CHAPTER-I

INTRODUCTION

The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that AIDS has killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive pandemics in recorded history. In 2007, between 30.6 and 36.1 million people were believed to live with HIV, and it killed an estimated 2.1 million people that year, including 330,000 children; there were 2.5 million new infections (Schoofs, 2008). Sub-Saharan Africa remains by far the worst-affected region, with an estimated 21.6 to 27.4 million people currently living with HIV. More than 64% of all people living with HIV are in sub-Saharan Africa, as are more than three quarters of all women living with HIV.

South-Africa & South East Asia are second-worst affected with 15% of the total deaths. AIDS accounts for the deaths of 500,000 children in this region. South Africa has the largest number of HIV patients in the world followed by Nigeria (UNAIDS/WHO/ CDC, 2007). India has an estimated 2.5 million infections (0.23% of population), making India the country with the third largest population of HIV patients.

On recent report of Nepal government the total number of HIV infected people in Nepal are 12,933 (8,626 males and 4,307 females) in 2008. The estimation also shows that 2,151 people are suffering from AIDS of which 1,529 are males and 622 are females (NCASC, 2008). The estimations showed 91.4% infections occurred among the age group 15 to 49 years, 6% infections among children and 2.7%% among adults above 50 years. Among population group of 15 to 49 years, clients of sex workers were the highest with 45% HIV infections, Housewives with 23.5%, , IDUs 18%, MSM 0.5%, female sex workers 6% and Blood or Organ recipients 0.3% (NCASC, 2008).

In a study carried out in the blood transfusion service centres seroprevalence of HIV were found as; Morang (0.019%), Nepalgunj (0.095%) and Kaski (0.05%) (Tiwari et al, 2008).

HIV-1 discovered in 1983, has been shown to be virologically and serologically associated with early and late stages of AIDS and has been described as more aggressive virus and responsible for global pandemic of AIDS. HIV-2 discovered in 1986, has been reported to be less pathogenic and rarely causing AIDS. HIV virus is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus (WHO, 2007). It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7 and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle (ESGHT, 1992). This is, in turn, surrounded by the viral envelope which is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle. This protein, known as Env, consists of a cap made of three molecules called glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle. Both these surface proteins, especially gp120, have been considered as targets of future treatments or vaccines against HIV (Chan et al, 1997).

The diagnosis of HIV infection is usually made on the basis of the detection of HIV antibodies and/or antigen. The most widely used screening tests for screening large numbers of specimens on a daily basis (e.g. blood donations) are ELISAs. The earliest

assays used purified HIV lysates (1st generation), and often lacked sensitivity and specificity. Improved assays based on recombinant proteins and/or synthetic peptides, which also enabled the production of combined HIV-1/HIV-2 assays, became rapidly available (2nd generation). The 3rd generation or sandwich ELISAs, which use labeled antigen as conjugate, are extremely sensitive and have reduced the window period considerably. In recent years, the development of enhanced ELISA assays that detect both HIV antibody and antigen (4th generation assays) has led to earlier detection of HIV seroconversion by further reducing the window period (UNAIDS/WHO, 2004).

A variety of simple, instrument-free rapid tests are now available, including agglutination e.g. Micro Red HIV-1/2, Serodia HIV-1/2, immunofiltration or flow through tests (e.g. HIV SpoT-1/2, HIV SAV-1/2) , immunochromatographic or lateral flow tests (e.g. Determine HIV-1/2, ORa Quick), and dipstick tests or Immunodot Comb (e.g. Dipstick HIV-1/2, Entebe HIV-Dipstick). Specimens and reagents are often added by means of a dropper to the test device. A positive result is indicated by the appearance of a coloured dot/line/agglutination patterns as specific to specific test format. Most of these tests can be performed in less than 20 minutes, and are therefore called simple/rapid (S/R) assays. The results are read visually. In general, these tests are most suitable for use in testing and counseling centres and laboratories that have limited facilities and process low numbers of specimens daily.

Until recently, the most commonly used confirmatory test was the Western blot (WB). However, its use has proven to be expensive and can, under some conditions, produce a relatively large number of indeterminate results. Similar assays, generically called Line immuno-assays (LIAs) (e.g INNOLIA, Pepti-Lav, and RIBA assays), based on recombinant proteins and/or synthetic peptides capable of detecting antibodies to specific HIV-1 and/or HIV-2 proteins, have been developed. In general, these assays produce fewer indeterminate results as compared to WB, but are equally expensive. Studies have shown that combinations of ELISAs or S/R assays can provide results as reliable as the WB at a much lower cost. WHO and UNAIDS therefore recommend that

countries consider testing strategies which use a combination of ELISAs and/or S/R assays rather than ELISA/WB for HIV antibody (UNAIDS/WHO, 2004).

The two main types of test that can be used for the direct detections of the viruses are viral culture and nucleic acid amplification technologies (NAT), such as polymerase chain reaction (PCR) tests. However, their usefulness is limited because levels of virus particles vary at different stages of infection and are not always detectable. Viral culture grows the virus from a sample of blood, in a laboratory. Nucleic acid amplification technologies (NAT), such as polymerase chain reaction (PCR) tests, work by detecting the genetic material of the virus. Viral culture and PCR testing are expensive, requires sophisticated facilities and highly trained technicians, and is not feasible in most developing countries (Yilmaz, 2001).

This study was carried out in NRCS, CBTS to evaluate three commercially available test kits. There is a well defined recommendations (table 3.2) and guidelines (figure 3.2) developed by UNAIDS/WHO that has to be followed during the screening of blood. In NRCS, CBTS these standard recommendations and guidelines has not been followed, NRCS, CBTS has developed its own working manual called as Standard Operating Procedure (SOP). The criteria included in this manual do not provide the enough validation for the blood samples to consider positive or negative against anti HIV antibodies.

This study shall help to eliminate the potential harms to individuals of false HIV diagnosis and unsafe blood banking procedures. A large number of new HIV kits are continuously introduced commercially. It is mandatory that the new kits be evaluated for their performance characters in terms of sensitivity specificity and predictive values, before they are released into the market.

CHAPTER-II

OBJECTIVES

2.1 General Objective

- To evaluate the commercially available ELISA based test kits for detection of anti-HIV antibodies.

2.2 Specific Objectives

- To determine the sensitivity and specificity of the different kits used in Nepal.
- To determine the positive predictive value (PPV) and negative predictive value (NPV) of the test kits.

CHAPTER-III

LITERATURE REVIEW

3.1 HIV

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that can lead to acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Previous names for the virus include human T-lymphotropic virus- III (HTLV-III), lymphadenopathy-associated virus (LAV), and AIDS-associated retrovirus (ARV) (Sowadsky, 1999).

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unprotected sexual intercourse, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth. Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world.

HIV infection in humans is now pandemic. As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that AIDS has killed more than 25 million people since it was first recognized on December 1, 1981. It is estimated that about 0.6 percent of the world's population is infected with HIV (UNAIDS, 2006). In 2005 alone, AIDS claimed an estimated 2.4–3.3 million lives, of which more than 570,000 were children. A third of these deaths are occurring in sub-Saharan Africa, retarding economic growth and increasing poverty (Greener, 2002). According to current estimates, HIV is set to infect 90 million people in Africa, resulting in a minimum estimate of 18 million orphans (UNAIDS, 2005). Antiretroviral treatment reduces both the mortality and the morbidity

of HIV infection, but routine access to antiretroviral medication is not available in all countries (Palella et al, 1998).

Unlike some other viruses, infection with HIV does not provide immunity against additional infections, particularly in the case of more genetically distant viruses. Both inter-clade and intra-clade multiple infections have been reported, (Smith et al, 2005). and even associated with more rapid disease progression (Gottlieb et al, 2004). Multiple infections are divided into two categories depending on the timing of the acquisition of the second strain. Coinfection refers to two strains that appear to have been acquired at the same time (or too close to distinguish). Reinfection (or superinfection) is infection with a second strain at a measurable time after the first. Both forms of dual infection have been reported for HIV in both acute and chronic infection around the world.

HIV primarily infects vital cells in the human immune system such as helper T cells (specifically CD4+T cells), macrophages, and dendritic cells. HIV infection leads to low levels of CD4+T cells through three main mechanisms: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells; and thirdly, killing of infected CD4+T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Eventually most HIV-infected individuals develop AIDS (Acquired Immunodeficiency Syndrome). These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system (Lawn, 2004). Without treatment, about 9 out of every 10 persons with HIV will progress to AIDS after 10-15 years. Many progress much sooner (Buchbinder et al, 1994). Treatment with anti-retrovirals increases the life expectancy of people infected with HIV. Even after HIV has progressed to diagnosable AIDS, the average survival time with antiretroviral therapy is estimated to be more than 5 years (Schneider et al, 2005). Without antiretroviral therapy, death normally occurs within a year (Morgan et al, 2002). It is

hoped that current and future treatments may allow HIV-infected individuals to achieve a life expectancy approaching that of the general public.

3.1.1 Classification

HIV is a member of the genus Lentivirus, part of the family of Retroviridae. Lentiviruses have many common morphologies and biological properties. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period (ICTV, 2006) Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses.

There are two strains of HIV known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed LAV. It is more virulent, relatively easily transmitted, and is the cause of the majority of HIV infections globally. HIV-2 is less transmittable and is largely confined to West Africa. The comparision of two different strains of HIV is given in table 3.1.

Table 3.1: Comparison of HIV species

Species	Virulence	Transmittability	Prevalance	Purported Origin
HIV-1	High	High	Global	Common Chimpanzee
HIV-2	Lower	Low	West Africa	Sooty Mangabey

3.1.2 History

Origin: HIV is thought to have originated in non-human primates in sub-Saharan Africa and transferred to humans early in the 20th century (Worobey, 2008) The first paper recognizing a pattern of opportunistic infections was published on June 1981. Two species of HIV infect humans: HIV-1 and HIV-2. Both species of the virus are believed to have originated in West-Central Africa and jumped species (zoonosis) from a non-human primate to humans. HIV-1 is thought to have originated in southern

Cameroon after jumping from wild chimpanzees (Pan Troglodytes) to humans during the twentieth century. It evolved from a Simian Immunodeficiency Virus (SIV) HIV-2, on the other hand, may have originated from the Sooty Mangabey (Cercocebusatys), an Old World monkey of Guinea-Bissau, Gabon, and Cameroon (Reeves et al, 2002).

