Addis Ababa University
School of Graduate Studies
Department of Biology

ASSESSMENT OF HIV-1 TRANSMISSION RISK FROM BLOOD DONORS SCREENED NEGATIVE FOR HIV-1 ANTIBODY/ ANTIGEN BY USE OF HIV-1 DNA PCR

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BY

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A Thesis Submitted to The School of Graduate Studies
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LIST OF ABBREVATIONS

AIDS	Acquired Immunodeficiency Syndrome
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EHNRI	Ethiopian Health and Nutrition Research Institute
EIA	Enzyme immunoassay
ELISA	Enzyme linked immuno sorbent assay
ENARP	Ethio-Netherlands AIDS Research Project
env	envelope
ERCS-NBTS	Ethiopian Red Cross Society-National Blood Transfusion Service
gag	group specific core antigen
gp	glycoprotein
HIV	Human Immunodeficiency Virus
ml	millilitre
MOH	Ministry of Health
NASBA	Nucleic Acid Sequence Based Amplification
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
UNAIDS	United Nations Joint Program for HIV/AIDS
WHO	World Health Organization
µl	microlitre

ABSTRACT

The aim of this study was to assess the potential risk of transmission of Human Immunodeficiency Virus type-1 (HIV-1) by transfusion of blood screened as negative for HIV antigen (Ag)/antibody (Ab) in the Ethiopian Red Cross Society-National Blood Transfusion Service (ERCS-NBTS) in Addis Ababa, Ethiopia. Assessment of risk factors, test performance and efficiency of the HIV screening methods of the ERCS-NBTS laboratory (Ag/Ab EIA, Vironostika HIV Uni-Form II Ag/Ab) was made. A total of 408 blood samples from unlinked one-time donations were collected and screened for HIV-1 infection by using Roche Amplicor V 1.5 DNA PCR and discrepant results were checked by Western blot and HIV RNA assays. Data analysis was done by using STATA software. The crude prevalence of HIV-1 infection in blood donors as screened by the ERSC-NBTS was 4.2%, while it was 3.4% as screened by nucleic acid testing. Thus, nucleic acid testing methods detected 3 false positive samples. All HIV-infected donors were replacement donors. The fourth-generation assay used at ERCS-NBTS had an excellent test agreement with HIV-1 DNA PCR assay. The validity of the Ag/Ab EIA was very high as depicted by its: sensitivity (100%), specificity (99.2%), test efficiency (99.3%), positive predictive value (82.4%) and negative predictive value (100%). Although no false negative blood unit was detected, it was shown that around 0.7% of the donated blood would be wrongly discarded as false positives. This study has also provided good evidence for the need to introduce strong and improved donor selection criteria. This would include, minimizing or exclusion of replacement donors, with an emphasis on recruitment of regular voluntary donors, and education of both donors and the general public on the need for safe blood. Similar studies should be done in other parts of the country before drawing a general conclusion about the quality/safety of blood donations in Ethiopia. Further studies on HIV incidence among blood donors, proper estimation of risk of HIV transmission through transfusion, and prevalence of HIV infection in transfusion recipients are essential for monitoring the safety of the blood supply. Studies on other transfusion transmittable infectious agents should also be undertaken.

1. INTRODUCTION

1.1. GENERAL FEATURES OF THE AETIOLOGIC AGENT OF AIDS

Acquired Immunodeficiency Syndrome (AIDS) represents only the end stage of a continuous, progressive pathogenic process, beginning with primary infection with Human Immunodeficiency Virus (HIV), continuing with asymptomatic phase, leading to progressively severe symptoms and ultimately profound immunodeficiency and opportunistic infections. AIDS was first recognized in the United States of America in 1981 among homosexual men (Gallo *et al.,* 1984), and since then has become a major worldwide pandemic. HIV is the sole causative agent of AIDS. By leading to the destruction and/or functional impairment of cells of the immune system, notably CD4 positive T-cells, HIV progressively destroys the body's ability to fight infections and predisposes to opportunistic infections and malignancies (Fauci, 1988).

The HIV-1 virus is an icosahedral, enveloped, and an RNA virus. Structurally, HIV is composed of two major parts: an outer envelope and an inner core (Figure 1). The external envelope components are embedded in the lipid matrix of the membrane and are involved in the binding of the virus to the host cells during infection (Moore, 1997). The outermost viral components are arranged as spikes or knobs of glycoproteins that extend from the lipid membrane.

The core components of the virus are located internal to the outer membrane and are bound by a protein coat encompassing two identical copies of the RNA genome. Also, within the core are three viral enzymes: reverse transcriptase (RT), integrase, and protease. These enzymes are responsible for transcription, integration of the virus into the host genome, and cleavage of other proteins, respectively.

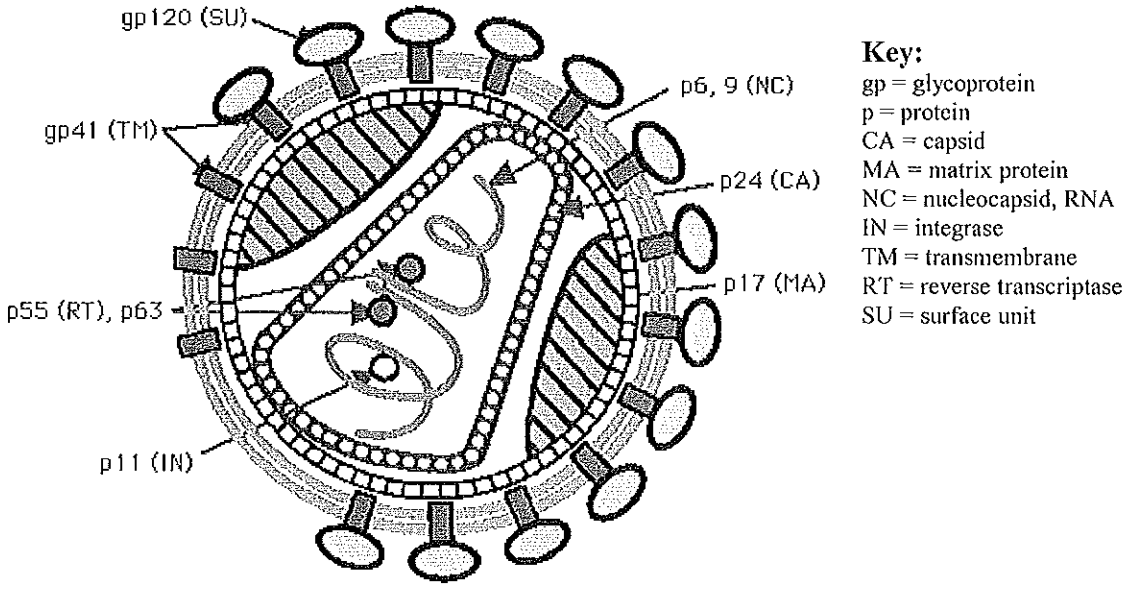


Figure 1. Schematic representation of the structure of HIV (After Heaphy, 1999)

