis :

Transmission of tuberculosis commonly occurs among drug users, facilitate by poor housing and other social conditions[5]. Pneumonias are also more common among drug users, but these do not carry the community risk inherent in tuberculosis. An epidemic within an epidemic is apparent, particularly in developing countries and among IDUs in the United States : the expanded tuberculosis problem facilitated by HIV related immunosuppression. In this way, the worldwide HIV epidemic becomes truly a threat to everyone because of the lack of personal control over aerosolized exposure compared with sexual or drug use exposure. It adds the need for chronic, directly observed chemotherapy and chemoprophylaxis among drug users with tuberculosis. In developing nations, isoniazid pro-phylaxis for HIV infected patients to prevent tuberculosis has proven efficacious. Although the special relationship of HIV and increased tuberculosis risk is an important issue, giving added incentive to prevent transmission of the former to inhibit transmission of the latter.

BLOOD AND BLOOD PRODUCTS :

In July, 1982, three recipients of factor VIII concentrates for treatment of hemophilia were reported to have developed P carinii pneumonia, which suggested that transfusion[61] of blood and blood products was a plausible mechanism for transmission., In December, 1982, a newborn who had received multiple blood transfusions for erythroblastosis fetalis was reported to have become progressively immunocompromised and developed opportunistic infections, including Mycobaterium avium complex. Investigation of the 19 blood donors revealed that one was later diagnosed with AIDS.

The risk of acquiring HIV infection through blood transfusion has varied by region, depending on the area's sero-prevalence among blood donors, and the sensitivity of the screening strategies. Circumstances in many developing countries remain more urgent. Transmission can occur from unscreened blood given for medical purposes, use of unclean needles in medical or traditional health care

settings, unsterile surgical procedures and births, anabolic steroid use, suboptimal hemodialysis, and scarification and tattooing. Resources are inadequate to guarantee a safe blood supply worldwide.

When untreated, whole blood, fresh frozen platelets, packed cells, and clotting factors have been demonstrated to carry HIV. Hepatitis B vaccines derived from human or recombinant sources, albumin and immune globulin (including Rh (D) immune globulin) have not been found to transmit HIV because of the purification processes which inactivates all known viruses.

Other related retroviruses are spread through donated blood and plasma, including HTLV-I and HTLV-II associated with adult T-cell leukemia and HTLV-I myelopathy. HTLV-II has been definitively shown to be caused by HTLV-II. Although the risk of acquiring HIV-1 infection in one large study was 3 per 100,000 units of blood product, the risk of acquiring HTLV-I infection was 24 per 100,000, suggesting the need to screen the blood supply for HTLV-I. In 1988, no cases of transfusion related acute T-cell leukemia were documented, although causality will be very difficult to demonstrate because of the long viral latency. Hepatitis B and C risk may presage HIV risk for blood and transplantation recipients.

Hemophilia :

A person with hemophilia was diagnosed with P carinii pneumonia at the end of 1981 in the United Sates, suggesting that transmission of an AIDS related infectious agent could be spread through blood products. Retrospective studies indicate that HIV antibodies were present in serum samples as early as 1978 and 1979, and fully 50% of one population had seroconverted by 1982. By the end of December 1994, adults and adolescents and 221 children (<13 years) with hemophilia or one of the rarer coagulation disorders had been reported with AIDS.

Among individuals with coagulopathies, persons with hemophilia A and B experienced the impact of the AIDS epidemic the most severely. According to CDC estimates in the late 1980s, 33% to 92% of persons with hemophilia B were infected with HIV, depending on their geographic region.

They require the use of factor VIII and IX concentrates in larger and more frequent doses than persons with milder clotting disorders. A typical vial of factor VIII concentrate contained clotting factors, alloantigenic compounds, and possibly, infectious agents from between 2000 to 20,000 blood and plasma donors. Exclusion of risky donors, modifications in the application of heat treatment (ie, increased temperature and longer exposure times), and the addition of detergent treatment and monoclonal antibody purified products have now virtually eliminated the risk of HIV transmission from the blood products of industrialized countries.

Allograft and Organ Transplantation :

Transmission of HIV through allograft transplantation has been uncommon and represents a minute proportion of all ADIS cases[62]. HIV or genomic fragments can be isolated from all bodily fluids and organs of an infected individual, including various blood compartments, tears, conjunctival epithelium, corneal tissue, saliva, semen, urine, sweat, vaginal secretions, breast milk, cerebrospinal fluid, alveolar fluid, and major organs. Every organ in a seropositive individual is likely to be infected with HIV. Donated kidneys have been the most frequently associated with HIV transmission. Several liver allograft recipients have been infected with HIV through transplantation. HIV transmission has also been documented through bone and bone marrow transplantation. Skin allografts can transmit HIV from an infected donor. In Australia, 4 of 8 women exposed to HIV transmission from a single infected semen donor sero-converted in 1985. Isolation of HIV from tears, conjunctival epithelium, and corneal tissue has been successful, but despite inadvertent transplantation of corneas from a seropositive donor, no seroconversion in recipients has been reported. Otologic homografts have also been identified as a potential vehicle of transmission, although no cases have been reported. Blood transfusion, common during the peritransplantation period, may have contributed its own risk for acquiring HIV, thereby overestimating the already small risks from transplantation.

Such episodes became exceedingly rare after institution of HIV testing in 1985. Organ donors, including semen and ova donors, undergo routine screening, including HIV antibody testing. The CDC and the American Fertility Society recommend that semen samples intended for artificial insemination should be frozen for at least 6 months, followed by another HIV - antibody test from the donor before sample use.

RARE OR PUTATIVE MODE OF TRANSMISSION :

Insects :

There is no evidence of transmission of HIV through inoculation of HIV infected blood by insect vectors. Biologic transmission does not seem to occur, because HIV is unable to multiply inside insects, and infection of mosquito cells in vitro has not been consistently successful. North American and European mosquitoes feed typically only once in 3 days, decreasing the probability that an infected lymphocyte on the mosquito mouthparts would be viably inoculated to a second host. Even mosquitoes that feed daily (eg, certain subspecies of Anopheles gambiae in Africa) do not feed on a second person immediately after feeding on the first, Small mouthparts limit the amount of potentially infective material that could be transferred. Mechanical vector studies of HIV and mosquitoes and bedbugs mimicking natural conditions have not demonstrated transmission through blood transfer from person to person.

In the early 1980s, it was suggested by Florida physicians that arthropod transmission explained the high HIV rates in Belle Glade, Florida, Epidemiologic investigations, by CDC did not support mosquito borne transmission[63].

Nonparenteral Bloodborne Transmission :

Nonparenteral transmission of blood in nonoccupational setting deserves consideration. In boxing, forceful blows often cause broken skin and bleeding, which could result in mixing of blood from and HIV infected competitor into his opponent's wounds[64], a noteworthy concern given IDU problems reported among

several boxers. One case of a bloody soccer accident suggested on field transmission, but no molecular evidence of HIV-1 strain similarity was offered.

One young traveler was infected in Rwanda after sustaining lacerations in a bus accident. Blood dripped into the traveler's wound from other injured passengers who tended to him. In this highly endemic area, with seroprevalence rates in blood donors from Rwanda approaching 18%, it was postulated that this resulted in HIV transmission, because no other risk factors were apparent and the traveler had tested negative for HIV antibody when donating blood a few months before the accident.

Saliva of human bites :

Transmission depends on many factors, including the concentration and viability of the agent within a given fluid or tissue, access to a port of entry for the fluid or medium, the presence of CD4+ cells or other target cells at the site of HIV entry, and natural host defenses near the site of entry. Although transmission through saliva is unlikely because of the extremely low viral load, transmission is conceivable by means of saliva through a bite wound although longitudinal studies and published report involving cases of bites do not offer this possibility much support.

In 1990, the CDC reported preliminary results of a study, including 89 household members living with 25 HIV infected children, mostly toddlers, who were infected through transfusion. No evidence of casual transmission among toddlers biting one another has been shown. None of the household members have tested positive for the virus, despite months of close contact before and after the child's diagnosis. Testing of 78 individuals household members with polymerase chain reaction methods revealed negative results[65].

Casual Contact :

There have been a few studies to assess the risk of transmission of HIV through casual household contacts as occur between family members. These studies failed to demonstrate a HIV infection in household members who did not have additional exposure to HIV through blood, sexual activity, or perinatal transmission.

Sharing of common household facilities such as beds, towels, toilets, baths, showers, kitchen utensils, dishes, dishwashers, and clothes washers and dryers was documented. Personal interactions included hugging and kissing of the cheek and lips, wiping of fecal matter of infants and children by parents, and other family casual fluid contact. May of the infected children were infected up to 2 years before becoming symptomatic with AIDS, and no specific precautions were taken before the HIV infection was recognized. It has been stated correctly that these studies remain too small to definitively rule out small risk form casual contact.

Swimming pool concern are insubstantial when the antiviral effects of chlorine are consider. Even unchlorinated pools have a huge dilutional effect. Food handlers would not be expected to be a public risk because of the transmission patterns of HIV. Fomites, such as bedclothes or dirty laundry, would be a risk if they were bloodied but would not otherwise be considered dangerous.

Health Care Worker to Patient Transmission :

The greatest mystery in the topic of casual transmission of HIV is the "Florida dentist" case. Well studied and documented with elegant epidemiology and molecular virology, it is clear that a dentist transmitted to five of his patients through an unknown route. Deliberate criminal inoculation, contaminated instrumentation, drooling or bleeding from the infected dentist into the mouths of the infected patients, sexual contact, or blood contamination all seem unlikely. It is unknown how these patients were infected. In subsequent look-back studies of over 22,000 patients from 51 HIV infected health care workers, all 113 HIV seropositive patients had identified risk factors or strong molecular virologic evidence that the health care worker was not the source of the patient's infection. The Florida dentist remains an isolated event, suggesting that spread from health care worker to patient is rare. This issue has implications for the day care industry and educational systems, as well as the rights and treatment of HIV infected individuals, and it can be expected to periodically reemerge in the press and the mind of the public[67].

VERTICAL TRANSMISSION :

(Mother to the fetus and infant)

The world health organization estimates that by 2003 a cumulative total of 37 million adults and 2.5 million children infected world wide with HIV/AIDS. Approximately 90% of infected children acquired the infection from their mothers. The rising prevalence of HIV infection in women of childbearing age, particularly in Africa, Asia, and Latin America, is expected to lead to a marked increase in vertical HIV infection.

Timing, Mechanisms & Risk factors :

Direct evidence for the timing of the vertical transmission HIV is difficult to obtain, but it appears that HIV may be transmitted from an infected women to her infant during gestation (in uteri), during delivery (intrapartum) of post partum through breast feeding. The isolation of HIV-1 from or the detection of HIV - 1 provirus in aborted fetal tissue as early as 10 weeks of gestation has been reported, but potential contamination with maternal blood was not always excluded, particularly in first trimester fetuses. The intrauterine transmission of HIV -1 also is suggested by the isolation of HIV - 1 from fluid and cells and by detection of P^{24} antigen in fetal blood obtained at 16-24 weeks by cordocentesis. The isolation of HIV - 1or the detection of HIV - 1 genome in blood samples obtained at birth from 30% to 55% of HIV - 1 infected infants also suggests the intrauterine transmission of HIV - 1.

The proportion of infants infected in each trimester of pregnancy is unknown, as are the routes or mechanisms of intrauterine infection. Potential routes of infecting include admixture of maternal - fetal blood or infection across the placenta. Although HIV - 1 has been detected in amniotic fluid, placental trophoblasts and Hofbaur cells, the detection of HIV - 1 in placental tissue has not correlated with transmission. The importance of the placenta as a barrier to infection is suggested, however, by an increased risk of transmission from women with

placentitis (eg, syphilitic) or women with illicit drug use (particularly vocative drugs cocaine) during pregnancy.

Indirect evidence suggests that the vertical transmission of HIV - 1 can occur during the intrapartum period. The routes and mechanisms of intrapartum transmission are unknown but probably include the admixture of maternal and fetal blood or mucocutaneous (eg. ocular, gastrointestinal tract) exposure of the infant to maternal blood and vaginal secretions. Several cases of the transmission of HIV - 1 through breast - feeding have been reported. HIV - 1 has been detected by culture and by the PCR method in the cellular and Acellular components of breast milk, the colostral viral load appears to be particularly high. The risk of breast milk transmission appears to be especially high during maternal primary infection, Particularly if maternal primary infection occurs in the first few months offer delivery for these reasons, the guidelines recommend that women infected with or at risk of infection with HIV - 1 abstain from breast - feeding their infants if local sanitary conditions and access to infant formulas are good. However, because studies in developing nations have demonstrated decreased morbidity and mortality in breast - fed infants of HIV - 1 infected women, the benefit of breast - feeding are thought to out weight the risks of vertical HIV - 1 transmission in those settings, and breast - feeding is currently recommended.

Working definitions regarding the timing of infection have been proposed for non breast fed populations. Infants are regarded as infected in uteri if HIV - 1 is cultured from or HIV - 1 DNA is detected in peripheral blood lymphocytes within 48 house of birth, Infants are regarded as infected during delivery (intrapartum) if HIV - 1 culture and DNA PCR results are negative for blood samples obtained within the first week of life and positive thereafter the application of these definitions to small chohort studies of non-breast fed populations suggests that 45%

to 70% of vertically infected infants are infected in utero, and the remainder are infected at delivery.

Several studies suggest that maternal blood viral load may be an important factor in transmission. A three fold increase in transmission was observed in the multicenter European Collaborative study when HIV - 1 P^{24} antigen was detected in maternal serum, suggesting an increased risk of vertical transmission with higher blood viral load. An increased risk of vertical transmission was observed with maternal AIDS. Rates of transmission increased markedly when the maternal CD4 count was less than for per mm^3 or the CD4 : CD8 ratio was less than 0.6 Although immunocompetence may be an important factor in vertical transmission, maternal HIV - 1 specific immunity may be especially important.

Natural history of Vertical infection :

The tempo of disease progression after vertical HIV - 1 infection is highly variable. As a group, however, vertically infected children experience more rapid disease progression than children infected at an older age or adults. More than 80% of vertically infected children manifest HIV - 1 related symptoms or CD4 T-cell depletion by 2 years of age.

Viral and host factors appear to contribute to the variability in the natural history of vertical HIV - 1 infection. Virus host dynamics in early infancy appear to be important determinants of infection outcome and may account for the particularly rapid disease progression observed after vertical HIV -1 infection. Virologic evaluation may differentiate infants infected in utero from those infected during delivery. However data demonstrate a rapid increase in viral load over the first month of life in most infants, whether infected in utero or during the intrapartum period. Other viral factors that may important in the pathogenesis of vertical infection are the genotypic and phenotypic variability vertically transmitted viruses. Host factors that may be particularly important in the pathogenesis of vertical HIV -

1 infection include host genotype, the ability of host cells to support viral replication, and virus - specific immune responses. Virus specific cytotoxic T-lymphocytes are important for the clearance of acute viral infections and suppression of viral replication in chronic infections.

Strategies to Prevent the vertical Transmission :

A variety of strategies to prevent the vertical transmission of HIV have been proposed. These include the prevention or therapy of venereal infections, maternal nutritional intervention, by passing the route of exposure, the reduction of viral load in maternal blood or vaginal secretions, and maternal or neonatal immunotherapy.

Attention must be given to concomitant infection and factor such as nutritional status that may affect vertical HIV - 1 transmission. Because concomitant maternal venereal infections may increase the risk of vertical transmission, efforts to prevent or treat infection are important. An increased risk of vertical HIV-1 transmission with maternal vitamin A deficiency. Vitamine A supplemention would offer an inexpensive and easily feasible intervention. The topical application of chlorhexidine reduces the vertical transmission of group B streptococcal infections. Because as many as 75% of infants may acquire HIV infection at delivery through mussel exposure to maternal blood or vaginal secretions, it has been suggested that cesarean section may reduce the risk of exposure, particularly if performed before the rupture of membranes. Perinatal antiretroviral therapy reduces maternal blood or vaginal HIV-1 load. Zidovudin was the first antiretroviral to be given to HIV-1 infected pregnant women and their infants to evaluate its effects on vertical HIV-1 transmission. Combination regimens that would potently inhibit viral replication. Evaluation of the passive administration of monoclonal or polyclinic antibody preparation to mother and their infants for the prevention of vertical transmission.

In addition HIV can be transmitted from a mother to her baby through breast milk. It has been found that breast-feeding approximately double the risk of transmission. So breast-feeding should be avoided. Moreover during intrapartum

period, invasive procedures like amidogen thesis, fetal scalp sampling, internal scalp electrodes, per umbilical cord sampling, should be avoided because of the risk posed to fetus by direct contact with maternal blood or secretions.

CLINICAL MANIFESTATIONS :

CLINICAL SPECTRUM OF HIV DISEASES :

Infection with HIV induces an insidious, progressive loss of immune system function, which ultimately results in the opportunistic infections and malignancies of the acquired immunodeficiency syndrome (AIDS). The median time from initial infection to the development of AIDS is approximately 10 years, although the rate of disease progression varies substantially among patients. In the early 1980s, the patients were staged primarily on the basis of whether they hand an AIDS-defining opportunistic disease process. Infection were catagorized as having asymptomatic HIV infection, AIDS related complex (ARC), or AIDS[71]. ARC symptoms and AIDS defining condition are listed in table - V and VI.

Table - V

CDC 1993 Classification System[72]

CDA count	*Symptoms*		
	A	***B***	***C***
> 500	A1	B1	C1
200-520	A2	B2	C2
< 20	A3	B3	C3

Symptom category :

A ***:*** Acute retroviral syndrome
Generalised lymphadenopathy
Asymptomatic disease

B ***:*** Symptoms of AIDS related complex
Condition, cervical dysplasia, constitutional symptoms, herpes zoster, idiopathic thrombocytopenic purpura, listeriosis, oral hairy leukoplakia, pelvic inflammatory disease, peripheral neuropathy.

C : AIDS defining conditions :

CD4 count < 200, condidiasis, cervical cancer; coccidiodomycosis, cryptosporidiosis, CMV infection, Herpes esophagitis, HIV encephalopathy, histoplasmosis, isoporiasis, Kaposis sarcoma, lymphoma, mycobacterial disease, P. carinii infection, pneumonia, progressive multifocal leukoencephalopenia and salmonellosis.

Table - VI

AIDS case definition for surveillance CDC 1987 (for persons above 12 years)[73]

1.	Two positive test for HIV infection (by ERS test)	
2.	Any one of the following criteria :	
	a.	Significant weight loss (10% of body wt. with last one month)
	b.	Chronic diarrhoea (intermittent or continuous)
	c.	Prolonged fever (Intermittent or continuous)
	d.	Tuberculosis : Extensive pulmonary TB, disseminated, miliary, extrapulmonary
	e.	Neurological impairment preventing independent daily activities, not known to be due to the condition unrelated to HIV infection.
	f.	Candidiasis of the oesophagus (oral with odynophagia)
	g.	Pneumonitis
	h.	Kaposi's sarcoma
	i.	Other conditions : Cryptococcal meningitis, Neurotoxoplasmosis, CMV retinitis, Penicillium marneffei, Recurrent herpes zoster and multi-dermatomal or disseminated molluscum.

Several clinical staging system have been proposed to characterize HIV disease Walter Reed classification system not in wide used. Other staging system have been based on CD4 counts, a marker that is the best predictor of the relative risk for developing HIV related opportunistic diseases. In keeping with this concept, the CDC revised the case definition of AIDS to include all patients with CD4 counts of less than 200 cells/mm^3, whether the patients had developed a true AIDS defining condition or not. The revised CDC classification also added three new AIDS-defining conditions: pulmonary tuberculosis, invasive cervical carcinoma and

recurrent bacterial pneumonia. Although the new CDC case definition staging system represents a substantial improvement over the previous version, it should be emphasized that the CDC staging system is intended primarily for use as an epidemiologic tool rather than an instrument for use in clinical practice. Although CD4 values are the best predictors of relative risk of disease progression. A reasonable approach to categorizing HIV disease is to divide the stages illness into six categories based on a combination of clinical features and CD4 counts : Initial infection, early HIV disease, intermediate HIV disease, late HIV disease, advance HIV disease and terminal HIV disease.

INITIAL INFECTION :

Acute seroconversion syndrome, the symptoms of acute serosconversion syndrome usually occur within 2-6 weeks after exposure to the virus[74]. The common symptom is fever with lymphodenopathy, pharyngitis, ophthous ulcerations, esophagitis, myalgia, arthralgias, diarrhea, nausea, vomiting and headache. Most distinguishing characteristic is the presence of flecting, morbilliform skin eruption. Initial infection may be asymptomatic. Most symptoms resolve within 14-21 days. Symptoms have been reported to have more rapid progression of AIDS.

Although this phase is characterised by marked CD4 lymphopaenia. Within 2-4 weeks after initial infection high levels of circulating virus can be detected by culture and PCR. P24 antigen also detected during this phase. During this period high level viremia, the immune system begins to recognised and respond to HIV antigens, and develop clinical symptoms. Within 1 to 3 weeks antibody become detectable. Routine use of antiretroviral treatment is not recommended during the acute seroconversion process.

EARLY HIV DISEASE (CD4>500 CELLS/MM^3) :

Most individual with high CD4 counts are asymptomatic. Those who have any symptoms of infection, lymphadenopathy is the most common. Dermatologic abnormalities are most common. Eosinophilic folliculitis, often characterised itchy red bumps manifest on the thorax, back and upper extremities. Herpes simplex

labialis may occur in early inffection[75]. Oral hairy leukoplakia caused by EB virus, and it may be indicator of impending disease progression. 100 million to 1 billion new viruses are produce in 24 hours period within an infected individual. Such high level production strongly suggests that antiretroviral therapy should be used.

INTERMEDIATE STAGE DISEASE : (200-500 cells/mm^3)

The relative risk of developing new opportunistic infection is higher, disease remain asymptomatic or demonstrate only mild disease manifestations. Skin and oral lesions more common, symptoms recurrent hepes simplex or varicella zoster infection, recurrent diarrhoea, fever weight loss and mild oropharyngeal or vaginal condidiasis represent the usual manifestations. Symptoms such as myalgias, orthralgias headache, and fatigue often present intermitantly. Bacterial sinusitic, bronchitis and pneumonia more frequent cause by S. pneumoniae, H. influenzae, M. cataralis, mycoplasma. Antiretroviral therapy with zodovudine monotherapy with zalcitabine or didanosine.

LATE - STAGE DISEASE : (50 - 200 cells/mm^3)

CD4 count less than 200 cells/mm^3 patients are having AIDs[76]. The most common opportunistic infection is PCP. Patients are also substantial risk of developing other opp. infections including toxoplasma gondii, encephalitis, cryptosporidiosis, isosporiasis, tuberculosis, β-cell lymphoma, Kaposi's sarcoma and esophageal candidiasis. Constitutional symptoms such as myalgia, arthralgia, fever and weight loss along with ARC. Neurological disorders such as mononeuritis, cranial nerve palsies, mylepathy, neuropathy and cervical cancer in women, carcinoma of rectum in man and other disorder pepillomavirus appears more frequently. Antiretroviral combination therapy continued throughout this disease stage.

ADVANCE HIV DISEASE : (< 50 cells / mm^3)

The risk of developing certain opportunistic infections that are associated with more profound immunosuppression becomes significantly higher when the CD4 count below 50 cells/mm^3.[77] MAC disease, cryptococal meningitis, cytomegaloretinitis, invasive aspergillosis progressive multifocal

leukoencephalopathy (PML) histoplasmosis, coccidioidomycosis, bartonellosis, infection become more common during this stage. Disorders of brain more common, PML, dementia. In wasting, weight loss with dirrhoea. is common, weight loss, weight loss includes the side effects of Addison's disease. Primary prophylaxis for opp. infection have been used. Standard pro-phylaxis for PCP and MAC disease and invasive fungal infection.

TERMINAL HIV DISEASE :

Although the rate of disease progression varies substantially among patients, will ultimately succumb to their disease. The diagnosis of terminal stage HIV disease is usually based on an ability to control the symptoms of disease because no treatments are available for the particular disorder or become ineffective. Provide the primary provision of comfort by psychological support, family support and aggressive pain management.

ORAL MANIFESTATIONS[79] :

Causitive organisms	*Disease*	*Clinical features*
Candida Spp	Candidiasis	Pseudomenbranous and crythematosis of mouth or pharynx
Criptococcus neoformans	Cryptococcosis	Ulcer on Palate
Histoplasma	Histoplasmosis	Lesion of mucosal surface
HP virus	HPV lesion	Oral, skin and genital warts
Herpes virus	HSV lesion HZV lesion	Painful lesion on tongue, skin and oral lesion
CMV	CMV infection	Ulcer on mucosal surface
EBV	Hairy leucoplakia	Oral lesion on tongue
MAC	Microbacterial infection	Palatal and gingival granulomatous mass
Other bacteria	Periodontal disease	Necrotizing ulcer, sever pain
Kaposis sarcoma	-	Multiple ulcer on oral mucosa
Lymphoma		Lymphoma on oral cavity
Idiopathic thrombocytopenic purpura		Petechiae, ecchymoses, hematoma on mucosa
Salivary gland disease and Xerostomia		Enlarged salivary gland. Dry mouth

DERMATOLOGICAL MANIFESTATIONS[80] :

Causative organisms	*Disease*	*Clinical features*
S. aureus	Impetigo, Ecthyma	Lesion grain and axilla, ulcerated lesions
Pseudomonas	Folliculitis	Follicular pustules bullous
Streptococcus Sp.	Cellulities	Impetigo, eczema, furuncles
Mycobacteria		Multiple papules or nodules
HSV		Indolant painful ulcerations
HIV exanthem		Maculopapular roseola.
EBV		Eruption with haemorrhagic necrotic center.
V. Zoster		Atypical zoster, particularly prolonged time or disseminated
Pox virus	Molluscum contagiosum	Flesh coloured umbillicated papules and nodules offace, warts.
HPV + EBV	Oral hairy leukoplakia	Whitish keratinised plagues on tongue
Candida	Candidiasis	Candidiasis in submammary areas, inguinal andaxilary area.
Tinea Try chophyton	Darmatophytosis	Keratodorma, Saborrhoeic dermatitis
M. Furfur	Pityrosporum infection	Hypo or hyper pigmented macules
Cryptococcus	Cryptococcosis	Papules, nodules, ulcers, cellulites
Spirochaetes	Syphilis	Ulcer
K. Sarkoma lymphoma		Neoplasia
Others	Saborrhoeic dermatitis, xeroderma, Psoriasis, Reiter's syndrome, photosensitivity, popular and follicular eruptions and valscular disease.	

NEUROLOGICAL MANIFESTATIONS[80] :

Abnormalities	*Organisms*	*Clinical clues*
Uncommon disorder dementia K. Sarcoma, Lymphoma, Mylopathy, Myopathy, Pleocytosis		Wasting, ganglia, calcification, headache, pain, dementia, loss of bladder and bowel control. painful dysesthesias, burning feet, weakness
Rare disorder : CNS lymphoma progressive multifocal leukoencephalopathy meningitis	Crypococci M. tuberculosis	Meningial irritation, headache, photophobia, confusion, lethargy, seizures.
Cerebral toxoplasmosis Polymyositis Neurosyphilis	Toxoplasma poliovirus T. Pellidum	Confusion, personality, change, lethargy genital ulcer.
Common disorder Seizures, encephalitis Cranial neuropathies Encephalopathy	Herpes zoster CMV	Mylitis, genital ulcer, encephalitis
Candidiasis Histoplasmosis Mucormycosis Aspergillosis Coccidiomycosis	Candia, Histoplasma Mucor Aspergillus C. immitis	Meningitis mental changes
Bacterial meningitides	Listeria, E.coli, S. pneumoniae, H. Influenzae	Headache, weakness, meningitis

RESPIRATORY MANIFESTATIONS[80] :

Causative organisms	*Disease*	*Clinical features*
Pneumocystis carinii	PCP Pneumonia	Non specific and insidious, fever, fatigue, weight loss, headache, diarrhoea and cough
Toxoplasma gondii	Pneumonitis	Mimic PCP, dyspnea, cough
Mycobacterium tuberculosis	Tuberculosis	Fever, lymphadenopathy, cough, weight loss, diarrhoea
Myco acium	Tuberculosis	
H. Influenzae Rhodococcus	Bacterial pneumonia	Fever, cough, dyspnea
Nocardia	Nocardiosis	Cough, fever, weight loss
C. neoformans	Cryptococal pneumonia	Cough, fever sweats fatigue
H. capsulatum	Histoplasmosis	Cough, fever sweats fatigue
B. dermatidis	Blastomycosis	Cough, fever sweats fatigue
Aspergillus Spp.	Aspergillosis	Cavity formation
CMV	Pneumonitis	Dry cough, dyspnea, interstitial infiltrates
Kaposis sarcoma		Dry cough, dyspnea, fatigue, fever, night sweat, Wt. loss
Non hodgkin's lymphoma		Pulmonary masses or nodules, hilar and mediastinal adenopathy

GASTROINTESTINAL MANIFESTATIONS[80] :

Causative organisms	*Disease*	*Clinical features*
HIV	Bowel disease	Diarrhoea, mucosal cellularity
CMV	GI syndrome	mononucleo like syndrome
HSV	Esophagitis proctitis	Painful, shallow, clean based perianal or perineal ulcer
Adenovirus		Diarrheal, disorders
Hepatitis virus	Viral hepatitis ABC & D	Liver disease, nausea, vomiting
Cryptosporidium	Cryptosporidiosis	Sever diarrhea, slim disease, wasting syndrome
Microsporidia	Microsporidiasis	Bowel syndrome
Isospora Belii	Isoporiasis	Cholangiopathy
Giardia Lamblia	Giardiasis	Gay bowel syndrome, diarrhea.
E. Histolytica S. Stercoralis Cyclospora B. Hominis	Amobiasis	Diarrhoea, hyper infection syndrome
M. tuberculosis M. Avium	Granulomatous hepatitis intestinal ulcer	Fever Progressive wasting and deilitation, diarrhoea.
Salmonella, Shigella, Campylobacter	Enteric fever, bacterial enteritidies	Abdominal pain, distension, diarrhoea
Candida	Candidiasis	Oral thrush
Histoplasma	Histoplasmosis	
Coccidiosis	Coccidioidomycosis	Chronic febrile illness
Actinomycetes	Actinomycosis	Esophageal ulcer
Kaposis sarcoma		Swallowing disorder, luminal obstruction
Lyphoma, Cancer of tongue, esophagus, stomach, colon and anus		Lymphoproliferative disease

HAEMATOLOGICAL MANIFESTATIONS[81] :

Anaemia is often present generally in advance stages of HIV illness. Several conditions may contribute to anaemia in HIV-infected patients. Concurrent infections, malignancies, malnutrition and toxic drug treatments, however, in most HIV-infected patients the cause of anaemia remains unclear. In AIDS patients, leukopenia and lymphopenia are also common and marrow hyperplasia with concomitant peripheral cytopenia is often found.

A severe type of autoimmune thrombocytopenia has also been described, it may occur in all stages of HIV infection. Physical findings in affected patients include ecchymosis and cutaneous or oral petechiae. Bone marrow aspirate and biopsy finding are consistent with peripheral platelet destruction.

RENAL MANIFESTATIONS[81] :

AIDS associated nephropathy is a renal syndrome characterized by proteinuria and glomerulosclerosis. The clinical presentation includes the nephritic syndrome with or without renal insufficiency. The syndrome produce rapid deterioration of renal function, leading to end-stage renal disease within a few weeks. On the other hand, nephrotoxic drugs, dehydration, sepsis, hypoxia and hypotension may contribute to acute renal failure in AIDS patients.

CARDIAC MANIFESTATION[81] :

Congestive cardiomyopathy may occur during HIV infection. Whether it is caused by HIV directly or by cardiotropic viruses or another mechanism remains to be established. Toxoplasma infection may occasionally cause myocarditis and Kaposi's sarcoma may involve the heart. Tuberculosis pericarditis is another condition that may complicate the course of HIV illness, and is a common cause of pericardial effusion.

REPRODUCTIVE SYSTEM MANIFESTATIONS[81] :

Hypogonadism in HIV infected patients has been reported. Amenorrhoea has been found in upto 40% of women with AIDS, it cause is unclear, but it is often associated with severe weight loss.

ARTHRITIS[18] :

A subacute oligoarthritis syndrome and Reiter syndrome associated with HIV infection have been reported. The cause of the arthritis is unknown, but HIV has been isolated from the synovial fluid.

INFECTIONS ASSOCIATED WITH HIV :

Atypical Fungal Infections :

✠ Pneumocystic Carinii :

Although more than a decade has passed since the recognition of the AIDS, Preumocystic carinii (PCP) continue to be acknowledged as the most common pulmonary infection in patients infected with HIV. During the early stages of the epidemic 60% cases of AIDS had PCP as their presenting illness in the USA and 80% of all AIDS cases had PCP during their lifetimes[82]. However, the prevalence of PCP has come down in the west with the institution of effective prophylactic regimes. Pneumocystic carinii is a single celled eukaryotic organism, which has been variously classified as protozoan, a fungus or an undifferentiated protist. However, molecular analysis has revealed that it is nearer to fungi than protozoa. P.Carinii exists in cyst form and trophozoite form which may be small 1.5 to 2 μ or large 3 to 5 μ. The trophozoites may have small or big nuclei depending upon size. The life cycle is not exactly known, continuous human exposure to this organism is a rule as indicated by the appearance of antibody in more than 80% of humans during early childhood. It is an intraalveolar extracellular parasite. Infection with P. Carinii is transmitted by the respiratory route among rodents. Epidemiologic studies of CD4 + T-lymphocyte (CD4 Counts) with onset of infection have begins with HIV, infection. CD4 counts are excellent predictors. PCP has been seen primarily in patients with recent CD4 counts lower than 200 cells / mm^3.