New World Monkeys are an interesting exception to the transmission of HIV. Their immunity is believed to be caused by retrotransposition of the cyclophilin gene into an intron of TRIM5. The result is fusion gene that provides the owl monkey with resistance to HIV-1 infection.

Discovery: Controversy surrounding the discovery of HIV was intense after French scientist Luc Montagnier and American researcher Robert Gallo both claimed to have discovered it, in 1983 and 1984 respectively. In 1987 the dispute was initially settled on a political level with both teams receiving equal credit (Brown et al, 1991). In 1991 a study confirmed that the samples in Gallo's laboratory had in fact originated in Montagnier's (Brown et al, 1991). In 1994 the US Government conceded that the French should receive the lion's share of the credit (Radhi et al, 2008).

The Karolinska Institute awarded half of the 2008 Nobel Prize in Physiology or Medicine to Montagnier and his colleague Françoise Barré-Sinoussi 'for their discovery of "human immunodeficiency virus"'. The other half went to Harald zur Hausen for unrelated work on Human Papilloma Virus (Karolinska, 2008). Gallo was reported to have said that it was "a disappointment" not to have been included, but that all three of the award's recipients deserved the honor (Ritter et al, 2008).

3.1.3 Transmission

Three main transmission routes for HIV have been identified. HIV-2 is transmitted much less frequently by the mother-to-child and sexual route than HIV-1.

Sexual: Globally, HIV is mostly transmitted through sexual contact. Both the fluid and cellular components of semen has been found to contain HIV, as do endocervical secretions. The rate of transmission of HIV depends upon the sexual behaviors and

sexual practices vaginal intercourse in conditions of other sexually transmitted infections or during menses, sexual mixing patterns and level of condom use, has been recognized as factors affecting spread (Folks et al, 1998). Similarly, the efficiency of transmission is also dependant on level of viremia, infectivity and virulence of particular HIV strain, and the presence of STDs such as genital ulcers (Plummer et al, 1991).

Blood or blood products: In general if infected blood comes into contact with any open wound, HIV may be transmitted. This transmission route can account for infections in intravenous drug users, hemophiliacs and recipients of blood transfusions (though most transfusions are checked for HIV in the developed world) and blood products. It is also of concern for persons receiving medical care in regions where there is prevalent substandard hygiene in the use of injection equipment, such as the reuse of needles in Third World countries. Health care workers such as nurses, laboratory workers, and doctors have also been infected, although this occurs more rarely. People who give and receive tattoos, piercing, and scarification procedures can also be at risk of infection. Since transmission of HIV by blood became known medical personnel are required to protect themselves from contact with blood by the use of universal precautions.

Mother-to-child: The transmission of the virus from the mother to the child can occur in utero during pregnancy and intrapartum at childbirth. In the absence of treatment, the transmission rate between the mother and child is around 25 percent (Coovadia, 2004). However, where combination antiretroviral drug treatment and Cesarian section are available, this risk can be reduced to as low as one percent. Breast feeding also presents a risk of infection for the baby.

Other routes: HIV has been found at low concentrations in the saliva, tears and urine of infected individuals, but there are no recorded cases of infection by these secretions and the potential risk of transmission is negligible.

3.1.4 Structure and genome

HIV is different in structure from other retroviruses. It is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus (WHO, 2007). It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7 and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle (ESGHT, 1992). This is, in turn, surrounded by the viral envelope which is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle. This protein, known as Env, consists of a cap made of three molecules called glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle. Both these surface proteins, especially gp120, have been considered as targets of future treatments or vaccines against HIV (Chan et al, 1997).

The RNA genome consists of at least 7 structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, INS) and nine genes (gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and tev) encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles. For example, env codes for a protein called gp160 that is broken down by a viral enzyme to form gp120 and gp41. The six remaining genes, tat, rev, nef, vif, vpr, and vpu (or vpx in the case of HIV- 2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease. The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The Rev protein (p19) is involved in shuttling RNAs from the nucleus

and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cell protein which deaminates DNA:RNA hybrids and/or interferes with the Pol protein). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) downregulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules (Stumptner et al, 2001). Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells (Chan et al, 1997). The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol.

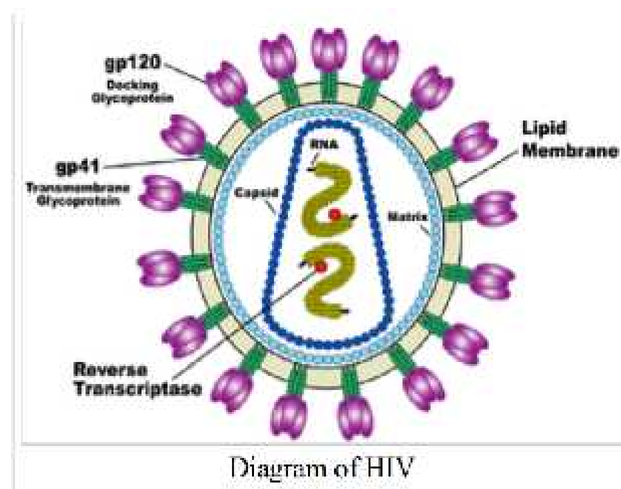


Fig 3.1 Structure of HIV(Source: www.stanford.edu/.../2005gongishmail/HIV.html)

3.1.5 Tropism

The term viral tropism refers to which cell types HIV infects. HIV can infect a variety of immune cells such as CD4+T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and CD4+T cells is mediated through interaction of the virion envelope

glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine coreceptors.

Macrophage (M-tropic) strains of HIV-1, or non-syncitia-inducing strains (NSI) use the -chemokine receptor CCR5 for entry and are thus able to replicate in macrophages and CD4+T cells (Coakley et al, 2005). This CCR5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when CD4+T cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system. In tonsils and adenoids of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amounts of virus.

T-tropic isolates, or syncitia-inducing (SI) strains replicate in primary CD4+T cells as well as in macrophages and use the -chemokine receptor, CXCR4, for entry. Dual - tropic HIV-1 strains are thought to be transitional strains of the HIV-1 virus and thus are able to use both CCR5 and CXCR4 as co-receptors for viral entry.

The -chemokine, SDF-1, a ligand for CXCR4, suppresses replication of T-tropic HIV-1 isolates. It does this by down-regulating the expression of CXCR4 on the surface of these cells. HIV that use only the CCR5 receptor are termed R5, those that only use CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection (Coakley et al, 2005) and HIV can also infect a subtype of myeloid dendritic cells, which probably constitute a reservoir that maintains infection when CD4+T cell numbers have declined to extremely low levels (Knight et al, 1990).

Some people are resistant to certain strains of HIV. One example of how this occurs is people with the CCR5- 32 mutation; these people are resistant to infection with R5 virus as the mutation stops HIV from binding to this coreceptor, reducing its ability to infect target cells (Tang et al, 2003).

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid which is passed from a male to his sexual partner. The virions can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway. How this selective process works is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXCR4 on their surface (Muciaccia et al, 2005) and that genital epithelial cells preferentially sequester X4 virus (Berlier et al, 2005). In patients infected with subtype B HIV-1, there is often a co- receptor switch in late-stage disease and T-tropic variants appear that can infect a variety of T cells through CXCR4 (Clevestig et al, 2005). These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse, and opportunistic infections that mark the advent of AIDS (Moore, 1997). Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS. A number of studies with subtype B-infected individuals have determined that between 40 and 50% of AIDS patients can harbour viruses of the SI, and presumably the X4, phenotype.

3.1.6 Genetic variability

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of 10^9 to 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase. This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day (Holzammer et al, 2001). This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will

generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes (Robertson et al, 1995). This recombination is most obvious when it occurs between subtypes.

The closely related simian immunodeficiency virus (SIV) exhibits a somewhat different behavior: in its natural hosts, African green monkeys and sooty mangabeys, the retrovirus is present in high levels in the blood, but evokes only a mild immune response, (Robertson et al, 1995), however, does not cause the development of simian AIDS, (Holzammer et al, 2001). and does not undergo the extensive mutation and recombination typical of HIV (Baier et al, 1991). By contrast, infection of heterologous hosts (rhesus or cynomologus macaques) with SIV results in the generation of genetic diversity that is on the same order as HIV in infected humans; these heterologous hosts also develop simian AIDS (Daniel et al, 1984). The relationship, if any, between genetic diversification, immune response, and disease progression is unknown.

Three groups of HIV-1 have been identified on the basis of differences in env: M, N, and O (Thomson et al, 2002). Group M is the most prevalent and is subdivided into eight subtypes (or clades), based on the whole genome, which are geographically distinct (Carr et al, 1998). The most prevalent are subtypes B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia); these subtypes form branches in the phylogenetic tree representing the lineage of the M group of HIV-1. Coinfection with distinct subtypes gives rise to circulating recombinant forms (CRFs). In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2 percent of infections worldwide were of subtype C, 26.7 percent were of subtype A/CRF02_AG, 12.3 percent were of subtype B, 5.3 percent were of subtype D, 3.2 percent were of CRF_AE, and the remaining 5.3 percent were composed of other subtypes and CRFs. Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes (Osmanov et al, 2002).