1.2. CHARACTERIZATION OF HIV

HIV belongs to the group of Lentiviruses in the family Retroviridae (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984). Lentiviruses are viruses that remain latent for a certain period of time before they cause disease, and infected individuals remain asymptomatic. Two distinct types of Lentiviruses with 40-50 percent homology between their genomes have been identified in man: HIV-1 and HIV-2 (Popovic *et al.*, 1984). HIV-1 is spread worldwide, while HIV-2 is found primarily in West Africa (Quinn *et al.*, 1986). As compared with HIV-1, HIV-2 is less virulent and less transmittable (Marlink *et al.*, 1994).

HIV-1 is a highly variable virus that mutates very readily resulting in many different strains. These strains are classified to groups and subtypes. Based on phylogenetic analysis of sequences of part/whole of a genome, the HIV-1 genetic variants are divided into three

groups: group M (Major), group O (Outlier) and group N (Non-M or O) (Robertson *et al.*, 2000).

Group M viruses are the most widely distributed all over the world and contribute to most infections. Group M viruses were further divided into eleven subtypes or clades (Ward, 1999). These subtypes are subtype A, B, C, D, E, F, G, H, I, J, and K. However, according to Robertson *et al* (2000), the previously subtypes E and I are found to be problematic and subtype E virus is designated as CRF01-AE and subtype I as CRF04-cpx.

In addition to the subtypes, more than 10 percent of the viruses circulating in the population are inter-subtype recombinant (Jetzt *et al.*, 2000). So far, four defined circulating recombinant forms (CRFs): CRF01-AE (CM240) in Southeast Asia (Carr *et al.*, 1996), CRF02-AG (IbNg) of West central Africa (Carr *et al.*, 1998), CRF03-AB (KAL153) in Russia (Bobkove *et al.*, 1998) and CRF04-cpx in Cyprus (Gao *et al.*, 1998) have been identified. However, recombinants between HIV-1 and HIV-2 have not been reported although dual infections occur.

Several phylogenetic analyses made on the Ethiopian HIV-1 isolate indicate that subtype C viruses dominated the epidemic (Abebe *et al.*, 1997; Ayehunie *et al.*, 1991; Hussien *et al.*, 2000). However, Abebe *et al.* (1997 and 2000) reported that the Ethiopian subtype C has a genetic sub-cluster designated as C'.

1.3. EPIDEMIOLOGY OF HIV/AIDS

Although sporadic cases in the United States and Europe had occurred since at least the mid-1970s (Huminer *et al.*, 1987), the epidemic of AIDS was first recognized among homosexual men in the United States in 1981 (Gallo *et al.*, 1984; Heyward and Curran, 1988). However,

the blood obtained in 1959 from an adult Bantu man in the Democratic Republic of Congo represents the oldest known case of HIV-1 infection in the world (Zhu *et al.*, 1998).

While initially limited, infection with HIV has literally exploded over the past two decades to become the worst epidemic of the twentieth century. The impact of AIDS on human suffering, cultures, demography, economics, and even politics has been felt in nearly every society across the globe. In some countries in sub-Saharan Africa, the AIDS epidemic is already having a dramatic effect on depopulation.

More than 60 million people have been infected with HIV since the start of the epidemic, of whom about one-third have died, and as of December 2002 an estimated 42 million people are living with HIV/AIDS worldwide (UNAIDS/WHO, 2002). Recent estimates show that 5 million people became newly infected with HIV in the year 2002; out of which 800,000 were children, and those who were hardest hit by the epidemic (4.2 million) were between the ages of 15 and 49, a period when people are in their most productive and reproductive phases of life. Of the 42 million people living with HIV/AIDS, around 90 percent live in developing countries, out of which 29.4 million living in sub-Saharan Africa alone, where the adult prevalence rate for the region is 8.8 percent (UNAIDS/WHO, 2002).

The HIV epidemic in Ethiopia started relatively late as compared to the neighbouring countries in East Africa. The HIV epidemic started in the mid-80s where the first sera with HIV antibodies were detected in 1984 (Tsega *et al.*, 1988) and the first Ethiopian AIDS cases were diagnosed from hospitalised patient in Addis Ababa in 1986 (Lester *et al.*, 1988). In 1988, high rates of HIV prevalence (13%) were detected among long distance truck drivers (Mehret *et al.*, 1990a) and commercial sex workers (17%) residing along the main trade routes of the country (Mehret *et al.*, 1990b). Since then the epidemic has spread at a fast rate throughout the country.

However, the epidemic has spread rather rapidly reaching prevalence as high as 7 percent among blood donors in 1994 (Fontanet *et al.*, 1998), 14 to 20 percent in 1999 among urban pregnant women (ANC attendees) (MOH, 2000), and 73.7 percent among commercial sex workers (Aklilu *et al.*, 2001). At the end of 2001, an estimated 2.2 million Ethiopians were living with HIV/AIDS, and the overall prevalence was 6.6 percent (MOH, 2002). According to UNAIDS (2002), Ethiopia is the country with the fifth largest estimated population of HIV-infected people in the world.

1.4. NATURAL COURSE OF HIV INFECTION.

The steps occurring in infection involve an interaction of HIV not only with the CD4 surface molecule on the cells but also with other cellular receptor (Weiss, 1996). Two co-receptors have been identified – CCR5 for macrophage-tropic virus or CXCR4 in the case of T-cell tropic virus (Moore, 1997). The usual ligands for CCR5 include macrophage inflammatory protein (MIP)-1 alpha and 1 beta and RANTES (regulated on activation, normal T cell expressed and secreted), while stromal cell-derived factor type 1 (SDF-1) is the usual ligand for CXCR4 (Cohen *et al.*, 2000).