PCP has a subacute presentation in HIV infected persons. The most common symptoms of PCP are fever and nonproductive cough, dyspnea, chest tightness,

shortness of breath, fatigue and weight loss. In some cases involving disjunction of the brain, the retina, the liver or the kidney.

The definitive diagnosis of P. Carinii requires the demonstration of cyst or trophozoites within tissue or body fluids, given that the organism cannot be cultured from clinical material. Three categories of specimens are collected (i) Induced expectorated sputum (nebulizer is preferred) (30% to 90% sensitivity) (ii) Broncho - alveolar lavage (BAL) (90 to 99% sensitivity) (iii) Pulmonary biopsy (95 to 100% sensitivity) Prepare the smears and doing staining. Various staining methods are :-

- Giemsa staining
- Immunofluorescence Staining
- Modified toludine blue o staining
- Silver impregnation staining.

Currently trimethoprim - sulfamethoxazole (TMP - SMX) is the first line agent for PCP. Intravenous pentamidine is highly effective, but highly toxic.

Protozoan Infections :

✠ Toxoplasma gondii :

Toxoplasmosis is a world wide zoonotic infection. Toxoplasmic encephalitis was one of the sentinel opportunistic infections observed early in the AIDS epidemic. Currently it is recognized as one of the most frequent and treatable opportunistic infections of the CNS. Toxoplasma gondii, an obligate intracellular protozoan whose definitive host is the cat, exists in three forms - tachyzoite, tissue cyst and Oocyst - all of which are potentially infections for humans. The infection is usually acquired by ingestion of cysts present in inadequately cooked meat or of Oocysts excreted in cat feces[83]. In AIDS patients reactivation of latent infection usually manifests as toxoplasmic encephalitis, chorioretinitis, pneumonia, and disseminated disease have also been reported, if there CD4 count decline below cells 100/mm^3. Transmission by oral route and by vertical transmission from mother to foetus during an acute infection.

Clinical presentation of toxoplasmic encephalitis usually consist of focal neurologic abnormalities. Altered mental status characterized by confusion, Psychosis or other neuropsychiatric disturbance, cognitive impairment or coma. Seizures and pulmonary disease reported, Ocular disease necrotizing retinochoroidinitis and encephalities has also been reported. Involvement of other organ system such as lungs, heart, muscles, gastrointestinal tract is seen at autopsy, but is asymptomatic during the life. Rarely, patients will present with severe symptomatic involvement of one or more of these organ system.

Definitive diagnosis is made by demonstration of organisms histopathologically in brain tissue. The presence of tachyzoites in cysts surrounded by an inflammatory reaction is diagnostic. Wright - Giemsa stain is useful to demonstrate tachyzoites in CSF and bronchoalveloar lavage fluid. Isolation of Toxoplasma by tissue culture techniques or animal inoculation. Show the trophozoite from culture, tissue or exudate by fluorescent antibody staining. Specific antigen detection peroxidase - antiperoxidase method used to identify antigen and tachyzoites in tissue. Serological test indirect fluorescent antibody test, Enzyme immune assay, compliment fixation test, Haemagglutination test, PCR, latex agglutination technique used for diagnostic testing.

First line therapy includes pyrimethamine in combination with sulfadiazine.

✠ Cryptosporidiosis :-

With the advent of the AIDS epidemic (1982) Cryptosporidium has been more widely appreciated as a cause of diarrheal disease[84]. It has been estimated that 30 to 50% of patients in the developing world have developed chronic cryptosporidiosis. Although out breaks are common among immunocompetent hosts. These episodes are predominantly self-limited. It has become an important contaminants found in drinking water and is an emerging cause of food-borne disease. In the 1980s, it was recognized as a cause of a traveler's diarrhea. Person to person transmission of cryptosporidium occurs frequently in day-care-centres.

Nosocomial transmission also occurs in hospital personnel and among patients in hospital.

The clinical course in HIV-infected patients can be varied. Patients with low CD4 counts, especially counts lower than 100 cells/mm3, experience sever, unremitting diarrhea. The diarrhea caused by is usually profuse, watery, and nonbloody with no leaucocytes but mucus may be seen. Although it may be cholera like in character. Diarrhea is often associated with crampy abdominal pain, fatigue, anorexia, nausea, and vomiting and it may lead to malnutrition and dehydration. Fever may be present. After it is ingested, the Oocyst excyst in the small intestine and sporozoite invade the small intestine. Although infection in the small bowel predominates, other site of the gastrointestinal tract have been involve, and invasion of extrahepatic bile ducts may result in acalculous biliary disease. It also causes chronic sinusitis and otitis.

Diagnosis of cryptosporidiosis is usually made by examination of stool with modified acid fast staining. If few Oocyst are present, concentration techniques employing sucrose flotation method may be useful. A monoclonal antibody directed against Oocyst wall and an enzyme - linked immunoabsorbent assay have become available. PCR used largely for the detection of environmental contamination.

Until recently, no treatment had been proven to be consistently effective in the treatment of diarrhea. Symptom - directed therapy with loperamide or Lomotil and diet modification may be helpful light microscopy and concentrated Giemsa staining of stool or a modified trichrome or chromotrope stain have been used to detect organisms.

✠ Microsporidosis :

Microsporidiosis has become increasingly recognized as a cause of refractory unexplained diarrhea in 15% to 20% of AIDS patients[85]. Microsporidia cause mild, self-limited diarrheal disease in immunocompromised patients. Microsporidia are primitive obligate intra cellular spore producing protozoa. Although several species have been recognized as causing disease in humans.Only two species have been

found to infect the gut : Enterocytozoon bieneuri and septata intestinalis. Microsporidia appear to invade the enterocyte of the intestine, which may serve as the portal of entry preceeding dissemination. Although infection with E. bieneusi is limited to the intestine, the organism has also been found in the biliary system and has been implicated in acalculous cholecystitis andd most recently, identified in bronchoalveolar lavage fluid in AIDS patients. S.intestinalis can cause more disseminated disease because of its propensity to invade macrophages in addition to enterocytes and to cause disease in the lungs, liver and kidneys.

Clinical presentation : Infection with microsporidia is characterized by non bloody diarrhea with cramping abdominal pain and gradual weight loss. Malabsorption may be present.

Laboratory diagnosis : Recently, light microscopy and concentrated Giemsa staining of stool or a modified trichrome or chromotroe stain have been used to detect organisms.

Treatment : No therapeutic agent has constantly been effective. Recently albendazole used as a p romising agent. Patients frequently improve with the use of antisecretory drug such as loperamide or lomotil. Nutritional therapy minimizing (fat and carbohydrates).

✠ Isosporiasis :

Infection of AIDS patients with Isospora belli, acquired by the ingestion of Oocysts in food or water contaminated with the faces of infected humans. Endemic areas include Indochina, S. America, & Southwestern Pacific. Developing nations, where the organism is much more prevalent in the general population.

Clinical Presentation : Particularly crampy abdominal pain and profuse watery diarrhoea of 8-10 stools per day along with weight loss, weakness, anoreixa, & low grade fever. Isospora infection may involve the pancreas and biliary tree in AIDS patients. Extraintestinal infection involving the liver, spleen, mediastinal and mesenteric lymphnodes rare.

The diagnosis of isosporiasis is made by the detection of large oval Oocysts (20 to 30 μ m by10 to 20 μ m) in the stool by modified acidfast method, or by wet mount[86]. Biopsy of the small intestine may show organisms within the lumen or within cytoplasmic vacuoles in enterocytes, localized mucosal inflammation and atrophy.

A 10 day course of therapy with TMP-SMX is highly effective. In patients also are intolerant of sulfa, pyrimethamine, metronidazole may be used.

✠ Cyclosporiasis :-

Cyclospora is an emerging worldwide cause of diarrhea in AIDS patients. The organism is classified in this genus based on in vitro sporulation and excystation studies and by electron microscopy. These organisms reported to cause prolonged but ultimately self-limited diarrheal illness in travelers. Epidemics in the USA have been associated with fecal-oral transmission through contaminated water and food.

Illness caused by cyclospora is characterized by watery diarrhea, abdominal cramping, flatulence, weight loss and nausea[87].

Microscopic examination of stool using modified ZN stain reveals abundant sperical bodies 8-10 μ m in diameter. Recently Giemsa, trichrome, chromotrope, Gram - Chromotrope, acid fast and safranin stain using as fast, reliable and easy to perform procedure. Detection of tissue stage HE stain on biopsy specimen.

The response with TMP - SMX. Patients has been also successfully treated with trimethoprim-sulfamethoxazole.

Fungal Infections :-

✠ Cryptococcus neoformans :-

C. neoformance is the major cause of meningitis in AIDS patients, and it can also cause local organ dysfunction and disseminated disease. C. neoformans is a yeast like fungus that reproduces by budding[86]. It produces no known toxins but it does have a large polysaccharide capsule that appears to play a role in protecting the organism from host defense mechanisms. There are four subtypes of C. neoformans

: based on several characteristics, including mating abilities, stereotypes A and D are classified as C. neoformans var neoformans and B and C are as C. neoformans var gattii. Stereotypes A and D cause disease in AIDS patients. C. neoformanse is a ubiquitous fungus that can be isolated from a variety environmental sites, including soil and is found in especially high concentrations in pigeon feces. Initial infection occurs by the respiratory route, through inhalation of aerosolized organisms after exposure to environmental sources. In immunocompromised patients, however, especially in-patients with AIDS, the organism in inadequately controlled and can cause life threatening extrapulmonary disease, primarily meningitis, or with many other opportunistic infections. Cryptococcosis can present in HIV-infected patients either as the initial, AIDS - defining opportunistic process or as a later process.

The most common site of infection with C. neoformans patients is the CNS. C.neoformans usually infects both the brain and the meanings diffusely, producing both meningitis and encephalitis. Occasionally, cryptococomas, which are large focal lesions, develop. Headache and fever are the most common manifestations, nausea, vomiting, mental status, seizures, or focal neurological abnormalities are seen frequently. Pneumonia and fungemia are the most common extraneural presentations. Patients with pneumonia may present with fever, productive cough, dyspnea and occasionally hemoptysis. Fungemia associated with minimal complaint; fever, malaise and fatigue are often the only symptoms suggesting a systemic infection. Other sites of infections are Eye, oral, Skin, stomach, colon, liver, pancreas and heart. Other processes commonly seen such as Kaposis sarcoma, herpes and molluscum contagiosum. The prostate may also be a site of asymptomatic infection.

Specimen for laboratory diagnosis sputum, CSF, blood, urine, autopsy material and pus from skin lesion examine for the India ink and culture examination. Diagnosis of extraneural cryptococcosis detecting histopathologically in biopsy or other clinical specimen, sections are stained with methanamine sliver stain. The

most effective test is the latex test for capsular antigen in CSF or blood. It give positive over 90% of meningeal infection.

Amphotericin B were routinely used. Important new drug are flucytosine, fluconazole, itraconazole have been used.

✠ Candida :

Candida are yeasts that exist predominantly in uniecellular forms. They are 2.5 to 6 μ m ovoid cells that reproduce by budding. More than 150 species exist, and at least 10 are pathogenic in humans. Candida organisms are ubiquitous they have been recovered from soil, food and hospital environment.

Candida species are the most common causative agents of fungal infection in HIV - infected persons. Fortunately, these infections are seldom invasive, can usually be easily treated, and with appropriate management can often be prevented. It was established early in the course of the HIV epidemic that oral candidiasis is a marker of an impaired immune system and a prognostic marker for the subsequent development of opportunistic infections. Candidal infections of the esophagus, trachea, bronchi or lungs are recognised as indicator disease for AIDS[89]. Whereas oropharyngeal candidal infections (and candidal vaginosis) regularly occur in HIV infected persons with CD4 counts higher than 200 cells/mm^3, esophageal candidiasis is indicative of more advanced immunodeficiency and seldom occurs with CD4 counts higher than 100 cells/mm^3. Candida spp. seldom cause disseminated infection in patients with AIDS unless there are associated factors such as the presence of chronic indwelling catheters or neutropenia.

Oropharyngeal candidacies (thrush) is usually diagnosed by the characteristic appearance of white plaques on the tongue, bouccle mucosa or palate. Candida esophagitis is frequently diagnosed on the basis of the characteristic clinical presentations of odynophagia, dysphegia, a feeling of obstruction, substernal chest pain or combination of these symptoms, often accompanied by oropharyngeal candidiasis. Smear from mucous, sputam, blood, scrapings, CSF are stained with PAS or by Gram's method, pseudo mycelium and budding cells examined. Scraping

are mounted in 10-20% KOH examination. Material is cultured on Sabouraud dextrose agar. Biopsy showing tissue invasion by candida. Detection of antibodies against candida are least helpful in AIDS patients. Constantly rising titres are suggestive of deep infections.

Prophylactic drugs are fluconazole, ketoconazole and Itraconazole frequently used.

✠ Histoplasma Capsulation :-

Histoplasmosis is the most frequently diagnosed systemic fungal disease in the UK. Histoplasma capsulatum exists in the soil in the mycelial phase but converts to the yeast form at the body temperature of mammals. The mycelial from has septate, branching hyphae with lateral and terminal spores. The yeast form is ovoid, 1.5 to 2 μ m by 3.0 to 3.5 μ m and reproduce by budding. Histoplasmosis primarily involving reticuloendothelial system. Histoplasmosis is a serious problem in AIDS patients.

The most common presentation is fever and weight loss. Granulomatous nodules in the lungs, hepatomegaly, splenomegaly, lymphadenopathy occur. Thrombocytopenia, neutropenia and anemia, septicemia has been reported. Cutaneous lesions like rashes, tender pustules, and papules, skin or oral ulcers and ulcerated palatal nodules. Gastrointestinal masses, chorioretinitis and pleural effusion have also been reported[90].

The definitive diagnosis by biopsy and culture from tissue. Culture from bone marrow, blood, lung biopsy or lavage, sputum, lymphnode, skin or CSF are obtained positive. Diff - quick, Wright - Giemsa or methanamine silver stains can lead to a rapid diagnosis from tissue, sputum or lavage specimens. Immunodiffusion or complement fixation anti Histoplasma capsulatum antibody test may helpful in positive cases. Radio-immunoassay from blood and urine has been used for therapy and relapse.

Amphotericin B is the highly effective drug. Itraconazole, fluconazole an alternative regimen.

✠ Coccidioides immitis :-

Coccidioides immitis is a fungus that lives in the soil as a mold. Arthroconidia, produced from the hyphae of the mycelial phase, infect the host after they are released into the air and inhaled. In tissues, the fungus grows as a spherules, large structures containing hundreds of endospores. Coccidioido mycosis is endemic in certain areas in America. This disseminated disease occurs in 1.2% of immuno-competent patients with coccidioidomycosis who require hospitalization is more common in patient with AIDS[91]. Either the disease reactivated or susceptibility to infection in enhanced in patients with AIDS.

Because the route of infection of C. immitis by inhalation of arthroconidia, it most frequently involves the lungs. The most common presentation is that of fever, weight loss and cough. Diffuse reticulonodular infiltrates on chest radiography are characteristic in patients on chest radiography are characteristic in patients with AIDS. Pulmonary cavities, hilar adenopathy, meningitis, peritonitis and fungemia caused by C. immitis.

The diagnosis is easy by culture. Visulization of the distinactive spherules in tissue is also diagnostic of invasive disease. Serology may be helpful but complement and precipitation serology may negative with 25% patients.

Standard treatment is Amphotericin B. Ketoconazole, triazole itraconazole and fluconazole are also used.

✠ Aspergillus :-

Aspergillus is a common mold found in the soil that frequently cause invasive pulmonary and obstructing bronchial disease in immunocompromised patients. Relatively uncommon complication of AIDS occurs as a late manifestation of the disease[92]. Spore (conidia) are approximately 3 μm in size hyphae 2 to 5 μm

wide, are often septet and exhibit Y-shaped branching. A. fumigates and A. Floavus are the most common cause of aspergillosis.

Common symptom was the incidious development of cough and fever in immunocompromised patients with neutropenia. Radiographycally cavitary lung disease and diffuse infiltrates were the most common findings. Other organs, including the heart, brain, pancreas and spinal cord may rarely infected in AIDS patients.

Examination of sputum, blood & skin sample by direct KOH microscopy. Isolation from sputum or biopsy sample appearance of hyphae suggestive the diagnosis.

Response to treatment has been poor. Amphotericin β, itraconazole have been used with limited success.

✠ Blastomyces dermatitidis :-

β. dermatitidis is an 8-15μm yeast cell with daughter cells forming a bud with a broad base. Pulmonary or miliary blastomycosis has only rarely been reported in persons with AIDS[93]. Reported other body sites such as skin and bone.

Direct microscopy from sputum, biopsy, pus, skin scraping by KOH. Characteristic large round cells with refractile wall and broad based single bud seen. Culture on sab. agar. serological diagnosis are limited useful.

Early therapy with Amphotericin B. Lifetime therapy with Ketoconazole or itraconazole may be of benefit.

✠ Penicillium marneffei :-

P. marneffei is a dimorphic fungus that grows in tissue as a 3 by 8 μm yeast. It is endemic in South east Asia and china and has been reported to be the third most common opportunistic infection associated with HIV infection in Thailand[94].

The disease most frequently present with generalized popular skin rash associated with fever, weight loss, lymphadenopathy and anemia.

Diagnosis can be made by bone marrow or lymph node biopsy or less commonly by blood culture.

Amphotericin B and itraconazole is the choice of drug.

Viral infections :-

Viral infections are very common in HIV infected individuals, especially those viruses where cell mediated immunity plays a major role in host defence.

✠ Herpes Simplex 1 & 2 :

The herpes viruses are a major cause of morbidity & mortality in patients with HIV/AIDS infections. Initial HSV-1 infection often occurs during childhood, with infection typically developing from infected droplets of orolabial or nasal secretion onto susceptible mucosal surface. HSV-2 infection is usually acquired from sexual activity. Anorectal HSV-2 infection is a frequent cause of protitis in homosexual men.

Clinical feature[95] :

Genital or oral infections with more extensive tissue damage, progressive HSV perianal ulcers, proctitis, colitis, oesophagitis, oral lesions, preumoniae and a variety of neurologic disorder have been observed in AIDS patients.

Virus isolation can be done in tissue culture cells like Vero, rabbit kidney or human diploid cells. Rapidly determined by electron microscopic examination. For rapid diagnosis of skin, mucous membrane lesion scrapings can be stained with Giemsa/PAS, immunoflourescence technique & immunoperoxidase stain also. Specific IgM can be detected by serology. PCR is useful.

✠ Herpes Viruses - 6[95] :-

HHV-6 has been associated with CNS complications like febrile seizures, encephalitis, meningitis and multiple sclerosis. The most reliable source of HHV - 6 is saliva. Clinically HHV - 6 B has been recognized as the cause of exanthema subitum in infant & other febrile illnesses in young children. Infection seen in retinal disorders in HIV infected patients. It has been found in the brain, particularly in the

oligodendrocytes of children with HIV related encephalitis. HHV 6 can be diagnosed by detection of anti HHV 6 IgG or IgM antibodies. Immunofluorescent assay or ELISA & viral load determined by PCR. Virus can be isolated from PBMCS of patients with AIDS, by co-culture with cord blood mononuclear cells.

✠ HHV-8[95] :

HHV-8 is non pathogenic but highly oncogenic in HIV infection and iatrogenic immunsupression. It is involved in the pathogenesis of Kaposi's sarcoma, primary effusion lymphoma and some cases of multicentre Cast leman's disease. The availability of B cell lines infected by HHV-8 permit serological studies in diverse populations. Those assay have strong correlation between HHV-8 and presence of Kaposis' sarcoma or B cell lymphomas of abdominal cavity. Therapy is Acyclovir & alternative treatment is Foscarnet.

✠ Cytomegalovirus (CMV)[95] :-

CMV infection is usually seen late in the course of HIV infection and is one of the most important & frequent cause of severe disease in this patients. Primary infection with CMV can be acquired at any time throughout life, infection evident by presence of CMV antibodies. 30-40% of homosexual men shed CMV in semen or urine irrespective of their status. Viremia may be present in AIDS or ARC patients. CMV retinitis and CMV infection of the gastrointestinal tract such as esophagitis, enteritis and colitis are the most important presentations. CMV retinitis seen in patients with CD4 count 55/mm^3 or less. CMV also causes acute pancreatitis, endocraniopathies & glandular involvement.

CMV can only be cultured in human fibroblast cells. For the demonstration CMV using immunofluorescence. CMV isolated from urine, throat swab, buffy coat, or other tissues Detection of CMV antigenaemia in circulating neutrophils is a sensitive and clinically useful method of detecting viremia. Antigen can detected either by ELISA or immunoflourescence. Detection of IgM antibody & PCR using for serological test.

Therapy Ganciclovir & Foscarnet used.

✠ Varicells zoster virus[95] :-

VZV or Herpes zoster is recognised as a frequent infection in HIV infected patients. VZV is a self limiting infection but in advance HIV severe complications can arise. There can be disseminated lesions of lungs, liver or nervous system leading to pneumonitis, hepatitis, retinitis and encephalitis. Cuteneous infectin is common, may menifest as hyperkeratotic, verrucous lesion. The diagnosis of usually clinical. Immunofluorescent assay used for the detection of virus. Acyclovir & foscarnet used as a treatment.

✠ Epstein barr virus (EBV) :-

The most common menifestation of primary EBV is infectious mononulcosis. Children with HIV/AIDS has discribed the development of aggressive muscle tumors during primary EBV infection[96]. These tumors are result of a clonal expression of smooth muscle cells. Reactivation associated with hematopoietic malignancies such as Burkitts lymphoma CNS lymphoma and nasopharyngeal carcinoma. Virus isolation is rarely used. The EBV Specific antibodies EIAS using. PCR may be useful.

✠ Human Papilloma Viruses : (HPV)

More than 85 types of HPV have been identified. Severe disease with HPV is frequent in patient with HIV infection. The clinical range includes anogenital warts, carcinoma in situ in women, intraepithelial neoplasia and cancer in homosexual men. Sexually transmitted HPVs are probably the most common of all STDs. Although most genital HPV infections are self-limited, & associated with an increased risk of developing genital cancers ie cervical, vaginal, valvar, penile and anal cancers. Endocervical carcinoma have also been associated with HPV infection. The diagnosis of warts is made clinically. As HPV can not be cultivated. Typing detection technique of HPV DNA exist, nucleic acid hybridization and PCR. Dot blot hybridization & Southern blot hybridization can only done in National centres.

✠ Hepatitis Viruses : HBV, HCV[97] :-

Worldwide coinfection with HIV is exceedingly common among persons with hepatitis virus infection. Viruses are present as available for transmission in blood and in genital tract secretions. The highest prevalence of HBV occurs among those engaging in high-risk sexual behavior or intravenous drug abuse or among those requiring frequent exposure to blood or blood products. Defects in CMI in AIDS result in a wide variety of hepatic complications including granulomas, cytomegalovirus hepatitis, multimicrobial AIDS cholangiopathy, Kaposis sarcoma and lymphoma. Kupffer cells are the major hepatic target cell population for HIV. In these patients the differential diagnosis includes opportunistic infections and neoplasms as well as chronic viral hepatitis due to viruses B,C,D, and G and drug hepatotoxicity. Among these chronic viral hepatitis due to hepatitis C virus is frequently seen. It presents a more accelerated course in HIV infected patients leading to cirrhosis and liver failure in a shorter period of time. Decompensated liver disease like encephalopathy, ascitis and jaundice or complications like gastrointestinal bleeding, hapatorenal syndrome and peritonitis are commonly diagnosed.

Other viruses like Adenovirus, Polyomavirus, Molluscum contagiosum, Measles virus & Respiratory synsytial virus are also dials with HIV/AIDS patients.

Bacterial infections :-

✠ Tuberculosis :-

Tuberculosis which had declined in the nineteenth and early twentieth century, has re-emerged during the last two decades of twentieth century on account of the advent of HIV all over the world[98]. The world wide epidemic of HIV infection has resulted in a major secondary epidemic of TB. The first report of TB occurring in patients with AIDS appeared in 1983 when an illness associated with sever immunosuppression was described in a group of Haitian patients in South Florida

24% patients had tuberculosis. Epidemiologic study explained that in many areas HIV infection is prevalent among groups already known to be at high risk for TB : drug users, the homeless and foreign born individuals, they also suggests that there is amore direct association between HIV infection and the development of TB. Overlap in at risk group, increased risk of reactivation of latent M. tuberculosis infection, high rates of infection after exposure and rapid progression to disease the connection between the HIV and TB is important at both individual clinical and societal public health levels. The CDC has recommended therefore that HIV testing should be offered to everyone diagnosed with TB[99]. Among adults, TB is the leading infectious cause of death worldwide and approximately go million new cases are expected during current decade. The majority of these infections occurred in the developing countries of Africa, and Asia, where dramatically high rates of HIV infection and AIDS have been observed. The high rate of coinfection has resulted in TB becoming the most common opportunistic infection associated with HIV disease in many part of the world. In some report more than 50% of AIDS patients in Africa, India and Thailand developed clinical TB[100]. Seropositivity highest among patients between 20-40 years old, especially women in that group & patients with extrapulmonary TB.

✠ Mycobacterium Avium Complex (MAC) :

MAC consists of M. avium, M. intracellular. Infection due to MAC was relatively rare in the pre AIDS era. The most common infection was pulmonary. However, with the advent of AIDS, disseminated infection with MAC is recognized as the most common systemic bacterial infection in patients of AIDS. Disease due to MAC is geographically widespread with reports of MAC in HIV infected patients from North & South America, Europe and Australia[101].

✠ Other nontuberculous mycobacteria :

Very few reports availbale on other NTM infection in patients of AIDS. M. haemophilum infections have been reported, mainly causing erythematous nodular skin lesions, subcutaneous abscesses, lymphadenitis and occasional joint

involvement. M. genavense cause infection rarely leading to bacteraemia in patients of AIDS. M. gordonae one of the least pathogenic mycobacteria also lead to pulmonary infection. Scattered reports are available on the occurrence of M. scrofulaceum and M. celatum infection in AIDS patients[102].

The clinical manifestations of tuberculosis in patients with HIV infection very considerably depending on the severity of the immunosuppression. The manifestation very from typical pulmonary presentation. A productive cough, hemoptysis, fever and weight loss are common symptoms. Signs of cervical, hilar, paratracheal or mediastinal adenopathy are highly suggestive of TB. In pulmonary disease bilateral lower lobe infiltration & plural effusion are more frequent in patients of TB connected with HIV than others. As compared only 10% HIV - TB cases have extra pulmonary manifestations. Lymphadenopathy is very common. Pericardial disease, meningitis and miliary disease may be the other manifestations.

Tuberculin skin test used for screening test. Aspirate, sputum, lavage, swab, blood, CSF, urine, stool all used for the microscopic examination. ZN stain, Kinyoun stain, Fluorochrome stain are used for the smear examination. Growth on solid media LJ and Middle brook 7H10 & 7H11 agar visualise mycobacterial colony. Advance detection by growth in liquid media with the use of radiometric methods (Bactec) Newer diagnostic techniques are DNA probe tech. PCR, Ligase chain reaction are recently used. Other serodiagnostic techniques are ELISA, RIA limited use.

The initial treatment for HIV & TB infection is rifampin, pyrazinamide & ethambutol used. Second line drugs are ofloxacin, Amikacin, Aagmentin, Ciprofloxacin, Capreomycin, Kanamyein, Rifabutin used.

✠ *Other bacterial infections :-*

Persons with HIV infection are more susceptible to bacterial infections because of defects in both cell-mediated and humoral immunity. Bacterial infections, multiple or recurrent, recurrent pneumonia and recurrent salmonella septicemia have been included in the 1993 AIDS surveillance case definition by

CDC. The true incidence of Bacterial infection in HIV infected persons is difficult to discern and varies with population. Disease like pneumonia, Sinusitis, bacteraemia and bacterial enteric infections occur at a rate many times higher than in the general population.

Respiratory tract infections :-

✠ *Pneumonia :-*

The lower respiratory tract is one of the most common sites of bacterial infection observed in HIV infected patients. Pneumonia may be community acquired or nosocomial. Most common bacterial pathogens are S.Pneumoniae, H.influenzae, Klebsiella Spp, S.aureus, P.aeruginosa & other Enterobacteriaceae. Other pathogens that have been moraxella catarrhalis, Nocardia Spp. Rhodococcu equi, Legionella pneumophila etc.

HIV associated respiratory disease includes upper respiratory tract infections, acute or chronic sinusitis, acute or chronic bronchitis and bacterial pneumonias. HIV infected patients having higher rate of complications including intra pulmonary cultivation, abscess formation, empyma and death. Bacterial lower respiratory tract infection are manifested as cough with sputum production, variable fever, chills chest pain etc. Other symptoms may include headache, nausea and myalgias. Bacterial pneumonia appears to be good with appropriate treatment.

Primary diagnosis by direct microscopy with the sputum, bronchial lavage, bronchial aspirate, samples to stain with Gram stain, modified acidfast stain, Gomoris methamine silver stain (Nocardia) and Toludin blue (Actinomycetes) are used. Direct fluorescent antibody staining can be done for Legionella. Bacteria can be isolated on routine culture media. For Legionella used charcoal yeast extract agar. specific bacterial infection conformed by the cultural characteristic.

Trimethoprim sulfamethoxazole choice of treatment. 2nd generation cephalosporins - cefuroxime & erythromycin have been used.

✠ *Bacteraemia :-*

Bacteraemia may indicate the presence of a focus of infectious disease or may merely present transient release of bacteria into the blood stream. Bacteraemia may be transient, continuous or intermittent. Cause of continuous bacteraemia include septic shock, bacterial endocarditis, early stage of typhoid fever, brucellosis, leptospirosis etc. Intermittent bacteraemia occurs in meningitis, pneumonia, Osteomyelitis, undrained abscesses etc.

The clinical manifestations of septicemia may include fever, chills, hyperventilation, skin lesion and lead to hypotension or shock, major organ system failure and disseminated intra vascular coagulation. AIDS patients have recovered bacterial agents are S.aureus, enterococci, Pseudomonas, Sligella, Salmonella, Bartonella Sp., corynebacterium, Listeria monocytogens. Enteric pathogens recovered from HIV patients includes Salmonella, sligella, campylobacter, E.coli, clostridium difficile, V.cholerae, S.aureus, plesiomonas, yarsinia and Aeromonas.

Blood culture is the main criteria for the bacterial infection. Trypticase soya broth, Brain heart infusion broth is used for culture. Isolation of fastidious and rarely isolated organisms recovered successfully from blood culture. For the shigellosis stool examination is required. Direct microscopy & stool culture are routine methods.

CNS infections :-

Infections of the CNS usually occur as part of disseminated disease. The major causes of CNS infection in HAV patients may be conventional organisms or unusual agent of meningitis including established opportunistic pathogens. S.pneumoniae, S.aureus, Nocardia Spp., Listeriamonocytogens, Various gram negative bacilli, Treponema pallium, Actinomycets Spp etc are infecting organisms. For the identification CSF should be examined for sugar, protein level & cell count, direct microscopy, culture & antigen detection tests using.

Some of the bacterial Spp. that are more likely to be associated with opportunistic infection in AIDS/HIV are Nocardia, Rhodococcus equi, Legionella Spp. Listeria, Corynebacterium, camplylobacter & Bortonella Spp.

Prevention of the bacterial infection use vaccinations and antibacterial prophylaxis.

TUMORS IN HIV INFECTION

✠ *Kaposi's Sarkoma & HIV :-*

In 1981 AIDS was initially recognized by the outbreak of Kaposi's sarkoma[103]. In 1972 the Hungarian physician Moriz Kaposis first described this disease as a multiple idiopathic pigmented hemangiaosarcoma. He recognised disease as a rare, chronic, coetaneous disorder affecting men, the nature of disease is multifocal, the occurrence of visceral involvement and the vascular nature of the tumor. This form of KS is known as classic KS, occurs mostly in Eastern Europe and N. America. HIV infected homosexual and bisexual men infected with an aggressive from of KS known as epidemic or AIDS associated KS. Endemic KS is most common in black Africans. Iatrogenic KS has been reported with increasing frequency in children & adults because of increased number of organ transplantation case & more frequent use of immune-suppressive therapy for autoimmune disorder and chemotherapy for cancer. Mucocutaneous KS has also been reported in children who developed HIV infection through blood transfusions. In Africa AIDS associated KS transmitted by heterosexual contact.

Four categories of KS are recognized : Classic, African endemic, iatrogenic and AIDS associated. Classic KS manifests with blue, reddish purple machules, plaques or nodules and tumors that produce fungating masses with ulceration, on the extremities, feet and lower leg, any where on the skin. In iatrogenic KS cutaneous, lymphatic, and visceral dissemination occur. Clinical course similar to classic KS. AIDS associated KS involve any location on skin, lymph nodes, gastrointestinal

tract & other visceral organs. KS is first sing of HIV infection. Pulmonary KS manifest with intractable cough, bronchospasm and respiratory insufficiency. Progression of KS very greatly with clinical form. Classic KS follow slow and indolent course, Endemic KS follows a more aggressive course, AIDS associated KS have a rapidly progressive course. Advance biotechnology have made it possible to investigate the cellular & molecular events leading to progression of KS.

No effective treatment is available for HIV - associated KS. May consist of vinblastine, vincristine, etoposide, doxorubicin and bleomycin, alone or in combination.

LYMPHOMAS AND OTHER CANCERS :

✠ *Non - Hodgkin's lymphoma (NHL)[104] :*

NHL occurs more frequently in AIDS patients than in other groups. These lymphomas are generally of B-cell origin and include Burkitt lymphoma. They are frequently localized in extranodonal sites, including CNS, the bone marrow, and the bowel. These lymphomas are generally high grade malignancies and respond poorly to chemotherapy. EB virus, together with the immunodeficiency associated HIV infection probably plays a major role in development of these B-cell lymphomas.