3.1.7 The clinical course of infection

Infection with HIV-1 is associated with a progressive decrease of the CD4+T cell count and an increase in viral load. The stage of infection can be determined by measuring the patient's CD4+T cell count, and the level of HIV in the blood. HIV infection has basically four stages: incubation period, acute infection, latency stage and AIDS. The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks. The second stage, acute infection, which lasts an average of 28 days and can include symptoms such as fever, lymphadenopathy (swollen lymph nodes), pharyngitis (sore throat), rash, myalgia (muscle pain), malaise, and mouth and esophageal sores. The latency stage, which occurs third, shows few or no symptoms and can last anywhere from two weeks to twenty years and beyond. AIDS, the fourth and final stage of HIV infection shows as symptoms of various opportunistic infections.

Acute HIV infection: The initial infection with HIV generally occurs after transfer of body fluids from an infected person to an uninfected one. The first stage of infection, the primary, or acute infection, is a period of rapid viral replication that immediately follows the individual's exposure to HIV leading to an abundance of virus in the peripheral blood with levels of HIV commonly approaching several million viruses per mL (Piatak et al, 1993). This response is accompanied by a marked drop in the numbers of circulating CD4+T cells. This acute viremia is associated in virtually all patients with the activation of CD8+T cells, which kill HIV- infected cells, and subsequently with antibody production, or seroconversion. The CD8+T cell response is thought to be important in controlling virus levels, which peak and then decline, as the CD4+T cell counts rebound to around 800 cells per µL (the normal blood value is 1200 cells per µL). A good CD8+T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo et al, 1997). During this period (usually 2-4 weeks post-exposure) most individuals (80 to 90%) develop an influenza or mononucleosis-like illness called acute HIV infection, the most common symptoms of which may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophagal sores, and may also include, but less

commonly, headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms. Infected individuals may experience all, some, or none of these symptoms. The duration of symptoms varies, averaging 28 days and usually lasting at least a week (Kahn et al, 1998). Because of the nonspecific nature of these symptoms, they are often not recognized as signs of HIV infection. Even if patients go to their doctors or a hospital, they will often be misdiagnosed as having one of the more common infectious diseases with the same symptoms. Consequently, these primary symptoms are not used to diagnose HIV infection as they do not develop in all cases and because many are caused by other more common diseases. However, recognizing the syndrome can be important because the patient is much more infectious during this period (Darr et al, 2001).

Latency stage: A strong immune defense reduces the number of viral particles in the blood stream, marking the start of the infection's clinical latency stage. Clinical latency can vary between two weeks and 20 years. During this early phase of infection, HIV is active within lymphoid organs, where large amounts of virus become trapped in the follicular dendritic cells (FDC) network (Burton et al, 2002). The surrounding tissues that are rich in CD4+T cells may also become infected, and viral particles accumulate both in infected cells and as free virus. Individuals who are in this phase are still infectious. During this time, CD4+CD45RO+T cells carry most of the proviral load.

AIDS: When CD4+T cell numbers decline below a critical level, cell -mediated immunity is lost, and infections with a variety of opportunistic microbes appear. The first symptoms often include moderate and unexplained weight loss, recurring respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis), prostatitis, skin rashes, and oral ulcerations. Common opportunistic infections and tumors, most of which are normally controlled by robust CD4+T cell-mediated immunity then start to affect the patient. Typically, resistance is lost early on to oral Candida species and to Mycobacterium tuberculosis, which leads to an increased susceptibility to oral candidiasis (thrush) and tuberculosis. Later, reactivation of latent herpes viruses may cause worsening recurrences of herpes simplex eruptions, shingles,

Epstein-Barr virus-induced B-cell lymphomas, or Kaposi's sarcoma, a tumor of endothelial cells that occurs when HIV proteins such as Tat interact with Human Herpesvirus-8. Pneumonia caused by the fungus *Pneumocystis jirovecii* is common and often fatal. In the final stages of AIDS, infection with cytomegalovirus (another herpes virus) or *Mycobacterium avium* complex is more prominent. Not all patients with AIDS get all these infections or tumors, and there are other tumors and infections that are less prominent but still significant (Clapham et al, 2001).

3.2 Laboratory Diagnosis of HIV

HIV infection/AIDS is a complex infectious disease since it is lifelong, the outcome is invariably fatal and no vaccine is available so far. Since HIV is acquired most frequently through unprotected sexual contact, a number of moral, ethical, legal, and psychological issues are related to HIV testing. It is important to understand that laboratory diagnosis is the only way to establish the HIV infection status of an individual.

3.2.1 HIV test

Many HIV-positive people are unaware that they are infected with the virus. For example, less than 1% of the sexually active urban populations in Africa have been tested and this proportion is even lower in rural populations. Furthermore, only 0.5% of pregnant women attending urban health facilities are counseled, tested or receive their test results. Again, this proportion is even lower in rural health facilities. Since donors may therefore be unaware of their infection, donor blood and blood products used in medicine and medical research are routinely screened for HIV (Kumaranayake et al, 2001).

HIV-1 testing consists of initial screening with an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result

are retested in duplicate (CDC, 2001). If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., Western blot or, less commonly, an immunofluorescence assay (IFA)). Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate result, which may be either an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person. Although IFA can be used to confirm infection in these ambiguous cases, this assay is not widely used. Generally, a second specimen should be collected more than a month later and retested for persons with indeterminate Western blot results. Although much less commonly available, nucleic acid testing (e.g., viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations. In addition, a few tested specimens might provide inconclusive results because of a low quantity specimen. In these situations, a second specimen is collected and tested for HIV infection (Celum et al, 1991).

The main purposes of HIV testing are:

1. To identify of asymptomatic and symptomatic HIV positive individuals

2. To assure safety of blood and blood-related products

3. To motivate for behavior changes through counseling among those high-risk behavior individuals who tested anti-HIV negative.

4. To induce behavior change and prevent further HIV transmission by counseling in individuals who tested anti-HIV positive.

5. To monitor trends of HIV epidemic.

Serological detection of HIV infection: Current routine laboratory diagnosis of HIV infection is mainly based on the detection of specific anti-HIV antibodies. Blood (whole

blood, serum or plasma) is the preferred specimen for the detection of anti-HIV antibodies since it has a higher concentration of antibodies than urine or oral fluids. Antibodies to HIV usually begin to be detectable 3 to 6 weeks (on average 22 days) after infection. The time from infection to first reactivity of screening tests (seroconversion) is called the "window period". During this period the infected individual is highly infectious but anti-HIV seronegative. The window period may be in some individuals somewhat shorter or several weeks longer. Persistently undetectable antibodies more than three months following infection are rare (1, 5-8). All samples are first screened for anti-HIV using basic screening serological methods such as the enzyme immunoassay or agglutination assay. Since the specificity of screening tests is limited, all anti-HIV reactive screening test results have to be confirmed, using confirmatory tests such as Western-blot or immunoblot tests.

3.2.1.1 Screening tests

Enzyme immuno assay (EIA) is the most commonly performed screening test for detection of anti-HIV antibodies. Briefly, in classical EIA immobilized HIV antigens are used to bind anti-HIV antibodies from the patient's sample. Bound anti-HIV antibodies are then complexed with enzyme-labeled anti-human IgG and are detected in colorimetric reaction. The resulting colour change is quantified spectrophotometrically and is proportional to the concentration of antibodies in the original sample. The improved EIA test formats used for anti-HIV antibody screening are the antibody capture assay and the double antigen sandwich assay (Palella et al, 1998).

Since the introduction of HIV screening assays in 1985, the performance of EIA has continued to improve. First-generation antibody screening assays were very sensitive but not specific since whole viral lysates were used as a source of HIV proteins (antigens). The time interval between infection and antibody detection has been substantially shortened by the introduction of 3rd generation double antigen sandwich assays, which use recombinant and synthetic HIV peptides as antigens. An even earlier laboratory diagnosis of HIV infection is possible by the detection of p24 antigen or HIV

RNA, which are present prior to or during the early stages of seroconversion. Thus, HIV infection can be detected on average 9 days earlier by p24 antigen testing than with 3rd generation assays. 4th generation EIAs or comboscreening assays, which are now available, enable simultaneous screening for anti-HIV antibodies and p24 antigen in a single assay. 4th generation EIAs shorten the window period to an average of 7 days and can detect antibodies against HIV-1 group O (Sowadsky, 1999).

Agglutination assays, which may be considered as a variant of the double antigens sandwich assay, incorporate a variety of antigen-coated carriers like red cells, latex or gelatin particles. HIV antigens that are based on viral lysate are attached to the carrier particles by nonspecific attachment. Since antibodies are multivalent, in the case of a positive result a sort of lattice network is formed which can be visualized macroscopically or microscopically. Agglutination assays are highly specific, sensitive, simple to perform and do not require sophisticated equipment (Buchbinder et al, 1994).

The reported sensitivity of licensed anti-HIV screening tests under optimal laboratory conditions is greater than 99% (Buchbinder et al, 1994). False negative EIA results can occur during primary HIV infection, in immuno-compromised patients and due to errors in specimen handling and labeling. The specificity of repeatedly reactive screening tests is approximately 99%. False reactive (better term than false positive) results can occur due to human error, in individuals with acute Epstein-Barr virus or human cytomegalovirus infection, autoimmune disorders, hipergamaglobulinemias, multiple myeloma, hemophilia, and hemodialysis patients. The percentage of false reactive EIA results decreases as the prevalence of HIV infection in a particular population increases (ICTV, 2006).

3.2.1.2 Supplemental or confirmatory tests

As mentioned before, anti-HIV antibody screening assays are highly sensitive but prone to false reactivity, while supplemental tests are both sensitive as well as specific and are mainly performed to rule out false reactive results of screening assays. However, supplemental tests do not always give conclusive results. In these cases other tests for

direct detection of HIV infection have to be performed or a follow-up strategy is used (Sowadsky, 1999).

Western blot (WB) and immunoblot (IB) tests are the most widely used supplemental or confirmatory tests for detection of anti-HIV antibodies. Both tests, are highly specific, but in comparison with screening tests more laborious and costly. Their high specificity is mainly based on the fact that they allow determination of the reactivity of anti-HIV antibodies with particular HIV proteins. In WB, electrophoretically separated natural HIV proteins derived from whole virus lysates are transferred (blotted) to a solid membrane. HIV viral proteins on WB membrane may contain contaminating human cell proteins. In contrast, recombinant or synthetic HIV proteins mechanically applied onto the solid membrane are used in the IB test. HIV proteins on IB membrane, however, do not contain contaminating human cell proteins and are highly specific.