Entry of the virus occurs via fusion of the viral and cellular membranes. Subsequently, internalisation and uncoating processes took place in the cytoplasm of infected cell (Grewe *et al.*, 1990). Following virus infections, a viral of intracellular mechanism determine the relative expression of viral regulatory and accessory genes leading to productive or latent infection (Figure 2).

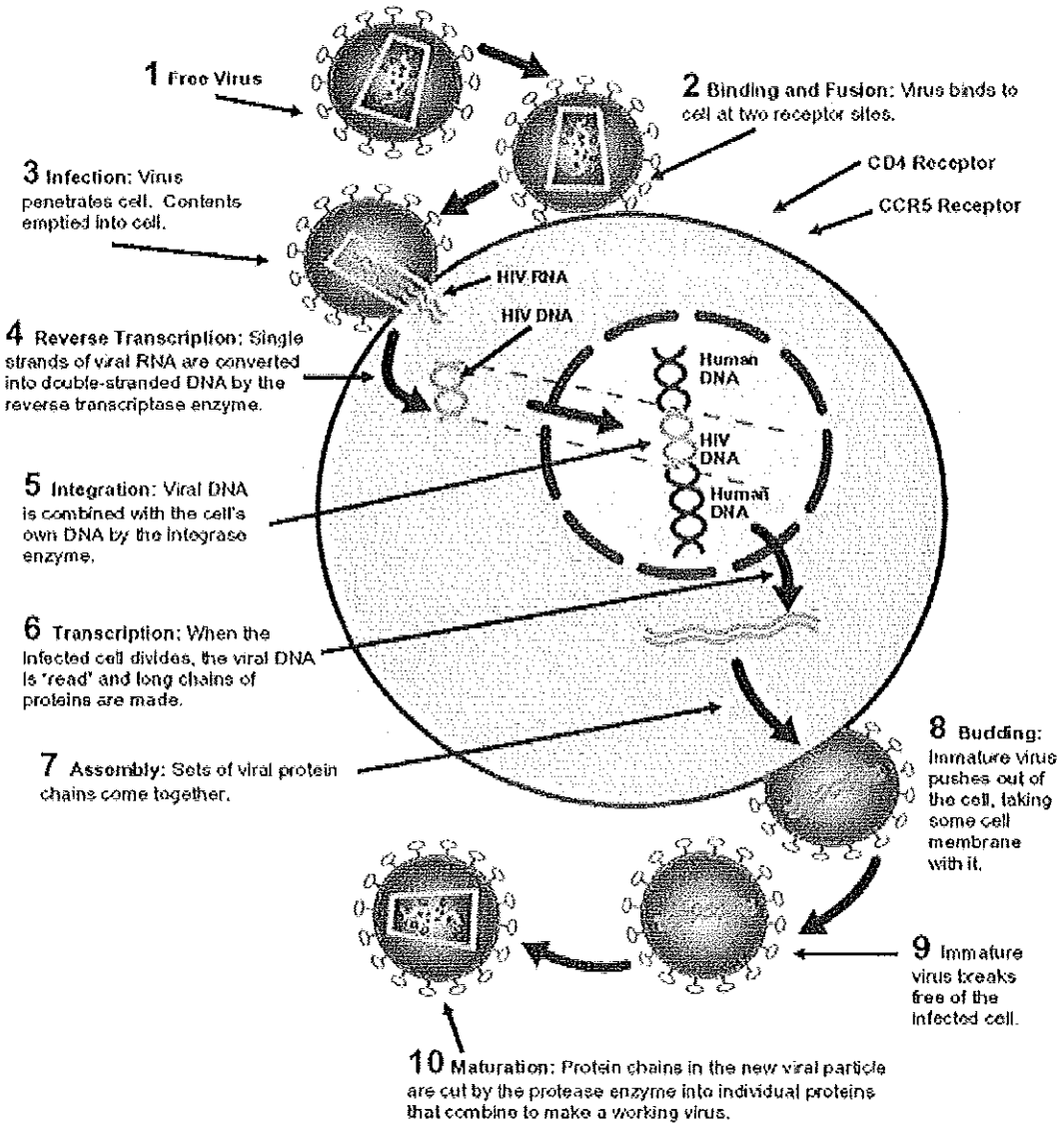


Figure 2. Life cycle of HIV (After New Mexico AIDS InfoNet, 2003)

Once virus enters the blood, wide spread dissemination to organs such as brain, spleen, and lymph nodes occurs. At the time of initial infection with HIV, infected individuals have a large number of susceptible CD4 positive cells and no HIV-specific immune response (Quinn, 1997; Kahn and Walker, 1998). Therefore, viral replication is rapid and p24 antigen levels also rise (Figure 3).

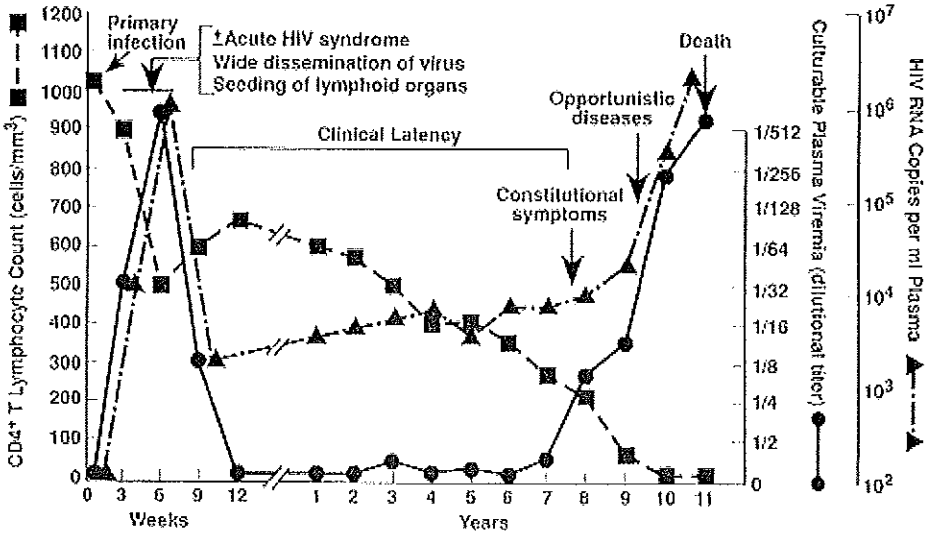


Figure 3. Schematic representation of the course of HIV infection (After Pantaleo *et al.*, 1993).