AIDS RELATED MALIGNANCIES :-

✠ *Primary CNS lymphoma :-*

Primary CNS lymphoma in 1 to 3% patients with AIDS[105]. Patients may have dramatic focal neurologic deficits, headaches, and seizures, including memory loss, confusion and lethargy. The primary therapy is radiation therapy, followed by procarbazine, lomustine, and vincristine chemotherapy.

✠ *Hodgkin's disease :*

An atypical aggressive form of Hodgkin's disease probably occurs with greater frequency in patients with AIDS. Bleomycin, vincristine, etoposide, and streptozoicn achieved a complete response.

✠ ***Cervical and Anal cancers :***

Cervical intraepithelial lesions associated with HPV infections more frequently among women with HIV infection[106]. There is evidence that homosexuality and receptive anal intercourse are important risk factors for anal rectal cancer. Associated between these tumor and HIV infection to be expected.

✠ ***Miscellaneous tumors :***

Numerous case reports of malignancies occurring among HIV infected adults, most notably cancers of lungs, testes and skin. Smooth muscle tumors rarely occur in children[107].

PEDIATRIC HIV INFECTIONS :

HIV infection is often more accelerated in children and in some infants, symptoms can manifest during the first months of life, because many organ especially brain, yet not mature. Pediatric infection should be considered a family disease. World wide about 2.5 million children living with HIV/AIDS, 700000 children are newly infected in 2003[108]. About 500 000 children's death due to AIDS in 2003. It's seventh leading course of death in children. Vertical transmission is main route, account 98% case pediatric infection. Transmission through blood product has become rare. Heterosexual transmission (13-19 year) adolescent is 41% with HIV infection. AIDS in children the most current definition of AIDS is age dependent decline CD4 counts, and differentiate mild, moderate and severe degree of clinical symptoms. New classification system ranges from a stage with no clinical impairment (No) to one of severe clinical & immunological suppression (C3) Table.

Table - VII
Pediatric HIV classification CDC 1994[109]

Immunologic Categories	***Clinical Categories, Based on Signs & Symptoms***			
	N (None)	***A (Mild)***	***B (Moderate)***	***C (Severe)***
1. No evidence of suppression	N1	A1	B1	C1
2. Moderate suppression	N2	A2	B2	C2
3. Sever suppression	N3	A3	B3	C3

Table - VIII

New definition : Based on age specific CD4 counts CDC 1994[109]

Immunologic Category	*Age of Child*		
	<12 months cells/mm^3	*1-5 years cells/mm^3*	*6-12 years cells/mm^3*
1. No evidence of suppression	≥ 1500	≥ 1000	≥ 500
2. Evidence of moderate suppression	750-1499	500-599	200-499
3. Sever Suppression	< 750	< 500	< 200

Category N **:** Not symptomatic : No sign & symptoms consider to be result of HIV infection or who have only one of the conditions listed in Category A.

Category A **:** Mild symptomatic children with 2 or more of the condition listed : Lymphadenopathy Hepatomegali, Splenomegali, Dermatitis, Parotitis, Recurrent URTI, Sinusitis or otitis media.

Category B **:** Moderately Symptomatic : Children who have symptomatic conditions other than A & C that are attributed to HIV infection. Anemia, Bacterial meningitis, pneumonia, candidisasis Cardiomyopathy, Diarrhea, Hepatitis, CMV infection, HSV Stomatitis, shingles, Leiomyosarcoma, LI, Nephropathy, Nocardiosis, persistent fever, Toxoplasmosis, Varicella.

Category C **:** Severely Symptomatic :Children who have any condition listed in surveillance case definition including serious bacterial infections. Fungal infections, Viral infections, protozoal infections, Kaposi's sarcoma, Lymphomas, mycobacterial infections, wasting syndrome & pneumocystic carini pneumonia.

Table - IX

AIDS Case definition : For surveillance 1987 CDC. (up to 12 years of age)[110]

1. Two positive tests for HIV infection (E/R/S) in children older than 18 months or confirmed maternal HIV infection for children <18 months.
2. Presence of at list two major and two minor signs in the absence of known causes of immunosuppression.

Major Signs :

(a) Loss of weight

(b) Chronic diarrhoea > 1 month duration.

(c) Prolonged fever > 1 month duration.

Minor Signs :

(a) Repeat common infections (pneumonitis, otitis, phargngitis)

(b) Generalised lymphadenopathy,

(c) Oropharyngeal candidiasis

(d) Persistent cough more than 1 month,

(e) Disseminated maculo popular dermatosis.

A Child older than 18 months of age can be diagnosed by ELISA, W. blot, IFA test, maternal antibodies measurable in the child for a median of 13.3 (10.4 to 15.6) months. PCR & virus culture can identify 30 to 50% shortly after birth & 100% by 3-6 months. Zodovudine approved for the use in children with symptomatic HIV infection.

EPIDEMIOLOGY :-

GLOBAL ASPECT :-

The epidemic of HIV infection and AIDS emerged in the last quarter of the 20th century, and within two decades has affected over 190 countries[111]. HIV distribution is characterized by a marked heterogenecity among continents and countries or even within a single country, with geographic areas of HIV prevalence of up to 30% of the adult population contiguous with areas of much lower prevalence[112]. These differences are producing a checkered pattern of visible impact, with AIDS the leading cause of adult deaths in some population and HIV effects not yet detectable in others. The study of trends in HIV spread has contributed to a better understanding of the dynamics and impact of the epidemic in various part of the world. The data indicate the emergence of new epidemic foci shifts in

transmission pattern in established epidemics and suggest that prevention efforts may be influencing the overall course of the pandemic.

The world health organization estimated that during 2000 some 5.3 million people became infected with HIV and about 3 million deaths from HIV/AIDS[113]. It is a higher global total than in any year since the beginning of the epidemic. Around half of the people who acquired HIV become infected before they turn 25 and typically die of the lifetime called AIDS before their 35th birthday. This age factor makes AIDS uniquely threatening to children. The overwhelming majority of people with HIV, some 95% of the global total, live in the developing world. They grew even further as infection rates continue to rise in countries where poverty, poor health system and list prevention and care fuel the spread of the virus. So it is clear that HIV will continue to be a global problem well in to the twenty first century.

The global AIDS epidemic shows no signs of abating 5 million people became indicated with HIV worldwide and 3 million died this year 2003[114] alone the highest ever. One in 5 adults across southern Africa are now living with HIV/AIDS, the higher rate since the beginning of the epidemic. While infection rate across Sub-Saharan Africa very widely, from less than 1% in Mauritanina to almost 39% in Botswana an Swaziland, the breadth of the epidemic indicates that HIV/AIDS now has a firm hot on most countries in the region.

According to the new report, an estimated 40 (between 34 and 46) million people are living with HIV worldwide, including 2.5 (between 2.1 and 2.9) million children under the age of 15. Globally, an estimated 5 (4.2 - 5.8) million people were newly infected and 3 (2.5 - 3.5) million people died of AIDS in 2003[114]. Sub Saharan Africa the most severely affected region of the world, accounted for over 3 million of these new infection and 2.3 million AIDS deaths. Everyday in 2003 an estimated 1400 people were newly infected with HIV. More than 95% of those live in low and middle-income countries (Table - X).

Table - X
Global summary of the HIV /AIDS epidemic, December 2003

Number of people living with HIV / AIDS	Total	40 million (34-46 million)
	Adults	37 million (31-43 million)
	Children under 15 years	2.5 million (2.1-2.9 million
People newly infected with HIV in 2003	Total	5 million (4.2-5.8 million)
	Adults	4.2 million (3.6-4.8 million)
	Children under 15 years	700000 (590000-810000)
AIDS deaths in 2003	Total	3 million (2.5-3.5 million)
	Adults	2.5 million (2.1-2.9 million)
	Children under 15 years	500000 (40000-580000

PATTERNS OF INFECTION AND TRANSMISSION :

The pandemic of HIV/AIDS reflects many coexisting subepidemics in different regions and populations. Many epidemiological, socio-demographic, behavioral, and biologic factors contribute to the differential spread of HIV. The character of each subepidemic remains determined largely by the extent to which people are exposed to HIV by one or more of the three primary modes of transmission : (a) sexual (vaginal, anal an oral); (b) parental, that is, by injection (including the sharing of drug insetting equipment), transfusion, or transplantation of HIV infected blood, blood components, tissues or organs, and (C) maternal infant, from a woman to her fetus or infant. The vast majority of HIV infections in the world is transmitted sexually, most often through heterosexual intercourse. Notable exceptions occur, particularly among certain populations in industrialized countries in North America, Europe, and the Pacific Basin, where transmission has occurred predominantly through male to male sexual contact and injecting drug use. The area characterized by these later transmission modes typically have had a predominance

of HIV -1 subtype B, the other subtypes appear to predominate in most of the areas where heterosexual transmission prevails[115].

Geographic distribution of recognised HIV-1 group M subtype are not well documented. By 1995 the relatively small number of patients reported with group O infection had come primarily from central Africa, particularly Camroon[116].

Many factors influence the risk of sexual transmission, including specific sexual practices, the presence of other sexually transmitted infections and the stage of HIV infection in the source partner.

Mother to infant transmission has become common in areas with high rates of HIV infection among women of childbearing age. In the absence of interventions, about one quarter to one third of infants born to HIV -1 infected women become infected, regardless of the subtype of HIV - 1 involved[117]. Most transmission occurs during pregnancy or at delivery, breastfeeding also has contributed to overall mother to child transmission, at least of HIV - 1[118].

The transfusion of HIV infected blood, notably to children with malaria-associated anemia accounted for up to 10% of new HIV infections earlier in the epidemic[119]. Although screening for HIV in transfused blood is generally available, the window period during which recent infection is not detectable as well as interruptions in the supply of HIV test kits and laboratory error continue to contribute to an HIV risk. The transmission risk posed by clinical injections is difficult to quantify but is thought to account for some infections and has been associated with significant outbreaks[120].

In relatively short time, the epidemic has evolved quickly and differently in various regions of the world.

EPIDEMIOLOGY BY REGION :-

✠ *Sub-Saharan Africa :-*

Africa, the global epicenter, continues to dwarf the rest of the world on the AIDS balance sheet. Since the start of the epidemic, 83% of all AIDS deaths so far have been in this region. At least 95% of all AIDS orphans have been African. While no country in Africa has escaped the virus, some are far more severely affected than others. The southern part of the African continent holds the majority of the world's hard hit countries.

As of end 2003, the WHO estimated that 25 to 28 million Adult and Children living with HIV/AIDS in sub Saharan Africa[121]; constituting 75% of the global total. Who also estimated that 3 to 3.4 million people are newly infected with HIV. East and Central Africa total 50% to 60% of the HIV infections that have occurred in sub-Saharan Africa, despite accounting for only about 15% of the total population of the region. Epidemiologic evidence indicates that heterosexual intercourse is the predominant mode of transmission with little transmission recognised from male to male sexual exposure or injection drug use. HIV seroprevalence rate in pregnant mothers was found to be over 20% in majority of sentinel sites in this country.

✠ *Western Europe, North America, Australia and New Zealand :-*

In western Europe 520000-680000 million people living with HIV/AIDS, and 30,000-40,000 Adults and Children were newly infected with HIV and 2600-3400 Adults and child deaths due to AIDS as of end 2003[121].

In North America 790000 - 1.2 million people living with HIV/AIDS and 36000-54000 people were newly infected in 2003[121].

HIV-Infection spread rapidly in this region in the early 1980S primarily associated with male to male sexual exposure and injecting drug use, and subsequently by heterosexual transmission.

Australia & New Zealand estimated 12000-18000 people infected with HIV/AIDS and 700-1000 was newly infected in the year 2003[121]. Infection with homosexual men predominantly affected.

✠ *Latin America and The Caribbean :-*

As of end 2003 WHO estimated 1.3 - 1.9 million people in latin America & 350000-590000 people living with HIV/AIDS in caribbean. Prevalence in recent year 2003 in Latin America is 120000-18000 and 3.50000-590000 in caribbean[121]. Most infections in Latin America were initially among homosexual or bisexual men, now it has been increasing heterosexual transmission. In Arjentina the prevalence of HIV infection among IDUs from 30% to 50% and in Brazil from 20% - 60%[122]. In most of the Caribbean, heterosexual transmission has been the predominant mode of transmission.

✠ *South and South - East Asia :*

Although the extensive spread of HIV in south & south east. Asia began only in the mid 80's, the progression of the pandemic in this region has been rapid in several populations. WHO estimated that 4.6 - 8.2 million people living with HIV/AIDS and 610000-1.1 million adults & children newly infected in the year 2003. The first epidemic in Thailand began with rapid increase in HIV-1 seroprevalence among IDUs in Bangkok from 1% to about 40% by the end of 1988[123]. The second wave of epidemic occurred primarily among female commercial sex workers in 1990. The median seroprevalence increased from 3 to 9% with more than 60% infected in some group of prostitute in northern Thailand[124]. Commercial sex was major source of both homosexual and heterosexual transmission. Studies in China have shown rapid heterosexual transmission. Studies in China have shown rapid spread of HIV-1 among injecting heroin users in Western Yunnan province, a region that shares a border with Myanmar (Barma)[125]. Myanmar, Vietnam, Cambodia and Indonesia show increasing HIV rates among commercial sex workers[126]. In Cambodia, the epidemic is spreading mainly through heterosexual contact, with prevalence among blood donors of 3.5%. In Vietnam predictably rapid increase in seroprevalence were observed among IDUs with more than tenfold increase from 2% to 30%, and there is concern that heterosexual

transmission of HIV will follow a pattern similar to that of Thailand. The extent of the epidemic is less clear in Bangladesh, Pakistan and Indonesia.

✠ ***East Asia and Pacific :***

The WHO estimated that there were 700000-1.3 million people living with HIV/ADS and 150000-270000 people new infected in East Asia and Pacific region by end 2003[121]. A large proportion of the reported AIDS cases in Japan have been among persons with hemophilia who were transfused with HIV-infected blood products[127]. There is diversity in transmission mode across the region, in Papua New Guinea more than 70% of those infected through heterosexual transmission. In Yunnan Provicne is an outbreak of HIV infection among IDUs.

✠ ***Eastern Europe and Central Asia :***

The magnitude of the HIV problem in this region poorly defined. Most infection were attributed to heterosexual transmission in St. Petersburg & Russia. In Russia[128] - Kalmykia outbreak in 1988 several hundred children infected through shared syringes Contaminated with HIV infected blood. In 1989 Romanian outbreak, in which several thousand children were involved transmission thorough transfusions of unscreened blood.

In some Eastern European countries injecting drug use is associated with very high rates of HIV infection.

In Warsaw, Poland found seroprevalence of 46% among IDUs[129]. Who estimate 1.2-1.8 million people are living with HIV/AIDS and 180000-280000 people were newly infected in 2003[121].

✠ ***North Africa and Middle East :***

As of and 2003 the WHO estimated that about 43000-67000 people newly infected with HIV. And 470000-730000 people living with HIV/AIDS in the region. In morocco HIV prevalence among blood donors was 0.01% in 1993[130]. In Nouakchott, Mauritania the prevalence among blood donors was 4% 1992[131]. In Eritrea, indicated prevalence 25% among sex workers, 2.7% among blood donors and 3% among antenatal clinic attenders (Table - XI, Fig. 4).

Table - XI

Regional HIV / AIDS statistics and features, end of 2003

	Adults and Children living with HIV/AIDS	Adults and Children newly infected with HIV	Adult prevalence rate (%)*	Adult and child deaths due to AIDS
Sub-Saharan Africa	25.0-28.2 million	3.0-3.4 million	7.5-8.5	2.2-2.4 million
North Africa and Middle East	470000-730000	43000-67000	0.2-0.4	35000-50000
South and South-East Asia	4.6-8.2 million	610000-1.1 million	0.4-0.8	330000-590000
East Asia and Pacific	700000-1.3 million	150000-270000	0.1-0.1	32000-58000
Latin America	1.3-1.9 million	120000-180000	0.5-0.7	49000-70000
Caribbean	350000-590000	45000-80000	1.9-3.1	30000-50000
Eastern Europe and Central Asia	1.2-1.8 million	180000-280000	0.5-0.9	23000-37000
Western Europe	520000-680000	30000-40000	0.3-0.3	2600-3400
North America	790000-1.2 million	36000-54000	0.5-0.7	12000-18000
Australia and New Zealand	12000-18000	700-1000	0.1-0.1	<100
Total	40 million (34-46 million)	5 million (4.2-5.8 million)	1.1% (0.9-1.3)	3 million (2.5-3.5 million)

*The proportion of adults (15 to 49years of age) living with HIV/AIDS in 2003, using 2003 population numbers.

✠ *Epidemiology of HIV/AIDS in India :-*

The presence of HIV was not noticed in Asia and India until the late 1980s, South East Asia has now become the epicenter of the HIV/AIDS pandemic. The onset of the HIV epidemic in India was heralded by the detection of an HIV-infected commercial sex worker and by a case of AIDS in Chennai and Mumbai, respectively in 1986. Several epidemiological studies conducted in India between 1986-1996 established that hetero-sexual transmission was the dominant mode of transmission of HIV infection. Other modes included transmission through blood and blood products, through sharing of needles and syringes among intravenous drug users and

from mother to baby. Evidence suggesting the presence of parallel epidemics of both HIV-1 and HIV-2 came from a study conducted Mumbai in 1990. Subsequent studies among sexually transmitted disease clinic attenders in Mumbai have shown that HIV strains were distributed as HIV-1 in 80%, HIV-2 in 6.0% and dual 14% of infected persons with subtype C being the dominant HIV-1 strain among heterosexual in southern India.

There phases of the HIV epidemic have already unfolded in several parts of India. Including Maharashtra, Tamil Nadu and Manipur. In the first phase, which took place during 1980s infection occurred among more venerable groups such as CSW and their clients, intravenous drug users, gaymen and single migrant workers. The second phase saw infection among population groups where cases of HIV infection could not be directly traced back to vulnerable group and occurred in the 1990s. In the third phase, paediatric HIV infection has emerged as a result of substantial levels infection among women of childbearing age.

Within a geographic area, as one epidemic matures there is the likelihood of waves of subepidemics, with variations in infections rates among Vulnerable groups. Across the regions, there is also uneven distribution of HIV infection rate. Factors such as the presence of vulnerable groups, the size of these groups, links to other population groups and socio-cultural and sexual practices influence the occurrence of HIV infection in different populations. The associated epidemics due to tuberculosis and STD are likely to compound the impact of the HIV pandemic.

In India has a population of one billion. The spread of HIV in India has been diverse, with much or India having a low rate of infection and the epidemic being most extreme in the Southern State. 96% of the total number of nationally reported AIDS cases were found in 10 of 28 state and 7 Union Territories, the worst being Maharashtra in west, Tamil Nadu and Pondicherry in the south, and Manipur in the north east. In Maharashtra and Tamil Nadu the infections are mostly due to heterosexual contact, while infections are mainly found amongst injecting drug a users and their sexual partners in Manipur.

NACO estimate about 5 million people are living with HIV/AIDS infections. NACO estimate about 5 million people are living with HIV/AIDS infections, and 62785 cumulative AIDS cases reported on Jan 2004[132].

✠ *AIDS in Gujarat :*

In Gujarat, the first HIV positive case was reported in August - 1986. The number of AIDS cases being reported has been on the rise since 1991 and today there are 3596 cases in Gujarat, reported to NACO Jan. 31st 2004. Gujarat State AIDS Control Society (GSACS) was registered in 1999 and is responsible for the programme management for HIV prevention, surveillance and control.

✠ *Global distribution of HIV-2*

Since 1986, several West African Countries have reported significant rates of HIV-2 infections. The discovery of HIV-2 in East Africa prompted numerous serologic surveys to identify its geographic distributions. During the past decade, significant HIV-2 infection has been well documented in most West African countries. A second epidemiologic pattern of HIV-2 infection has been suggested from reports of HIV-2 in Portugal and in Mazambique, Angola, Southwestern India, and Brazil, low prevalence rates have been documented in other parts of Africa, Europe, the American, the middle East and Asia. Guinea Bissau and Cape Verde both HIV-2 endemic regions. Aside these two exceptions HIV-2 has been virtually absent in Central & East Africa. The rates of HIV-2 infection in Portugal and Spain are distinctly higher than other European countries.

In Portugal HIV-2 accounted for 10% of 12% HIV infections and 48% of HIV-2 infected persons had like with Angola. In the HIV-2 infected persons whom no link with Africa was established, presumed risk factors were transfusion of blood or blood products (74% of cases), heterosexual contact (21% of cases), IDUs (4% cases). France was one of the first countries to institute HIV - 2 testing of blood donors, and several HIV-2 cases were identified. In North America, only sporadic cases of HIV-2 infection have been reported. Some HIV-2 infections were identified in Cuban soldiers returning from campaigns in Africa.

India : The rate of HIV-2 is significant in the Maharashtra State of India. Of the HIV-Positive samples from patients with STDs or other conditions in Bombay, 4% were reactive to HIV-2 and 20% to both viruses. A later study men attending an STD clinic with a history of homosexual behavior reported a 4.8% rate of HIV-2 infection and 3.2% of dual HIV infection. Goa, reported a 4.9% rate of HIV-2 infection among STD patients.

GENETIC DIVERSITY OF HIV :

Human Immunodeficiency virus is a primate retrovirus of the Lentivirinae sub family a group that shares the ability to infect their hosts chronically and progressively damage the host's immune system. Genetic variation of HIV is extremely high, with rapid turnover of HIV virions, and infected persons maintain a substantial viral burden during the entire course of infection[134]. Related strains share genetic similarity, as expressed by the sequence of component nucleotides, and methods have been developed to infer the phylogenetic relationship between different strains. Two major viral types have been characterised in humans : HIV type -1, the predominant HIV type throughout the world, and HIV type - 2, first reported and still found primarily in persons from West Africa[135].

Based on viral genetic sequences, HIV-1 isolates have been classified in to a number of subtypes alternatively termed clades or genotypes). This subtypes, designated by alphabetical letters, Constitute the major group of HIV-1, group M (10 subtypes A through K)[136] and group O (g subtypes) and group N (New Virus). HIV-2 has also been classified in to at least five subtypes (A to E)[137] (Fig. 5).

Most of the HIV-1 subtypes have been found in Sub-Saharan Africa. subtypes A, C, and D have bee found more frequently than others, although others have occasional isolates. In Thailand HIV-1 subtypes B was detected in IVDU's during the mid 80's. During the late 1980's subtype E was first detected. Mid 1990's subtype E had spread very rapidly among heterosexuals[138]. HIV-1 subtypes B never spread to cause a major heterosexual epidemic as did subtype E.

A similar situation occurred in India, with HIV-1 subtypes B and C. Although B apparently was introduced earlier and expanded among IVDU'S, this subtype did not appear to spread as rapidly among heterosexuals as did HIV-1 C. Previously associated with a massive heterosexual epidemic in southeastern Africa, subtypes C also caused a rapidly - spreading heterosexual epidemic in Western India, evidently originating from the Bombay region[139]. The results in Africa and Asia suggest that HIV-1 subtypes A, C, D and E are well adapted for heterosexual transmission but subtype B is less efficiently transmitted by this route. Whenever HIV-1 moves very rapidly through a new population, as has happened in Asia for subtypes E and C, the viruses isolated show relatively less diversity.

An even more distant subtype, designated HIV-O, has been detected in Cameroon[140]. The viruses isolated from this subtype are less related to HIV-1 subtypes. A through H than the other subtypes are related to each other, yet, HIV-O is more related to HIV-1 than to HIV-2[141]. To emphasize this distance, HIV-1 subtypes A through H are designated the major group, and HIV-O is designated the out group. Although HIV-1 subtypes A through H probably entered independently from a chimpanzee host, and HIV-2s almost certainly entered independently from Mangabey monkey species West Africa. Although some HIVS apparently entered human population independently from subhuman primate hosts, others presumably emerged as recombinants from within a single human host. HIV-1 subtype E is a recombinant with a gag and pol gene region from HIV-1 subtype A but a distinctly different envelope, presumably from a different human progenitor virus that has not yet been identified. Although rare, dual infections have been described with different

clades of HIV-1 in the same human host. Instances of dual infection with HIV-1 and HIV-2, have also been described, although infection with one type appears to offer some protection against subsequent infection with another.

Simon F et al describing identification of a new group of HIV-1 designated from an isolate YBF 30 obtained from Cameroonia Woman suffering from AIDS. Her serum did not show anti HIV-1 antibody reactivity to specific HIV group M EIA but was reactive to the third generation EIAs and HIV-1 Western blot. They developed a V3 loop peptide enzyme immunoassay. The genomic studies revealed that YBF 30 structural genes are equidistant from HIV - 1 M and more distant from HIV - 1 O. So they suggested that this new group should be name as "N". N falls between M and O and also can imply that it is a new strain[143].

The Global dispersion patterns are :

- Clade B predominants in united state, Haiti, and Western Europe, Clade B also predominants in Brazil, but clade F and C is not uncommon.
- Eastern Europe have been other than B, including clades A,D,C,F and G.
- Clades A and D predominate in most of Sub-Saharan Africa but in Central Africa Six other clade recovered.
- Clade C predominates in Southern Africa, the Horn of Africa and in West Asia (Bombay).
- Clade B predominates in most of East Asia.
- Clade E predominates in Thailand.

The clear predominance of distinctive genotype i particular countries does permit some inferences about the global spread of HIV-1. The HIV-1 epidemics in Latin America, Europe, and East Asia may have originated in the United State[145], because clade B predominates in all these regions. However, it is highly unlikely that the HIV-1 epidemics in India (C genotype) originated in the United States or Europe[146]. A more likely source is one of the C clade epidemics in Southern Africa. The E clade epidemic in Thailand and Southeast Asia may have originated in the equatorial Africa, where clade E viruses are also found.

EPIDEMIOLOGY OF DISEASE PROGRESSION[147] :

HIV disease is a continuum of progressive damage to the immune system from the time of infection to the manifestation of severe immunologic damage by opportunistic infections, neoplasms, wasting or low CD4 lymphocyte count that define AIDS. The time it takes to traverse this spectrum varies greatly, ranging from 1 year or less in some persons to a still unknown upper limit in others that has reached nearly 20 years in a few individuals. The period from infection to development of AIDS is known as the incubation period. The period from an AIDS diagnosis to death has been studied separately as AIDS survival time. The epidemiology of HIV disease progression has attempted to characterize the distribution of possible lengths of the incubation period and the AIDS survival period, to identify laboratory test useful for prognosis and treatment decisions, and to determine cofactors accelerate or retard the rate of disease progression.

✠ *Incubation Period :-*

The median incubation period from HIV infection until development of AIDS is estimated at approximately 10 years for young adults. The estimate varies with the age at which infection occurs and is significantly shorter in infants and in older adults and varies even between infection at age 20 and infection at age 40. Whether the incubation period varies by mode of HIV acquisition has been more difficult to determine, but the preponderance of evidence now indicates that, after adjustment for age, the incubation period is similar in injecting drug users, those infected sexually, and hemophiliacs, whereas incubation time in transfusion recipients is shorter, probably because of the large HIV inoculums in infected blood transfusions. The incubation period does not appear to very significantly in men and women or in different racial groups.

Rates of progression to AIDS are very low in the first 2 years after infection and increase thereafter. Although patients infected by transfusion, especially infants, have developed AIDS in the first year after infection, progression to clinical AIDS

in healthy adults is rare within 2 years of seroconversion. The progression rate increasing for the first 7 years, after which they level off or even drop slightly.

✠ ***AIDS Survival Time :-***

The time from first diagnosis of AIDS to death has been characterized separately from the incubation time from infection to AIDS as AIDS survival time. Incubation time has been altered by the introduction antiviral therapies and prophylaxis for opportunistic infections, but AIDS survival time was affected earlier by treatments. AIDS survival time is a mean or median value of survival times among the large of disease diagnoses that define AIDS. Knowledge of median AIDS survival time may not be prognostic for survival in an individual patient. AIDS defining diagnoses have a wide range of average survival times. The median survival after a single AIDS - defining condition ranged from 3 to 51 months, its further broadened in lower CD4 count is on average nearly 2 yrs. The rate of long-term survival after an initial AIDS diagnosis has been very low. Before 1986 showed median survival time past initial AIDS diagnosis of 10 to 13 months. Overall median survival was 11 months. For patients with opportunistic infections, median survival was 9 months. Median survival after an initial Kaposis Sarcoma diagnosis was 16 months.

Age is a cofactor for shorter survival. Survival was significantly shorter among all patients over 40 yrs old.

Improved median AIDS survival time was observed in diverse geographic locations. Some early improvement in AIDS survival occurring early diagnosis & greater awareness of symptoms and increased use of HIV testing. It is also increased by anti retroviral therapy. The use of the combination therapy is having a significant effect in lengthening survival time.

✠ ***Laboratory Markers :***

The length of the AIDS incubation period means that laboratory tests to identify persons at high risk of disease progression are needed to guide clinical decisions in asymptotic seropositive persons, such as when to begin antiviral therapy

and prophylaxis against OI. Because depletion of CD4 + T lymphocyte is the hallmark and the apparent source of the central immune defect of HIV disease, determination of the CD4 lymphocyte count has been the most important laboratory marker of disease progression. Absolute CD4 lymphocyte count correlates strongly with AIDS - defining disease, has been included in the surveillance case definition of AIDS since 1993, and has been used to set indication for therapy.

Virus accurate measurement of the quantity of viral RNA in the peripheral blood has become as important laboratory marker as CD4 lymphocyte count and is now the primary marker for antiretroviral treatment decisions. The concentration of HIV in peripheral blood is very high during the primary infection / seroconversion phase, but it stabilize shortly after seroconversion and changes relatively slowly thereafter. The result of these analyses has been to make HIV RNA blood levels the primary surrogate marker for the effectiveness of therapy in controlling HIV disease.

In addition to the CD4 lymphocyte count and the quantity of HIV in peripheral blood, other laboratory test, primarily measures of generalized immune activation, have been shown to predict AIDS in asymptomatic HIV infected persons. Serum levels of β2 - microglobulin, serum and urine levels of neopterin, soluble CD8, Soluble interleukin - 2 receptor, interferon - α, and serum levels of IgA predict development of AIDS.

✠ ***Cofactors :-***

Endogenous biologic or psychologic factors, other infections, behaviors, or other environmental factors that alter the natural history of HIV infection may be cofactors for disease progression. Many potential cofactors for HIV have been investigated. They include genetic factors, age, gender, route of HIV infection, drug use, smoking, nutrition, and other infectious diseases.

Genetic differences in HLAs have been studied as potential cofactors. Age was identified as a cofactor for HIV progression. The estimated effect of age on the risk of AIDS was 1.5 per every 10 years of age. Opportunistic disease that may

occur in the course of increasing HIV immunosuppression are largely caused by infectious agents.

Whether the occurrence of disease from OI, either reactivated or newly acquired, is simply a marker of HIV mediated immunosuppression or whether these infections act, in turn, as cofactors changes host control of the infections agent, resulting in disease, and disease process in turn activates HIV.

Other cofactors are behaviors such as drug use, smoking and poor eating habits. Evaluating Psychological measures, such as depression, as cofactors has an analogous problem.

LABORATORY DIAGNOSIS OF HIV INFECTION :

The HIV continues to spread around the world, causing nearly 1600 new infections a day, with infection rates rising rapidly in much of Asia. Laboratory diagnosis is the only method determining the HIV infection status of an individual during the long asymptotic period.

COLLECTION & TRANSPORT OF SPECIMEN[148] :

Almost all the laboratory procedures for HIV testing are performed on patients blood, serum or plasma, hence collect the blood aseptically. Allowed clotting for 30 min. and centrifuge of 3000 rpm for 10 minutes. Preservative should not be added. If necessary 5 bromo, 5 nitro, 1-3 dioxane in propylene glycol at a final concentration of 0.05% is recommended. Sample are place in leak proof containers at 4° C for storage. Transport : The specimen tube in which serum is to be transport, should have leak proof, plastic screw capped. Disinfect outside area of container. Tube is labeled, place in second tightly capped unbreakable, labbeled container. This is placed in tharmocol box having ice packs. Performa with detail name, age, sex, risk factor, history of previous testing etc should accompany the specimen. A biohazard symbol must be fixed outside the box. Transport of whole blood for virus isolation& CD4, CD8 studies : Same as above, transported in wet ice

so as to reach the laboratory within 24 hrs. For virus isolation, if the specimen cannot be transported immediately after collection, it should be frozen (Fig. 6).

fig. 6

HIV testing must always be undertaken after pre test counseling and informed consent. The confidentiality of the test result should be strictly maintained in most cases.

PURPOSE OF HIV TESTING[150] :

1. Information is useful for prophylaxis, medical management and treatment.
2. To assure blood safety and donation safety.
3. To monitor trends of epidemic.
4. Identification of asymptomaticv individuals.
5. To plan personal and family's future if the result is positive.
6. Counseling amongst those who test negative and who practice high-risk behaviors.
7. To induce behavior change and prevent transmission by counseling in those who test positive.
8. To diagnose clinically suspected cases.
9. For peace of mind of individuals practising high risk behaviour.

KINETICS OF HUMORAL IMMUNE RESPONSE :

An understanding of the sequence of events that follow the entry of virus into the body will help to understand the optimal usage of various HIV tests during different stages of HIV disease. Viral entry into the body leads to transient period of plasma viremia and p24 antigenaemia. However, the levels of these components comedown with concomitant immune response. Humoral response is evidenced by formation of antibodies of different classes against different structural proteins (gag : P^{15}, P^{17}, P^{24} , P^{55}; env : gp^{41}, gp^{120}, gp^{160} and pol : P^{31}, P^{51} and P^{66}), regulatory proteins (nef, rev, tat) and accessory proteins (vif, vpu). All structural components are strongly immunogenic and induce formation of antibodies, whereas immunogenicity of regulatory and accessory proteins is variable.