Briefly, in both WB and IB patient's serum is incubated with a nitrocellulose membrane strip containing separated different HIV proteins. Antibodies directed against particular HIV proteins are identified with enzyme-anti-human IgG, similarly to the EIA method (Worobey, 2005). In addition to greater specificity in comparison to WB, IB allow also a reliable differentiation between HIV-1 and HIV-2 infection since type specific recombinant proteins and peptides from HIV-1 and HIV-2 are present on the IB membrane.

Patients with repeatedly reactive EIA and indeterminate WB or IB results require serologic and clinical follow-up to determine whether HIV infection is present. There are no clear patterns of antigen reactivity that predict a higher likelihood of seroconversion in patients with indeterminate WB/IB results. Additional testing for HIV infection using methods for direct detection of HIV may be indicated, depending on the clinical picture and risk factors for HIV infection (Sowadsky, 1999).

3.2.1.3 Direct detection of HIV infection

Serology represents a highly sensitive, cheap and quick method to screen blood for the presence of HIV infection. However, in certain situations such as in newborns born to HIV-infected mothers, in individuals with indeterminate WB/IB results, in order to determine the HIV status during "window period" and to monitor HIV infected individuals a direct detection of HIV is required. For these purposes detection of the p24 antigen, virus isolation or detection of viral DNA or RNA by different molecular amplification methods is the most widely used (Sowadsky, 1999).

Detection of p24 antigen: The HIV *gag* gene encoded core protein or p24 antigen can be detected in serum or plasma during the acute phase of primary HIV infection ("window period"), during very late symptomatic stages of infection in the newborns born to HIV-infected mothers (Sowadsky, 1999).

Detection of p24 antigen is mostly performed using EIA. The test principle consists of binding the p24 antigen present in a sample to anti-p24-specific, usually monoclonal, capture antibodies, which coat a solid support. Unbound sample components are washed away, and bound antigen reacts with another p24-specific antibody conjugated with enzyme. For a confirmation of a reactive result, the sample must be subjected to an additional confirmatory neutralization assay (Sowadsky, 1999).

Levels of free p24 antigen decline after the appearance of anti-p24 antibodies due to formation of immune complexes. Only 4% of asymptomatic HIV-infected adults have detectable p24 antigen using standard assays. However, a recently developed assay based on heat denatured p24 antigen tyramide-mediated signal amplification-boosted EIA, coupled with neutralization allowed the detection of p24 antigen in up to 90% asymptomatic HIV-infected adults (Gao et al, 1999).

The overall sensitivity of standard p24 antigen testing for the detection of HIV infection in infants is 50 to 75% and the specificity is greater than 95% (19, 20). The sensitivity

of the assay decreases in asymptomatic children and children younger than 6 months of age and ranges 0 to 20% in the first month of life (Goodier et al, 2008).

Although p24 antigen assays are very specific, they are relatively insensitive compared with molecular amplification methods and negative result for p24 antigen does not rule out the HIV infection.

Isolation of HIV: HIV can be isolated from peripheral blood mononuclear cells (PBMC) or plasma and other body fluids. Briefly, the patient's PBMCs are isolated and incubated phytohaemagglutinin- and interleukin-2-stimulated from healthy blood donors (Sowadsky, 1999). The presence of HIV in the culture supernatant is detected by demonstration of the presence of p24 antigen, reverse transcriptase or HIV RNA (Brown, Phyllida, 1991). The majority of cultures from HIV-positive untreated patients become positive within two weeks (Thomas and Maugh, 2008).

Isolation of HIV is a time-consuming and costly procedure requiring specialized containment facilities and a high degree of expertise. HIV isolation therefore remains mostly an important research and not diagnostic tool.

Molecular amplification methods: Recent studies on the dynamics of HIV-1 replication have enhanced our understanding of HIV/AIDS pathogenesis (Karolinska, 2008). The discovery of a persistently high level of viral turnover in all stages of disease, even during the period of clinical latency, suggests that sensitive measurement of viral load (reflecting rapid or unchecked viral replication) may be useful in assessing HIV disease stage, disease progression, response to therapy, and risk of transmission (Smith et al, 2005). HIV RNA quantification or determination of plasma viral load is presently considered the best method for monitoring disease progression and response to antiretroviral therapy (Buchbinder et al, 1994).

Three methods for determination of HIV-1 plasma viral load are widely used at present: two are based on use of enzymatic amplification of target nucleic acids (polymerase chain reaction (PCR) and nucleic acid sequence-based amplification) and the third

utilizes branched-chain DNA hybridization signal amplification (bDNA assay). With "ultrasensitive" protocols developed for all three methods the detection limit of 20-50 HIV -1 RNA copies/ml has been already reached (Buchbinder et al, 1994).

The HIV-1 viral load usually ranges between 102 and 107 HIV RNA copies/ml in untreated individuals. Persistently detectable viremia and high baseline levels are predictors of poor prognosis, while risk of progression of HIV infection to AIDS is relatively low if the load is below 10,000 HIV RNA copies/ml. Apart from being an extremely useful predictor of disease progression, viral load measurement is a key laboratory method for monitoring antiretroviral therapy. Determination of HIV RNA has been used by some clinicians also as a diagnostic tool in patients with acute HIV infection, in newborns born to HIV-infected mothers and in individuals with reactive results from screening tests and indeterminate WB/IB results, although this approach is not approved by the Food and Drug Administration (FDA) (Sowadsky, 1999).

3.3 Treatment

A large number of antiviral drugs are approved for treatment of HIV infections. Current drug regimens can prolong the survival of patient but not cure HIV. These include nucleoside analogue reverse transcriptase inhibitor (NRTIs), protease inhibitors (PIs), non nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors. The fusion inhibitors are newest class of drugs that block virus entry into cell. Researches have shown that by taking three or more antiretroviral drugs at the same time, each attacking HIV in different points in its cycle of replication, then treatment is more effective than one or two drugs alone. So the common treatment is to use combination of three or more drugs which include drugs from different classes. This is called HAART (highly active antiretroviral therapy). Such combination of drug has been shown to reduce the risk of developing resistance to any individual drug. HAART often can suppress viral replication below limits of detection in plasma, decrease viral load in lymphoid tissues, allow the recovery of immune responses to opportunistic infections, and prolong patient survival (Brooks et al, 2004). Moreover, it would take more than a

lifetime for HIV infection to be cleared using HAART (Dybul et al, 2002). Despite this, many HIV-infected individuals have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world (Blankson et al, 2002). One study suggests the average life expectancy of an HIV infected individual is 32 years from the time of infection if treatment is started when the CD4 count is 350/µL (Chene et al, 2003). In an study, the mean CD4 cell count in patients at first visit to National Public Health Laboratory,Kathmandu (NPHL) (155/mm3) increased to (297/mm3) significantly after six months of ART (Tiwari et al, 2008).

3.4 EVALUATION OF COMMERCIAL TEST KITS

3.4.1 Background Information

In 1988, the World Health Organization (WHO) Global Programme on AIDS (GPA), conscious of the need to advise Member States on the laboratory diagnosis of HIV, initiated a programme to provide objective assessments of commercially available assays for detecting antibody to both types of HIV, HIV-1 and HIV-2. The programme has been extended to include assays which also detect HIV antigen (HIV Ag/Ab assays). The laboratory aspects of this continuing programme is carried out by the WHO Collaborating Centre for HIV/AIDS Diagnostic and Laboratory Support in the Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium and coordinated by the Department of Essential Health Technologies of WHO in conjunction with UNAIDS (UNAIDS/WHO, 1996). The assessments focus on the operational characteristics of these assays, such as ease of performance and their sensitivity and specificity on a panel of well-characterized sera of diverse geographical origins, and indicate their suitability for use in small laboratories, e.g. many blood-collection centres in developing countries. Additionally, the sensitivity of the assays on eight commercial seroconversion panels, one anti HIV-1 mixed titer performance panel and one HIV-1 p24 antigen mixed titer performance panel are assessed. The assessments are published in the form of reports which are intended for use by health

policy-makers, directors of blood banks, and managers of national AIDS prevention programmes**.** They may be used in conjunction with consideration of other factors, such as experience with a given test, availability, cost, service and troubleshooting provided locally by manufacturers, etc., to help select HIV antibody and/or HIV Ag/Ab assays appropriate to local needs.