The natural course of HIV infection is outlined from primary HIV infection, the acute HIV syndrome, and the HIV-specific immune response through a period of clinical latency to clinically apparent disease or AIDS-defining illness, and finally death from AIDS (Mellors, 1999). Of course, the duration of clinical latency following primary HIV infection varies widely among individual persons depending upon mode of transmission, portal of entry, viral pathogenicity and mutation, host resistance and intrinsic and specific immune responsiveness, and new treatment strategies (Jordan *et al.*, 2003).

1.5. MODE OF TRANSMISSION OF HIV

Although HIV has been isolated from a variety of body fluids (Vernezza *et al.*, 1994; Levy and Greenspan, 1988), only contact with blood, semen, other genital secretions and breast milk has been implicated as sources of infection. Laboratory and epidemiological studies indicate that the infectivity of saliva from HIV-infected persons is extremely low. The low

risk of saliva-mediated HIV transmission is probably attributable to the very low concentrations of HIV in the saliva of infected persons (Levy and Greenspan, 1988), as well as the presence of HIV inhibitory activity in saliva (Yeh *et al.*, 1992).

More than a decade after the initial studies were conducted to determine the ways in which HIV is transmitted, surveillance and epidemiological data throughout the world continue to strongly support three primary modes of transmission: sexual contact; perinatal transmission from infected mothers to their infants; and exposure to contaminated blood, largely through transfusion and injecting drug use.

1.5.1. SEXUAL TRANSMISSION

Unprotected sexual contact is the predominant mode of HIV transmission throughout the world. However, the geographic distribution of cases attributable to homosexual and heterosexual transmission varies markedly (Padian, 1987). Heterosexual transmission is the major mode of spread of HIV infection in developing countries, and albeit growing proportion of AIDS cases in North America and Europe, where male-to-male sexual transmission continues to account for a major proportion of reported cases of AIDS (Coates *et al.*, 1996). In the Ethiopian situation, most HIV-infection is acquired through heterosexual transmission (MOH, 2002).

The likelihood of acquiring or transmitting HIV infection through a single sexual contact is directly related to certain correlates of exposure, such as the number of partners and the prevalence of HIV infection in the population. Sexual transmission of HIV is relatively inefficient, but behavioural and biologic factors influence the likelihood of HIV transmission in a given sexual encounter (Beck *et al.*, 1996). Anal sex has been consistently found to be more risky than vaginal sex, which in turn is higher risk than oral sex (Samuel *et al.*, 1993), and the coexistence of a sexually transmitted infection greatly increase the infectiousness as

well as the susceptibility of the individual (Cohen, 1998). Vaginal and anal intercourse is the predominant way in which persons are exposed to HIV-infected semen and cervicovaginal fluids.

1.5.2. PERINATAL TRANSMISSION

Perinatal transmission of HIV can occur during pregnancy, labour and delivery, and through breastfeeding after birth. Increasing evidence indicate that most perinatal transmissions to live-born infants probably occur late in gestation or during delivery. A study conducted in Zaire indicated that 23 percent of transmissions occur in uterus, 65 percent intrapartum, and 12 percent via breastfeeding (Bertolli *et al.,* 1996). A meta-analysis of studies comparing transmission rates in breastfeeding contributed an additional 14 percent risk (Dunn *et al.,* 1992). Prior to the use of antiretroviral medications, estimates of the frequency of perinatal transmission ranged from a low of 13 percent in Europe to a high of more than 60 percent in Africa, with frequencies of 14 to 33 percent reported in the United States (European Collaborative Study, 1992).

1.5.3. BLOOD-BORNE HIV TRANSMISSION

Molecular techniques have allowed quantitation of virus in the blood and have established a correlation between viral burden, disease stage, and CD4+ cell count (Piatak *et al.,* 1993). Viremia is highest during the initial acute infection, then drops dramatically during the asymptomatic stage and rises steadily throughout progression to disease. During symptomatic stage, levels of more than 2 million virions/ml can be detected in the blood of an HIV-infected person (Piatak *et al.,* 1993). Between 1 in 1000 and 1 in 10,000 PBMCs are believed to be infected in asymptomatic HIV-seropositive individuals (Ho *et al.,* 1989). With the ability to produce thousands of viral particles per day, the virally infected cells constitute the substantial source of infectious virus in the blood (Levy, 1993).

Blood, containing infected cells and particles, transfused from an infected person is an extremely efficient means of HIV transmission. Yet the risk of transmission is a consequence of the interdependent factors of disease stage, viral load, and perhaps the phenotype of the predominant host virus strain (Levy, 1993). Recipients of unscreened blood or blood products from HIV-infected donors are at high risk of HIV infection. The likelihood of a person becoming infected with HIV after receiving a single-donor blood product documented to be HIV positive approaches 100 percent (Donegan *et al.,* 1990). Transmission of HIV-1 can occur following transfusion of a blood product derived from an infected person's blood and processed into a blood component (whole blood, packed red cells, fresh-frozen plasma, platelets and clotting factors) (Donegan *et al.,* 1994).

HIV-1 infection after blood transfusion has been documented repeatedly since the first case was reported in late 1982 (Ammann *et al.,* 1983; Curran *et al.,* 1984). In 1987, an estimated 12,000 living persons were infected with HIV through transfusion of contaminated blood products (Busch *et al.,* 1991). In San Francisco alone, an estimated 2,135 transfusion recipients were infected with HIV-1 during the 7 years preceding HIV-1 testing of blood donations. HIV-1 transmission via transfusion peaked in that city in 1982, when probably 1.1 percent of transfused units were HIV-1 contaminated (Busch *et al.,* 1991).

Among injecting drug users, HIV is transmitted by parenteral exposure to HIV-infected blood through the use of contaminated needles, cutting and piercing equipment. Specific factors that have been associated with HIV infection among injecting drug users include the duration of injecting drug use, frequency of needle sharing, number of needle-sharing partners, number of injections, and prevalence of HIV infection in the area of residence (Des Jarlais *et al.,* 1989; Prevots *et al.,* 1996). Poor socio-economic conditions, homelessness, and minority

race/ethnicity are associated with an increased frequency of risk behaviour and higher rates of HIV infection among injecting drug users (Kral *et al.*, 1998).