The antibodies appear in the blood within 2-8 weeks, after infection but usually become detectable after 3-12 wks with the assays available presently. This period following the entry of HIV in to the body and the appearance of detectable levels of antibodies with the available test kit is called the "Window Period". During this period the individual is infected, infectious and non-reactive with the antibody detection tests. The antibodies to gag protein (P^{24} & P^{55}) are first to appear usually, though antibodies to env proteins and pol proteins may also be produced simultaneously. An infection progress to AIDS antibody to P^{24} usually declines as P^{24} antigen levels rise concomitant with progression of disease to AIDS. However, antibodies to env proteins persist throughout the infection (Fig. 7).

fig

Anti - HIV antibodies may be IgA, IgM, IgG. The IgA and IgM responses are inconsistent. IgG response is consistent and better understood. IgM response appears earlier than IgG but the sensitivity of assays available to detect this class of immunoglobulins is low and also IgM is detectable for a short period. Detection of IgM is valuable for identifying early seroconversion particularly following needle stick injury. (2-11 days) and infection in newborn, IgA is the predominant immunoglobulin in seromucous secretions (Saliva, colostrum, genito urinary secretion etc). Since IgA does not cross placenta and therefore detection of anti HIV IgA by HIV - IgA assay in a newborn is diagnostic of congenital HIV infection.

The diagnosis of HIV infections can be made on the basis[149] :

1. Detection of specific antibodies to HIV.
2. Detection of Viral antigens.
3. Viral isolation and culture.

1. DETECTION OF HIV SPECIFIC ANTIBODIES[150] :

Detection of anti HIV Antibes is the mainstay of testing for HIV and diagnosis of HIV. Serological tests to detect specific HIV antibodies can be classified into :

(A) Screening tests (ELISA/EIA Rapid, Simple)

(B) Supplemental tests.

(A) SCREENING / INITIAL TESTS :

Screening tests are performed to screen units of donated blood and blood products and for surveillance. Most common specimen is blood/serum/Plasma Screening assays are :

(a) ELISA (2-3 hrs)

(b) Rapid test : (minutes)

- (i) Dot blot assays
- (ii) Particle agglutination
- (iii) HIV spot & comb test.
- (iv) Flurometric microparticle technologies.

(c) Simple : Based on ELISA principle (1/2 hrs.)

(a) <u>ELISA</u> :

ELISA is the most commonly performed screening test. It is easy to perform, adaptable to large number of samples, is sensitive and specific and cost effective.

Different types of ELISA developed; First generation : used antigens derived from detergent disruption of viruses grown in human lymphocytes. Second generation : use artificially derived recombinant antigens expressed from bacteria or tungi. Third generation : use chemically synthesized oligopeptides of about 15-40 amino acids (synthetic peptides).

✠ ***Principles of ELISA :***

On the basis of the principle of ELISA can be divided into : Indirect, competitive, sand which, and capture assays.

All ELISA consist of either HIV antigen or antibody (depending upon the principle) attached on a solid phase and in corporate a conjugate and substrate detection system. Viral antigens may be whole virus lysate, recombinant or synthetic peptides. The matrix can be wells or strips of a microplate, plastic beads or nitrocellulose paper. Conjugates are most often antibodies (IgG sometimes IgM or IgA also) coupled to enzymes (alkaline phosphatase or horseradish peroxidase), fluorochromes or other reagents that will subsequently bring about a reaction that can be visualised. In case of enzyme conjugates the signal generated is a colour reaction and in case of fluorochrome it is fluorescence. The substrate used a 4-nitrophenylphosphate for alkaline phosphatase and O-phynxylencdiamine dihydrochloride (OPD) and TMB for horseradish peroxidase, which produce colour on being acted upon by the respective enzymes and the colour can be either detected visually or measured on a ELISA reader as OD values.

✠ ***Indirect ELISA :***

HIV antigens are attached covalently to the solid phase support allowing HIV antibodies present in the specimen to bind, and these bound antibodies are subsequently detected by enzyme labeled anti human immunoglobulin and specific substrate system. If the test specimen contained antibodies colour reaction will take

place. Indirect ELISA is the most commonly used system. The indirect ELISA produces a colour change directly proportional to the concentration of specific antibodies in the specimen.

✠ ***Competitive ELISA :***

In this assay the HIV antibodies present in the specimen compete with the enzyme conjugated antibodies in the reagent for binding to the antigen on the solid phase. If the test specimen contains HIV antibodies, these will compete with the labeled antibodies in the reagent for binding to antigen. So that less or no labeled antibodies can attach to the solid phase. Hence, faint or no colour is produced on addition of substrate if specimen contained HIV antibodies. Here reduction and or absence of colour indicates the presence of HIV antibodies in the test specimen. Development of strong colour means specimen is non-reactive for HIV antibodies.

✠ ***Sandwich ELISA :***

This is modification of indirect ELISA to improve sensitivity and specificity of the test. Antigen bound to the solid phase binds antibody in the test specimen in first step. Since antibody molecules are bivalent they are still able to bind to another molecule. The next step is addition of similar enzyme labeled HIV antigen ie. Same antigen as on solid phase. This will attach to the antibody molecule which is already bound to the solid phase antigen with one arm. Thus forms a sandwich of antigen + antibody + enzyme labeled antigen complex. The next step is addition of specific substrate which result in development of colour which is measured by ELISA reader one big advantage of sandwich ELISA is that all classes of HIV antibodies can be detected. There is also antibody sandwich ELISA which is done to defect P^{24} antigen.

✠ ***Antigen - antibody capture ELISA :***

Antigen capture ELISA can be based on principle of indirect or competitive ELISA, only difference being in the initial step of attaching antigen to the solid phase. A monoclonal antibody directed against an HIV antigen is bound to the solid support. Than addition of HIV antigen supplied as reagent. This antigen is captured

by the monoclonal antibody bound to the solid phase. Test specimen appropriately diluted is added next HIV antibody if present in the specimen bind to HIV antigen on solid support. Remaining principle is same as indirect ELISA. Only advantage of antigen capture ELISA is that it is more specific than indirect assay.

Anti body capture assays were developed to test specimens with low concentration of HIV antibodies or to detect specific class of antibodies (eg. IgG, IgM, or IgA.) In this test an antihuman immunoglobulin (IgG, IgM or IgA) is attached to solid support. The patient specimen is added. The concentrated immunoglobulin in patients specimen binds to the antiglobulins on solid phase. Next labeled antigen is added which bind to HIV antibodies of the patient bound to solid support. Next, the substrate is added and the OD value is read on the ELISA reader.

(b) <u>RAPID ASSAYS</u> :

A number of rapid assays based on principle of agglutination and ELISA have been developed for ease of performance and quick results. These assays generally require less than 30 minutes to perform and do not require special equipment's.

✠ ***Agglutination assays :***

Agglutination assays incorporate a variety of antigen coated carriers like red cells, latex particles, gelatin particles and microbeads. These particles are used to support or carry the antigen. HIV antigens are attached to the carrier particles by non specific attachment. Agglutination assays have good sensitivity. However, specificity is somewhat compromised and prozone reaction may be seen. To overcome the prozone reaction, diluted specimen is used to perform the test. During the agglutination reaction HIV antibody combines with HIV antigen on the carrier particles and since all antibodies are multivalent, a sort of lacttice network is formed which can be visualised macroscopically or microscopically.

✠ ***Dot blot assay/Comb test :***

These assays are rapid, easy to perform, can usually discriminate between HIV-1 and HIV-2. The results are read by development of colour. Sensitivity and

specificity of these assays compares with ELISA. The assays utilize recombinant or synthetic peptides spotted on to nitrocellulose paper/micro particles. The antigen containing matrix is housed in a plastic device containing adsorbent pads underneath to collect reactants or made as a comb and the antigens are spotted onto the tooth of the comb card. Each assay contains an immunoglobulin capture control to validate the result. These assays are very good for single test application i.e. in emergency, autopsy room and peripheral blood banks. Immunocomb test is similar to blot assay based on principle of indirect ELISA.

✠ ***HIV antibody detection in other fluids :***

The standard specimens for detection of HIV antibodies are serum/plasma/blood. However, detecting HIV antibodies can be detected in oral fluids (saliva/oral mucosal transudates). The levels are less than 1% of the levels in serum, but can be detected by sensitive ELISA technology. The isotype of antibody detected is secretary IgA, So appropriate ELISA should be used. HIV antibodies can also be detected in urine by ELISA.

(c) **SIMPLE TESTS** are also based on eia principle but take a little longer time (> 1/2 hr) compared to rapid tests.

(B) SUPPLEMENTAL/CONFIRMATORY ASSAYS :

Supplemental tests are performed on serum sample reactive in screening test for the purpose of diagnosis and identification of the individual. When a serum specimen is reactive by any one of the screening tests it has to be tested again by a different system using different HIV antigens or different principle of test to confirm the diagnosis. If a specimen is reactive in 2 different systems it has to be tested again using one of the supplemental tests which may be a third ELISA/Rapid/Simple test or a western Blot test as the case may be. A healthy individual reactive in three different system of testing is confirmed to be having HIV infection.

Supplemental assays are sensitive as well as specific and are performed to rule out false positive reactions. Supplemental E/R/S using different antigen and / or principle of test are performed to fulfil the strategy II and III of HIV testing policy.

Various supplemental assays are : ELISA with different antigen system (recombinant or synthetic peptides) or with different principle of fest which makes the test more specific. Western blot (WB), Immunoblot (IB), Line immunoassay, Indirect fluorescent antibody test (IFA), and Radioimmunoprecipitation Test (RIPA).

✠ ***E/R/S test as supplemental assays :***

Supplemental E/R/S are recommended for specimen reactive in the screening assay for strategy II & III of HIV testing policy. If the screening assay contained virus lysate as antigen the second and third assays should have recombination or synthetic peptides as antigens. Use of recombinant and synthetic peptides makes the assay more specific while retaining the sensitivity. Alternatively the second and third assays can also be based on different principle of ELISA, again done to increase the specificity of the screening assay when used as supplemental assays.

✠ ***Western blot[151] :***

The WB was developed as a method for separating proteins obtained from HIV harvested from cell lysates and there by analysing sera for antibody content to these specific proteins. HIV is allowed to replicate in continuous lymphoid cell lines usually HUT - 78 H-9 cells. Cells are lysed to release viral and Precursor protein yields. Virus is then partially purified by centrifugation. The viral protein is than placed in the multiple, small aliquotes at the top of a thin slab like polyacrylamide gel and electrophoresed from the negative to positive electrodes. When complete, the lower molecular weight proteins migrate for in the gel and the higher molecular weight. Proteins remain near the origin. The proteins are then blotted to a nitrocellulose paper electrophoretically by applying a current across the thin slab of gel and nitrocellulose paper. The paper is dried and cut into the strip of 5 mm width. The strips are subsequently exposed to dilutions of patient serum washed and

incubated with antihuman IgG labeled with an enzyme that produce a colored band upon exposure to its substrate.

✠ ***Indirect immunofluorescence assay :***

This is a rapid and reliable test using uninfected and HIV infected H-9 (or HUT - 78) cells in the long phase. The cells are air dried and fixed to a fluorescent glass microscope slide. A small quantity of a 1:10 - 1:20 test serum dilution is applied to each well, incubated to allow antibody to react with antigen. Then washed, and air dried. Then antihuman IgG labeled with fluorescein isothioacyanate is applied to each well, followed by incubation, washing, drying and mounting. Then specimens are evaluated for fluorescence intensity, percentage of fluorescent cells and fluorescent pattern. IFA can be used for differentiate HIV-1 from HIV-2.

✠ ***Radioimmunoprecipitation assay :***

RIPA is infrequently used as a confirmatory / supplemental test because it involves a rigorous procedure, requiring the use of radio activity labeled antigens and facilities to propagate/culture HIV in continuous cell lines. HIV infected lymphocytes are grown in the presence of radio labeled amino acids to permit their incorporation into HIV proteins. Soluble viral lysates prepared are reacted with the test serum and the mixture is then incubated with protein. A coated sephedex beads. The antigen antibody complex is eluted, subjected to gel electrophoresis and the gel exposed to auto-radiography.

✠ ***Line immunoassays :***

LIA is a variant of the WB test in which optimal concentrations of recombinant proteins or synthetic peptides are applied in a band pattern on plastic backed nitrocellulose strips. Antigens from HIV-1 and HIV-2 can be added to use as a combination assay. LIA makes reading and interpretation of results easier and minimises the problem of indeterminate results seen with conventional WB test that use viral lysates as antigens.

2. DETECTION OF VIRAL ANTIGENS :

Persons infected with HIV, particularly early in the course of infection detection of viral antigens may be useful.

(i) P^{24} Antigen detection :

P^{24} antigen detection was earlier used for prognostic staging and management of the infected individual. In USA P^{24} antigen testing has recently been added into the routine blood donor screening for HIV infections, in an attempt to reduce the diagnostic Window period. Test or control sera are allowed to react with P^{24} antibody to a micro well. After incubation and washing, goat anti HIV P^{24} antibody is added and will bind to the P^{24} antigen captured on the solid phase. Washing is followed by the addition of an enzyme labeled anti goat immunoglobulin and the appropriate substrate to produce a calorimetric reaction which is measured spectrophotometrically. By using a positive control with a know concentration P^{24} antigen a standard curve of OD viruses concentration is generated to quantitative the P^{24} antigen present in test sera.

(ii) Polymerize chain reaction[152] :

The method of PCR was first descried in 1985 by scientist at Centre Corporation. The principle of PCR is based on a three step cycling process at different temperature. Step one is the separation or denaturations of the double stranded DNA sample at 95c. If an RNA sequence is to be amplified, it is first necessary to convert the RNA sequence into DNA using reverse transcripts. Step two is the annealing of two primers to complementary strands of the dissociated DNA at a lower temperature (40 to 50c). Each primer usually consist of synthetic single strand of DNA (20 to 40 nucleotides in length) and is complementary to the positive or negative strands flanking the sequence of interest to be amplified, the associated original DNA strands are more likely to anneal to the primers instead to each other due to their relatively low concentrations and the excess of primer sequences. Step three is called primer extension and involves DNA plymarase mediate incorporation of nucleotides complementary to the unpaired or template

DNA strand to the 3' end of the primer. The PCR is infect to sensitive that it has been possible to detect a single cell containing the target B-globin gene.

(iii) bDNA assay :

Discussed after words.

3. VIRAL ISOLATION AND CULTURE :

HIV may be isolated from the blood and other body fluids. Isolation of HIV is a specialized procedure requiring sterile facilities. HIV isolation has therefore remained mostly for research. HIV is isolated from the peripheral blood mononuclear cells (PBMC) or plasma and other body fluids. The activated CD4 + Cells are susceptible for HIV infection. Hence autologous or heterologous PBMCs activated with mitogen phytohaemagglutinin (PHA) are cultured with the infection material. The cultures are maintained at 37c in 5% Co2 atmosphere for up to 28 days. The cultures are fed with fresh activated PBMCs at regular intervals. The presence of virus in the culture supernatant is detected either by demonstration of the presence of P^{24} antigen of enzyme reverse transcripts. The infected cells may also demonstrate syncitia in culture. Virus infected cells may also be detected by HIV-Specific immunofluorescent assay.

Two methods normally used for virus isolation are direct method or co-culture method. In the direct method, PBMCs from the patient are cultured in vitro in presence of PHA. In the co-culture method PBMCs from heterologous HIV uninfected donor are stimulated with PHA, and after 48-72 hours the stimulated cells are cultured along with the PBMCs from the patient (Fig. 8).

fig.8

STRATEGIES OF HIV TESTING IN INDIA :

Because of the enormous risk involved in transmission of HIV through blood, safety of blood and blood products is paramount importance. Since the positive predictive value (PPV) is low in populations with low HIV prevalence, WHO/GOI have evolved strategies to detect HIV infection in different population groups and fulfill different objectives. The various strategies, so designated, involve the use categories of tests in various permutations and combinations.

1. ELISA/Simple/Rapid test (E/R/S) used in strategy I, II, III.
2. Supplemental test like WB and LIA are used in problem cases eg. in case of interment/discordant result of E/R/S.

UNAIDS and WHO recommendation for HIV testing strategies according to test objective & prevalence of infection in the sample population.

Objective of Testing	***Prevalence of infection***	***Testing strategy***
Transfusion / transplant safety	All Prevalence	I
Surveillance	> 10% ≤ 10%	I II
Diagnosis : Clinical sings/symptoms of HIV infection	> 30% ≤ 30%	I II
Asymptomatic	> 10% ≤ 10%	II III

Strategy I :

Serum is subjected once to E/R/S for HIV. If negative, the serum is to be considered free for HIV and if positive, the sample is taken as HIV infected for all practical purposes. This strategy is used for ensuring donation safety (blood, blood products organ, tissues, sperms etc.). This unit of blood testing reactive (Positive) is discarded. Donor is not informed.

Strategy II :

A serum sample is considered negative for HIV if the first ELISA report is so, but if reactive, it is subjected to a second ELISA which utilized a system different from the first one. It is reported reactive only if the second ELISA confirms the report of the first. This strategy is used for surveillance and for diagnosis only if some AIDS indicator diseases is present.

Strategy III :

It is similar to strategy II, with the added confirmation of a third reactive ELISA test being required for a sample to be reported HIV positive. The test to be utilized for the first ELISA is one with the highest sensitivity and for the second and third ELISA, tests with the highest specificity are to be used. Strategy II & III are to be used for diagnosis of HIV infection. ELISA 2 and ELISA 3 ought to be tests with the highest PPV possible to eliminate any chance of false positive results. Strategy II is used to diagnose HIV infection in asymptomatic individuals including in high risk behaviour.

DIRECT EVIDENCE OF HIV INFECTION[153] :

The diagnosis of infectious diseases can be made with certainty by the direct demonstration of the Presence of infecting organisms in the clinical specimen collected from the patients. While isolation of the infecting organisms is possible in many infectious disease, the diagnosis may also be made by detection of nuclear material or antigens specific for the infecting organisms in the clinical specimens. HIV infection is diagnosed by the presence of anti HIV antibodies in the blood. However there are situations where the serology is negative although there is definitive evidence of exposure to HIV as seen in patients who are in the window period and in health care workers following accidental exposure to contaminated bold etc. Also children born to HIV infected mothers present dilemma as antibody positively seen in the infants upto 18 months, may be due to maternal antibodies in circulation. The diagnosis in these situation may be made by :

- Detection of P^{24} antigen
- Detection of HIV-Specific DNA by PCR.

- Isolation of HIV.

  (All ready discussed before)

- Hetroduplex Mobility Assay (HMA) : HIV-1 specific DNA PCR may also used to in Heteroduplex Mobility Assay for determining to which subtype the infecting virus belongs.

LABORATORY DIAGNOSIS OF HIV INFECTION IN NEWBORN :

Transplacental transmission of HIV can occur from infected pregnant mother to the foetus as early as 8 weeks of gestation or may be even earlier. Diagnosis of HIV infection in infants born to seropositive mothers is difficult because maternal antibody (IgG) to HIV crosses the placenta and can persist for upto 15 months making the distinction between maternal and neonatal IgG difficult. The test which can be undertaken to diagnose HIV infection in neonates before 15 months of age are :

✠ *Detection of IgA and / or IgM anti HIV antibodies :*

These antibodies do not cross placenta. The IgA class of HIV antibody assay using W.B. technique in infected children at 3 months of age. IgM class of antibodies are produced by infected infants by 6 months of age. Production of IgM is erratic, false positive results are obtained due to rheumatoid factor and polyvalent nature of IgM leading to nonspecific binding.

✠ *Estimation of P^{24} antigen :*

The immune complex dissociation assay which involve pretreatment of serum / plasma to librate P^{24} antigen complexed with P^{24} antibody prior to performance of ELISA are quite sensitive in identifying HIV infected infants.

✠ *PCR :*

The technique specifically amplifies viral DNA sequences of interest. It is possible to identify one infected cell in the specimen as also latent HIV infections. Various reports indicate the specificity of PCR to be invariably > 95% regardless of age of testing while sensitivity range from 15% in neonates (within 48 hrs. after birth) to more than 95% in infants over 1 months of age.

✠ ***In-vitro antibody production assay :***

The method is tedious and involves in vitro culture of antibody producing β lymphocytes from peripheral blood. The HIV antibodies are detected in culture suppurate from truly infected infants.

✠ ***In-Vitro isolation of Virus : (blood, tissues)***

The method is time consuming, expensive and not sensitive. This is due to the lower number of available cells cultured and paucity of infected cells in the sample that are present. Additionally, it may take upto 6 weeks or longer to obtain a result.

✠ ***Indirect indicators of HIV infection[154] :***

This includes various non specific markers like hypergamaglobubulinemia, absolute lymphopenia, hematologic abnormalities, low CD4 count and clinical markers like wasting syndrome, recurrent bacterial infections, Opp. infections, malignancies, Pneumonia etc. Which point towards AIDS. However, these parameters are employed to supplement the conventional serodiagnosis of HIV infection.

LABORATORY TEST FOR MONITORING STAGE AND PROGRESSION OF HIV INFECTION :

Infection with HIV may develop to AIDS at different rates in different individuals, with a spectrum varying from rapid progression to long term non progression. This variability marks it essential to have test which can accurately assess the stage of infection in an individual, as well as predict its course and monitor its progression. These test are very valuable during the period of clinical latency and subsequently, supplement various clinical parameters, which are also extremely important in categorizing the infection / disease stage. Response to anti-retroviral therapy is also monitored using this prognostic test. The tests are classified into :

(A) Viral markers : Plasma HIV RNA load.

Viral antigenaemia eg. P^{24} antigen.

(B) Surrogate markers : Virus specific markers.

(C) Non Specific markers : Cellular markers.

Soluble markers of immune activation.

(A) VIRAL MARKERS :

(a) Plasma HIV RNA load :

Plasma viral load (HIV RNA) Quantification is presently considered the best method for monitoring progression and response the best method for monitoring progression and response to anti-retroviral therapy. Active replication of virus occurs in all clinical stages of infection. It is possible to detect and quantify virus throughout the course of HIV infection. The viral load usually ranges between 10^2 and 10^7 HIV RNA copies/ml in untreated individuals though it may be lower in those on treatment. The techniques available for quantifying viral RNA are : (i) Quantitative RNA - PCR, (ii) b DNA assay, (iii) Nucleic acid sequence based amplification.

(i) Quantitative RNA PCR :

This test is based on parallel amplification of HIV RNA (142 bp sequence from gag gene) from the sample and a known amount of quantitation standard (QS) RNA, using RT-PCR with the same set of biotin-labeled primers to give different products (amplicons). These are detected in separate well of a micro well plate by hybridization with immobilized HIV and QS specific probs. The bound biotinylated amplicon is quantified colorimetrically by measuring the optical density (OD), obtained by using avian hose radish peroxidase (HRP) conjugate and an appropriate substrate. The HIV RNA copy number can be then calculated by extrapolating from the known input number of copies of QS RNA. To cover a while range of HIV RNA copies possible in plasma of infected persons, a number of dilutions are put up.

(ii) Branched DNA assay :

The DNA assay is based on amplification of the signal instead of the target. A target probe complementary to both the target sequence and the capture probe

hybridizes with both of these. Another sequence on the target probe then hybridizes with an "amplifier" branched DNA. An enzyme labeled probe, which can bind to the multiple branches of the amplifier branched DNA, is allowed to hybridize with it. This amplified label is detected by a luminometer and quantified by its action on a chemiluminiscent substrate. The quantity of the target in the sample can be determind from this.

(iii) Nucleic acid sequence based amplification : NASBA.

This technique uses 3 enzymes reverse transcriptase, RANASE H & T7 RNA polymerase at a single temperature (37c), to generate multiple copies of the target HIV RNA, by a strategy similar to that used by retroviruses for their replication. RT enzyme and a primer designed with the T7 RNA polymerase promoter sequence at one end, are utilized for synthesis of an RNA - CDNA hybrid containing the T7 promoter from the target. From this, the original RNA target strand is removed by addition of RNAse H and dsDNA is generated by the DNA polymerase activity of RT and a second primer. Multiple copies of RNA are then generated by T7 RNA polymerase enzyme. This RNA enters another similar cycle and the amount of RNA multiplies expotentially with each cycle.

Present status of HIV RNA load - are more than 10 fold lower in long-term non-progressors than in individuals with progressive disease. A viral load of > 100,000 copy equivalents/ml by bDNA assay, within 6 months of seroconversion increases the risk of progression to AIDS within 5 years by more than 10 fold. Persistently detectable viremia & high baseline levels carry a poor prognosis, while risk of progression is low at copy numbers < 10,000/ml. Apart from being an extremely useful predictor of progression, viral load responds very well to anti retroviral therapy & can be used to monitor it.

(b) Viral antigenaemia - P^{24} :

Already discussed before.

(B) SURROGATE MARKERS :

✠ *Virus specific markers :*

A viral characteristic associated with progression is the conversion of strains from non-syncytium inducing (NSI) phenotype to a syncytium inducing (SI) phenotype. This change of character has now been associated with the ability to infect cells with certain chemokine coreceptors. HIV specific antibodies have also been used as markers of progression, though they are not considered particularly sensitive. Decline in or absence of antibodies to various HIV antigens including $P^{24+,}$ $P^{17,}$ gp^{120}, gp^{41} and nef gene product have been used as surrogate markers in the past.

(C) NON SPECIFIC MARKERS :

(a) Cellular markers :

A number of non HIV - Specific cellular markers have been used for staging, monitoring progression and assessing response to therapy. One of the most useful is the absolute CD4 + lymphocytes in the blood. Other lymphocyte phenotypic markers associated with progression include an in crease in indicators of immune activation on T lymphocytes like CD38, HLA - DR, IL - 2R, CD45RO and markers of apoptosis.

CD4 count :

The most commonly used cellular marker is the CD4 lymphocyte count. Its decline is the hallmark of HIV infection and the rate of loss in each person is unique. CD4 cell number changes during HIV infection in the following stages :

- Rapid diction for 6-18 months at the time of seroconversion.
- Plateau of gradual decline that can last at least several years during the asymptomatic period.
- Sleeper decline for several months just be for AIDS develops.
- Continued CD4 cell decline until death.

CD4 cells count is doing by flow cytometry, microsphere assay and by Enzyme immunoassay (EIA).

(b) Soluble markers :

A large number of soluble markers of immune activation have been evaluated as prognostic indicators in HIV infection.

(i) Neopterin :

Neopterin (6-D-erythro-trihydroxy propylpterin) is a product of guanosine triphosphate catabolism which is derived from macrophages and B cells stimulated by interferon gamma and reflects its activity, while being stabler. Neopterin is present in both urine and serum. Urine and serum neopterin levels are elevated in patients with asymptomatic HIV infection as compared to seronegative individuals. It is an early marker of HIV infection. The levels rise further on progression from pre-AIDS to clinical AIDS. Since neopterin levels are stimulated by HIV infection, measurement of neopterin level can be useful in monitoring progression and evaluating antiviral therapy. However, since it is a non specific marker, besides HIV infection, high neopterin levels in urine and serum may be found in numerous infections and inflammatory disorders, collagen vascular diseases and in advance stage of certain malignancies.

The tests are based on competitive ELISA. The plates are coated with polyclonal anti-neopterin antibody raised in animals eg. Sheep/rabbit. The neopterin in the test sample competes with enzyme (alkaline phosphatase or peroxidase) conjugated neopterin. The enzyme conjugate is detected by addition of the substrate and a lower OD corresponds to a higher level of neopterin in the sample.

(ii) Beta - 2 microglobulin :

It is a nonspecific but relatively sensitive marker for immune activation. Elevated levels are also seen in certain HIV high risk groups such as haemophiliacs and drug abusers. High serum beta 2 m levels especially during the first year after seroconversion to HIV positively, were associated with progression to AIDS. Its measurement may be useful in evaluating anti-viral therapy.

Beta - 2 microglobulin is present in most biologic fluids at low concentration and can be measured in serum or plasma by using ELISA test. Other soluble receptors TNF and IL -2 are also present in kits.

Alternatives to classic tests :

As technology evolves alternative to the classics tests and testing arise. Each offers one or more attractive features that may simplify collection, testing or interpretation of results.

Saliva tests :

Non envasively collected specimens, such as oral fluids (saliva), have been used for HIV testing as an alternative to blood samples. These fluid, containing crevicular fluid from capillaries beneath the tooth-gum margin, are transudates of blood, therefore, they include the same fluid (plasma) that is used for serum base testing. The concentration of antibodies in oral fluid is about 1/400 of that is plasma; however because of the dilutional effect of fluids from the salivary glands and fluid testing necessitates extremely sensitive tests that are able to detect small quantities of antibodies the testing technology to detect these low quantities is now availables is oral fluid test both ELISA and rapid tests are accurate.

As of 1998 USA FDA has approved ELISA and W.B. specifically for testing oral fluid. The use of oral fluids for testing offers advantages, such as ease of collection, group collection, collection from persons in whom blood is difficult to obtain and an increase in collection compliance.

Urine test :

Intact IgG antibodies are found in urine, but their exact origin is unknown. Urine collection is simple, noninvasive and inexpensive and the sample can be stored at RT for extended period of time. The use of urine for testing is appropriate for physicians officers, health clinics and developing countries where health care personnel may not be professionally trained or where clean needles for drawing

blood may not always be available. Recently FDA approved ELISA and WB for use to test urine for antibodies to HIV.

Home collection testing :

Recently, home collection, but not home testing, has been approved by the FDA for HIV. These collection devices are filter paper for the collection of whole blood via fingerstick. The samples are mailed to a lab. eluted, and used for ELISA testing. Result and Cons. are made available by telephone.

QUALITY ASSURANCE PROGRAMME[156] :

The diagnostic test to dated antibodies to HIV have sensitivity and specificity which are not absolute. In all these tests we have false negative or false positive results, which are inherent and cannot be avoided. The percentage of false positive results will increase as the prevalence rate of person with HIV antibodies in a population decreases. So consistent production of reliable results requires a stringent overall assurance programme, which would control technical conditions before, during and after each assay. The quality Assurance Programme ensures that the results reported by the laboratory are correct, reliable and accurate as far as possible. It also ensures use of the most reliable tests for the dianosis of HIV infection.

Guidelines to improve quality of testing :

(i) **Condition of specimen :** All specimens must be suitable. Use of lipaemic, haemolysed and contaminated sera should be avoided. All samples include information like name, date, identification number, test request form which include Age / Sex, physician name, risk group, reason, route. Anonymous testing only code number and date mentioned. The sample serum/plasma to be tested should be at 37c & well-mixed before testing. Presently all the test kits distributed by the NACO, are sent for testing serum or plasma only. Therefore, these kits may not be reliably detect presence of antibodies in other body fluids.

(ii) Controls used in the tests : Each test run requires a set of controls to validate the results. Controls must be treated in the same manner as unknown samples. The assay is valid and the results are reliable when the controls produce acceptable results.

(a) Internal Control : The controls (positive & negative) are included in each test kit, and to be included in each test run. These are essential for quality control measures.

(b) External Control : These should be included with each test-run to monitor consistent performance and lot to lot variation which can not be detected using internal control. These controls are made from pooled test kit controls or made from pooled sera from HIV positive or negative individuals in each laboratory.

(c) Standardization of Controls : Reproductibility and quality of internal & external controls must be standardized by intra run reproducibility and inter run reproducibility. The control samples (Internal or external) are tested at least three times on the same test run. This will indicate intra run reproducibility. This exercise is then carried out on test runs on three consecutive days to determine inter run reproducibility. These samples may also be evaluate at the National Reference Centers.

(iii) Accuracy of EIA : Since no assay can be 100% sensitive & specific, it is likely to give false negative as well as false positive results.

✠ ***Any EIA may false negative :***

(1) during early stage of HIV infection. Generally 2-24 weeks also as long as 42 months.

(2) On late stage disease.

(3) Technical error.

✠ ***The EIA may false positive :***

(1) Certain condition increase false positively rates of ELISA, Auto immune diseases, multiple preganancies, multiple transfusions, hyper a gammaglobulinaemia, chronic alcoholics, Hepatitis B immunisation & antipolystyrene antibodies.

(2) Technical error

(3) Human error.

(iv) Reporting : All test results must be kept confidential and should never be discussed in public, & followed by pretest and post test counseling.

Proficiency testing of Laboratory :

Proficiency testing is synonymous with External Quality Assessment (EQA). Recently National AIDS Control Organisation has formulate Quality Assessment Programme. Under this coded panels of known HIV antibody positive and negative sera are provided, handle exactly the same manner as the routine HIV testing in ELISA.

Quality Control of HIV kit :

(a) Monitoring different lots of kit : This is done by a technique called 'parallel testing' in which performance of new lots of kit with the previous lots via a common control material (external & controls of previous lots) is compared. If all controls produce expected results, the new lot has passed the parallel tests and may be used for routine testing.

(b) Monitoring different kits : For safe blood banking kit should be correctly identify all antibody positive sera. Test kit should 100% sensitive. For sero surveillance and serodiagnosis kit with a high sensitivity and high specificity is needed.

BIO-SAFETY IN HIV LABORATORY[155] :

There is no vaccine for prevention of HIV/AIDS & treatment is expensive. Hence prevention of infection is the cornerstone of control of this epidemic. It is extremely important that the laboratory workers should be aware of the risks involved in their day to day work and apply effective infection control practices to reduce the possibility of transmission of these fatal infections to a minimum. Bio-safety in a HIV testing laboratory may include (A) Precautions in relation to blood and body fluids (B) effective use of sterilisation and disinfection and (C) safe disposal of hospital waste.