3.4.2 HIV testing strategies

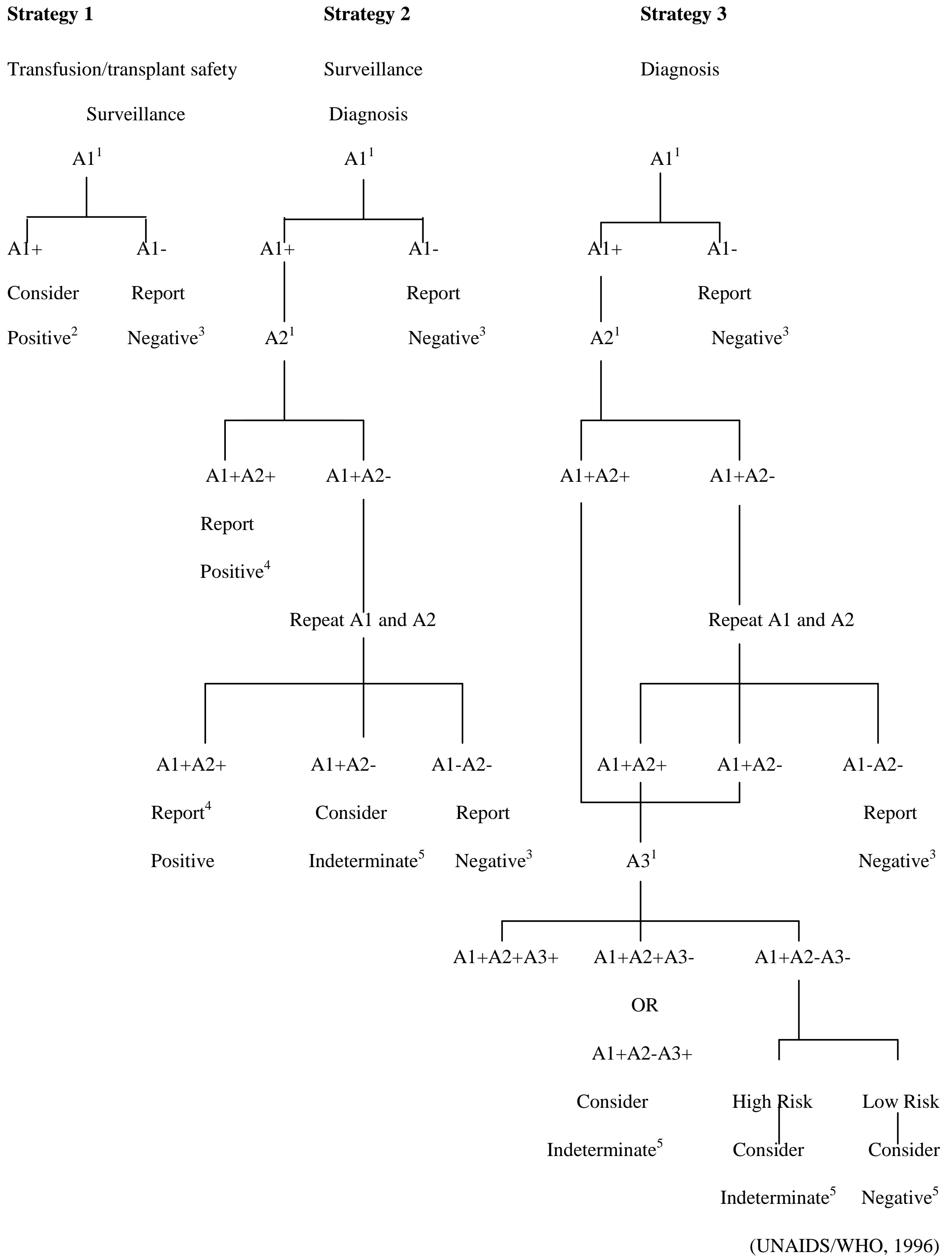
UNAIDS and WHO recommend three testing strategies, which have been recently updated, to maximize accuracy while minimizing cost. Which strategy is most appropriate will depend on the objective of the test and the prevalence of HIV in the population, as shown below in table 3.2. and figure 3.2.

Table 3.2: UNAIDS and WHO recommendations for HIV testing Strategies according to test objective and prevalence of infection in the sample population

Objectives of testing			Prevalence of Infection	Testing strategy
Transfusion/transplant safety			All prevalances	I
Surveillance	i.		>10%	I
	ii.		10%	II
Diagnosis	i.	clinical signs/ symptoms of HIV infection	>30%	I
			30%	II
	ii.	Asymptomatic	>10%	II
			10%	III

(WHO modified form of Interim proposal for a WHO staging system for HIV infection and disease 1990)

Figure 3.2: Schematic representation of the UNAIDS and WHO HIV testing strategies



(UNAIDS/WHO, 1996)

1. Assay A1, A2, A3 represent 3 different assays.
2. Such a result is not adequate for diagnostic purposes; use strategies II or III.Whatever the final diagnosis, donations which were initially reactive should not be used for transfusions or transplants.
3. Report: result may be reported.
4. For newly diagnosed individuals, a positive result should be confirmed on a second sample.
5. Testing should be repeated on a second sample taken after 14 days.
6. Result is considered negative in the absence of any risk of HIV infection.

3.4.2.1 Strategy I

Each serum/plasma specimen is tested with one ELISA or simple/rapid assay. Serum that is reactive is considered HIV antibody positive. Serum that is non-reactive is considered HIV antibody negative.

Transfusion/transplant safety: When the objective is safeguarding the blood supply, the test selected for this strategy should preferably be a combined HIV-1/HIV-2 assay which is highly sensitive. Units of donated blood yielding reactive or indeterminate test results must be considered as probably infected with HIV and should be discarded according to universal safety instructions two. With the advent of combined HIV antibody and antigen assays, many blood centres have commenced using these tests although in situations of very low HIV prevalence and incidence, they may not provide any greater sensitivity. Strategy I is meant for testing the donations, but must not be used for notifying donors of a positive test result. If a blood or tissue donor is to be notified of a positive test result, testing strategies II or III for diagnosis must be applied. In situations where the blood centre does not have the capacity or facilities for further testing, the donor should be referred to their physician or to appropriate referral health services.

Whatever the final diagnosis, donations which were initially reactive should not be used for transfusion or transplants. Several studies have shown that careful selection of donors is more efficient than HIV antigen testing in minimizing the risk of transfusion related infections. To prevent further spread of HIV, it is recommended that systems are put in place to notify donors of their HIV status. Such systems should include referral for counseling and confirmation of HIV status where these facilities are not available on site.

Surveillance: Sensitivity is less crucial for surveillance purposes; however, for this and the above application the assay chosen should have a specificity of at least 98%. It is recommended that the same assay(s) be used over time to monitor fluctuations in HIV prevalence.

3.4.2.2 Strategy II

All serum/plasma is first tested with one ELISA or simple/rapid assay. Any serum found reactive on the first assay is retested with a second ELISA or simple/rapid assay based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive). Serum that is reactive on both tests is considered HIV antibody positive. Serum that is non-reactive on the first test is considered HIV antibody negative. Any serum that is reactive on the first test but non-reactive on the second test, should be retested with the two assays. Concordant results after repeat testing will indicate a positive or negative result. If the results of the two assays remain discordant the serum is considered indeterminate.

Surveillance: When testing low HIV prevalence populations for surveillance purposes, even if one uses a test of high specificity, the PPV will be very low. Therefore, an additional test is necessary in order not to overestimate the HIV prevalence in such regions. All samples remaining discordant after repeat testing with the two assays are considered indeterminate; unlike for diagnosis, no further testing is needed. The indeterminate results should be reported and analysed separately in the annual surveillance overviews.

3.4.2.3 Strategy III

As in strategy II, all serum is first tested with one ELISA or simple/rapid assay, and any reactive samples are retested using a different assay. Serum that is non-reactive on the first test is considered HIV antibody negative. Serum that is reactive in the first test but non-reactive in the second assay should be repeated with both tests. Strategy III, however, requires a third test if serum is found reactive on the second assay or is reactive on the repeated first assay. The three tests in this strategy should be based on different antigen preparations and/or different test principles. Serum reactive on all three tests is considered HIV antibody positive. Serum that remains discordant in the second assay, or is reactive in the first and second tests but non-reactive in the third test, is considered to be indeterminate. Serum that is reactive on the first assay and nonreactive on the second and third assays is considered indeterminate for individuals who may have been exposed to HIV in the last three months and negative for those who have not been exposed to any risk for HIV infection. Where a positive result is obtained from a combined HIV antigen/antibody ELISA but HIV antibody only tests are negative then an HIV antigen test should be carried out where applicable or a further sample obtained to examine for seroconversion.

An additional blood sample should be obtained and tested from all persons newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible technical or clerical error.

3.4.2.4 Uncertain diagnosis: indeterminate result

Serum from people with clinical signs meeting the WHO criteria3, stages III or IV, may have an indeterminate result due to a decrease in antibodies. In this case serum does not normally need to be retested.

For diagnosis of HIV infection in asymptomatic individuals with an indeterminate result, a second blood sample should be obtained after a minimum of two weeks following the first sample and should be tested using the appropriate strategy. If the

second serum sample also produces an indeterminate result, it should be tested with a confirmatory assay. However, if this result is also indeterminate longer follow-up may be required (3, 6, 12 months). If the results remain indeterminate after 1 year, the person is considered to be HIV antibody negative.

3.4.2.5 General remarks about Strategies I-II-III

Strategy I can only be used to confirm the clinical diagnosis of individuals meeting the WHO criteria of stage III or IV of HIV infection and when the HIV prevalence in the sample population is above 30% (e.g. a sample of patients from a tuberculosis ward). In lower prevalence populations, strategy II should be used to diagnose persons with the above-mentioned clinical symptoms.

In the selection of HIV antibody tests for use in strategies II and III, the first test should have the highest sensitivity, whereas the second and third tests should have a similar or higher specificity than the first. As tests have continued to increase in quality, it is now frequently found, however, that the tests employed have both high sensitivity and specificity values. The number of initial discordant, indeterminate results should not exceed 5%. If it does, quality assurance procedures should be checked and/or a new test combination should be adopted (WHO, 1991).

3.4.3 Follow up after diagnosis

A number of other assays have been introduced in recent years which assist in the establishment of the diagnosis of HIV infection and may also be used to monitor the progress of the infection and the response to therapy. These include assays that detect virus particles e.g. the HIV p24 antigen ELISA, or the presence of HIV viral nucleic acid sequences (RNA or DNA) by means of nucleic acid amplification techniques. The first assays capable of detecting free circulating HIV particles were the HIV p24 antigen ELISAs. Circulating p24 antigen appears early in the course of HIV infection, is detectable for 1-2 weeks, and then disappears or falls to very low levels until the onset of clinical illness. Rising titers of HIV p24 antigen late in the illness are correlated with

a poor prognosis. The presence of circulating p24 antigen is also associated with increased levels of infectious virus particles, as the probability of isolating HIV from an infected person is highest when p24 antigen can be detected.