1.6. HIV SCREENING OF BLOOD SUPPLY

In most developed countries of the world, extraordinary strides have been made over two decades to reduce the risk of HIV transmission by transfusion of blood and blood products. Prior to the discovery of HIV and the development of effective screening and testing measures to prevent its transmission, thousands of persons in the United States and worldwide became infected after receiving contaminated blood or blood components. In the United States, the first screening of all blood donations for antibody to HIV was implemented in March 1985 (Chamberland *et al.*, 2001).

Although transmission of HIV through blood transfusion and diagnosis of infection in hospitals, blood banks and public health settings continue to be a worldwide concern; considerable progress has been made in serologic detection of HIV infection over the past 18 years. The first-generation of HIV assays relied on the detection of antibody to HIV viral proteins. These assays used a solid phase coated with viral antigens and polyclonal antibodies to human immunoglobulins conjugated to an enzyme for detection of HIV-specific antibodies (Ward *et al.*, 1986). The second-generation assays used HIV recombinant antigens instead of viral lysates as the source of antigen on the solid phase and also incorporated recombinant antigens for HIV-2 (Dawson *et al.*, 1988). These assays had improved specificity although the overall sensitivity remained similar to that of the first generation assays.

Third-generation assays used the solid phase coated with recombinant antigens and/or peptides and similar recombinant antigens and peptides conjugated to a detection enzyme or hapten that could detect HIV-specific antibodies bound to a solid phase (Gallarda *et al.*, 1992;

Zaaijer *et al.,* 1992). These assays could detect immunoglobulin M, the early antibody to HIV, in addition to immunoglobulin G, thus resulting in a reduction of the seroconversion window period (Thorstensson *et al.,* 1998). At the same time, assays were also developed to detect HIV-1 antigen by using anti-p24 antibodies on the solid phase and p24 specific antibodies conjugated to an enzyme (Busch *et al.,* 1990; Courouce *et al.,* 1992).

During the acute period of infection, tests for p24 antigen can detect HIV infection earlier than antibody tests. p24 antigen is detectable 2-3 weeks after HIV infection during the initial burst of virus replication associated with high levels of viremia (Daar *et al.,* 1991; Clark *et al.,* 1991). During this time, the blood of infected persons is highly infectious, and tests for p24 antigen are usually positive. On average, p24 antigen is detected an estimated 6 days before antibody tests become positive (Gallarda *et al.,* 1992; Busch *et al.,* 1995). Two studies have demonstrated that detection of HIV antigen prior to the development of detectable immune response results in a further reduction of the seroconversion window period by approximately 9 days (Busch *et al.,* 1990; Busch and Statten, 1997). When antibodies to HIV become detectable, p24 antigen is often no longer detectable because of antigen-antibody complexing and viral clearance (Gallarda *et al.,* 1992). The use of these two independent assays (Ag and Ab) could reduce the seroconversion window period significantly (Busch *et al.,* 1990).

While some developed countries use HIV antigen assays for blood screening, there is also a demand to reduce the number of tests that have to be preformed in order to identify and eliminate contaminated blood. The development of fourth-generation assays that would detect both antigens and antibodies in a single assay would be preferable over performing two separate assays, one for antigen and the other for antibody. The benefits of testing for both antibody and antigen are justifiable due to the need to identify individuals with both established and early HIV infection (Saville *et al.,* 2001). Several studies have demonstrated

that the Ag/Ab combination assays are more sensitive than antibody assays alone (Ly *et al,* 2001; Weber *et al.,* 1998; Saville *et al.,* 2001; Ly *et al.,* 2000; Weber, 2002; Weber *et al.,* 2002).

Antibody is detected in a majority of individuals within 6 to 12 weeks after infection with the earlier generation of assays, but antibody levels can be detected within 3 to 4 weeks after infection when the newer third-generation antigen sandwich assays are used (Constantine *et al.,* 1994). This window period can be shortened to about 2 weeks using p24 antigen assays or to 1 week with the implementation of nucleic acid detection assays (Feinberg, 1996). Consequently, the window period between infection and detection of infection may be less than 2 weeks if a comprehensive testing (fourth-generation Ag/Ab combination assays) approach is utilized (Constantine, 1999). The performance of this fourth-generation assay, however, has not been evaluated in Ethiopia.

Observations from Ethio-Netherlands AIDS Research Project (ENARP) cohort study demonstrated 3/14 (21.4%) seroconvertors, a detectable viral load by HIV RNA assay at the visit preceding seroconversion (Rinke de wit *et al.*, 2002). Of particular interest was that the window period in these HIV-1 seroconverting patients has been calculated to be relatively longer, when compared to those from the western countries. Thus, the mean duration of the HIV RNA positive window period has been estimated to be 34.4 days (Rinke de wit *et al.*, 2002), which is longer than the 22.5 days of window period reported from western countries (Brookmeyer and Quinn, 1995). However, pre-seroconversion infection with HIV-1 was identified in 4 of 20 (20%) seroincident participants in the ENARP Cohort study (ENARP, unpublished data). These four individuals had a high degree of viremia (ranging from 3,300 to greater that $1X10^7$ HIV-1 RNA copies/ml plasma), but negative for HIV p24 antigen at their last HIV-1 seronegative visits. Another patient was positive for HIV p24 antigen, but with

undetectable viremia at his last HIV-1 seronegative visit. The results indicate that in an area where the prevalence of HIV infection is very high, there could be a substantial residual risk of transfusion-transmitted HIV from blood donors screened negative by conventional HIV ELISA, even those employing HIV p24 antigen detection.

Assuming that blood donors would be infectious only during the PCR-positive period, the rate of potentially HIV positive blood donations screened as negative for HIV antibody could be substantial in areas where HIV-1 prevalence is very high. The immune system of Ethiopians has a reduced ability to build effective immune responses to newly encountered infections and may contribute for the prolonged seroconversion among Ethiopians (Messele *et al.,* 1999). Taken together, the data emphasize that there is a substantial residual risk of transfusion-associated HIV infection as prolonged window period has been associated with increased risk of transmission of HIV (Ward *et al.*, 1988; Julien *et al.*, 1988).

Therefore, this study was initiated to assess the potential risk of transfusion transmission of HIV by blood units screened as negative for HIV antibody/antigen, and to determine the HIV-1 prevalence and risk factors among blood donors screened negative for HIV by the Ag/Ab EIA in the Ethiopian Red Cross Society-National Blood Transfusion Service (ERCS-NBTS) in Addis Ababa.