Universal precautions apply for : Blood, all other body fluids containing visible blood, semen & vaginal secretions, CSF, synovial fluid, pleural fluid, peritoneal fluid, Pericardial fluid and amniotic fluid. Universal precautions do not apply for the risk of HIV transmission is extremely low or negligible these includes faeces, nasal secretions, Sputum, Sweat, tears, urine and vomitus. Universal Precautions include : Barrier protection, hand washing, safe techniques, safe handling of sharp items, safe handling of specimen, safe handling of spill of blood/body fluid, use of disposable/sterile items, safe techniques including mechanical pipetting device & immunisation with Hepatitis β Vaccine.

(A) General bio-safety guidelines for laboratory workers :

(i) The laboratory door should have a symbol of BIOHAZARD; NO ADMITTANCE. Laboratory should be well ventilated so personnel do not breath in contaminated air.

(ii) Eating, drinking, smoking and application of cosmetics are prohibited in Laboratory. Sandals and open style shoes do not afford proper foot protection and are not to be used. Contact lenses should not be worn.

(iii) Laboratory and work tables should be scrupulously cleaned with liquid detergents and disinfectants. Laboratory work surface should be decontaminated once a day after completion of day's activity and immediately after spill of viable material with disinfectant.

(iv) Biological safety cabinets and other devices, caps should be used whenever handling hazardous specimens.

(v) Blood & other specimen containers should be labeled with a warning sing. Outside of container should be cleaned with sodium hypochloride in case of visible contamination.

(vi) Gloves/Gown should be worn in laboratory working with infective materials.

(vii) Hand should be washed immediately after contact with blood and before leaving the laboratory.

(viii) Mechanical pipetting devices should be used. Mouth pipetting is strictly prohibited. Careful techniques should be followed to minimise the creation of aerosols.

(ix) Accidental wounds from sharp instruments should be avoided.

(x) Paper work should not be done on potentially contaminated surface.

(xii) All potentially contaminated materials and wastes from the laboratory should be disposed after decontamination preferably by autoclaving. A label with globally accepted biological hazard sigh should be applied.

(B) Effective use of sterilisation and disinfections :

Thorough cleaning and drying with detergents and water remove most organisms from an object / surface and should be carried out meticulously before sterilization. All contaminated glass wares/items after prior disaffection should be dismantled before cleaning. Cold water is used for protein materials and prevents the release of aerosols by the surface of the water, after drying it ready for sterilization.

Environmental Cleaning :

Floors, surface, skin and drains should be cleaned with warm water & detergent. If there is spillage of blood, body fluids or sputum, disinfections before cleaning is recommended. Surface cleaned with freshly prepared 0.5 - 10% sodium hypochlorite.

Disinfections :

Either thermal or chemical processes are used for disinfections. Thermal disinfections is preferred whenever possible. It is more reliable, leaves no residue, is more easily controlled & is non toxic. Boiling (100c) for 20-30 mins is very simple & reliable method for inactivation of all micro organisms including HIV, HBV and mycobacteria. Chemical disinfectants is for heat labile equipment's. Alcohol, chlorine, 2% glutaraldehyde & phenol are used.

Sterilisation :

Sterilisation is carried out by stem under pressure, dry heat, gas or liquid chemicals. HIV is a fregile virus and is adequately inactivated by autoclaving at 15 lbs 20 min at 121c or hot air oven at 160-180c for 1 hour.

(C) Safe disposal :

Laboratory wastes are potential hazards. Infectious waste can transmit numerous diseases in the community and put those who handle waste and live on its proximity, at risk.

Guidelines for Waste disposal :

(i) All biomedical wastes should be treated and disposed of strictly in accordance with the options mentioned in the table.

(ii) Waste which cannot be incinerated (Plastics) should be pretreated by disaffection and disposed of in an environmentally sound manner.

(iii) Waste should not be dumped, discharged or disposed in any place.

(iv) All precautions and personal safety measures should be taken (gloves, masks, clothing, gumboots, goggles etc). HBV vaccine is recommended again from handling or exposure.

(v) All treatment and disposal facilities should be located at a specified area away from hospital public place & residential areas.

(vi) When the treatment option is burial, the pits should be located away from agricultural land, residential areas and safe water sources.

(vii) All plastic should be disinfected, shredded and disposed of Recycling of disposable should be prevented.

(viii) All liquid waste should be disinfected & flushed in the sinks at the point of generation.

(ix) Biomedical waste should not be disposed of on open land and municipal dustbins.

Table - XIII

Container and Colour Coding for disposal of bio-medical wastes

Waste Calgary	***Waste Class***	***Type of Container***	***Colour Coding***	***Treatment/ Disposal Option***
No.1	Human anatomical waste, blood & body fluids	Single use containers/plastic holding bags	Red.	Incineration/Deep Burial.
No. 2	Animal & Slaughter house waste	Single-use containers/plastic holding bags/sacs	Orange	Disinfections & Deep Burial.
No. 3	Microbiology & bio-technology waste	Single-use containers/plastic holding bags.	Yellow	Autoclaving/ Microwaving & incineration.
No. 4	Waste Sharps	Re-usable/single use sturdy containers of plastic, glass or metal.	Blue	Shredding & Deep Burial.
No. 5	Discarded Medicines	Re-usable/sturdy card board/glass/ plastic holding bags	Blue	Shredding & Deep burial.
No. 6	Solid Wastes (linen)	Plastic bags/sacs.	Yellow/ Black	Disinfections and machine cleaning.
No. 7	Disposable (Other than sharps)	Reusable/sturdy containers/plastic holding bags.	Yellow/ Black	Disaffection-chemical/ Autoclaving, Shredding, Burial.
No. 8	Liquid wastes	NA	NA	
No. 9	Chemical Wastes	Sturdy containers/ plastic holding bags.	Yellow / Black.	

TREATMENT :

The development of therapy for HIV infection is one of the most remarkable stories in modern science.

The effectiveness of current therapy is reflected by the observed decline in HIV mortality. These drugs have improved survival times, they do not halt the progressive destruction of the immune system that characterised by HIV infection. The HIV infection is associated with viral replication and progressive immunodeficiency. Therefore treatment should be targeted both to restore the immune system and to inhibit viral replication.

(I) Antiviral agents[157] :

(A) <u>Drugs</u> :

(a) Nucleoside reverse transcriptase inhibitors (NRTI) :

Zidovudine (AZT) Didanosine (ddI)

Stavudine (d&T) Zalcitabine (ddc)

Lamivudine (3TC)

(b) Non NRTI : Delvirdine, Nevirapine Efavirenz.

(c) Protease inhibitors : Indinavir Saquinavir

Ritanovir Nelfinavir

Amprenavir.

(B) <u>Monoclonal antibodies</u> :

Monoclonal antibodies to viral envelop components

Monoclonal antibodies to viral receptors.

Recombinant Soluble CD4 (rs CD4)

(C) <u>Interferons</u> : IFN - α

IFN - β

(D) <u>Oligonucleotides</u> :

Antisense : directed against tat and rev sequences.

Sense/decoy

Ribozymes (HIV-specific)

(E) **Dominant negative mutants :**

For Tat, Rev, Gag and Env.

(II) Immunorestoration / immunopotentiation :

(A) Cellular engineering :

Bone marrow transplantation.

(B) Genetic engineering :

Augmentation of anti HIV immune response by : transfixion of patients fibroblast with murine retrovector expressing gPl 160 and reintroduction into the patient.

(C) Engineering with biologic molecules :

- Interferon's
- Interlukin - 2
- Thymic hormone
- IL - 12
- Intravenous Y-globulin.

(D) Pharmacologic engineering :

Isosprinosine Imuthiol

Enkephaling Azimexon

Retinodis.

(III) Combination treatments :

(A) Cellular engineering and antiviral agents.

(B) Engineering with biologic molecules and antiviral agents.

(C) Pharmacologic engineering and antiviral agents.

(D) Chemotherapy and interferon.

(E) Combination of two or more antiviral agents.

(IV) Gene therapy :

It is generally believed that gene therapy would be directed to treat persons already infected with HIV, rather than as a prophylactic measure. The strategy will involve introduction of gene into cells that specifically interferes with HIV replication or that causes the death of infected cell, thereby preventing virus spread.

Zidovudine alone was recommended in the past. New anti-retroviral drugs with various modes of action become available in the 1990s, & it was shown that combinations of drugs belonging to different groups could greatly reduce the chances of drug resistant HIV mutants emerging. Drug such as zidovudine, combined with a group NRTl drug such as didanosine has a much greater effect than each drug alone and limited to the emergence of drug resistance. The most dramatic improvement in therapeutic efficacy followed the introduction of protease inhibitors and the use of a suitable triple combination that included on of these drugs with two NRTI drugs. The latter type of triple combination drug therapy is sometimes referred to as highly active anti-retroviral therapy (HAART) & is increasingly becoming recommended as standard therapy rather than double drug combination therapy. HAART has a particularly potent beneficial effect when administered to a previously untreated patient who is symptomatic with established but not too advanced HIV disease. Such as patent typically has a high plasma HIV load derived mainly from HIV actively replicating in a short lived population of CD4 lymphocytes and the CD4 count is falling. When HAART is administered there is a marked fall in viral load, the CD4 lymphocyte count increases with restoration of immune function, there is clinical improvement, delayed clinical onset of AIDS and substantially longer survival.

The current main objective of HAART is to obtain the maximum suppression of HIV replication for the longest duration possible. The limitation of HAART involves a lack of effect on 'resting' HIV infected lymphocytes, and other cells where the HIV genome is integrated as HIV DNA in the human DNA. Resting cells may become activated in due course to cause HIV replication and release of virus to infect other cells, so even HAART is given early on in HIV disease and continued for a few years, infection may appear if the treatment is stopped. Also there may be sanctuary sites, such as the brain, where there may be many such resting cells and also where some if not all the drug in a combination may have

difficulty in penetrating the tissue and suppressing the replication of HIV - drug - resistant mutants might emerge after therapy. Lifelong drug therapy is currently recommended.

When to start anti-retroviral drug treatment of HIV infection :

1. Established symptomatic HIV disease any base line viral load and CD4 count test - strongly recommended starting therapy.
2. Asymptomatic HIV disease & CD4 count < 500 per mm^3 HIV RNA > 5000 copies per ml. plasma. strongly recommended starting therapy.
3. Asymptomatic HIV infection & CD4 count > 500 per mm^3
   - CD4 count rapidly decline & HIV RNA > 500 copies/ml
   - CD4 count not yet rapidly decline but HIV RNA > 30000 copies/ml. Consider starting therapy.
4. Asymptomatic HIV infection & CD4 count in normal range (600-1200/mm^3) HIV RNA < 5000 copies/ml plasma.

Therapy not yet recommended in current guidelines but considered by some it HIV RNA detected.

PREVENTION[158] :

WHO has recommended the following prevention measures :

(1) Sexual Contact :

Most HIV infection is transmitted through sexual intercourse with an infected partner. The risk increases with the increase in No. of sexual partners. It can be prevented by :

- having only one uninfected partner.
- always using condoms with new partner.
- availing STDs.
- establishing comprehensive STD treatment programmes[59].
- educating children about safer sex before they become sexually active.
- teaching HIV positive about sasfe 'sex'.

(2) Blood[160] :

All blood & blood products are to be screened. This also applies to donation of semen, cornea, bone marrow, kidney and other organs since, HIV can spread through any one of these tissues. This can be prevented by. :

- Screening blood for HIV
- Discouraging donation from people known to be HIV positive or to be at high risk.
- Prohibiting donation by professional blood donors.
- Only giving B.T. when absolutely necessary.

(3) Sharing needles :

Contaminated needles, syringes or other skin piercing equipments should not be shared with others. HIV transmission by sharing needles can be prevented by :

- Ensuring that staff uses a sterile needle & syringes for each patient.
- Disinfecting, cleaning and sterilizing all needles and syringes before reuse.
- Ensuring clinics having adequate supplies of needles and syringes to meet patients need.

(4) Mother to child transmission :

The children born to HIV infected mothers are at risk of acquiring HIV. This can be prevented by :

- educating women about HIV
- Counseling HIV positive women about the risks and providing good contraceptive advice and materials.

(5) Drug abuse :

The drug abuse are at high risk of acquiring HIV infection. This can be minimized by :

- educating children about the risk of using intravenous drugs.
- organizing drug rehabilitation programs.
- Providing addicts with clean syringes and needles to avoid the sharing of equipment between addicts.

(6) Health Care workers[161] :

Well documented studies show no increased risk to health worker to acquire HIV. Any risk of HIV can be virtually eliminated through good clinical and laboratory practice.

- handling all the specimens carefully.
- availing needle stick injuries.
- following safe laboratory practice by using plastic wares, wearing gloves, cleaning up accidental spillage with Na DCC, disposal of all waste carefully.

POST EXPOSURE PROPHYLAXIS[162] :

Most exposures do not result in infection. The risk of infection varies with type exposure and other factors such as : The amount of blood involved in the exposure, The amount of virus in patient's blood at the time of exposure and whether post exposure prophylaxis was taken within the recommended time. Prevention is the mainstay of strategy to avoid occupational exposure to blood/body fluids.

An occupational exposure that may place a worker at risk of HIV infection is a percutaneous injury, contact of mucous membrane or contact of skin with blood, tissue or other body fluids to which universal precautions apply. At the time of exposure immediately follow:

(i) Needle sticks and cuts should be washed with soap & water.

(ii) Splashes to the nose, mouth or skin should be flushed with water.

(iii) Eyes should be irrigated with clean water, saline or sterile irritants.

(iv) Pricked finger should not be put into mouth, reflexly.

In some cases, HIV postexposure prophylaxis (PEP) may be recommended and it should be started as soon as possible, preferably within a few hours, late PEP (after 72 hrs) may still be useful as early treatment of HIV infection, in case infection has occurred. The decision to start PEP is made on the basis of degree of exposure to HIV and HIV status of the source from whom exposure/infection has occurred. Person should be provided with pre test counseling and AZT be started &

taken sample for testing. In case the test non reactive, 2nd sample collected after 6 weeks and 3rd at 12 weeks after exposure, and tested for HIV antibodies by RT-PCR.

Recommendation for preventing transmission of HIV include refraining from blood, semen, organ donation, abstaining from sexual intercourse, women should not breast feed their infants. Drugs recommended in India is Zodovudine (200 mg shly) and lamivadine. Used in combination, ZDV & 3TC are very effective. The optimal course of treatment is unknown; Since 4 week of ZDV appears to provide protection against HIV infection. In case of pregnancy ZDV taken during 2nd and 3rd trimesters.

AIDS VACCINES :

Identification of a new pathogen can result in the development of accurate diagnostic test, therapies and preventive vaccines. Since HIV-1 and HIV-2 were identified as a cause of AIDS a preventive vaccine however has not been identified, despite intense academic, pharmaceutical and government research.

Ideally such a vaccine would[163] prevent HIV infection or limit it to a transient asymptomatic infection that is unlikely to be transmitted to others. Recent evidence from human suggests that transient infections can occur and studies in nonhuman primates suggest that some vaccines allow only transient infection or may reduce the severity of subsequently established infection.

Challenges to the development of an HIV vaccine include : The vast and rapidly increasing genetic diversity of HIV.

- Lack of understanding about the biology of the virus host interaction.
- The complexity of the required clinical trials.

However, the reasons for optimism that a vaccine will ameliorate, it not end, the HIV epidemic are the long term, if temporary control of viral replication by the human immune system during HIV infection, successes in vaccinating primates against HIV, SIV and SHIV, and the possibility that a partially effective vaccine might significantly diminish the epidemic.

One of the most important problem is that the virus is often intracellular and can be transmitted from cell to cell without entering the extracellular fluid. This means that immunological mechanisms that may be capable in removing circulating viruses, will be powerless once the virus is intracellular. Moreover, the HIV attacks the T-helper cells, which are necessary for an effective immune response. Once infected these cells contain an integrated proviral genome for life. Thus to be effective a vaccine against HIV must prevent the virus from establishing infection[164].

The second problem is that the major neutralizing antigenic area of the virus (Known as principal neutralizing determinant PND) is subject to mutation. The PND is located on the outer coat of the virus envelop on a surface glycoprotein called gp120. The V3 loop of the gp120 has a hypervariable region in a sequence of 24 aminoacids. This means that antibodies elicited be one strain of the virus may not be able to neutralize another strain. There are still major gaps in our under standing of the biology of HIV infection in man. We knew that the outer viral surface contained epitopes which stimulate a protective immune response, that this immune response can be measured in terms of titres of antibodies, that these antibody titres were protective and could be evaluated in experimental animal models and these models provided an excellent predictor of human response. None of this is available for a vaccine against HIV. It is not known of the antibodies produced after vaccination are protective or not, and role of cell mediated immunity in protection against disease is largely unknown. Moreover, there is no animal model on which the vaccine can be tested. Though the chimpanzee has been used as a model. This animal does not develop the disease AIDS, though it can be infected with HIV.

At present 3 different approaches to developing a vaccine against HIV. The preventive vaccines are preventing the infection before exposure. The therapeutic vaccines is to reinvigorate the already infected immune system allowing it to combat more effectively the HIV induce damage to the immune system. Parinatal

vaccination has aim to blocking the transmission from mother to baby. Of the three preventive vaccination has made the greatest progress :

- **Killed vaccine :** Killed SIV vaccine do not induce protective immunity but may prevent the emergence of clinical disease.
- **Live attenuated vaccine :** These are prepared from genetically engineered strains lacking some crucial genes, so that the resulting virus produce a harmless infection this technique has been successfully used with SIV.
- **Recombinant viral particles :** Vaccine prepared by recombining env protein with vaccinia virus has been tried in Zaire in a group of healthy volunteers. A cytotoxic T-lymphocytes (CD8 T cells) response was obtained. The overall effect of recombinant vaccine has not been established in human.

The Global programme on AIDS of WHO in collaboration with two vaccine manufacturers and national scientists, is conducting trial of the HIV-MN gp120/alum vaccine in Thailand and trials of HIV-MN gp120/alum vaccine in Thailand and trials of HIV-MN V3 peptide/alum vaccine in Thailand and Brazil.

A consortium consisting of the U.S. department of defense (DOD) scientists a vaccine manufacturer and Thai Public health leader and scientists is developing a bivalent clade β HIV Sf2 gp120/mg 59 and clade E gp120/MF 59 vaccine to be tested in Thailand.

In the case of HIV/AIDS infection prophylaxis of opportunistic infection also has increased life expectancy. Live virus OPU, MMR and bacterial BCG vaccine has not been recommended for immunocompromised persons because replication of live attenuated agents may be enhanced, where as inactivated bacterial and Viral vaccines DPT, influenza, Pneumococcal, HbcV and IPV are recommended. In the case of children with HIV/AIDS OPV and varicella not recommended, DPT, IPV, MMR, Penumococcal, influenza, Hepatitis β and Hib are recommended.

WORLD AIDS DAY[165] :

World AIDS day has a special place in the history of the AIDS pandemic. Since 1988 December 1st has been a day bringing messages of compassion, hope, solidarity and understanding about AIDS to every country in the world, North & South, East & West. World AIDS day emerged from the cell by the world summit of Ministers of health on programmers for AIDS prevention in January 1988 to open channels of communication, strengthen the exchange of information & experience and forge a spirit of social tolerance. Since then, world AIDS day has received the support of the world health assembly, Governments, communities and individuals around the world. Each year, it is the only international day of coordinated action against AIDS. Each year there is a particular theme chosen for World AIDS Day.

✠ ***The themes have been as follows :***

1988	Communication.
1989	Youth
1990	Women & AIDS
1991	Sharing the challenge
1992	Community Commitment
1993	Time to Act.
1994	AIDS and the family
1995	Shared Rights, shared Responsibilities
1996	One world, One Hope.
1997	Children Living in a World with AIDS.
1998	Force for change : World AIDS campaign with young people.
1999	Listen, Learn, Live
2000	AIDS : Man make difference.
2001	I care do you
2002	Live & let live (Stigma & discrimination)
2003	Live & let live (Stigma & discrimination)

Young people can & have played a vital role in AIDS & HIV prevention and support work. Young people can make a huge difference in arising awareness of AIDS of HIV by talking about AIDS & HIV in & outside school and college, by holding fund raising events for AIDS charities or by wearing a red ribbon to show support.

The red Ribbon is an international symbol of AIDS awareness that is increasingly being worn by people all year round to demonstrate their care and concern about HIV and AIDS, and to remained others of the need for their support and commitment.

MATERIAL AND METHODS

Diagnostic testing for any infectious agent is an important aspect of disease control. Since the recognition of AIDS in 1981, in the very next year virus was isolated; and a diagnostic test was formulated soon after. Through these newer techniques, the immunologic foot prints of the viral infection are detected rather than the virus itself. Out line testing of all donated blood was initiated in most developed countries by 1955. In the year since, detection method have been refined to be more specific, more sensitive and more rapid.

In 1985 only a first generation test was available using an HIV lysate as antigen in both ELISA and W.B. by 1988 recombinant sub unit antigens of HIV brought up more specific test. This was second generation tests. Then was the advent of ear of third generation test based on sub cellular fractions like synthetic peptides genetic probes, PCR, nuclei acid hybridization as also recombinant DNA technology, for large scale production of gag, pol and env proteins of HIV, which subsequently became the basis of most commercial test[166]. We in India reacted timely to detect whether this virus has reached our country also. 1985 the Indian council of medical research and in 1987 National AIDS Control Programme established a task force and initiated HIV screening of presumed risk group to detect the presence of this virus in India. Since that time we have come a long way having established an extensive surveillance network in the whole country[167].

In the present study the HIV seropositivity prevalence was carried out in various groups as follows :

Group - I Symptomatic group

Included patients who presented with signs and symptoms which were suggestive of AIDS, viz. Unexplained fever, weight loss more than month duration's, diarrhea more than 1 months, generalized lymphadenopathy,

disseminated fungal infections, multiple non healing ulcers. And the cases of pulmonary tuberculosis : who were admitted in the infectious ward of the G.G. Hospital and T.B. Hospital, Jamnagar.

Group - II Asymptomatic group

Included persons who were at very high risk of getting HIV infection but who had no clinical signs and symptoms suggestive of AIDS viz. STD patients, promiscuous heterosexual groups people whom had sexual intercourse with multiple partners, homosexual and Haemophiliacs. Cases coming to the skin and V.D. opp. of G.G. Hospital, Jamnagar. And Multitransfused patients from paediatric ward of G.G. Hospital Jamnagar.

Group - II Healthy group

In this group we included blood donors of G.G. Hospital Jamnagar, and Antenatal mothers attending the PPTC of G.G. Hospital Jamnagar.

Collection of Study material :

- Sterile plain bulb for collection of blood.
- Disposable syringe and needle.
- Storage vials
- Disposable gloves.

5 C.C. of blood was collected from each patient ascetically in plain bulb. Details as regarding the patients age, sex, education, address, marital status, history of exposure, history of drug addiction, STD and Jaundice and other necessary data were obtained from the cases.

Processing of the clinical material :

On arrival of the laboratory, the blood sample was placed at 4c for 1 hrs to facilitate better separation of serum. The separated serum was centrifuged at 3000 rpm for 10 minutes to obtain a clear serum sample. The sample were transferred to storage vials and preserved at 20°c until used. (for longtime storage at 70°c is advised).

Screening for HIV antibodies :

All samples were tested by any three of the following assays. As per WHO/GOI strategies.

- Different ELISA.
- Immuno Comb II - HIV – 1 & 2 Bispot.
- HIV TRI - DOT
- Comb AIDS - RS
- Capillus HIV - 1 / HIV - 2
- Bio Line SD HIV - 1/2.3.0
- Western blot.

We also send the sample at NARI pune, for final confirmation.

Now we are using following three tests :

(1) ELISA : ENZ aids HIV 1 + 2.

(2) Comb Aids - Rs : HIV 1 & 2 Immuno Dot test.

(3) HIV TRI - DOT.

(1) ENZ AIDS HIV 1 + 2[168] :

Enzaids used synthetic and for recombinant antigens, ensures highest level of specificity and sensitivity of the detection system (Fig. 9).

✠ ***Principle :***

The antigenic peptides/proteins are absorbed onto the wells of a microplate. These antigens are selected from immunodominant regions of HIV - 1 & 2, So as to provide maximum level of sensitivity. A special sample diluent is developed using proprietary formulation to minimise the non-specific binding. Test serum and control,s along with sample diluent are added to the respective wells and incubated. If antibodies to HIV - 1 or / and 2 envelope proteins are present in the specimen, they, will bind to the antigens coated on the solid surface. A wash step removes non specifically bound material. A conjugate consisting of enzyme horse radish

peroxidase, chemically coupled to anti human immunoglobulin is added to each well and incubated. The conjugate binds to HIV antibodies that are already bound to the immobilized antigens. A wash step removes nonspecifically bound conjugate. The substrate for the enzyme peroxidase and the chromogen 3,3', 5,5' Tetramethylbenzidine (TMB) are added to all the wells and incubated further, resulting into a blue coloured complex. The reaction is terminated by the addition of a stopping solution yielding a stable yellow colored end point. The intensity of colour is proportional to the concentration of anti HIV - 1 and / or HIV - 2 present in the test specimen or control.

✠ ***Sample :***

Serum or plasma can be used. Specimen can be kept at 2-8°c for four weeks only. Grossly haemolysed or contaminated samples should not be used in the test. Use of fresh sample is recommended. Do not use heat inactivated sample.

✠ ***Reagents :***

1. Sample Diluent :

   Contains Tris buffer, proteins and preservatives.

2. Conjugate :

   Peroxidase labelled anti human immunoglobulin containing protein stabilizers and 0.01% Thimerosal as preservatives.

3. Washing Buffer :

   Concentrated (10X) buffer containing Tween - 20 and 0.01% Thimerosal preservative. Before use, dilute by adding one volume of concentrate to 9 volumes of distilled water.

4. Negative Control :

   Anti - HIV negative human serum, negative for Anti - HIV -1 and Anti HIV - 2 and containing 0.01% Thimerosal as preservative.

5. Positive Control :

   Inactivated serum containing anti - HIV antibodies and 0.01% Thimerosal as preservative.

6. Colour reagent :

Buffer containing hydrogen peroxide and 3,3', 5,5' Tetramethyl benzidine (TMB) in solution.

7. Stopping Solution : Mineral Acid.

8. Microwell Strips :

Coated with HIV 1 + 2 Recombinant and synthetic peptides.

✠ *Storage and Stability :*

All kit components are stable until the expiration date, when stored between 2-8°c. Unused strips should be stored along with silica gel bag. Once diluted, the washing buffer is stable for one week when stored at 2-8°c.

✠ *Setting up the test :*

(1) Washing buffer : Dilute the concentrated buffer 1:10 (1+9) with distilled or Deionized water. 500 ml of diluted buffer is enough to wash all the 96 wells. If all the strips are not to be used at a time, prepare proportionate amount of diluted washing buffer.

(2) General blank, Positive and Negative controls must be included with each run. All liquid reagents must be gently mixed before use, & kept at room temperature except colour reagent.

✠ *Assay Procedure :*

1. Bring all the reagents to room temperature before use, except colour reagent (to be stored at 2-8°c, till use). Remove the required number of Microwells/Strips from the packet. Label the wells appropriately.
2. Leave the Reaction Blank well empty.
3. Dispense 200 µ of sample diluent to rest of the required wells. Use three Negative and one Positive control in each run. Add 10 µ of Negative, Positive and test serum samples to the respective wells. Mix properly and cover the strips with adhesive strip cover. Incubate for 30 minutes at room temperature (20-30°c)

4. Remove and discard the adhesive strip cover. Decant the contents of well into a waste container. Fill the wells with diluted washing buffer (approx. 350 µl) allow soak time of 30 seconds and then decant it in a waste container. Repeat for 4 more times. Drain wells on a disposable absorbent pad or towel and tap firmly to remove excess of fluid. Take care not to scratch the inner surface of well with pipette tips or tissue paper. (Add next reagent immediately)
5. Add 50µl of conjugate to each well, except the one used for the Reaction Blank Control.
6. Mix the contents of Micro wells by agitating the strips gently for 5-10 seconds.
7. Cover the strip with fresh adhesive strip cover and incubate for 30 minutes at room temperature (20-30°c).
8. Remove and discard the adhesive strip cover. Wash strips as in step 4, five times with diluted washing buffer.
9. Add 100µl of colour Reagent into each well including Reaction Bank Control.
10. Leave at room temperature for 15 minutes in dark.
11. Stop the reaction by adding 100 µl of stopping solution in all wells.
12. Mix the contents of microwells by agitating the strip gently for 5-10 seconds.
13. Using either monochromatic (450 nm) or bichromatic (450 + 630 nm) mode, absorbance reading must be taken after blanking with A1 well.
14. Calculate the cutoff value & report the results.

✠ ***Determining Cutoff Value :***

1. Negative control mean : (NCX) The absorbance of individual Negative control must be grater than 0.010 OD units and less than or equal to 0.200 OD units.

2. Positive Control : To consider the assay run to be valid, the absorbance value obtained for the positive control should be at least 1.00. If not, the assay must be repeated making sure that the procedure is being properly followed.

3. Blank : The absorbance value (OD) of reagent blank should fall between 0.000 to 0.100 in bichromatic mode.

4. Cutoff Value : The cutoff value is the mean O.D. of the Negative control plus 0.225 ex : NCX = 0.065 cut off value = 0.065 + 0.225 = 0.290.

For the assay run to be valid, the positive and negative controls must be within the acceptable range. It they are not, technique or reagents should be suspected to be at fault and the assay should be repeated.

✠ ***Interpretation of Results :***

All the samples with the absorbance less than the cutoff value should be considered Negative for anti HIV and the absorbance equal to or more than cutoff value must be considered positive for anti HIV. Non reactive results in a test for detecting antibodies against HIV - 1 and / or HIV - 2 do not exclude HIV infection with absolute certainty, if the patients happens to be in the window period. Further conformation of a reactive result with another techniques.

✠ ***Sensitivity and Specificity :***

The test system is a 100% sensitive & specificity.

(2) HIV - 1 + 2 IMMUNO DOT TEST - COMB AIDS – RS[169] :

Comb aids - Rs test is an vitro, visually read Dot immunoassay intended for the qualitative detection of IgG/IgM antibodies to the HIV - 1 & 2 in human serum or plasma (Fig. 10).

✠ ***Principle :***

Dot immunoassay employs the same principle as EIA, whereby immobolized antigen - antibody complex is visualized by means of colour producing (chromogenic) reaction. In combaids - Rs the coloured end - point is developed by a colloidal Gold - Protein - A signal reagent. Each arm of the comb is spotted with a

circular spot near the tip, by an optimally standardised blend of HIV - 1 and HIV - 2 recombinant antigens and synthetic peptides.

When incubated with a specimen containing HIV - 1 and HIV - 2 antibodies, these antibodies bind specifically to the peptide antigens. The antibody peptide complex is directly visualized after incubation with the protein - A - Colloidal Gold Signal Reagent. A positive result is indicated by the presence of magenta red coloured dot on the surface of the comb where peptides have been spotted.

✠ ***Sample :***

Serum or plasma can be used. For short-term storage, specimens can be stored at 2-8°c, However, they should be frozen (-20°c or lower) for a long term storage. Grossly haemolyzed and contaminated samples should not be used.

✠ ***Kit Contents :***

1, Washing Buffer 2, Colloidal Gold Signal Reagent, 3. Sample diluent, 4. Negative control, 5. Positive control, 6. Antigen Coated combs.

✠ ***Storage & Stability :***

All kit components are stable until the expiration date shown on the label, when stored between 2-8°c. Combs are highly sensitive to moisture so unused comb should be stored along with the silica gel bag. Once diluted, the washing Buffer is stable for one week it stored at 2-8°c.

✠ ***Setting up the test :***

Washing solution : Dilute the concentrated washing buffer 1:5 with distilled water by adding 15ml Con. buffer to 60 ml distilled water. Fill the wash tray with washing solution. Taking care to avoid foaming. Use 75 ml. diluted washing solution to wash four Combs.

✠ ***Assay Procedure :***

All kit components and samples to be tested should be at room temperature before starting the test clearly mark all samples to be tested and record their identity before starting the test.

1. Mark the sample numbers on the microtest wells and add two drops (0.1ml) of sample diluent to each well.
2. Add two drops (0.1ml) of sample or control to each of the above wells containing sample diluent. Mix the sample with diluent.
3. Cut foil pouch and carefully remove the required number of combs. Mark the sample numbers on the comb and place it into rows of corresponding microtest wells. Incubate at room temperature for 10 minutes.
4. In the mean time dispense the Colloidal Gold Signal Reagent into another set of wells which have not been previously used. Add 4 drops (0.2 ml) of colloidal gold signal reagent to each well.
5. Remove the comb from the sample containing wells and blot the tips of the arms on absorbent material. Do not blot the reactive surface of the comb. Hold the comb vertically with tips painting down and immerse into the wash solution. Wash by carefully moving the comb forward and backward in the wash solution for a total of ten times. Blot the tips of the arm again.
6. Place the comb into wells containing colloidal Gold signal reagent. Incubate at room temperature for 10 min. After incubation repeat the washing procedure as above.
7. Place the comb on a clean surface, reactive side up. Do not blot or wipe the surface of the comb. Allow the comb to air dry completely before reading the results.

✠ ***Interpretation :***

A positive result is indicated by the presence of magenta red coloured spot/dot near the tip of the arm of the comb. The absence of coloured spot indicate that the sample is free of HIV - 1 & 2 antibodies.

Positive Control - must produce coloured spot on tooth of the comb.