New technologies based on the amplification of viral nucleic acids, such as PCR and NASBA or amplification of the probe binding signal as in branched-DNA tests, have made it possible to detect minute amounts of viral material. In theory, as little as a single viral genome can be detected - the detection limit for most assays is around 300 copies/ml. In practice the technique can have limited specificity. These sensitive procedures are well suited to early diagnosis of mother-to-child transmission and for monitoring the viral load of patients who are taking antiretroviral therapy. Although prices have recently decreased, the tests remain expensive, need sophisticated equipment, rigorous laboratory conditions and highly trained staff, and are still largely a research tool. Many of these tests need further refinement since not all HIV-1 subtypes are equally well detected, nor is HIV-2. Therefore, it would be unwise to base a diagnosis of HIV infection on a single positive PCR test result, in the absence of any other detectable marker.

3.4.4 Quality assurance

All laboratories and testing sites carrying out HIV tests, should have a well-functioning quality management programme. It is most important that quality control and assurance procedures be stringently complied with, so as to maximize the accuracy of the laboratory results. Procedures for detecting both (technical) laboratory and clerical errors must be included in all protocols. For example, procedures that guarantee the correct identification of initially reactive units of donated blood, which must be discarded, are essential to the maintenance of a safe blood supply. It is recommended that laboratories submit to an external quality assessment at least once a year, but preferably more regularly.

3.4.5 Safety

The testing of all clinical specimens should be performed in such a manner as to minimize occupational risk. Guidelines for good laboratory practice have been developed that, if followed, will ensure safety and keep laboratory accidents to a minimum (WHO, 1993).

3.4.6 Assay selection

There are various operational factors that influence the selection of assays, including:

- laboratory infrastructure
- access to a reference laboratory
- desired characteristics of the test (antigen, antibody)
- simplicity of test procedure
- equipment necessary to perform the test
- performance time
- shelf-life of the reagents
- price
- storage conditions
- technical skill of laboratory staff
- Laboratory logistics (continuous supply of kits, stability of electrical source, maintenance of equipment, spare parts, availability of service, etc.).

For use in small blood-collection centres and hospitals in developing countries, assays are needed that have the following specific characteristics:

- high level of sensitivity and specificity
- long shelf life at ambient temperatures
- reasonable cost (generally not exceeding the per-test cost of the most readily available ELISA)
- ease of performance
- rapidity of performance.

The WHO evaluations take these factors into account in assessing suitability for use in small centres. They show that some of the S/R assays now available, which need no or relatively simple equipment and can be read visually, are more suitable than ELISAs in small centres where there are only a limited number of sera to be screened (< 90 sera at a time). For testing large series of sera, ELISAs are still the most rapid and most appropriate assay type. However, they require expensive equipment which has to be well maintained.

The aim of the HIV assay assessment programme is to supply managers who will decide which tests to use, and the potential users of the tests, with enough comparative data to apply their own criteria and choose the best tests for their particular circumstances. The choice of the most appropriate HIV tests also depends on the HIV variants present in a particular geographical region (e.g., HIV-1 group O). It is clear, for example, that in areas such as West Africa, where HIV-2 is prevalent, a test capable of detecting antibodies to HIV-2 as well as HIV-1 will be required. Therefore, test combinations should always be evaluated in the context in which they will be used before large-scale implementation. An HIV test kit bulk-purchase programme has been established by WHO in collaboration with UNAIDS in order to provide national AIDS control programmes with tests giving the most accurate results at the lowest possible cost. This list of HIV test kits is updated annually. Tests other than those bulk-purchased through WHO, but meeting the minimum standards in terms of sensitivity and specificity, are also suitable for use with the testing strategies.

3.4.7 Analysis of the results of the assays under evaluation

Table 3.3: Sensitivity, specificity and predictive value of HIV serological tests

True HIV status				
Result of tests under evaluation		Positive	Negative	
	Positive	True positives (a)	False positives (b)	a + b
	Negative	False negatives (c)	True negatives (d)	c + d
		a + c	b + d	

Sensitivity = a/(a+c) Positive predictive value = a/(a+b)

Specificity = d/(b+d) Negative predictive value = d/(c+d)

(UNAIDS/WHO, 1999)

3.4.7.1 Sensitivity: Is the ability of the assay under evaluation to detect correctly sera that contain antibody to HIV (reference assays positive). Thus sensitivity is the number of true positive sera identified by the assay under evaluation as positive (a), divided by the number of sera identified by the reference assays as positive (a+c), expressed as a percentage.

3.47.2 Specificity: Is the ability of the assay under evaluation to detect correctly sera that do not contain antibody to HIV (reference assays negative). Thus specificity is the number of true negative sera identified by the assay under evaluation as negative (d), divided by the number of sera identified by the reference assays as negative (b+d), expressed as a percentage.

Indeterminate results, obtained with the assays under evaluation, were not included in the calculation of sensitivities and specificities.

3.4.6.3 Positive Predictive Value (PPV): The probability that when the test is reactive, the specimen does contain antibody to HIV. This may be calculated in two ways:

1. Using the simple formula a/(a+b) which will give an approximate value.

2. Using the more precise formula which takes the prevalence of HIV in the population into account:

PPV=

(Prevalence) (Sensitivity)/ {(prevalence)(sensitivity)+(1 - prevalence) (1 - specificity)}

3.4.6.4 Negative Predictive Value (NPV): The probability that when the test is negative, a specimen does not have antibody to HIV. This may be calculated using:

1. The simple formula d/(c+d) which will give an approximate value.

2. The more precise formula which takes the prevalence of HIV in the population into account:

NPV=

{1-prevalence)(Specificity)}/{(1-prevalence) (specificity) + (prevalence)(1-sensitivity)}

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative (UNAIDS/WHO, 1999).

CHAPTER-IV

MATERIALS AND METHODS

4.1 Materials

The materials used during the study period included various equipments, test kits, reagents glasswares and accessories enlisted as below.

4.1.1 Equipments

i. Automated ELISA processor (BEP III ELISA Processor, Germany)

ii. Centrifuge (Rotina 35, Hettich Zentrifugen, Germany)
   Speed range used: 2000 to 3000 rpm

iii. Refrigerator (White-Westinghouse, USA)
   Temperature range used: - 2^0C to 8^0C

iv. Deep Refrigerator (White Westinghouse, USA)
   Temperature range used: -20^0C to 8^0C

v. Incubator (Ambassador, India)
   T emperature maintained: 37^0C

vi. Micropipettes (Human, Germany)
   Range used: 10 µl to 1000 µl

vii. Microplate washer (Platos W96, AMD Diagnostic, Austria)
   Washing times: 4 to 5 times

viii. Microplate reader (Platos R496, AMD Diagnostic, Austria)

   Wavelength used: 450 nm

4.1.2. Test kits and Reagents

1. **HIV –Enzygnost Anti –HIV ½, Dade Behring, Germany**

Manufacturer: Dade Behring, Germany

Presentation: ELISA

Detection: anti HIV 1&2 IgG and IgM against recombinant HIV (env) proteins, gp 41 (HIV 1), gp 41 (HIV 1 Subtype O), gp 36 (HIV 2)

Lot no: 37803

Expiry Date: January 2009

2. **RecombiLISA HIV 1+2 Ab Test**

Manufacturer: CTK, Biotech Inc.6748 Nancy Drige Drive, San Diego, USA

Presentation: ELISA

Detections: anti HIV 1&2 IgG, IgM and IgA against HIV-1 and HIV-2 viral proteins from different parts of the genome, ENV, GAG and POL antigens

LOT No: EO513E2

Expiry Date: May 2010

3. **Eliscan^{TM} HIV^{Advance}**

Manufacturer: RFCL Ltd. SIDCUL, Haridwar

Presentation: ELISA

Detections: anti HIV-1 (group O and M) against HIV-1 gp41 antigen, anti HIV-2 against recombinant HIV-2 gp36 antigen and p24 antigen against monoclonal anti HIV-1 p24 antibody.

LOT No: HHIB 0607

Expiry Date: October 2008

4. **DETECT-HIV^{TM} (v.2)**

Manufacturer: Adaltis Inc.10900 Harmon Street, Montreal, Canada

Presentation: ELISA

Detections: anti HIV 1&2 IgG, IgM and IgA against HIV-1 and HIV-2 peptide antigens

LOT No: 080301

Expiry Date: January 2009

In this study HIV –Enzygnost Anti –HIV ½ test kit has been considered as the baseline kit for the evaluation of the other test kits viz. RecombiLISA HIV 1+2 Ab Test, Eliscan^{TM} HIV^{Advance} and DETECT-HIV^{TM} (v.2). The selection of the standard test kit is based on its credibility, performance and listing in WHO approved list of HIV testing Kits where it's sensitivity of 100% and specificity of 97.4% has been shown (UNAIDS/WHO, 2004).

4.1.3 Glasswares and others

i. Test tubes (Borosil, India)
ii. Disposable examination gloves (Powdered latex examination gloves, Handsafe products, Malaysia)
iii. Micropipette tips (Human, Germany)
iv. Sodium hypochlorite solution (Biolab diagnostics, India)
v. Distilled water (prepared in the laboratory)
vi. Test tube rack, tissue paper, marker, bar codes for microplate, adhesive seals, watch, mixing vessels, bottles (locally produced)

4.2 METHODOLOGY

4.2.1 Study design

This study has been designed to evaluate the commercially available test kits used in NRCS for testing of anti HIV antibodies in donated Blood. The study was carried out during a period of 6 months (March-September, 2008). Blood samples asiquoted from each donated blood bags has been used for testing.

The flow chart of the methodology is shown given in fig. 4.1.