2. OBJECTIVES

2.1. GENERAL OBJECTIVE

To assess the potential risk of transmission of HIV by blood transfusions screened as negative for HIV antibody/antigen in the ERCS-NBTS in Addis Ababa.

2.2. SPECIFIC OBJECTIVES

A. To determine the rate of false negativity of HIV infection among blood donors screened as negative for HIV antibody/antigen.

B. To identify the risk factors for HIV-1 infection among blood donors.

C. To assess the level of agreement of screening between the Ag/Ab EIA and HIV-1 DNA PCR.

D. To determine the sensitivity, specificity, test efficiency, and predictive values of HIV screening method of the ERCS-NBTS laboratory.

3. MATERIALS AND METHODS

3.1. BLOOD DONORS AND DONATIONS

ERCS-NBTS collects whole blood from donors and distributes it freely for transfusion purpose. The Addis Ababa Center of ERCS-NBTS collects 16,000 to 17,000 blood units (one unit has a volume of 350/450ml) annually from volunteer and replacement donors. Currently donor remuneration is not allowed. Blood donation is collected from donors at the Center or through mobile campaigns (carried out primarily at secondary schools, higher training institutions and different organizations). Both volunteer and replacement donors donate blood in the Center; while in the mobile program donors are only volunteers. All donors are required to complete a health questionnaire during confidential interview and undergo physical screening by nurses to meet strict donor selection criteria. In the prescreening room, an enrollment form is filled and checked by the blood bank staff. Those donors accepted for donation will donate whole blood in the blood-drawing room while unqualified donors will automatically be deferred.

Donated blood is routinely screened for hepatitis B surface antigen, hepatitis C antibody, antibody to *Treponema pallidum,* HIV-1 and HIV-2 antibodies/antigen at the ERCS-NBTS according to the routine protocol of the ERCS-NBTS laboratory. Any blood unit found to be positive for at least one of these screening tests would be discarded and incinerated. HIV infection was screened by using fourth-generation Ag/Ab EIA (test protocol is attached as Appendix I), Vironostika HIV Uni-Form II Ag/Ab (BIOMÉRIEUX, The Netherlands). All blood found to be reactive in the first test were retested in duplicate for confirmation. Non-reactive blood was processed and stored appropriately for distribution.

3.2. WHOLE BLOOD SAMPLE COLLECTION AND PROCESSING

For this particular study, blood samples were collected from 14 May to 11 July 2003 from ERCS-NBTS. 7 ml of whole blood sample was taken from 408 blood bags of unlinked one time donation. Just before sealing the blood bag, whole blood from the lines was drawn to fill in Vacutainer® Blood Collection Tubes with EDTA as anticoagulant (Becton and Dickinson, USA) and transported to the ENARP central laboratory. From the sample 2 ml of whole blood was taken into a vial and temporarily stored at 2-25 °C for qualitative HIV-1 DNA PCR analysis. Plasma was isolated from the remaining portion by centrifuging at room temperature for 10 minutes at 2500 rpm and stored at -80 °C until assayed.

Data on demographic information (age, gender, address, occupation) and results of HIV serostatus were collected from ERCS-NBTS enrolment form (attached as Appendix II) and standardized laboratory data sheets, respectively.

3.3. LABORATORY INVESTIGATIONS

3.3.1. QUALITATIVE HIV-1 DNA PCR

Detection of viral nucleic acid for HIV infection in the whole blood was done by using AMPLICOR HIV-1 DNA Test, Version 1.5 (Roche Molecular Systems, USA). It is a qualitative *in vitro* test for the detection of HIV in human whole blood. This version 1.5 PRC test has been shown to be highly sensitive and specific for the detection of HIV. The test utilizes amplification of target DNA by PCR and nucleic acid hybridization for the detection of HIV-1 DNA in human whole blood.

SAMPLE PREPARATION: Tubes of whole blood were inverted 10-15 times to mix thoroughly. 1.0 ml Specimen Wash Solution (BLD WS) was added into 2 ml labeled screw-cap tubes and 500 µl of whole blood was pipetted. Tubes were inverted 10-15 times to mix,

incubated for 5 min at room temperature and once again inverted and incubated. The tubes were shaked on a minishaker, Mini-Secoueur IKA MS1 (OMNILABO INTERNATIONAL BV, The Netherlands) and then spun in a microcentrifuge (Gerätebau Eppendorf GmbH, Germany) for 3 min at maximum speed. The supernatant part was aspirated carefully by vacuum pumping system without disturbing the pellet. 1.0 ml BLD WS was added to each tube and pellets were washed twice with the same step. The leucocytes in the form of dry pellets were isolated and extracted. 200 µl of Working Extraction Reagent (prepared as mixture of 6 ml HIV-1 Extraction Reagent [HIV-1 EXT] and 80 µL HIV-1 (-) Control [HIV-1 IC] for 24 tests) was added to each cell pellet and into two tubes labeled as HIV-1 (-) C and HIV-1 (+) C, and 50 µl of each control was added to the corresponding tube. All tubes were vortexed for 3-5 sec, incubated for 30 min at 60 °C ± 2 °C and then at 100 °C for 30 min in a Drying-heat Block, Type DHB-161 (BIOzym, The Netherlands). All tubes were shaked briefly and spun. HIV-1 DNA was extracted from leucocytes by lysing in a detergent solution containing proteinase K.

PCR AMPLIFICATION: Labelled reaction tubes were placed in the MicroAmp Tray and locked in place with retainer. 50 µl of Working Master Mix (prepared by mixing 100 µL HIV-1 Manganese Solution [HIV-1 Mn^{2+}] and the entire vial of HIV-1 Master Mix [HIV-1 MMX]) was added into each reaction tubes. A supernatant of 50 µl of samples and controls were pipetted to the appropriate reaction tubes. Tubes in the MicroAmp Tray were locked with Tray Retainer and placed into the thermal cycler block. PCR Amplification was done using Thermal Cycler GeneAmp® PCR System 2700 (Applied Biosystem, Singapore), and the following programs were used for amplification: HOLD program for 2 min at 50°C; CYCLE program (5 cycles) for10 sec at 95°C, for 10 sec at 52°C, for 10 sec at 72°C; CYCLE program

(35 cycles) for 10 sec at 90°C, for 10 sec at 55°C, for 10 sec at 72°C; and HOLD program for 15 min at 72°C.