Negative Control - absence of any coloured spot, indicating absence of antibodies to HIV - 1 & 2.

When reading the combs, examine them in moderate light, preferably against white surface. The surface of the comb should be perpendicular (at a 90° angle) to the eye. Faint uncolored spot may be visible which does not represent true reactivity. Sensitivity & specificity indicated 100%.

(3) HIV TRI – DOT[170] :

The HIV Tri-Dot test is a visual, rapd sensitive and accurate immunoassay for the differential defection of HIV 1 & HIV - 2 antibodies in human serum or plasma (Fig. 11).

✠ ***Principle :***

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent. As the patient's sample pass through the membrane, HIV antibodies, if present, bind to the immobilized antigens. Conjugate binds to purple DOT (S) against a white background.

✠ ***Kit Contents :***

1. HIV tridot test device - packed individually. Device has membrane with 1 control and 2 test dots, one each for HIV - 1 & HIV - 2.
2. Buffer solution - containing BSA, Sodium azide.
3. Protein - A - Conjugate in liquid form containing sodium azide.
4. Negative Control : lyophilized normal human serum tested non reactive for HBsAg, HCV, HIV - 1 & 2, contains 0.05% sodium azide, Reconstitute with diluent B (add whole of it).
5. Positive control : lyophilized rabbit serum positive for antibodies to HIV - 1 & 2 contains 0.05% sodium azide. Reconstitute with entire of diluent C.
6. Diluent - B & C.

✠ ***Storage:***

Store the kit at 4°C - 8°c. The components are stable for 12 months from the date of manufacturing, when stored at 4-8°c. Do not freeze the kit.

✠ *Test Procedure :*

Bring all the reagents and specimens to room + temperature (20-30°c) before beginning the test.

1. Add 3 drops of buffer solution to the center of the device. Allow it to soak.
2. Hold the dropper vertically and add 1 drop of patients sample. (serum of plasma).
3. Add 5 drops of buffer solution; allow it to soaked.
4. Add 2 drops of liquid conjugate directly from the vial. Again allow it to soak.
5. Add 5 drops of buffer solution and read result.

✠ *Interpretation of Results :*

- Negative result : If only one dot (only the control dot) appears, the specimen is non reactive for antibodies to HIV - 1 & 2.
- Positive result : 1. If two dots, one for the control and the other for HIV - 1 appears, the specimen is reactive for antibodies to HIV -1.
- If two Dots, one for control and other for HIV - 2 appear, the specimen is reactive for antibodies to HIV - 2.
- If all three dots, one each for control, HIV - 1and HIV - 2 appear, the specimen is reactive for antibodies to both HIV - 1 & HIV - 2.
- Invalid test : If no dot appears after the test is complete, either with clear background or with complete pinkish/purple background, the test indicates error. The specimen should be retest on a new device.
- Every Samples retested irrespective of positive or negative for quality control. As well as 20% HIV seropositive & 5% HIV seronegative sample sent at NARI Pune for confirmation.

OBSERVATIONS & RESULTS

A total of 53,671 blood samples from blood donors and 12,165 serum samples from clinically suspected cases of HIV, Tuberculosis patients, Sexually transmitted disease patients, Multitransfused thallassaemic children and Antenatal women were screened for HIV infection as under :

The samples were classified in symptomatic, Asymptomatic is healthy groups and detect HIV seropositivity by ELISA, Comb-Aids, and HIV Tri-dot test.

TABLE - XIV

GROUP WISE THE NUMBER OF CASES SCREENED

Group No.		Group	No. of tested Samples	No. of HIV Seropositive	Percentage
I	A	Symptomatic: Pt's with clinical sign & symptoms which were suggestive of HIV infection & patients.	4265	1103	25.86%
	B	TB patients.	2000	124	6.2%
II		Asymptomatic :-			
	A	Pt's attending STD clinic at G.G. Hosp. Jamnagar	2700	126	4.6%
	B	Multitransfused patients (Thallassaemic Children)	200	05	2.5%
III		Healthy Group :-			
	A	Pregnant women attending the antenatal clinic at G.G. Hosp. Jamnagar.	3000	07	0.23%
	B	Blood donors who donate blood at G.G. Hosp. Jamnagar	53671	199	0.37%

		TOTAL	65836	1564	2.3%

GROUP - I : TUBERCULOSIS (TB) PATIENTS

Table - XV

Incidence of HIV seropositivity in TB patients

Total No. Screened	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Percentage Positivity*
2000	124	1876	6.2%

Table - XV shows HIV seropositivity among clinically suspected cases of tuberculosis with the over all prevalence rate of 6.2%, a total 124 cases being positive out of 2000 screened (Graph - 1).

Table - XVI

Gender wise distribution of HIV seropositivity Aamong TB patients

Gender	*Total No Screened*	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Rate of Positivity (%)*
Male	1565	110	1455	7.02%
Female	435	14	421	3.2%
TOTAL	2000	124	1876	6.2%

Table - XVI shows the HIV seropositivity in TB patients in relation to sex with positivity rate of 7.02% among males, a total of 110 being positive out of 1565 screened and positivity rate of 3.2% among females, a total of 14 cases being positive out of 435 screened. Rate of positivity is more in male patients than female patients.

Table - XVII

Incidence of HIV-seropositivity in TB patients with relation to age

Age Group	*Total No Screened*	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Rate of Positivity (%)*
0-20	151	10	141	6.62%
21-30	770	59	711	7.66%
31-40	595	29	566	4.87%
41-50	340	17	323	5.00%
> 50	144	09	135	6.25%
TOTAL	2000	124	1876	6.2%

Table - XVII shows the incidence of HIV Seropositivity among clinically suspected TB pt's in relation to age, with the higher positive rate of 7.66% in the age group 21-30 years, a total 59 being positive out of 770 screened. This age group is more prone for TB & HIV infections (Graph - 2).

GROUP - II : STD PATIENTS (SEXUALLY TRANSMITTED DISEASE)

Table - XVIII

Incidence of HIV Seropositvity in STD patients :

Total No. Screened	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Percentage Positivity*
2700	126	2574	4.66%

Table - XVIII shows HIV seropositivity among STD patients who attend the STD clinic at G.G. Hosp. Jamnagar with over all prevalence of 4.66%, a total of 126 being Seropositive out of 2700 screened.

Table - XIX

Incidence HIV Seropositivity in STD pt's in relation to sex :

Sex	*Total No Screened*	*No. of HIV Seropositive*	*Rate of Positivity (%)*
Male	795	45	5.66%
Female	1905	81	4.25%
TOTAL	2700	126	4.66%

Table - XIX shows that out of 795 male tested 45 were HIV Seropositive (5.66%) and out of 1905 female tested 81 were HIV Seropositive (4.25%) Rate of positivity is high in male STD patients than female.

Table - XX

Incidence of HIV Seropositivity in STD patients relation to Age & Sex

Age	*Male*			*Female*			*Total*		
Years	*Tested*	*+ve*	*+ve%*	*Tested*	*+ve*	*+ve%*	*Tested*	*Positive*	*+ve%*
< 20	45	02	4.44	135	03	2.22	180	05	2.77
21-30	321	23	7.16	810	37	4.56	1131	60	5.30
31-40	279	13	4.65	695	25	3.59	974	38	3.90
>40	150	07	4.66	265	16	6.03	415	23	5.54
TOTAL	795	45	5.66	1905	81	4.25	2700	126	4.66

Table - XX Shows HIV Seropositivity more in male patients and is more in 21 to 30 age group. Out of 795 male screened 45 (5.66%) were positive & age group 21 to 30 years (7.16%) is more prone for infection. In female out of 1905 screened 81 (4.66%) were HIV Seropositive & age group 21-30 (5.30%) is more effective (Graph - 3).

Table - XXI

Local distribution of STD patients

Local	*Male*	*Female*	*Total*
Rural	290	695	985
Urban	505	1210	1715
TOTAL	795	1905	2700

Table - XXI shows urban people are more infective than rural. Out 2700 screen for STD 1715 were urban as 985 were rural.

Table - XXII

Incidence of STD and types of STD

Disease	*Male*			*Female*			*Total*		
	Tested	*+ve*	*+ve%*	*Tested*	*+ve*	*+ve%*	*Tested*	*Positive*	*+ve%*
Genital Uncler	435	25	5.74	145	05	3.44	580	30	5.17
Urethral Discharge	205	04	1.95	-	-	-	205	04	1.95
Genital Discharge	-	-	-	1554	61	3.92	1554	61	3.92
Genital Ulcer & Discharge	16	02	12.5	80	03	3.79	96	05	5.20
Genital Warts	139	14	10.07	126	12	9.52	262	26	9.81
TOTAL	795	45	5.66	1905	81	4.66	2700	126	4.66

Table - XXII shows different types of sexually transmitted disease. Out of 795 STD male screened 16 were infective with genital ulcer & discharge 2 (12.5%) were HIV Seropositive & 139 infective with genital warts 14 were (10.07%) HIV Seropositive. In female out of 1905 patients screened 126 were infective with genital warts, 12 (9.52%) were HIV Seropositive. So in STD group genital discharge with ulcer & warts are more common (Graph - 4).

GROUP-II : MULTITRANSFUSED PATIENTS (THALLASSAEMIC CHILDREN)

Table - XXIII

Incidence of HIV Seropositvity in Thallassaemic patients :

Total No. of Tested Sample	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Percentage %*
200	05	195	2.5%

Out of 200 multitransfused thallassaemic children 05 were seropositive for HIV by ELISA, Tri-dot or Comb-Aid test. The rate of positivity is 2.5%.

Table - XXIV

Incidence of HIV Seropositivity in thallassaemic children with relation of Age & Sex

Sex	*Age*					
		<2 yrs	*2 to 4*	*>4 to 8*	*> 8 to 13*	*TOTAL*
Male	Tested	46	40	49	22	157
	Positive	0	1	2	0	3
Female	Tested	14	12	09	08	43
	Positive	0	0	1	01	02 (4.65%)

Above table shows that out of 157 male patients, 3 (2.36%) were HIV Seropositive. Out of 3 Seropositive 2 belonged to age group >4 to 8 and 1 belonged to age group 2 to 4 No positivity found in other age group. Out of 43 female patients 2ee (4.65%) were found HIV Seropositive. Out of 2 Positive patient 1belonged to age group >4 to 8 & 1 belonged to >4 to 13 age group. HIV Seropositivity is more in male patient and in >4 to 8 years age group (Graph - 5).

Table - XXV

Incidence of HIV-Seropositivity in thallassaemic children with relation to number of blood transfusions

No of blood transfusions	*Total Tested*	*Positive*	*Percentage*
0-50	61	1	1.63%
51-100	70	2	2.85%
101-150	39	2	5.12%
151-200	22	0	0.0%
<200	08	0	0.0%
TOTAL	200	5	2.5%

Table - XXV shows HIV Seropositive patients who had received about 50 blood transfusions. Two patients had received about 100 & 2 about 150 blood transfusions, indicating that the incidence of HIV infection increases with transfusions.

Table - XXVI

Distribution of Thallassaemic children according to the Age of the number of transfusions

No. of Transfusions	*Age*				
	<2	*2 to 4*	*> 4 to 8*	*> 8 to 13*	*TOTAL*
0-50	18	23	15	5	61
51-100	2	08	54	6	70
101-150	0	02	14	23	39
151-200	0	02	06	14	22
<200	0	00	00	08	08
TOTAL	20	35	89	56	200

Table - XXVI shows numbers of transfusions in relation with the age. HIV Seropositivity is more in > 4 to 8 age group Children & out of 200 children 70 children had received 51-100 blood transfusions (Graph - 6).

GROUP-III : ANTENATAL MOTHERS

Table - XXVII

Incidence of HIV Seropositvity in Pregnant women

No. of Pregnant Women Screened	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Percentage %*
3000	07	2993	0.23%

Table - XXVII shows HIV-Seropositivity among pregnant women with overall prevalence of 0.23%. A total of 7 cases being HIV Seropositive out of 3000 screened.

Table - XXVIII

Incidence HIV-Seropositivity in relation to Age

Age of Group	*No. of Sample Screened*	*No. of HIV Seropositive*	*Percentage Positivity*
16-20	812	00	0.0%
21-30	1513	06	0.39%
31-40	495	01	0.20%
>40	180	00	0.0%

Table - XXVIII shows age wise distribution of 07 HIV Seropositive cases with highest incidence in age group 21-30 years of 0.39% (Graph - 7).

Table - XXIX

Incidence HIV Seropositivity in relation to different trimester of pregnancy

Trimester	*No. of Sample Screened*	*No. of HIV Seropositive*	*Percentage Positivity*
I	641	1	0.15%
II	859	1	0.11%
III	1500	5	0.33%

Table - XXIX shows that maximum number of cases were positive in third trimester of pregnancy. 5 were positive out of 1500 (0.33%) screened (Graph - 8).

Table - XXX

Incidence HIV Seropositivity in Pregnant women whose husband has a HIV.

No. of Pregnant women screened	*No. of HIV Seropositive mother*	*No. of HIV Seropositive husband.*
3000	7	3

Table - XXX shows that out of 3000 cases screened for HIV Seropositivity 7 Pregnant women were HIV Seropositive. Out of 7 positive women only 3 husbands were HIV Seropositive.

Table - XXXI

Incidence of HIV Seropositivity according to the education status in pregnant women

Education	*No. of Sample Screened*	*No. of HIV Seropositive*	*Percentage Positivity*
Illiterate	1190	04	0.33%
Up to Primary	864	02	0.23%
UP to Higher Secondary	830	01	0.12%
Graduate & above	116	00	0.00%

Table - XXXI shows HIV - Seropositivity is more in Illiterate pregnant women. Out of 1190 women 4 were HIV Seropositive (0.33%).

GROUP-III : BLOOD DONORS

Table - XXXII

Incidence of HIV Seropositvity in Blood Donors

No. of Blood Donors Screened	*No. of HIV Seropositive*	*No. of HIV Seronegative*	*Rate of Positivity*
53671	199	53472	0.37%

Out of 107343 blood donors 199 were Seropositive for HIV by ELISA, Tri-dot or Comb-Aid test, The rate of positivity in blood donors is 0.37%.

Table - XXXIII

Incidence HIV-Seropositivity in blood donors relation to Age

Age Group in years	*No. of Sample Screened*	*No. of Sample HIV-Seropositive*	*Percentage %*
18-20	4021	09	0.22%
21-30	24375	101	0.41%
31-40	18230	72	0.39%
>40	7045	17	0.24
TOTAL	53671	199	0.37%

Table - XXXIII shows age group wise Seropositivity for HIV in blood donors, the highest positivity in age group 21-30 years is 0.41%, which shows in table (Graph - 9).

Table - XXXIV

Incidence of HIV-Seropositivity in blood donors relation to gender

Sex	*Total No Screened*	*No. of HIV Seropositive*	*Rate of Positivity (%)*
Male	50796	197	0.38%
Female	2875	02	0.06%
TOTAL	53671	199	0.37%

Table - XXXIV shows sex wise HIV Seropositivity in blood donors. HIV-Seropositivity in male is 0.38% and in female is 0.05%. So the Seropositvity rate is more in male patients than female.

Table - XXXV

Incidence of HIV Seropositivity according to category of blood donors

Category		***No. of Sample Screened***	***No. of Sample HIV-Seropositive***	***Percentage Positivity***
Replacement Donors	M	36414	189	0.51%
	F	460	01	0.21%
Voluntary Donors	M	14382	08	0.05%
	F	2415	01	0.04%

Table - XXXV shows that HIV Seropositivity is found more in replacement (0.51% male and 0.21 female) donors as compared to voluntary donors (0.05% male and 0.04% female). Out of 199 HIV Seropositive sample 190 (0.51%) are replacement donors and 8 (0.05%) are voluntary donors (Graph - 10).

DISCUSSION

The HIV/AIDS epidemic represents the most serious public health problem in the world / India. There is no denial of the enormity of the problem. The prevalence of the infection in all parts of the country highlights the spread from urban to rural areas and from high risk to the general population. It is estimated that 4.5 million Indians were infected with the virus. Migration of labor, low literacy levels leading to low awareness, gender disparities, prevalence of sexually transmitted disease and reproductive tract infections are some of the factor attributed to the spread of HIV (AIDS).

A large variety of assays are available today to test the presence of anti-HIV antibodies. In present study was undertaken to find out the incidence of HIV seropositivity in different groups like :

Group - I : Symptomatic :

Patients who presented with signs and symptoms which were suggestive of HIV/AIDS and - TB patients.

Group - II : A symptomatic :

Person who were at very high risk of getting HIV/AIDS but who had no clinical signs and symptoms suggestive of HIV/AIDS.

- Sexually transmitted disease patients &
- Multitransfused Thallassaemic children

Group - III : Healthy individuals :

- Antenatal mothers &
- Blood donors.

The present study results are compare with other places and workers are as under.

Group - I TUBERCULOSIS

In April 1993, WHO declared tuberculosis as a global health emergency to curb the epidemic and called for immediate action. Tuberculosis is responsible for 27% of preventable deaths worldwide and in one of the most common preventable infections disease in India[171].

Tuberculosis has becomes a major threat to HIV infected people all over the world. This impact has been experienced in many African, Asian and American countries. The association of TB with HIV infection was first recognized in Haitians and intravenous drug users[172]. The growing epidemic of HIV has breathed new life into an old enemy-tuberculosis. Over the past few years, with the increase in HIV infection, TB has become a common clinical presentation of HIV infection. The HIV epidemic spurs the spread of TB and increases the tuberculosis risk for the whole population. For those who are HIV positive, the TB risk is especially great and the outcome often fatal.[173-175]

India has an enormous reservoir of tubercular infection, thus co-infection with HIV increases the rate of TB reactivation and mortality. Knowing the HIV status of TB patients has important benefits. TB & HIV are strongly associated and can be thought of as interacting synergistically. Most importantly, TB can be the 'yellow flag' that leads to the discovery of an unsuspected HIV infection, opening the door to the benefits of anti-retroviral treatment, preventive therapy for other opportunistic infection and counseling to reduce HIV transmission[176].

In India, under National Tuberculosis Control Programme 1.5 million cases are detected every year but still 1200 cases die due to it daily[177]. TB had re-emerged in countries were it was supposed to be on a decline. Global explosion of HIV infection coupled with chaotic with TB, the world today is threatened with untreatable epidemic of TB. India has an enormous reservoir of tubercular infection, thus co-infection with HIV increases the rate of TB reactivation and mortality. Here describe the prevalence of HIV infection in TB patients in Jamnagar.

The present study carried out a total of 2000 suspected cases of tuberculosis were screened to detect HIV antibodies, ELISA, HIV seropositive sample conformed by another test HIV - Tridot & Comb Aids.

In the present study 124 out of 2000 clinically suspected patients of tuberculosis were found HIV seropositve. With the prevalence rate of 6.2%. The incidence of HIV seropositivity in TB Pt's in various parts of world and the India is as :

Incidence of HIV seropositivity in TB Pt's from different parts of world

Sr. No.	***Author***	***Year of Publication***	***Place***	***Percentage positivity***
1.	Pitchenic AE[178]	1987	-	31.0%
2.	Onorato et al[179]	1994	UK	7.4%
3.	Shandera et al[180]	1997	Africa	20.0%
4.	Ackah et al[181]	1998	Abidjan	440.7%
5.	Horsburgh et al[182]	1998	US	41.0%
6.	Faggiano F[183]	1999	Itali	25.6%
7.	Bowen et al[184]	-	London	23.0%
8.	Robert J. et al[185]	2000	Paris	28.0%
9.	Punnotok J et al[186]	2000	Thailand	22.0%
10.	Nicholosn O et al[187]	2000	UK	10.0%
11.	Kiwanuka J.P. [188]	2000	Uganda	48.9%
12.	Salgueiro [189]	2004	Santiago	2.4%

The highest rate of incidence of HIV seropositivity in clinically suspected cases of tuberculosis is found in Sub-Saharan Africa 202 & it is found as high as 70%.

The researchers recorded pt's with tuberculosis at Grady Memorial Hospital Atlanta by Horsburgh[182] out of 1203 patients identified with TB, 493 (41%) were HIV seropositive similar high rate also detected at Uganda by Kiwanuka JP[188] 48.9%, and Ackah at Abidyan 44.7%.

In a hospital in Paris Robert J[185], the prevalence of HIV infection among TB patients increased from 2% in 1983 to 28% in 1993. Same prevalence rate also detect by Punnotok J Thailand,[186] Bowen[184] at London, Faggiano F Itali[183], Shandera Africa[180], and Pitchenic AE[178], 22%, 23%, 25.6%, 20% and 31% respectively.

In the present study, out of 2000 tuberculosis patients screened only 124 (6.2%) were found to be HIV seropositive. This rate of Seroprevalence is comparable with the rate in UK & it is found (7.4^%) very near by, and Nicholson UK is 10.0%.

In the Santiago the rate is as low as 2.4% study by Salgueiro et al[189].

India also conducted the study among patients with tuberculosis and HIV seropositivity have shown wide variation in the prevalence of HIV seropositivity.

Incidence of HIV seropositivity in TB Pt's from different part of India.

Sr. No.	***Author***	***Year of Publication***	***Place***	***Percentage Positivity***
1.	Kulshreshtha R. [190]	1997	Lucknow	1.04%
2.	Mohanti KC [191]	1998	Mumbai	33%
3.	Mohanti KC [192]	1999	Mumbai	74%
4.	Jain S.K. [193]	2000	Delhi	1.52%
5.	Deivanayagum CN[194]	2002	Chennai	4.42%
6.	Talib SH [195]	2002	Aurangabad	4.70%
7.	Kumar P [196]	2002	Delhi	13.95%
8.	Solomon S [197]	2002	Madras	1.6%
9.	Dey SK [198]	-	Calcutta	0.67%
10.	Ramchandra R [199]	2003	Chennai	4.7%
11.	Sharma S.K. [200]	2003	Delhi	9.4%
12.	Prasad R [201]	2004	Lacknow	2.8%
13.	Present Study	-	Jamnagar	6.2%

A study conducted by KEM Hospital in Mumabi by K.C. Mohanty[191] from 1987 to 1999 HIV seropositivity shows 2.4% to 74%, Jain SK et al[193] at Delhi from sentinel surveillance of the tuberculosis patients clinic. Showed prevalence of 0.68 to 1.52% in 1998. This data clearly indicate that percentage of the disease increased by the year.

Present study, HIV seropositivity rate 6.2% also comparable with Deivanayagum chennai[194], Talib SH at Aurangabad[195] and Ramchandra R Chennai[199] showed 4.42% and 4.7% respectively.

Sharma S K[200] showed slightly high 9.4% prevalence at Delhi. Some studies from other parts of India shows very low prevalence rate. Dey SK[198] Culcutta, Solomon S[197] Madras and Prasad R[201] Lucknow showed very low HIV seropositivity among TB patients it is 0.67%, 1.6% and 2.8% respectively.

This deviation of HIV seropositivity may be due to co-infection, substantially under estimated because of under diagnosis of HIV infection or un-responding an un co-operative nature of patient. In the region such as the Western Pacific & South East Asia, WHO data indicates that HIV/TB co-infection is much less common than it is in Africa, although it is likely that the incidence of co-infection is substantially under estimated because of under diagnosis of HIV infection[203].

In the present study as shown in table, out of 2000, 1565 male TB patients screened for HIV seropositivity 110 were found to be HIV seropositive and out of 435 females of TB patients, only 14 were HIV seropositive. The rate of incidence for male (7.02%) is higher than females (3.2%). The result shows the males are more prone than females. Because males are generally labour & have a nutritional deficiency.

The results are also comparable with Salgueiro[189], showed that 568 male out of 946 and Horsburgh[182], showed that 380 out of 493 were males. Onorato IM[179] in his study found that the median age of all 337 persons with TB screened was 33 (range 24-67 years) 10 (3%) being co infected with HIV & all were males.

In present study incidence of HIV seropositivity is more in the age group of 21-30 years. (7.66%) Other age groups shows only slightly low infectivity rate, so all the age groups of TB patients were susceptible for HIV infection.

So the infection with TB and HIV represents a major public health problem in many parts of the world[204].

The association of TB with HIV was so compelling that WHO included TB in HIV positive individuals to AIDS case definition. Hence TB in HIV infected person falls in high priority area and deserves better attention that present[205].

Group - II : SEXUALLY TRANSMITTED DISEASE (STD).

Heterosexual contact has emerged as the single largest cause of the spread of HIV in south East Asia. The growing evidence available from all over the world undoubtedly indicates that the incidence of HIV infection is higher in conditions of presence of sexually transmitted diseases. It is established that the presence of STD'S increases the risk of HIV by 8-10 times[206].

Women are at greater risk of being infected by their male partner because transmission from male to female is more efficient than from female to male. During sexual intercourse, damage to the lining of sexual organs can transmit HIV from the infected partner to the uninfected on by exchange of body fluids. It is easier for the virus to be transmitted if the uninfected partner is already suffering from STDs because in this case the lining is already damage. It is also possible that the virus is transmitted to WBC which are normally found on the lining of various openings of the body such as the genital areas. Irritation, infection and damage to the lining spurs the WBC to rich here in large number to repair damage and offer protection from infection thereby increasing their concentration locally. This is turn increases the risk of HIV transmission. STDs remain serious public health problem in Asia and rank among the top five diseases in adult who seek health care services In India the prevalence of HIV in STD varies from 1.5% to 23%.

HIV is sexually transmitted disease so why the STD is stated in high risk group. For the prevention of HIV the rate of STD should be reduced. So NACO

started STD surveillance clinic at every state. State stared center in their cities. Gujarat Started Sentinel Surveillance from 1994 in the 8 centres. The prevalence rate of last five years was 2.5 to 6.17% in STD patients. So the Gujarat is in medium prevalence state[207].

Incidence of HIV seropositivity in STD Pt's by different Indian workers

Sr.No.	Author	Year of Publication	No. Tested	Percentage Positivity
1.	Quinn et al[208]	1988	4028	5.20%
2.	Kamat H.[209]	1989	599	5.17%
3.	Chandra M.[210]	1991	2086	14.0%
4.	Malvika Kohli[211]	1991	2500	2.07%
5.	Jivrajani H.[212]	1992	1150	5.40%
6.	Dr. Parasuram[213]	1992	2293	5.40%
7.	John J.J.[214]	1993	832	2.64%
8.	Brajachand SN[215]	1994	-	4.8%
9.	Sharma V.K.[216]	1996	4539	1.12%
10.	Srikrishnan A K[217]	1997	774	5.9%
11.	Godkari D.A.[218]	1998	302	23.5%
12.	Elmy S. Rasul[219]	1999	-	29.0%
13.	Bajaj J K et al[220]	2000	3124	10.9%
14.	Kumar B. et al[221]	2001	53	5.66%
15.	Present Study	-	2700	4.66%

In present study 2700 blood samples of STD patients were tested for HIV seropositivity. Out of that 126 samples were observed HIV seropositive. The rate of HIV seropositivity is 4.66% out of 2700 patients 795 were male is 45 (5.66%) and in female 81 (4.25%). The females were more attendees of STD clinic than males, but seropositivity rate is found higher in males.

The maximum number of patients was between 21-30 years of age maximum positivity is also found in this group. This group is sexually active group so positively rate higher than other age group.

Brajachand S.N.[215] the sentinel surveillance for HIV infection at Manipur. The aim of study was to seen the paint prevalence of HIV infection among the various risk group in selected sentinel population and to see the trend of HIV infection. The sample were collected from STD clinic and tested for HIV antibody by ELISA. The seropositivity in STD patient was 4.8%. Our finding is exactly correlate with this findings.

Mallika Kohli[211] in span 21/2 years, approximately 2500 STD cases attending the out patient & their spouses were screened for HIV infection using ELISA. A total 124 patients (118 male, 6 female) were ELISA positive, 66 conformed by W.B. test. HIV seropositivity rate 2.07% observed.

T. Jacob John[214] at Vellore (1993) screened 832 patients of STD clinic. 22 (2.64%) were HIV seropositive. While in 1986 (0.16%) were positive. The rise in the prevalence rate has been gradual at vellore, where as it has been more steep in Bombay. In Bombay rate during 89-90 was 4.3% thereafter the rise was more rapid in 1993 26.0% Sharma V.K.[216] also screened 4359 STD cases they got 1.12% rate of HIV seropositivity. So all these workers results were lower than our study.

Quinin et al[208], Kamat H[209], Jivrajani[212], Dr. Parsuram[213], Shriskrishna AK[217] and Kumar B[221] screened STD patients. Their seropositivity should 5.2%, 5.17%, 5.4%, 5.4%, 5.9% and 5.66% respectively. This seropositivity rate was slightly higher than our study rate.

Chandra M[210] showed that 2086 patients suffering from all type of STD were screened for HIV seropositivity. Of these & out of 57 patients presenting with genital ulceration showed HIV antibodies indicating incidence of 14% HIV infection in genital ulcer disease.

Bajaj J K[220] at Aurangabad also screened 3124 STD patients. HIV seropositivity rate 10.9% was observed. Among 3124 patients 266 (78%) were male

and 75 (22%) females. Males were more than female in positively rate. These ratio similar like our study. Overall HIV seropositivity higher than our study.

Godkari D.A.[218] detected HIV seropositivity 23.5% at Pune. Elmy S. Rausl at Gawahati also found 29.0 rate in STD patients. There results showed very high prevalence rates than our study rate.

Our results are comparable with the global study results.

Incidence of HIV seropositivity in STD patients in different parts of the world

Sr.No.	***Authors***	***Years***	***Place***	***Percentage Positivity***
1.	Simonsen et al[222]	1988	Nairobi	11.20%
2.	Komatsu R. et al[223]	2000	Tokyo	0.9%
3.	Kjm AA et al[224]	2000	Santrancisco	7.1%
4.	Sullivan P.A, et al[225]	2001	Australia	0.0%
5.	Rest V et al [226]	2002	Germany	0.17%
6.	Sugihantono A.[227]	2003	Indonesia	0.5%
7.	Klausner JD[228]	2004	OUSA	40.6%
8.	Belza M J et al[229]	2004	Spain	0.7%

Sullivan PA[225] at Australia, Studied 427 pregnant women with STD, but the rate of HIV seropositivity was 0%.

Komatsu R[223] at Tokyo, Resl. V.[226] at Germany, Sugihantono A at Indonesia[227] and Belza M. J.[229] at Spain also studied the HIV seroprevalence rate in STD patients and it is 0.9%, 0.17%, 0.5% and 0.7% respectively. Prevalence rate of HIV seropositivity is very lower than our study rate.

Klausner J.D.[228] USA screened 1361 patients of STD. Out of them 553 (40.6%) were HIV antibody positive. This rate is very high.

Kim A A[224] et al at San Francisco 564 msm (men who have sex with men) surveyed. HIV seropositivity rate found 7.1% this is slight higher than our rate.

The STD associated with genital ulcer diseased was studied by Simonsen et[222] al 1988 in Nairobi, demonstrated that men who reported frequent sexual contact

with prostitutes and who has currently diagnosed genital ulcer were significantly more likely to infected with HIV antibodies. He detected 11.2% HIV antibodies positive out of 340 men. This rate is higher than our results.

GROUP - II MULTITRANSFUSED PATIENTS (Thallassaemic children)

Thallassaemia is the commonest single gene disorder in India[223]. They from a heterogenous group of conditions resulting from a wide variety of mutations of genes which code for hemoglobin synthesis. Beta thallassaemia major is the hemozygous from, inherited recessively and resulting in reduced or absent beta chain production. It's distribution is concentrated in a belt stretching from the mediterranean through the Middle East, India and South East Asia. It also occurs in parts of West Africa[231].

In India regional carriage rates have been estimated at between 0.6 and 15% Maximum number of cases belonged to Sindhi, Punjabi and Gujarati Community[232].

The mainstay of management of thallassaemia patients is regular blood transfusion and iron chelation. Transmission of various infections is a known complication of chemotherapy regimens. Thallassaemia major patients are exposed to the risk of various transfussion transmitted infections (TTI) due to repeated blood transfusions. It has been calculated that for every unit of blood transfusion, there is 1% chance of transfusion associated problems, including TTIS[233]. A linear correlation between increased number of transfusion and the increased incidence of TTIS, has also been reported[234]. The major transfusion transmitted agent are HBV, HCV, HIV, T-Pallidum and plasmodium Spp. Also it may give rise to transfusion acquired iron overload.

Transfusion of HIV via infected blood and blood products is among the major routes of HIV transmission[235]. HIV is a potentially dangerous virus transmitted through blood and children receiving multiple blood transfusions are at a greater risk to get it. In fact transfusions may be responsible for as many as 22% of all cases of HIV infection in children[236].

Beta thallassaemia major children, being transfusion dependent, constitute an important risk group for HIV infection. The reported HIV seroprevalence rate in such children very from 1.09-38.5% in different part of the world.

Parenteral transmission due to blood & blood product is the most efficient route, the risk being over 90% in a single episode. So screening of blood/blood product recipients has attracted wide attention in India.

In the present study 200 serum samples of thallassaemic children were screened for anti HIV antibodies. 5 children were seropositive (2.5%) for HIV infections.