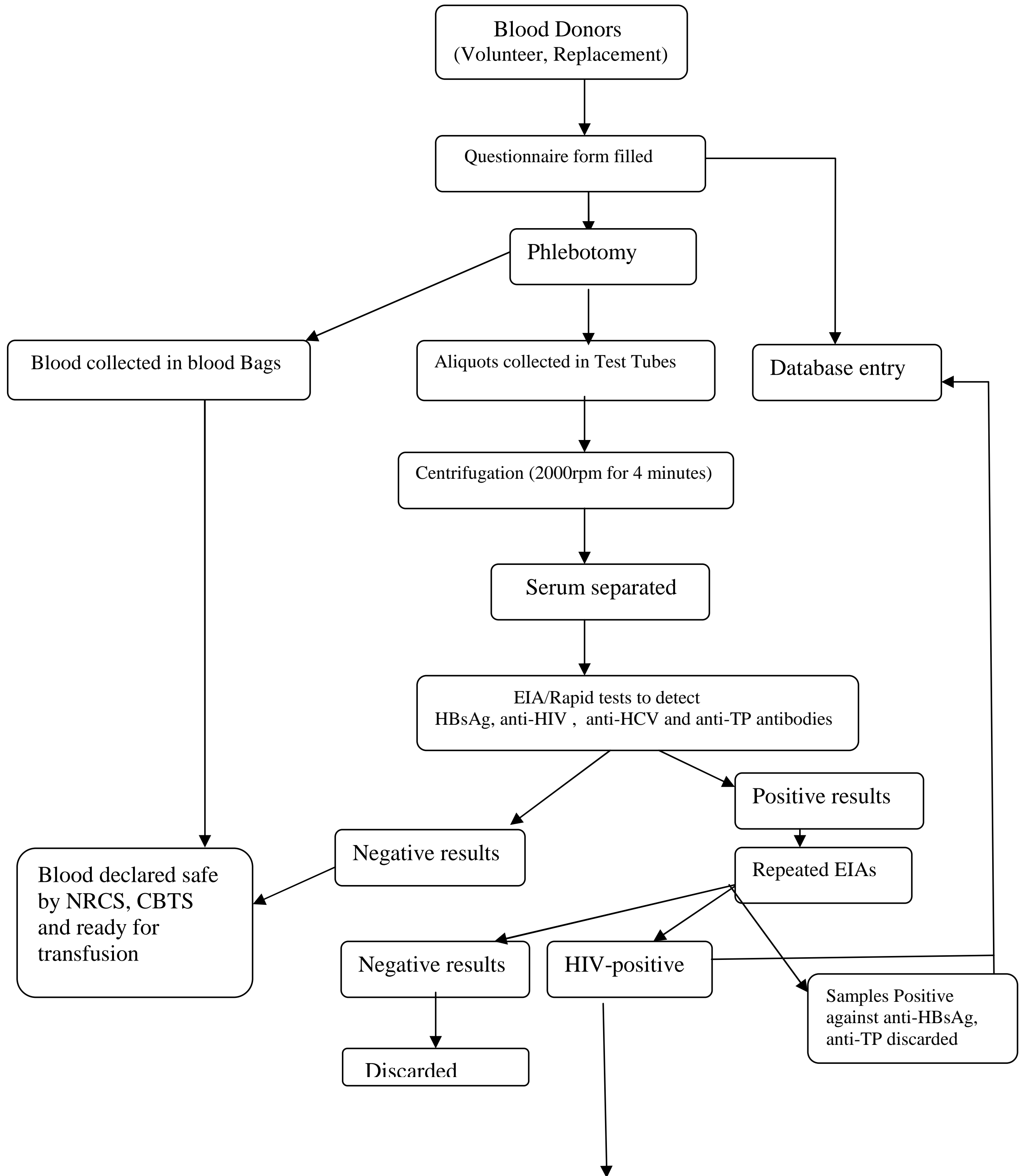


Figure 4.1: Flowchart showing blood screening procedure followed by the evaluation.

4.2.2 Study population

The study population was blood donors. Donors included in this study were donating blood in Blood Transfusion Center, Exhibition Road or in Mobile camps organized in Kathmandu Valley. The categories of donors that were present in this study were volunteers and replacement who may be first time or repeat donor. All the individuals selected by donor screening criteria as of NRCS, CBTS (Standard Operating Procedure, 2006) were requested to fill a donor questionnaire, and each donor was given a donor number and for all later investigations the donor number was used as identification.

Volunteer and Replacement Blood Donors: A volunteer (non-remunerative) donor is one who is not paid for the donated blood. A replacement donor is again a non remunerative donor who donates blood for a particular patient admitted in hospital in replacement.

First time and Repeat Donors: Donors who have made a recorded donation (by blood donor card/Register) or who described themselves as repeat donors were defined as repeat donors. Donors who described themselves as first time donors were defined as first time donors.

4.2.3 Sample size

A total of 21,716 blood samples were screened to detect anti-HIV antibodies of which 27 were found to be HIV positive. These 27 seropositive HIV samples and 3 additional HIV seropositive samples from previous lot of confirmed HIV positive samples provided by NRCS, CBTS were used later for the evaluation of the test kits. Along with these 30 HIV positive samples 30 other negative samples were also taken in order to calculate the specificity values as well as to find out if any false positive results are shown by the test kits under evaluation.

4.2.4 Collection of Blood sample

Blood samples were collected by medical professionals (Laboratory Technicians and Nurses) using aseptic technique. Before collection of sample each donor was requested to fill the donor's Record form/Questionnaire (Appendix I). With the help of a sterile syringe, 350 ml blood was drawn in blood bag labeled with sample number. At the same time, about 5 ml of blood was drawn and dispensed in a test tube having labeled with corresponding sample number.

4.2.5 Sample processing

All the collected blood samples were first incubated at 37^0C for 15-30 minutes and then centrifuged at 2000 rpm for 4 minutes. Serum thus separated from the blood samples were tested for the presence of Anti HIV-1 and 2 antibodies using the Enzygnost Anti HIV ½ Test kits following all the standard operating procedures (NRCS,CBTS 2006) developed by NRCS, CBTS Laboratory.

The Serum samples and Reagents of all the Elisa test kits were brought to room temperature before testing. Testing for anti HIV 1 and 2 using HIV –Enzygnost Anti –HIV ½ was done. The Test serum sample with positive result was further tested for confirmation using SD Bioline HIV-½ 3.0 rapid test kit and another repeated test by Enzygnost Anti –HIV ½. The positive samples in all these three assays are to be used later in the evaluation process, therefore, were preserved in the deep refrigeratior at -20^0C.

All the collected positive samples were evaluated by RecombiLISA HIV 1+2 Ab, Eliscan^{TM} HIV^{Advance} and DETECT-HIV^{TM} (v.2) ELISA Test Kits following standard manufacturer's protocol. (Appendix II)

Test samples were considered reactive or non-reactive based on the Optical Densities (ODs) in comparison with the Positive Control (PC)/ Negative Control (NC), cut-off ODs were noted for record (Photograph 1 HIV –Enzygnost Anti –HIV ½), (Photograph

2: RecombiLISA HIV 1+2 Ab) (Photograph 3: Eliscan^{TM} HIV^{Advance}), (Photograph 4: DETECT-HIV^{TM} (v.2)).

4.2.6. Recording of Test Results

All the test results were recorded in Microsoft Access 2007 based spread sheet for the further analysis using available statistical tools.

4.2.7. Statistical Analysis

SPSS 11.5 was used to determine the seroprevalance of HIV among the blood donors of Kathmandu Valley and Chi Square Test was used in order to find out whether there was a significant difference between the sensitivity, specificity and the predictive values of the kits under evaluation with respect to the standard test kit or not (Mahajan, 1997).

CHAPTER-V

RESULTS

5.1 Background information obtained prior to evaluation

During the study period, blood samples from 21,716 blood donors were screened for the presence of anti-HIV antibodies. Among the blood samples that were screened for anti-HIV antibodies, 18,434 samples were from male donors and 3,282 samples were from female donors. Seroprevalance of HIV in males was found to be 0.13% (25/21,716), while that in females was found to be 0.06% (2/21,716). The overall seroprevalance was found to be 0.12%.

Table 5.1: Seroprevalance of HIV

SN	Anti-HIV test	Male		Female		Total		P-Value
		No.	%	No.	%	No.	%	
1	Positive	25	0.13	2	0.06%	27	0.12%	P>0.05
2	Negative	18,409		3,280		21,689		
		18,434		3,282		21,716		

Out of 21,716 blood samples screened for Anti-HIV antibody 27 were found to be seropositive against antibodies to HIV by HIV –Enzygnost Anti –HIV ½, test kit. For the evaluation 30 blood samples seropositive against HIV antibodies are taken. 27 of which are obtained from the screening procedure. The three additional samples were provided by NRCS, CBTS. For evaluation along with 30 HIV seropositive samples 30 HIV seronegative samples were subjected. Followings ELISA test kits were used for the evaluation:

1. **RecombiLISA HIV 1+2 Ab Test**
2. **Eliscan^{TM} HIV^{Advanc}**
3. **DETECT-HIV^{TM} (v.2) Test kit**

5.2 Evaluation of RecombiLISA HIV 1+2 Ab Test:

The 30 seropositive samples detected by the standard kit and 30 other seronegative samples were subjected to evaluation by RecombiLISA HIV 1+2 Ab Test Kit. Out of 30 seropositive samples 28 of them were found to be positive to anti HIV antibody by RecombiLISA HIV 1+2 Ab Test kit. Among the 30 seronegative samples used for evaluation none of them were found to be false positives.

Table 5.2: Results from Enzygnost Anti –HIV ½ and RecombiLISA HIV 1+2 Ab Test

Test Results	Enzygnost Anti –HIV ½	RecombiLISA HIV 1+2
HIV seropositives	30	28
HIV seronegatives	30	32

5.3 Evaluation of Eliscan^{TM} HIV^{Advance} Test Kit

Under evaluation by Eliscan^{TM} HIV^{Advance} Test Kit all the 30 seropositive samples were found to be seropositive and there were no false negative or false positive results

Table 5.3: Results from Enzygnost Anti –HIV ½ and Eliscan^{TM}HIV^{Advance} Test

Test Results	Enzygnost Anti-HIV ½	Eliscan^{TM}HIV^{Advance}
HIV seropositives	30	30
HIV seronegatives	30	30

5.4 Evaluation of DETECT-HIV^{TM} (v.2) Test Kit

When subjected to evaluation under DETECT-HIV^{TM} (v.2) Test Kit out of 30 seropositive samples used for evaluation 29 were found to be positive with 1 false negative result. All the 30 seronegative samples were found to be negative therefore no false positive results were obtained.