The tray was removed from the thermal cycler at any time during the final HOLD program and was placed in the MicroAmp Base. The caps were removed from the reaction tubes carefully to avoid creating aerosols of the amplification products. Amplification reaction was performed using the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase and a biotinylated primer pair (SK145 and SKCC1B) specific for both HIV-1 and HIV-1 Internal Control in the presence of excess dNTPs (dATP, dCTP, dGTP, dTTP, dUTP), Bicine buffer, Potassium acetate, AmpErase® uracil-N-glycosylase and Sodium azide. Both primers were used to define a sequence of 155 nucleotides within the highly conserved region of the HIV-1 *gag* genes. The HIV-1 Internal Control (a cloned DNA fragment with primer binding regions identical to those of the HIV-1 target sequence, and a unique probe binding region that differentiates it from HIV-1 target amplicon) was used in the identification of processed samples containing substances that may interfere with PCR amplification. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase prior to amplification of the target DNA. AmpErase recognized and catalyzed the destruction of DNA containing deoxyurine by opening the deoxyribose chain at the C1-position.

HYBRIDIZATION REACTION: 100 µl Denaturation Solution (DN) was immediately pipetted to the reaction tubes and mixed by pipetting up and down to chemically denature the HIV-1 amplicon and the HIV-1 Internal Control amplicon so as to form a single-stranded DNA. Tubes were incubated for 10 min at room temperature to allow complete denaturation. HIV-1 Microwell Plate (HIV-1 MWP) [coated with HIV-1 specific (SK102) oligonucleotide probe] and HIV-1 Internal Control Microwell Plate (HIV-1 CT MWP) [coated with HIV-1 Internal Control-specific (CP35) oligonucleotide probe] were allowed to warm at room

temperature before removing from the foil pouch and then appropriate number of 8-well MWP strips were removed from the foil packages and set into the MWP frame. 100 µl HIV-1 Hybridization Buffer (HIV-1 HYB) was added to each well on the MWP. 25 µl of denatured amplicon was pipetted to the corresponding wells. The MWP was gently tapped 10-15 times until the colour changes from blue to light yellow. The MWP was covered with MWP lid and incubated for 1 hour at 37 °C ± 2 °C. The biotin-labeled HIV-1 and HIV-1 Internal Control amplicon were hybridized to the target-specific oligonucleotide probes bound to the wells of MWP. MWP was washed 5 times by using an automated MWP washer, Washer 430 (Organon Teknika Microwell System, Austria) using Working Wash Solution (40ml of Working Wash Solution for each 8-well MWP strip was prepared by adding 1 volume of 10X WB to 9 volume of distilled water).

Then the plate was tapped to dry. 100 µl Avidin-Horseradish Peroxidase Conjugate (AV-HRP) was added to each well. The MWP was covered and incubated for 15 min at 37 °C ± 2 °C. AV-HRP binds to the biotin-labelled amplicon hybridized by the target-specific oligonuceotide probe bound to the MWP. MWP was washed again as described before to remove unbound conjugate. 100µl Working Substrate (prepared by mixing 2.0 ml Substrate A (SUB A) and 0.5 ml SUB B for two 8-well MWP strips) was added into each well. Working substrate contains hydrogen peroxide and Tetramethylbenzidine (TMB). The plate was allowed to develop colour for 10 minutes at room temperature in the dark. In the presence of hydrogen peroxide, the bound AV-HRP catalyzes the oxidation of TMB to form a coloured complex. 100 µl STOP reagent (containing sulfuric acid) was added to each well to stop the reaction. Within 30 minutes of adding stop reagent, absorbance at 450 nm was measured using an automated microwell plate reader, Reader 530 (Organon Teknika

Microwell System, Austria). Finally the absorbance value was recorded for each sample and control tested.

INTERPRETATION: If the absorbance value of HIV-1 test is <0.2 and the absorbance value of IC test is ≥0.2; it indicates that HIV-1 DNA is not detected and hence the sample is negative. If the absorbance value of HIV-1 test is ≥ 0.8 and the absorbance value of IC test is any; it indicates that HIV-1 DNA is detected and hence the sample is positive. If the absorbance value of HIV-1 test is between 0.2 to 0.8 and the absorbance value of IC test is any; it indicates that the test is equivocal, that means the result is inconclusive.

3.3.2. SUPPLEMENTARY TESTS

All specimens yielding discrepant results were reassessed by using other supplementary tests in order to confirm presence or absence of HIV infection. The HIV Ag/Ab EIA (Blood Bank)/ HIV DNA PCR (ENARP) assay discrepant samples were assessed by using HIV-1 Rapid test, Ab EIA, Western blot and HIV-1 RNA assays.

RAPID TEST: Rapid test was used to detect antibodies for HIV infection in the 27 plasma samples and it was done by using Determine™ HIV-1/2 (Abbott Laboratories, Japan). Determine HIV-1/2 has been shown to be 100 percent sensitive and 99.8 percent specific (Meless *et al.*, 2002). After removing the protective foil cover from each test pad, 50 µL of plasma was pipetted to the sample pad and then results were read after 15 to 60 minutes. The added plasma migrates through the solid phase selenium colloid-antigen conjugate to the immobilized recombinant antigens and synthetic peptides at the patient window site. Red lines on the patient and control windows indicate HIV positive test results.

Ab EIA: A qualitative determination of antibodies to HIV-1 and/or HIV-2 plus O was performed in 15 plasma samples by using Vironostika® HIV-Uni-Form II *plus* O

(BIOMÉRIUX, France). It is an EIA based test used to detect antibodies to HIV-1 and plus group O, and/or HIV-2. This third-generation Ab EIA has been shown to be 100 percent sensitive and 99.1 percent specific (Tegbaru *et al.,* 1999). The required number of microelisa strips was fitted to the strip holder and 100 µl specimen diluent (contains stabilizing protein and detergent) was pipetted into all wells, including control wells. 50 µl plasma or control was pipetted into the corresponding wells. The strips were incubated at 37 ±2 °C for 60 ±5 minutes. Each well was washed and soaked six times with phosphate buffer (prepared as 1:25 of phosphate buffer concentrate to distilled water) by using an automated washer. 100 µl TMB substrate was pipetted into each well. The strips were incubated at 15 to 30 °C for 30 ±2 minutes. 100 µl sulfuric acid was added to stop the reaction. Within 15 minutes the absorbance of the solution was read at 450 nm and 620nm as reference using an automated reader. A test result is reactive if the absorbance is greater or equal to cutoff value. The cutoff value is calculated as the summation of the mean value of negative controls and 0.100.