Incidence of HIV seropositivity in thallassaemic children as discovered by different workers

Sr. No.	***Authors***	***Year os Publication***	***No. Tested***	***Positivity %***
1.	M.A.F. ElHazami et al[237]	1989	212	2.35%
2.	Manorama Bhargava et al[238]	1991	185	0.54%
3.	Khan M.A.[239]	1992	203	8.37%
4.	Amrapurkar D.N. et al.[240]	1992	40	2.5%
5.	S.K. Bichite et al.[241]	1992	50	6.0%
6.	Surjitsingh et al.[242]	1993	100	0.0%
7.	V.P. Chaudhury et al.[243]	1993	91	0.0%
8.	A.P. Dubey et al.[244]	1993	75	9.3%
9.	S. chandra et al.[245]	1993	22	0.0%
10.	S. Sen et al.[246]	1993	203	8.9%
11.	Sudarshan Kumar et al.[247]	1994	223	3.5%
12.	Chaudhury N. et al.[248]	1995	19	0.0%
13.	Renu Vohra et al.[249]	1995	89	8.9%
14.	Sur D. Chakraborty AK[250]	-	330	0.9%
15.	Present Study	-	200	2.5%

In the present study 5 out of 200 samples of patients with thallassaemia major syndrome were positive for anti HIV antibodies showing an incidence of

2.5%. Out of five positive patients 3 were males & 2 were females, belonging to the age groups 3 were in > 4 to 8 & 1 in age group of 2-4 years and 1 of > 8 to 13 years of age group. Out of five seropositive patients 1 had received more than 40 blood transfusion, 2 were more than 50 and 2 were more than 100 transfusion.

All the blood units have been screened for anti HIV before issuing, as HIV screening is mandatory in all blood banks. All the blood units were anti HIV seronegative. The parents of all the HIV positive children were screened for HIV infection. They all were HIV seronegative. So one reason for the HIV seropositivity in the thallassaemic children could be due to the presence of a seronegative infective stage in the HIV infected blood donor called the window period.

Frequency of HIV markers were investigated in multi transufused thallassaemic patients by M.A.F. EL. Hazmi et al[237] who reported only 5 thallassaemic out of 212 (2.3%) were positive for HIV, and D.N. Amrapurkar (1992)[240] studied 40 thallassaemic in which 2.5% were HIV seropositive. Both the study is similar to our results.

Khan M.A.[239] screened 203 multi transfused children with thallassaemia attending the thallassaemia clinic of Charak Plika hospital, New Delhi for anti HIV antibodies by ELISA test. All positive cases were confirmed by western blot test. of the 203 children screened 17 (8.37%) were HIV seropositive. A.P. Dubey et al[244] found 7 out of 75 multi transfused children seropositive for HIV antibody (9.3%) Renu Vohra[249] screened 89 thallassaemic 8 (8.9%) were HIV seropositive. S. Sen et al[246] screened 203 thallassaemic children 18 (8.9%) were seropositive. These all studies shows the very high prevalence rate than present study. The reason for such a high rate of seropositivity is speculative, since most of the blood these children received was from voluntary donors. S.K Bichile et al also showed very high rate (6%) of HIV seropositivity in thallassaemic children.

Surjitsingh et al[242] and V.P. Chaudhary et al[243] screened blood samples of thallassaemic patients for anti HIV antibodies by ELISA. All patients were negative for anti HIV antibodies. Surjitsingh screened 100 patients. These patients had been

on a regular transfusion programme for periods varying from 1-12 years. All the children were seronegative. Similar results also shown from S. Chandra et al[245] and Chaudary N. et al[248]. They screened 22 and 19 samples. They all were seronegative. These results indirectly reflected the absence of, or a very low HIV seropositivity in voluntary blood donors of the region. The patients were transfused with blood from the blood bank society with obtained its supplies from voluntary donors only.

Sudarshan Kumar[247] screened 223 multitransfused patients in the age group of 1-25 years. 8 children (3.5%) showed HIV seropositivity. One reason for this seropositivity could be that all these positive cases had received blood from other blood bank also. Possibly unscreened ones. Which would have been transfused as.

In the present study, low incidence of HIV seropositivity (2.5%) is seen. The reason for the low incidence is that routine screening of blood units for HIV infection has become mandatory before issuing. So, this routine screening will detect HIV infection (unless the donor is in window period) and thus save the recipient from HIV infection. Other reason of low incidence in a hospital proper selection of healthy blood donor is undertaken before transfusion.

GROUP - III PREGNANT WOMEN ATTENDING THE ANTENATAL CLINIC AT G.G. HOSPITAL JAMNAGAR. (Antenatal Group)

Over the past decade, the global epidemic of HIV infection has become a major focus of preventive, therapeutic and community health care in all parts of the world. The importance of the problem cannot be over state when we consider the situation in a developing country like ours where the major route of transmission is heterosexual. This leaves the providers of care to women with a particularly acute problem and the responsibility to care for two patients the prospective mother and the fetus. Transmission of HIV infection from mother to infant are called 'vertical transmission."

Perinatal transmission of HIV affects nearly 5,00,000 infants each year world wide, most of them born in developing countries[251]. Recent sentinel surveys

amongst pregnant woman in Maharashtra has shown a seroprevalence rate of 0.25% to 1%[252]. Considering 25 million births per year in our country, a seroprevalence amongst pregnant women is of 1% and a vertical transmission rate from mother to child is around 30%[253], we would expect to have 7500 HIV infected neonates born every year. Reducing burden of pediatric morbidity from mother to child transmission of HIV is important. Mother to child transmission rates have shown to have wide variations amongst different populations[254] Transmission rates ranging from 14% to 33% have been reported in the USA and Western Europe[255]. In developing countries rates are as high as 43% have been reported[256].

HIV transmission from mother to child can occur during antipartum (in utero), intrapartum (during labor and delivery) or postpartum (breast feeding) period.

Most studies suggest that 25% to 35% of transmission occurs during antepartum[257-259]. 7,89. Although transmission does take place even in early pregnancy[260], transmission is more frequent at late pregnancy, mostly at the time of labour or delivery[261].

Evidence suggest that 70% to 75% of vertical transmission occurs during labor and delivery[262]. Increased transmission is noted with increased duration of rupture of membranes prior to delivery[263].

Prospective studies have suggested an increased risk of transmission associated with postpartum (breastfeeding)[264,265]. Dunn et al[266] in a meta analysis have estimated that the postpartum transmission world wide from HIV is 14% Particularly high when maternal primary infection occurs in the first few months after delivery[267].

By the May 1986 the ICMR established a nation wide HIV testing scheme. In 1987 Oct. first HIV seropositive infant was detected[268]. In 1998 NACO started ANC Sentinal surveillance in Gujarat they reported 0.00% to 0.50% of HIV seroprevalence rate[269]. In 2003 started (PPTC) (Prevention of Parents to child trans) programme.

The present study was under taken to Findus the seropositive rate among women attending antenatal clinic at G.G. Hospital, Jamnagar. A total of 3000 cases were studied for the presence of HIV-antibodies, out of which 7 cases were found HIV seropositive, indicating 0.23% positivity rate.

Several studies have been conducted in world or India to investigate the prevalence of HIV in pregnant women.

Prevalence of HIV seropositivity among pregnant women in India

Sr. No.	***Author***	***Place***	***Percentage Positivity***
1.	Singh et al[270]	Manipur	0.86%
2.	Lakshmi et al[271]	Tirupati	1.5
3.	Khorsed et al[272]	Bombay	0.1
4.	T. Jacob John[273]	Vellore	0.05
5.	Pal A. et al.[274]	Allahabad	0.0%
6.	Ramanamma et al[275]	Visakhapattnam	1.83%
7.	Ravindra Kumar et al[276]	Delhi	0.16
8.	Prabijot et al[277]	Bombay	8.04
9.	Lals et al[278]	Miraj	4.5
10.	V. K. Sharma et al[279]	Delhi	0.4
11.	Present Study	Jamnagar	0.23%

The results suggest that HIV seropositivity rate among pregnant women ranged between 0% to 8% and average rate was 1.5%

In present study HIV-Seropositivity rate is 2.3% in pregnant women, In comparison to our results lower incidence of HIV seropositive have been shown by Pal et al[274]. 0%, Ravindra Kumar[276] 0.16% at Delhi, T. Jacob John[273] at Vellore 0.05% and Khorsed et al[272] at Bombay 0.1 rate.

Slightly high prevalence rate then our study shown V.K. Sharma at[279] Delhi was 0.4% and Singh et al shown 0.86% positivity among pregnant women at Manipur. More over geographical area also effects overall HIV prevalence. The

Subjects of this study reside in the vital geographical zone through which the international trafficking of drugs occurs from golden triangle. (Myanmar, Thailand Loas) to Manipur via the international border of Manipur and Myanmar 232 cases were screened by Singh et al out of which 2 were HIV seropositive. The husband of both was IVDUs and both were HIV positive. It is likely that seropositive mother got infection through heterosexual transmission.

In present study 7 female were HIV seropositive 4 husbands of seropositive female were HIV positive. By the counseling the detail history, it was apparent that the women were monogamous, but their husband had sex with multiple partners. Thus the prevalence of HIV seropositivity in majority (90%) of infected women is a reflection of the prevalence of HIV infection due to sexual promiscuity of their husband.

Ramanamma et al[275] study result shows the HIV seropositivity among antenatal women was 1.83% can be consider higher, probably it may be representing the logarithmic trends in the increase of AIDS in all risk groups, women of reproductive age being one of them. More ever Vishakhapattnam is a major port city in India, and as such large number of floating population can be expected for rapid spread of HIV.

Lakshmi et al[271] 1.5% was the prevalence rate in Tirupati, which was higher in comparison to our study. Tirupati is a place of pilgrimage which is frequented by 20,000 to 30,000 people a day from various parts of the country. More ever sexual promiscuity is also prevalent as a result there is steady rise in HIV positivities in high-risk group and hence the rise in percentage positivity among general population[281].

Lals et al[278] also showed the higher result 4.5% at Miraj Maharashtra. Prabijot et al[277] showed that women attending antenatal clinic is 8.04% which is highest among all the studies. Bombay is over flooded population due to the population movement towards these large urbanized areas to secure job[282]. Moreover large number of prostitutes provides sexual services in cities like Bombay, it is

reported that prostitutes have the highest and their male clients the next highest prevalence rates and they constitute the primary chain of transmission to general population[282].

As regard to age, in present study most of the cases were in age group 21-30 years. Out of 1513 cases screened 6 (0.39%) were HIV seropositive in this group. In the study done by Ramanamma et al[275], majority of seropositive were in the age group 21-30 years. Which correlates with other our findings. The high prevalence of HIV seropositivity in the age group 21-30 years is due to large numbers screened because this age group is reproductive age group.

In present study HIV seropositivity rate is shown in different trimester of pregnancy, More seropositivity 0.26% is shown in 3rd trimester of pregnancy. This is because of the more number screened in 3rd trimester in PPTCT.

In the present study 0.23% HIV seropositivity found among pregnant women. It can be considered low in comparison with other studies but it is important to note that HIV infection has already affected such a healthy group.

Prevalence of HIV seropositivity among pregnant women in the world[280]

Sr. No.	Country	Percentage Positivity
1.	California USA	0.8%
2.	U.K.	0.6%
3.	Paris (France)	4.14%
4.	Norway	0.07
5.	Nigeria	3.0
6.	Nairobi (Africa)	17.0
7.	Sweden	0.09
8.	Thailand	1.6%
9.	Vietnam	0.8%
10.	Pakistan	0.0%
11.	Japan	0.0%
12.	Indonesia	0.0%
13.	China Hong Kong	0.1%
14.	Cambodia	3.5%
15.	Burma	2.3%
16.	India [3]	1%
17.	Cameroon	2.0

The prevalence of HIV seropositivity among pregnant women in the world is range from 0% - 17% shows in the table[280]. Pakistan, Japan, Indonesia shows the 0% rate but in the Nairobi it is very high 17%. In the China Hong Kong rate is 01% but Z Huang K studied a survey in highly epidemic villages it is very high 32.2% seropositivity rate in pregnant women[284].

The increase in the number of HIV in pregnant women is continuously increasing. Increase in the number of HIV among pregnant women indicates, the increase in vertical transmission. Virtually, all the HIV infection that now occur in children mostly by vertical transmission, i.e. from mother to child. Vertical transmission of HIV to the foetus occurs is 15-33% of pregnancies[285].

Early diagnosis of HIV status of pregnant women is required, for the women, herself, her baby and obstetrician, NACO started the antenatal survey for pregnant women. They started 200 sites for the antenatal surveillance all over the India[286]. Out of 200 sites 8 sites started in Gujarat. Out of 8 sites in Gujarat one is at Jamnagar. Gujarat is a low prevalence state categorised by NACO. HIV prevalence rate in antenatal women is less than 1%.

Those women who desire to continue pregnancy can be told that there is no evidence that babies born of such mother are at increased risk of prematuring or growth retardation[287]. All succumb to the disease by 5 years. The uninfected 75% are likely to be orphaned by 5 years of age and suffer all its adverse consequences. To prevent this calamity MTP in the first trimester should be performed.

Motherhood is women's desire, women who desire to continue pregnancy should be provided with adequate perinatal care, intensive neonatal care and precautions should be taken to prevent accidental spread of HIV infection while providing health care. Zodovudin & ACTG 076 proved to be effective in reducing perinatal transmission, should be offered to all HIV infected women.

Moreover, routine testing by voluntarily is necessary, strict universal Bio-safety precautions should be followed, proper health education and counseling also advocate to prevent further spread of HIV transmission in antenatal women.

GROUP-III BLOOD DONORS

There is no fluid, which can to tally substitute blood in the human body. In many cases transfusion of blood becomes necessary to save the life of an individual. Therefore, blood should be pure and free from contamination. In case of transfusion of infected blood it is almost sure that such blood would carry transmissible diseases like HIV, HBsAg, malaria and syphilis. HIV is one of the latest additions to the long list of already existing diseases that can be transmitted through transfusion.[288]

The use of blood transfusion in clinical practice has brought untold benefits to countless individuals. But the risk transmitting an infectious disease is associated with every transfusion. With the report increase in HIV Seropositivity amongst blood donors in the state Tamil Nadu and Maharashtra, Govt. of India recommended mandatory screening of all blood and blood products before transfusion. India's first HIV Seropositive blood donor was detected in 1987 in Tamil Nadu. Since then serosurveillance centers were established in major cities. By the end 1993 100 Zonal blood testing center were established mainly in urban areas to screen all the blood donors for HIV antibodies[289-292].

Based on it some workers have undertaken isolated studies of prevalence of HIV Seropositivity in blood donors. Replacement donors, majority of whom are men, are considered as an indicator of the trend of HIV infection in the general population and these figures for blood donors can be used as a proxy for prevalence figures for general population. On similar basis a trend analysis is carried out on infections of HIV / AIDS, Hepatitis syphilis three main disease, transmitted by transfusion, may reflect the prevalence status of these disease in general population[293-298]. So transfusion transmitted diseases (TTDs) are the most common cause of transfusion associated undertaken to find out the prevalence and trend of HIV Seropositivity in blood donor population attending Guru Gobind Singh Hospital, Jamnagar.

HIV has been transmitted by whole blood, cellular components, plasma and clotting factor concentrates as well as in cellular fraction of blood. Seroprevalence

dater from different parts of India has shown ELISA reactivity in the donated unit of blood. Blood of these donors have been used for making blood products by manufactures without appropriate HIV neutralization methods[299]. Consequently in India person receiving blood or blood products repeatedly would be at risk for HIV infection.

In the present study a total of 53671 donors blood samples were screened for the presence of HIV. Commercially available solid phase immunoassay was used for the detection of HIV antibodies. Only negative blood allowed to be transfused. If blood is found to be positive for HIV, it should be discarded. The donor is not to be informed. If donor wants to know the result, pretest and post-test counseling regardless of the test result should be carried out and then only result should be informed[300].

The presence of HIV Seropositivity in the blood of healthy donor poses serious risk for recipients. This study was undertaken to determine the incidence of HIV Seropositivity in blood donor population.

Incidence of HIV Seropositivity in Blood donors from different parts of India

Sr. No.	***Author***	***Year***	***Place***	***No. tested***	***Positivity***
1	Lina Deodhar[301]	1990	Bombay	47922	0.9%
2	Chandra M.[302]	1991	Bombay	30632	0.6%
3	Kulshreshta R.[303]	1992	N. Delhi	11256	0.17%
4	Bhushan at al[304]	1993	Vellore	79591	0.10%
5	Ramani T. V.[305]	1994	Vishakhapttam	394	5.06%
6	Brajachand S. N.[306]	1994	Pune	-	2.2%
7	Purnima Rao[307]	1994	Pune	44190	0.69%
8	Joshi S. R.[308]	1994	-	34098	1.2%
9	R. Sambasiva Rao[309]	1995	Pondicherry	19023	0.55%
10	Gupta et al[310]	1996	Chundigarh	2787	0.82%
11	Kumars et al[311]	1996	-	85500	0.23%

12	Patil A. V. et al[312]	1996	-	38776	3.59%
13	Shashikala S. Tallur[313]	1997	Hubli	19705	1.74%
14	Sujater V. apur[314]	1998	Delhi	-	0.06%
15	Rajesh Kulshrestha[315]	1999	Lacknow	39965	0.53%
16	Chaudhari N.[316]	2000	Lacknow	62288	1.28%
17	Varma N. et al[317]	2001	Delhi	144	1.06%
18	Kapur S.[318]	2002	Delhi	-	0.68%
19	Rasheed M.V. [319]	2004	Hydrabad	-	0.14%
20	Present Study	-	Jamnagar	107343	0.37%

Incidence of HIV seropositivity among blood donors in world

Sr. No.	***Author***	***Year***	***Place***	***No. tested***	***Positivity***
1	Aberle Grasse J.[320]	1995	USA	-	1.8%
2	Sohmunis G. A.[321]	1997	Argentina	-	2.4%
3	Yan Y. Zhengz[322]	1999	Fujian	569873	0.12%
4	Shandera W.[323]	1999	Africa	-	0.39%
5	Brain F.[324]	2000	-	10000	0.17%
6	Williams A. E.[325]	2000	USA	100000	0.03%
7	Osterhaus A. D.[326]	2000	Somalia	256	0.00%
8	Chiavetta J. A.[327]	2001	Canada	2 Million	0.10%
9	Zacharian[328]	2001	Malawi	-	2.2%
10	Kiwanuk N.[329]	2002	Uganda	6868	3.92%
11	Durosinmi M. A.[330]	2002	Nigeria	16080	2.30%
12	Kwesigabo G.[331]	2002	Tanzania	454	11.9%

In our present study, of the 53671 blood donors tested during study. HIV, Seropositivity were observed in 199 (0.37%) cases.

The age group most commonly affected by HIV infection is 21-30 years i.e. sexually active group

This is comparable with the studies by Purnima Rao et al Pune[24] and R. Sumbasiva Rao[26] Pondicherry where maximum number of HIV Seropositive blood donors are between age group of 21-30 years.

Maximum prevalence of HIV Seropositivity in 21-30 years age group could be, because the individuals in this group are more independent and economically stable as compared to lower age groups.

The HIV Seropositivity in this study is less in female than in males. In all the studies also males are more than female. Males are considered more independent and they go outside their abode to earn their living. They have more chances of getting exposed to unprotected sexual practices and acquired HIV infection, thus blood donated from these high risk. Male donors are more likely to transmit the infectious agent to the recipient. But with todays modernization, female are also getting exposed to the outside world and the prevalence of HIV Seropositivity in female may increase in the near future.

HIV Seropositivity in this study is less in voluntary donors (0.04%) than replacement donors (0.52%). Y. Munde at al[332] Thailand have observed more prevalence of HIV infection replacement donors than in voluntary donors. Similar study of A. Moniatis et al[333] also shows more prevalence of HIV among replacement donors than volunteer. Because replacement donors are patients relatives only so there is no choice in donor selection. These findings correlates with the observation of the present study.

Brain F. (2000)[324], Shandera W. (1999)[323], Africa, Lina Deodhar (1990)[301], Bonbay, Chandra M. (1991)[302] Bombay, Kulzhreshtha R. (1992)[303] New Delhi, Purnima Rao (1994)[307] Pune, R. Sambashiva Rao (1995)[309] Pondicherry, Gupta et al (1996)[310] Chandigarh, Kumars et al[311] (1996) and Rajesh Kulshreshtha (1992)[315] Delhi all the studies shows the similar prevalence of HIV Seropositivity, .0.17%, 0.39%, 0.9%, 0.6%, 0.17%, 0.59%, 0.55%, 0.82%, 0.23% and 0.53% respectively from world and India.

Williams A. E. (2000)[325] USA, Yan Y. Zhengz (1999)[322], Chiavetta J. A. (2001)[327] Canada, and Bushan et al (1993)[304] Vellore shows a very low prevalence of 0.03%, 0.12%, 0.10% and 0.10% respectively.

Aberle Grasse J. (1995)[310] USA, Schmunis G.A. (1997)[321] Argentina, Kiwanuk N. (2002)[321] Uganda, Durosinmi M.A. (2002)[326], Nigeria, Zacharian (2001)[328] Malawi Brajachand SN (1994)[306] Pune, S.R. Joshi (1994)[308], Patil A.V. (1996)[312] Shashikala Tallur (1997)[313] Hubkli shows a slightly and higher prevalence of 1.8%, 2.4%, 3.92%, 2.30%, 2.2%, 2.2%, 1.2%, 3.59% and 1.74% respectively than present study.

Kwerigabo G. (2002)[331] from Tanzania shows the very high prevalence rate 11.9% and Osterhaus A.D. (2000)[322] Somalia shows very low 0.00% rate from blood donors.

Indian studies by Ramani TV (1994)[305], Visakhapattam shows very high rate 5.06%b and Sugata Kapur (1998)[31] Delhi shows very low 0.06% HIV Seropositivity in blood donors. So prevalence of HIV Seropositivity in Indian range is from 0.05 to 5.5% present studies shows that there is low rate of HIV Seropositivity in blood donors, and it is because of selection of blood donor is proper manner

HIV infected donor represents the major source of transmission of HIV infection, and they are the largest group who are not aware that they are infected.

The present study was therefore carried out to assess such an incidence of HIV infected cases among blood donors.

SUMMARY

For a decade the world has struggled to come to terms with HIV/AIDS. Most of the human faces of this epidemic are of young people, including pregnant women & their children.

In the present study three groups were studied

Group - I : Symptomatic

Patients who presented with signs and symptoms which were suggestive of HIV/AIDS - TB patients.

Group - II : Asymptomatic

Persons who were at very high risk of getting HIV/AIDS but who had no clinical signs and symptoms suggestive of HIV/AIDS - Sexually transmitted disease patients, - Multi transfused Thallassaemic patients

Group - III : Healthy individuals - Antenatal mother and Blood donors

- HIV antibodies were detected ELISA & Rapid Tests.
- Samples for transfusion safety purpose one E/R/S is used; for surveillance and diagnosis of full blown AIDS cases two E/R/S are used; and for asymptomatic individual three E/R/S are used.
- Out 12,165 cases were studied for the presence of HIV antibodies by three different methods 1365 were found HIV seropositive. So the over all incidence rate of HIV seropositivity is 11.22%

Group I :

- 2000 cases of clinically suspected cases of tuberculosis were screened 124 were seropositive for HIV antibody So the prevalence of HIV-Seropositivity in tuberculosis patient is 6.2%.
- HIV seropositivity rate is more in male 7.02% than female 3.2% case of tuberculosis.
- 21-30 age group is more susceptible (7.66%) for HIV infection.

Group II :

- 2700 cases of STD were screened out of these 126 were seropositive for HIV antibodies.
- So the prevalence of HIV seropositivity among STD patients is 4.66%
- HIV seropositivity rate is more in male (5.66%) patients than female (4.25%) patients.
- 21-30 years of age group is more (5.80%) susceptible for STD.
- Urban people are more infective than Rural.

❖ 200 cases of multi transfused thallassaemic children were screened and 5 were seropositive for HIV antibody. Rate of HIV seropositivity is 2.5%.

- Out of 5 HIV seropositive children 3 were male & 2 were female.
- Out of 5 HIV seropositive 3 were from age group of > 4 to 8.
- Out 200 cases 70 were transfused by more than 51-100 transfusion HIV seropositivity rate was more in 101-150 transfusion group (5.12%).

Group III :

- Out of 3000 cases of pregnant woman only 7 were found HIV seropositive. So incidence of vertical transmission is 0.23% is low in general population.
- Out of 7 HIV seropositive mothers 6 were from age group of 21-30 years.
- Antenatal women are more seropositive in the III trimester of pregnancy 0.33%.
- Three husbands were HIV seropositive from 7 positive women.
- Illiterate women were more positive (33%) than illiterate one.

❖ A total of 53671 blood donors were studied. Out of them 199 were HIV - seropositive, Prevalence rate is 0.37% in blood donor.

- HIV seroprevalence more in male (0.38%) than female (0.06) donors.
- HIV Seropositivity is more in replacement donors than in Voluntary donors.
- 21-30 years of age group is more prevalent (0.41%).

CONCLUSION

In Indian context, it is difficult to estimate the exact prevalence of HIV because of the varied cultural characteristics, traditions and values with special reference to sex related risk behaviours. The Western African model of making estimates cannot be easily applied the Indian scenario. However, it is possible to have an estimate at different levels of risk and the HIV prevalence, It may be used for planning of HIV/AIDS prevention and control. This estimation can also be useful for mapping of specific vulnerable groups & areas.

We concluded that the prevalence of HIV seropositivity is entered from high-risk group to lower risk group and in general population. Our group wise conclusion is :

Group - I : Tuberculosis Patients :

The present study reveals the rising trends of HIV infection among tuberculosis patients, so the urgent upgrading of microbiology laboratories to help in early diagnosis and prompt treatment of tuberculosis is extremely relevant in Indian context.

- All the persons with tuberculosis should be questioned about risk factors for HIV infection and whether or not risk factor are elicited, urged to have HIV test.

Group : II Sexually transmitted disease patients :

HIV infection is increasing dramatically in patients of sexually transmitted diseases.

- Screening of HIV antibody prevalence in STD cases is likely to help in the understanding of the reality of the spread of HIV infection. There is an immediate need for comprehensive and national efforts to control STDs and changing high-risk behaviors.

- Treatment and control of STD offers a rational approach to the control of HIV.
- To raise awareness levels regarding HIV/AIDS in rural and slum areas and other vulnerable group of the population.
- Wide spread voluntary testing of individuals who are practicing high risk behaviour together with counseling of infected individuals is recommended.
- To make people aware about the services available under the public sector for management of reproductive infection RTI / STD.

Multi transfused Thallassaemic Children :

Patients with Thallassaemia are at higher risk of HIV transmission, through blood transfusion.

- Therefore to prevent HIV infection from blood transfusion all but absolutely essential, transfusion should be avoided.
- Mobilising people for voluntary donation, self exclusion technique among donors, and Judicious use of blood/blood products will go a long was in helping to control transfusion associated HIV infection in India.

Group : III Antenatal Mother (Vertical transmission).

A relatively lower antenatal rate for HIV Seropositivity but has been detected in our study.

- Screening should important so urged to have HIV testing.
- The major advantage for screening, if positive is the counseling for MTP (medical termination of pregnancy) in early phase of pregnancy.
- The other advantage is to relive them from anxiety by giving them the proof, if they are uninfected. For those with HIV infection could take precautions that they do not spread the disease to other.
- Identification of seropositive women and follow up of their children is the only means available by which children requiring special care in future could be identified long before the need arises.

- The screening also helps the doctor to plan adequate and appropriate antenatal intrapartum and postnatal care and intensive neonatal care facilities.
- However, looking towards the magnitude of the problem, health education and proper counseling of seropositive mother is a must to prevent the further spread of the disease.

Blood donors :

A relatively lower seropositivity rate found among blood donors, but the positivity indicates the infectivity in healthy general population.

- Screening of blood donor is mandatory, but seropositivity may account because transfusion of HIV antibody negative blood during the window period.
- A thorough evolution of the transfusion practices and rethinking of single bottle transfusion need to be done since HIV testing alone does not eliminate the risk of transfusion spreading AIDS.
- Behavioural risk reduction and education of donors continue to remain the only effective tools to limit the progression of this pandemic among the blood donors. Measures should be initiated to end the practice of commercial paid blood donations and to rehabilitate such donors.
- HIV testing of donated blood should not culminate in discarding the HIV positive blood alone. Proper counseling and education of the effected donors should necessarily follow.
- Efforts should also be infected to prepare HIV test kit and reagents indigenously, based on the subtypes prevailing in the region, So that detection rates are improved.

The study is expected to generate, that will help in evolving timely intervention strategies for control of HIV infection, and also provided valuable tools for impact assessment of specific intervention programmes.

We need to act fact before we are left powerless against AIDS !!

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STUDY OF HIV 1 & 2 SEROPOSITIVITY IN PATIENTS AND HEALTHY SUBJECTS IN G.G. HOSPITAL AT JAMNAGAR

- R. D. Jadeja

2004

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By

R. D. Jadeja

Under guidance of

Dr. (Mrs.) R. J. Joshi

M.D.

A THESIS SUBMITTED TO THE

Saurashtra University

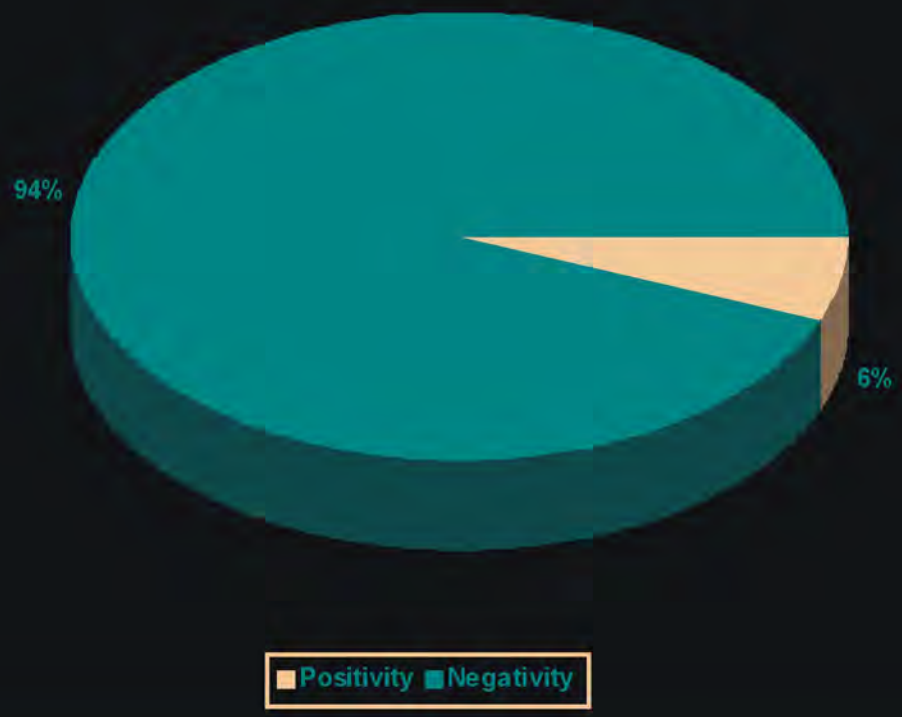
FOR THE DEGREE OF

Doctor of Philosophy

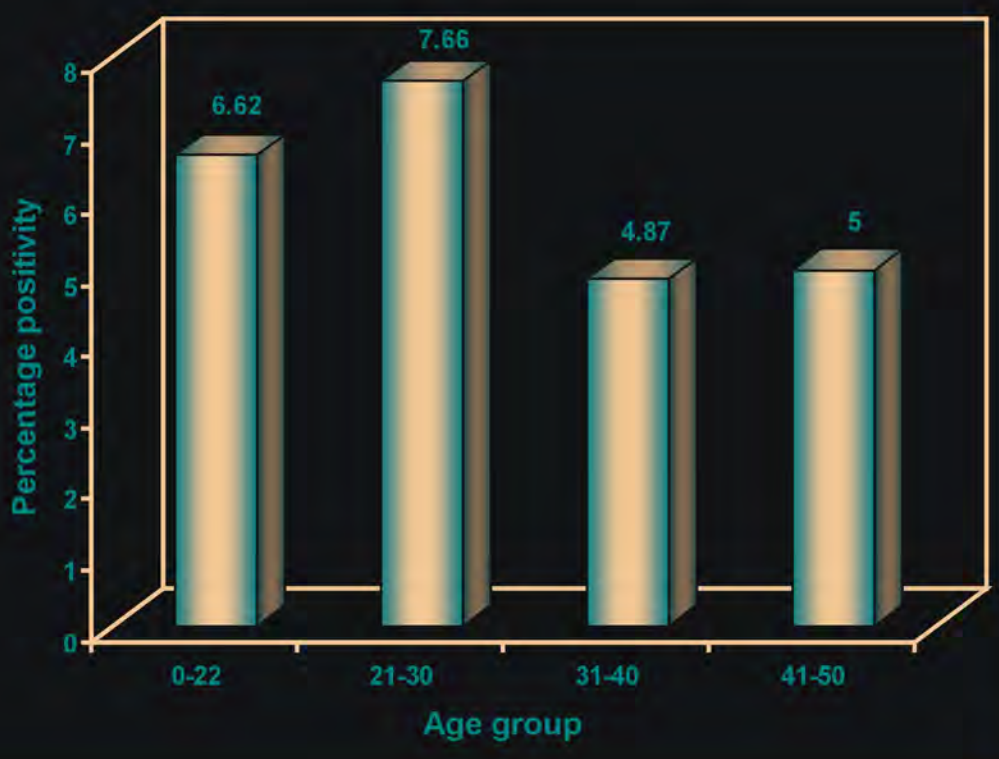
(Medical Microbiology)