Table 5.4: Results from Enzygnost Anti –HIV ½ and DETECT-HIV^{TM} (v.2) Test

Test Results	Enzygnost Anti –HIV ½	DETECT-HIV^{TM} (v.2)
HIV seropositives	30	29
HIV seronegatives	30	31

On the basis of the results obtained by the kits under evaluation the Sensitivity Specificity and the Predictive values were calculated (Appendix III). In case of RecombiLISA Sensitivity, specificity, PPV and NPV were determined to be 93.33%, 100%, 100% and 93.75% respectively. Sensitivity, specificity, PPV and NPV of Eliscan^{TM} HIV^{Advance} Test Kit were determined to be 100% each. DETECT-HIV^{TM} (v.2) showed the sensitivity of 96.7%, specificity of 100% PPV of 100% and NPV of 96.8%.

Table 5.5: Sensitivity, Specificity and the Predictive values of the kits under evaluation

NAME	**Eliscan^{TM} HIV^{Advance}**	**RecombiLISA HIV 1+2**	**DETECT-HIV^{TM}**
Sensitivity	100%	93.33%	96.7%
Specificity	100%	100%	100%
PPV	100%	100%	100%
NPV	100%	93.75%	96.8%

CHAPTER-VI

DISCUSSIONS AND CONCLUSIONS

6.1 DISCUSSIONS

During the study period, a total number of 21,716 blood samples were screened for the presence of anti HIV antibodies. Out of 21,716 blood samples, 27 blood samples were found to be HIV seropositive with the overall seroprevalance of 0.12%. Seroprevalence was found to be higher in males (0.13%) than that in females (0.06%). The test kit used in the NRCS, for the purpose was HIV-Enzygnost Anti-HIV ½ test kit. HIV–Enzygnost Anti-HIV ½ test kit was found to be in accordance with the bulk procurement programme as well as meeting the minimum standards in terms of sensitivity and specificity as mentioned in UNAIDS, WHO bulk procurement scheme (UNAIDS/WHO, 2004).

Considering HIV-Enzygnost Anti-HIV ½ test kit as the standard test kit, three commercial test kits namely: RecombiLISA HIV 1+2 Ab, Eliscan^{TM} HIVAdvance and DETECT-HIV^{TM} (v.2) were subjected for evaluation. For this purpose, along with 30 true seropositive samples (27 from the study population and 3 provided by NRCS, CBTS) 30 seronegative (true negative) samples were also taken for the investigation so as to determine the specificity values of the kits under evaluation.

In the standard working manual provided along with the kit, the sensitivity and specificity of RecombiLISA HIV 1+2 Ab test kit is mentioned to be 100% but in this study the sensitivity was found to be 93.33% with the specificity of 100%. The sensitivity of DETECT-HIV^{TM} (v.2) was determined to be 96.7% with the specificity of 100% while that mentioned in the standard working manual provided along with the kit showed the sensitivity to be 100% and specificity 99.7%. The specificity in present study was in concurrent with those in the standard manual but the sensitivity was found to be much lower.

The statistical analysis showed no significant difference between the sensitivity, specificity and the predictive values of the test kits used for evaluation.

The sensitivity and specificity values of the kits under evaluation can be affected by the sensitivity and specificity values of the standard test kit. For example the specificity of the standard test kit is 97.4% (UNAIDS/WHO, 1997). This indicates that there is the probability that not all the samples that are detected to be seropositive by HIV–Enzygnost Anti –HIV ½ test kit (standard kit) are actually seropositive. Therefore, sometimes false positive results can be obtained by the standard kit. If such false positive samples are taken for evaluation in the other kits there is a very high possibility that the kits under evaluation will give otherwise result thus lowering the sensitivity of the kits under evaluation. This might be one reason for the lower sensitivity values of DETECT-HIV^{TM} (v.2) and RecombiLISA test kits.

The probability that when the test is reactive, the specimen does contain antibody to HIV is PPV. The PPV of all the test kits used for evaluation was found to be 100%. This clearly indicates that all the seropositive samples did contain the antibody against HIV; this is because all the samples were positive in one or the other kit.

Negative Predictive Value (NPV) is the probability that when the test is negative, a specimen does not have antibody to HIV. In the current study the NPV of RecombiLISA HIV 1+2 Ab test kit was found to be 93.75% that of Eliscan^{TM} HIV^{ADVANCE} test kit was 100% and that of DETECT-HIV^{TM} (v.2) was 96.8%. The lower NPV of the two kits are attributed to the low risk population that was used for study as well as the small sample size.

Fresh sera are the preferred specimens for evaluation of serum-based tests and preliminary evaluation of whole blood tests when whole blood is not immediately available. Specimens should be stored uniformly aliquoted and stored in a polypropylene tube. Specimen identifiers should be labeled directly on the tube, and not on the screw-cap top. Specimen inventories should be maintained for storage freezers that are specifically reserved for reposited specimens. Every effort should be made to

limit the number of freeze-thaw cycles, since repetitive thaws may result in loss of antibody titer and formation of serum flocculates. For long-term storage, specimens should be frozen –70ºC. If sera are frozen before the evaluation, there should be some standards and practices to ensure that the quality of the thawed serum has not been impaired by freeze/thawing, contamination, excess particulate matter, etc. (UNAIDS/WHO/CDC, 2001).

In this study, since the positive samples were collected over a period of 6 months the samples had to be stored, therefore sera were aliquoted in separate vials to avoid multiple freezing/thawing. For monitoring the quality of frozen storage, a percentage of specimens were retested with standard test kit prior to performing the evaluation to ensure that test results do not change.

The revised recommendations of UNAIDS and WHO state that HIV antibody tests used for surveillance need to have a specificity of at least 95% (UNAIDS/WHO, 1998). Currently, most tests have a specificity of 98% or higher, but the actual sensitivity and specificity of the tests when conducted in the field may vary by the geographic origin of specimens (UNAIDS, 1997).

The reason for the lower sensitivity might be the small sample size. A test evaluation should include a minimum of approximately 200 HIV-positive and 200 HIV-negative specimens to provide 95% confidence intervals of less than ± 2% for both the estimated sensitivity and specificity. Lower numbers of HIV positive and HIV negatives specimens when used increases the confidence interval for sensitivity and specificity (UNAIDS/WHO/CDC, 2001)**.** In this study only 30 positive and 30 negative samples were used, so the above explanation might be a vital reason for lower sensitivity.

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected

(i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative.

Infection with human immunodeficiency virus, which causes AIDS, has become a worldwide epidemic since its first documentation in 1981, and it is a major public health concern for all countries. Diagnosis of HIV infection is important for prevention and patient management. Laboratory diagnosis is the mainstay in identifying HIV infection in an individual. The wide range of diagnostic kits available from both the international and local diagnostic manufacturers, for the detection of anti HIV antibodies, makes it mandatory that the kits are evaluated before they are made available in the market and put to routine use by the laboratories.

6.2 CONCLUSIONS

In this study three commercially available test kits RecombiLISA HIV 1+2 Ab Test, Eliscan^{TM} HIV Advance and DETECT-HIV^{TM} (v.2) were evaluated in comparison to Enzygnost Anti –HIV ½ test kits. Eliscan^{TM} HIV Advance was found to be equally sensitive and specific as Enzygnost while two other assays were less sensitive.

The evaluation of the commercial kits is mandatory and must be carried out at regular intervals before procurement and use in order to improve the test quality, ultimately benefiting the transfusion system.

CHAPTER-VII

SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

This study was carried out at the NRCS, BTC and the primary goal of the study was to evaluate the commercially available test kits that are commonly used in the detection of antibodies against HIV. The aim was also to study the effectiveness of various test kits in the detection of anti-HIV antibodies.

1. Among the 21,716 blood samples screened against anti-HIV antibodies 27 of them were found to be seropositive to anti-HIV antibody. The test kit that was used for the screening purpose was Enzygnost Anti –HIV ½, test kit.
2. Enzygnost Anti –HIV ½, test kit was considered as the standard test kit. Considering this kit as the standard test kit other test kits viz: RecombiLISA HIV 1+2 Ab Test Kit, Eliscan^{TM} HIV^{Advance} Test Kit, DETECT-HIV^{TM} (v.2) Test Kits were evaluated.
3. Along with 30 seropositive samples (27 from the screening procedure and additional seropositive samples provided by NRCS, CBTS) 30 other seronegative samples were also taken under study so as to find out the false positive results if any.
4. The sensitivity of RecombiLISA was determined to be 93.33% with the specificity of 100%. Similarly, the PPV was found to be 100% with NPV being 93.75%.
5. The sensitivity and specificity of Eliscan^{TM} HIV^{Advance} Test Kit were determined to be 100% wiht the PPV and NPV being 100%.
6. The sensitivity of DETECT-HIV^{TM} (v.2) was determined to be 96.7% with the specificity of 100%. The PPV was found to be 100% with NPV being 96.8%.

7. When the performance of these test kit was evaluated the Eliscan^{TM} HIV^{Advance} Test Kit was found to be the most efficient test kit in the detection of anti-HIV antibodies.
8. Among the test kits used for evaluation the RecombiLISA HIV 1+2 Abs Test Kit was least effective in the detection of antibodies against HIV.

7.2 RECOMMENDATIONS

1. In a region where Enzygnost Anti –HIV ½, test kit is deserved to be used for screening procedure, but unavailable, equally sensitive and specific test kit such as Eliscan^{TM} HIV^{Advance} can be used as substitute.

2. Evaluation of the commercially available test kits should be carried out at regular intervals to assure that the test kits used are the most sensitive and specific one.

3. Implementation of a full quality assurance programs is strongly recommended to eliminate the potential harms to individuals of a false HIV diagnosis and unsafe blood banking practices.

CHAPTER-VIII

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