2004

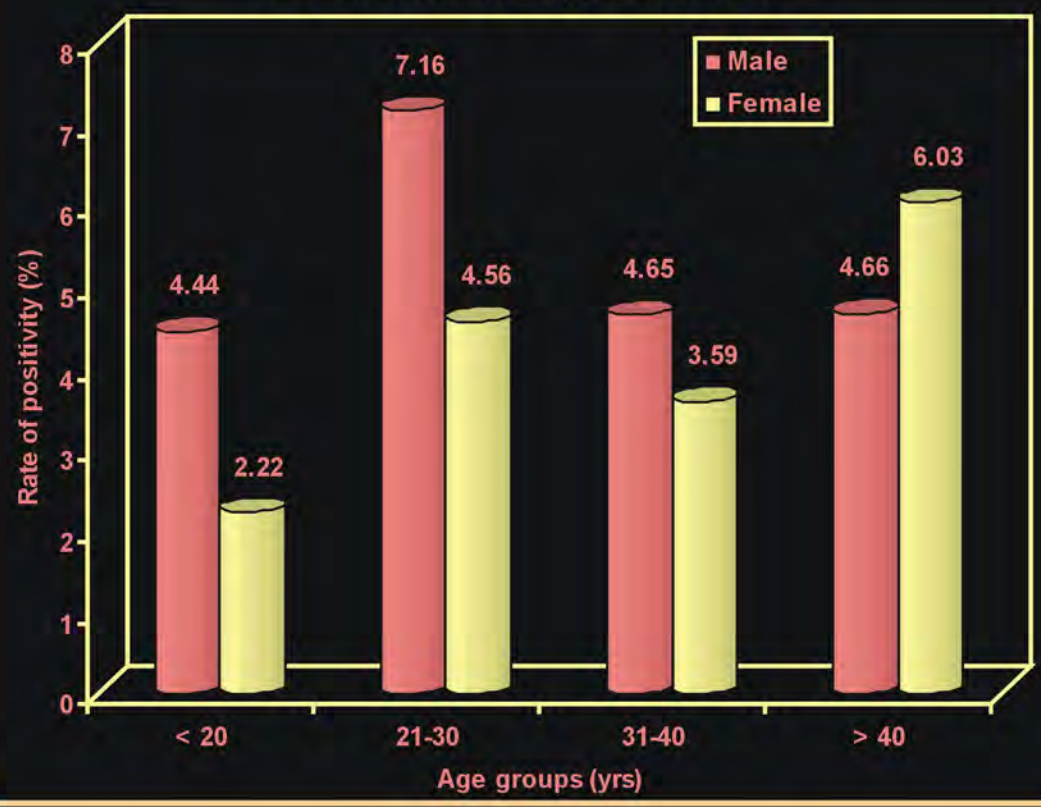
Graph - 1
Incidence of HIV seropositivity in TB patients



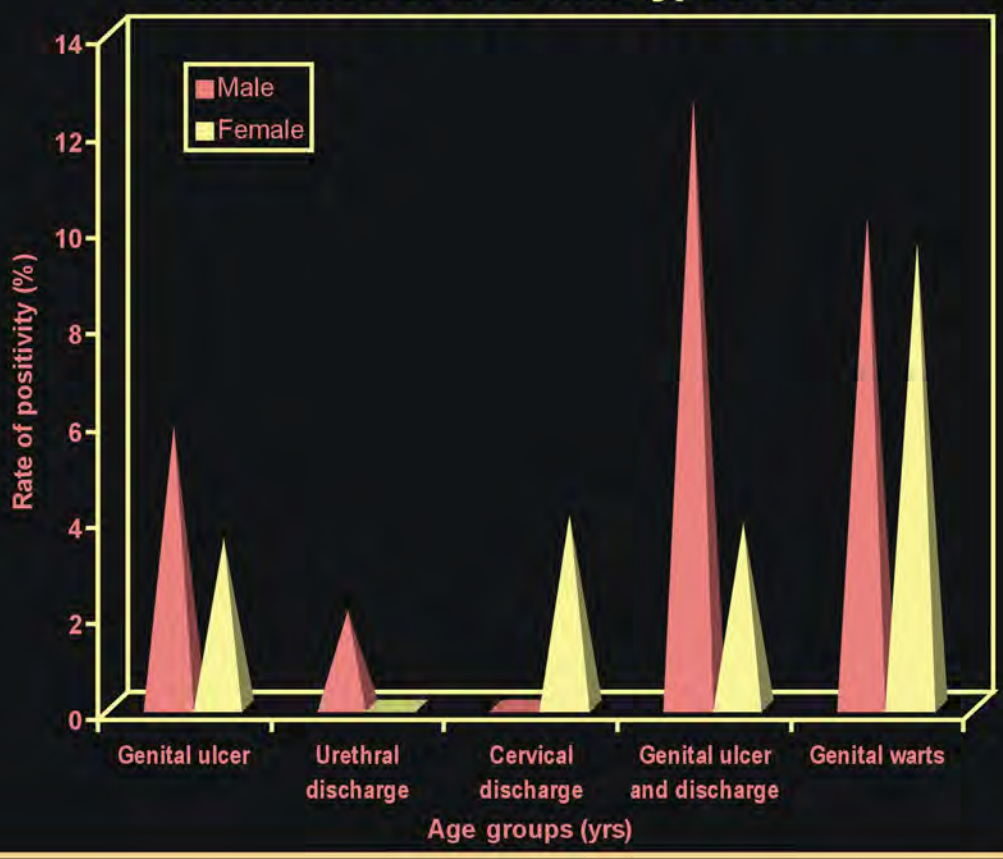
Graph - 2
Incidence of HIV seropositivty in TB patients with relation to age



Graph - 3
Incidence of HIV seropositivity in STD patients in relation to age and sex

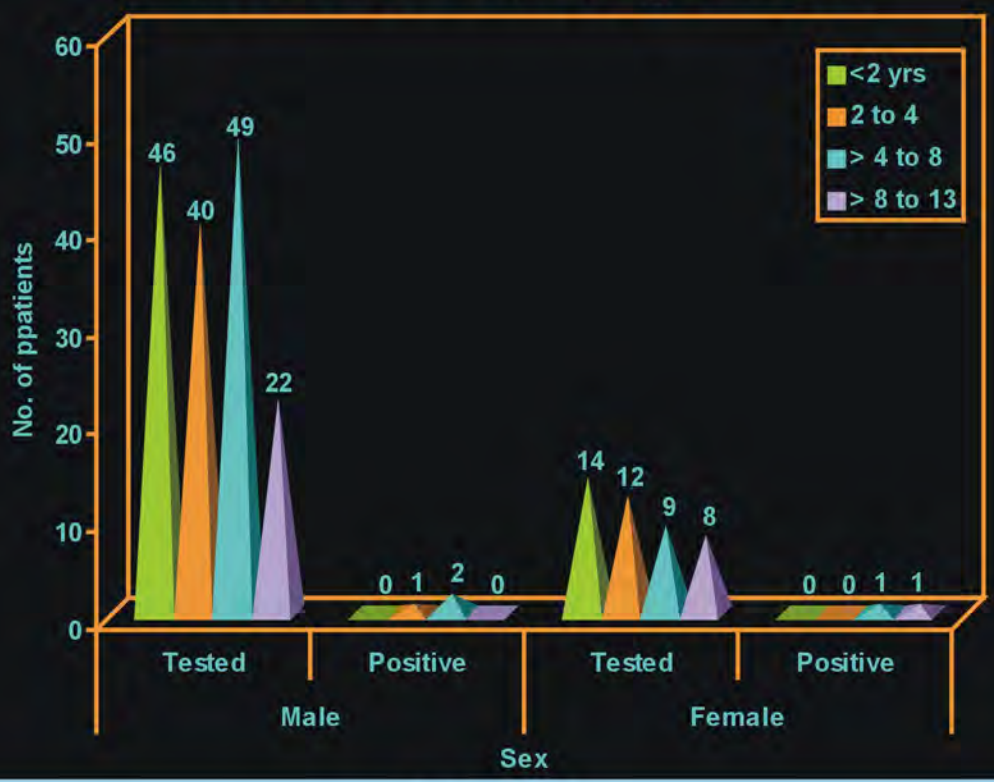


Grap - 4
Incidence of STD and types of STD



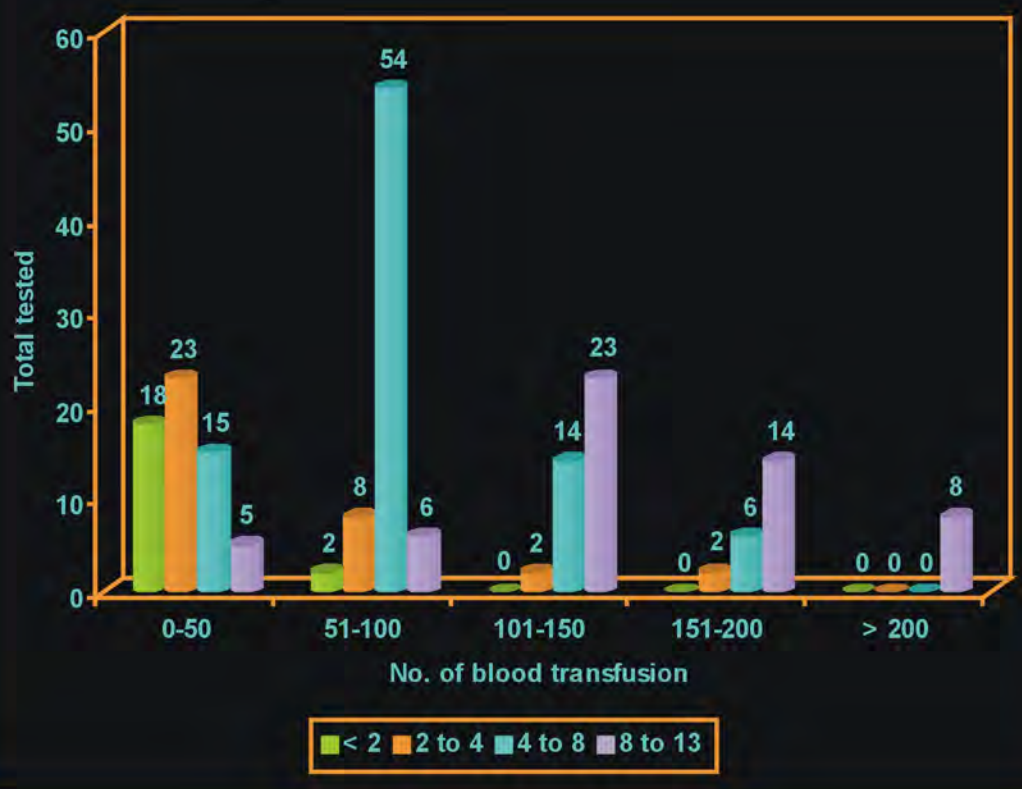
Graph - 5

Incidence of HIV sropositivity in thalassaemic children with relation to age and sex

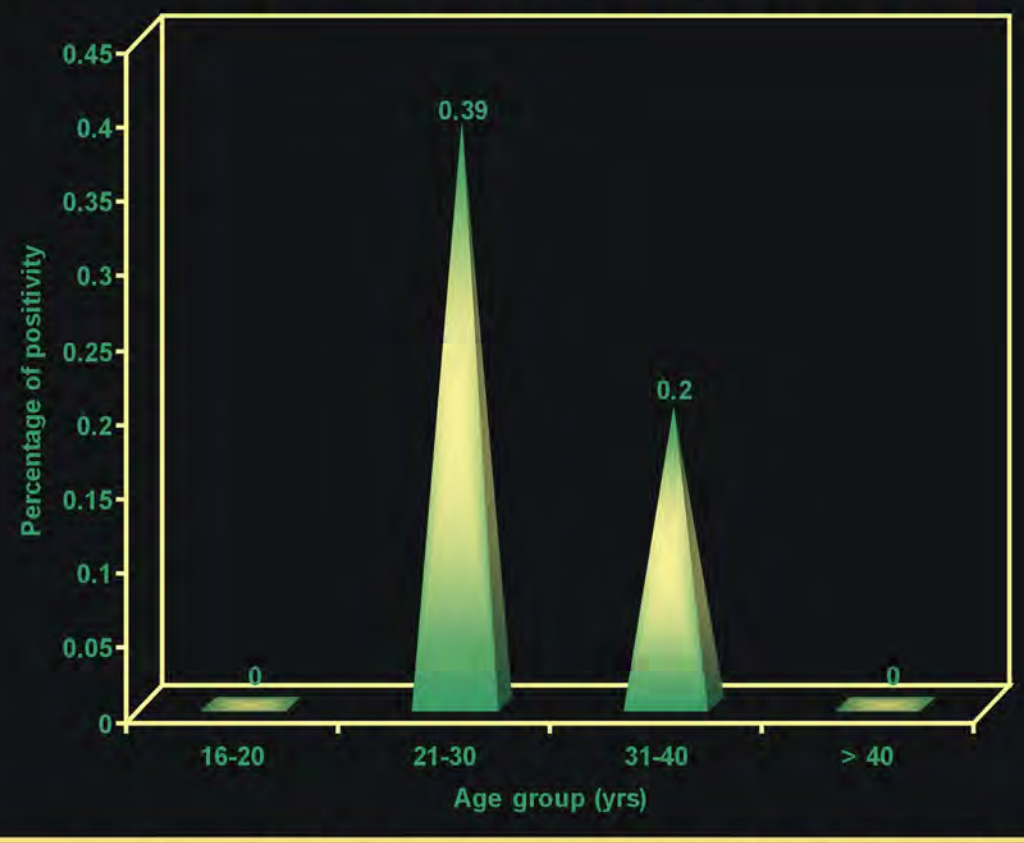


Graph - 6

Distribution of thallassaemic children according to the age as the number of transfusion



Graph - 7
Incidence of HIV-seropositivity in relation to age



Incidence of HIV seropositivity in relation to the different trimester of pregnancy

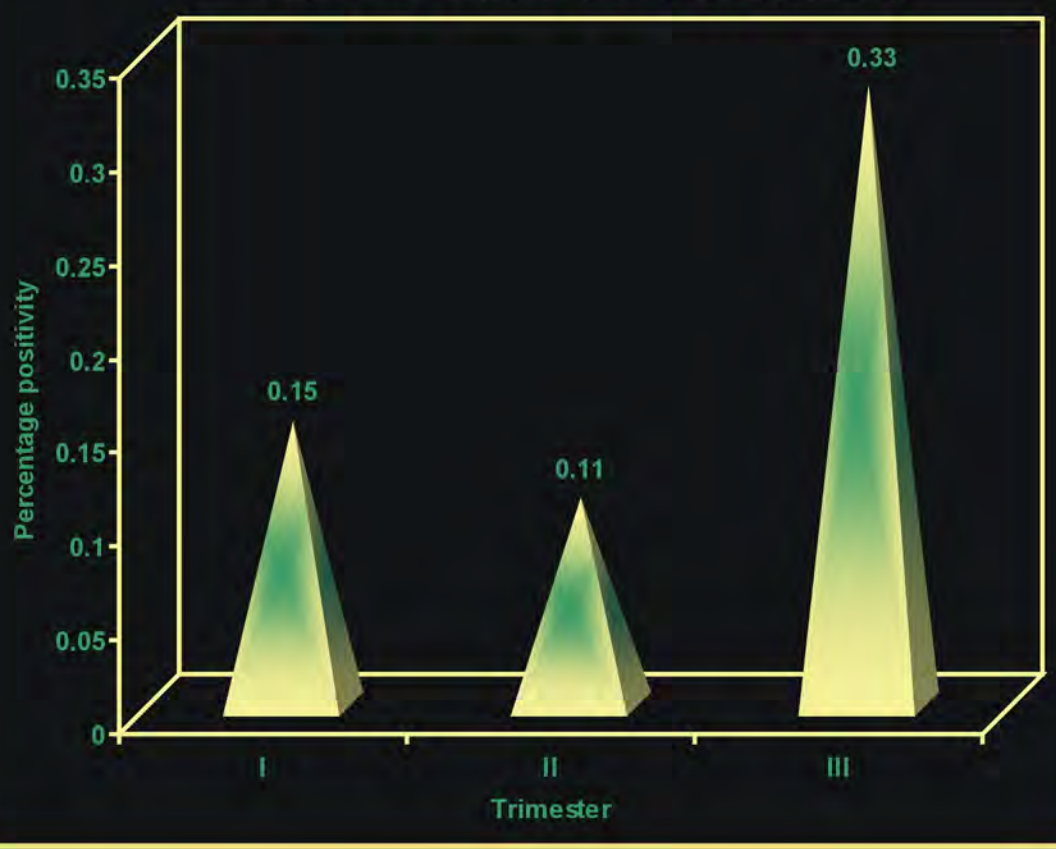
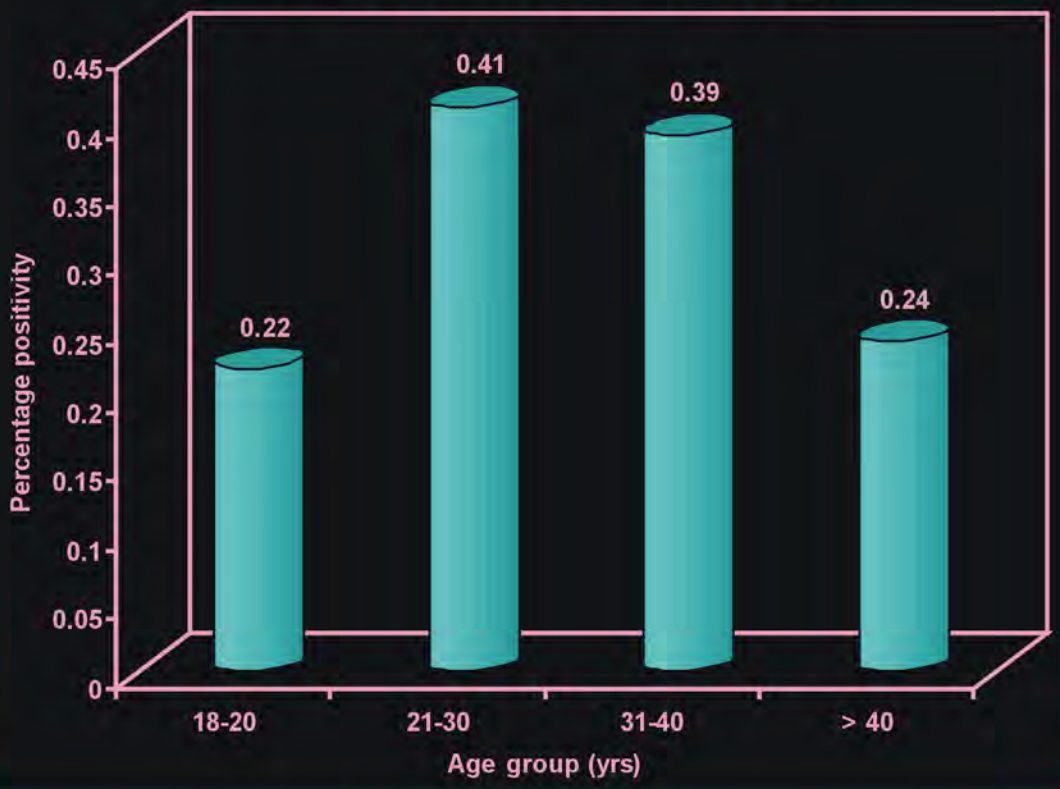
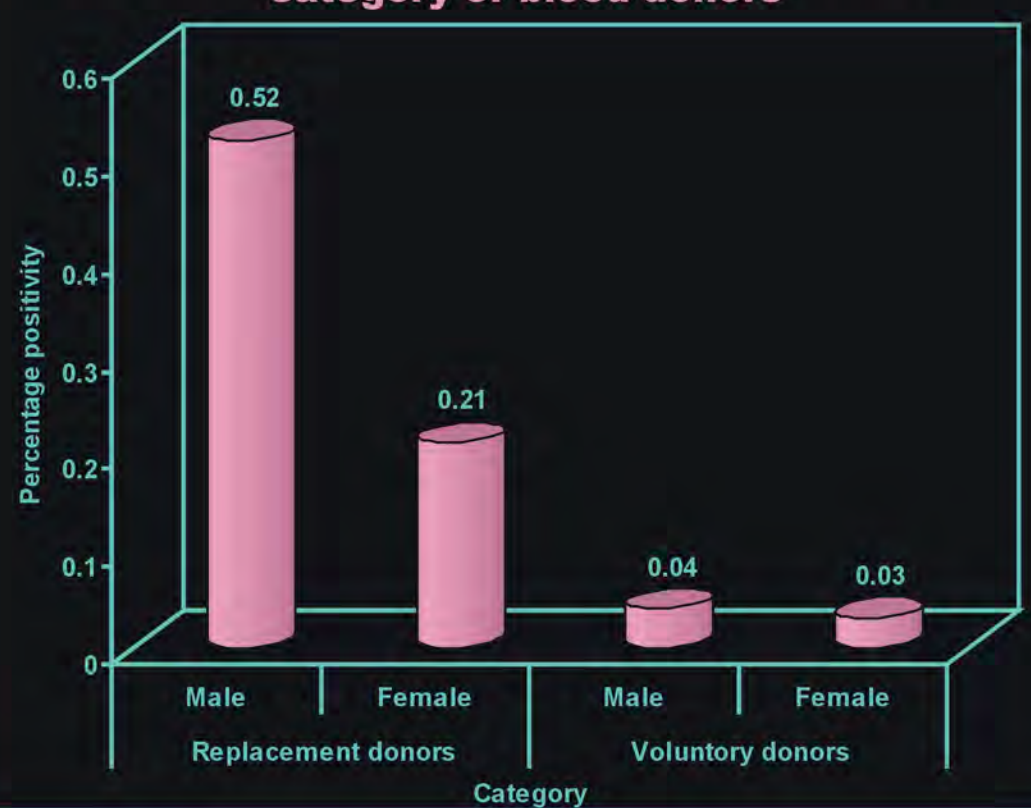
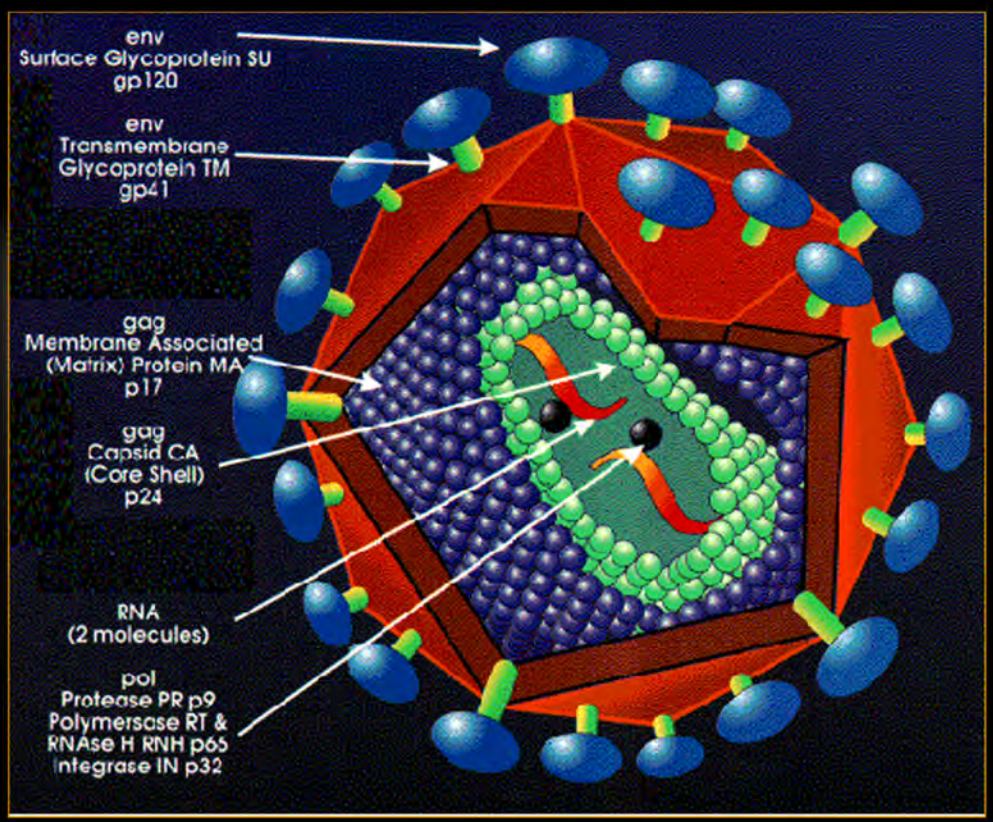


Table - 9
Incidence HIV-Seropositivity in blood donors relation to Age



Graph - 10
Incidence of HIV seropositivty according to category of blood donors





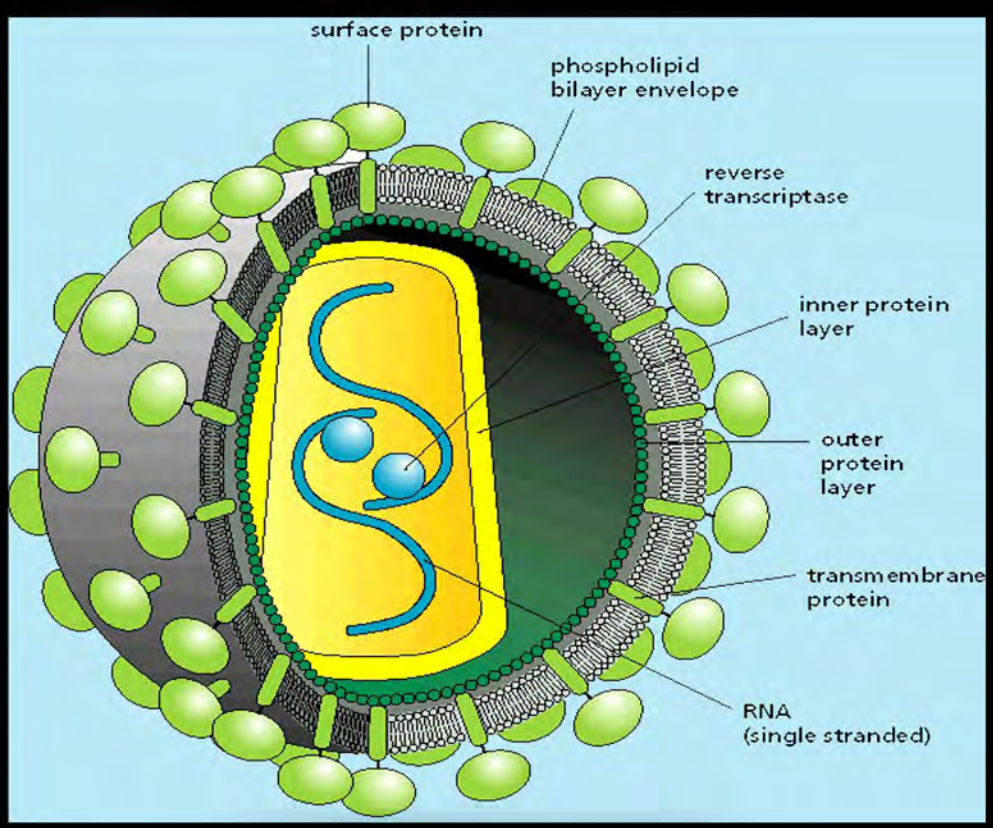


Fig. 1 Structure of HIV

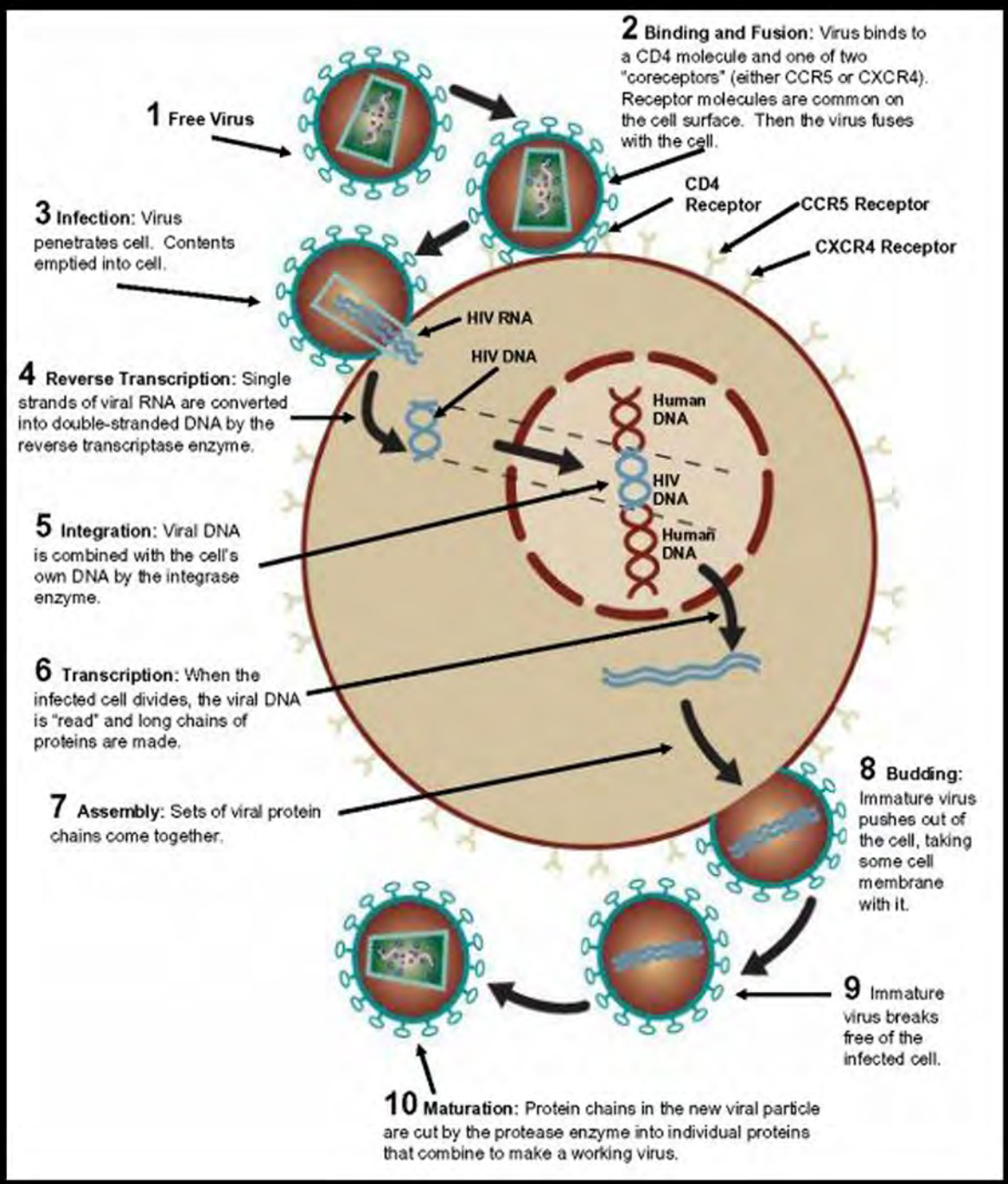


Fig. 2 Life cycle of HIV

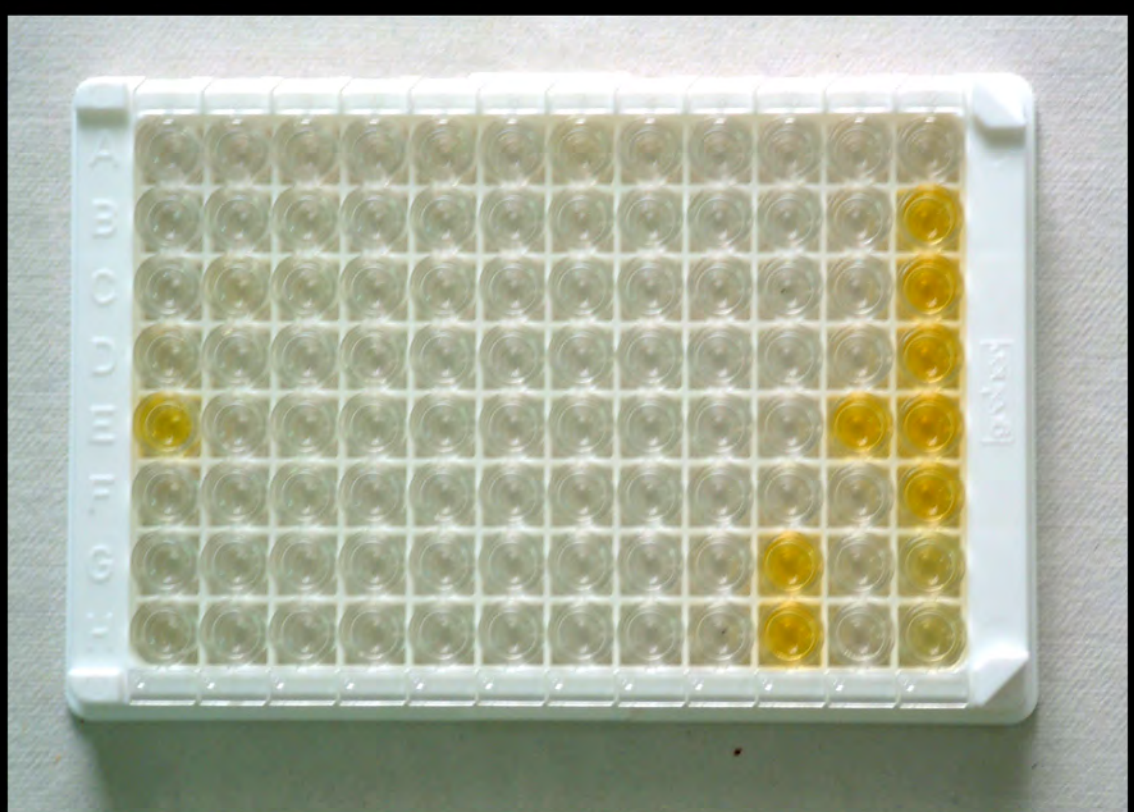


Fig. 9 Enz AIDS HIV 1+2 ELISA test kit / Results

Fig. 10 Comb AIDS-RS HIV 1 & 2 Immuno Dot test / results

Fig. 11 HIV TRI-DOT test kit / results