WESTERN BLOT ASSAY: Western blot assay was used as a specific supplemental test on 7 plasma samples that were found reactive using EIA Ag/Ab or HIV-1 DNA PCR assays. Western blot confirmatory test was done using an HIV BLOT 2.2 (Genelabs® Diagnostics, Singapore). It is a qualitative detection for the antibodies to HIV-1 and HIV-2 in plasma.

The required numbers of strips were removed using forceps from the tube and placed into each well. Strips for Strong Reactive, Weak Reactive and Non-Reactive controls were included. 2 ml of Diluted wash buffer (diluted as 1 volume of Wash Buffer Concentrate with 19 volume of distilled water) was added to each well. Strips were incubated for 5 minutes at room temperature on a rocking platform, Platform Shaker STR6 (Stuart Scientific, UK). Buffer was removed by aspiration. 2 ml of Blotting Buffer (1 volume of Stock Buffer Concentrate was diluted with 9 volume of distilled water, and then 1 g of Blotting Powder

was added to 20 ml of the diluted Stock Buffer) was added into each well followed by adding 20 µl of plasma or controls to appropriate wells. The tray was covered with cover and incubated for 1 hour at room temperature on the rocking platform. The tray was carefully uncovered and mixture was aspirated from the wells. Each strip was washed 3 times with 2 ml of Diluted Wash Buffer allowing 5 minutes soak on the rocking platform between each wash. 2 ml of Working Conjugate Solution (diluting Conjugate 1:1000 into Blotting Buffer) was added to each well. The tray was covered and incubated for 1 hour at room temperature on a rocking platform. Conjugate was aspirated from the wells and each strip was washed as described before. 2 ml of Substrate Solution was added to each well. The tray was covered and incubated for 15 minutes on the rocking platform. The substrate was aspirated and the strips were rinsed several times with distilled water to stop the reaction. Using forceps, strips were gently removed onto paper towels, covered with paper towels and air-dried. Strips were mounted on worksheet and taped over the developed bands. The bands were observed and the results were graded. The presence or absence of antibodies to HIV-1 in a test is determined by comparing each nitrocellulose strip to the assay control strips tested with the Non-reactive, Strong reactive and Weak reactive controls. Results were interpreted as negative, positive or indeterminate according to the criteria given by the American Red Cross Society (Constantine *et al.,* 1992).

HIV-1 RNA DETERMINATION

Assessment of HIV-1 RNA determination in 15 plasma samples was done using NASBA by NucliSens® HIV-1 QT (BIOMÉRIEUX, The Netherlands). NucliSens HIV-1 QT is a nucleic acid amplification assay for the quantitative determination of HIV-1 RNA.

Nucleic acid isolation: 225 µl plasma aliquot was taken into sterile Eppendorf tubes labeled with blood bag ID. After spinning for 5 minutes at maximum speed, 200 µl plasma

supernatant was pipetted into the corresponding Lysis Buffer tubes. The content was mixed thoroughly to make sure that any crystals present have dissolved. 20 µl calibrator solution was added into each Lysis Buffer tube. Tubes were vortexed briefly and the content was spun down. 50 µl Silica suspension was added into each tube. Tubes were vortexed and left for 10 minutes at room temperature. Tubes were shaked at every two minutes to resuspend any silica that has settled on the bottom. After tubes were centrifuged, the supernatant was removed.

Silica pellet in the tubes was washed by adding 1 ml of Wash Buffer and the tube was vortexed until the pellets have completely resuspended. Tubes were centrifuged and supernatant was removed. This wash procedure was repeated four times –once more with Wash Buffer, twice with 70% ethanol, and once with acetone. After the final wash, residual acetone was carefully removed. The silica pellets were dried in open test tubes at 65 °C for 10 minutes in a heating block. When dried, 50 µl Elution Buffer was added to each tube. The tubes were vortexed until the pellets are resuspended completely. The resuspended silica was left for 10 minutes (after five minutes resuspend by vortex) at 56 °C to elute the nucleic acid. Tubes were centrifuged for 2 minutes at 10 000 g. 5 µl of each of the nucleic acid supernatants was transferred to a fresh tubes.

Amplification and Detection: 10 µl of Primer solution was added into each tubes containing 5 µl nucleic acid supernatant. Tubes were incubated for 5 minutes at 65 °C. Tubes were then cooled at 41 °C for 5 minutes. 5 µl of Enzyme solution was added to each tube and flicked to mix well. Tubes were returned immediately to 41 °C and incubated for 5 minutes. Tubes were transferred to the detection room within five minutes.

Tubes were incubated at 41 °C waterbath for 90 ±5 minutes. For each amplified sample, four fresh 5 ml polypropylene tubes (referred to as hybridization tubes) were placed ready. One

fresh 5 ml polypropylene tube was also ready for use as blank (only Detection Diluent to be added). Hybridization solutions 1 to 4 were prepared as mixing 130 µl Bead-Oligo and 130 µl respective (WT, Qa, Qb, Qc) probes. Hybridization solutions were vortexed until a complete suspension has formed. 20 µl of hybridization solution was added to each hybridization tube 1 and to the blank. Each of 20 µl hybridization solution 2, 3, and 4 were added to each respective hybridization tube 2, 3 and 4. 200 µl Detection Diluent was added to a fresh tube. 5 µl of the amplified samples were added to the corresponding 200 µl Detection Diluent.

The diluted amplified samples were vortexed and spun down. 5 µl diluted amplified sample was added to each of the four hybridization tubes. 5 µl Detection Diluent was added to the blank. All hybridization tubes were covered with adhesive tape and mixed until a complete suspension has formed. All hybridization tubes and the blank were incubated for 30 ±2 minutes at 41 °C and mixed every 10 minutes by hand shaking for 10 seconds. 300 µl Assay Buffer was added to each hybridization tubes and the blank. The Instrument Reference Solution was vortexed to ensure complete suspension and 250 µl was added to a fresh 5 ml polypropylene tube. The tube with Instrument Reference Solution was placed on position 1 of the instrument carousel. Blank on position 2, and the hybridization tubes (for each sample in the order WT, Qa, Qb, Qc) in the following positions of the instrument carousel. Detection of amplified RNA was performed on NucliSens Reader (Organon Teknica, The Netherlands). The lower detection limit of the assay is 80 RNA copies/ml.

3.4. DATA ANALYSES

All laboratory-generated data were recorded and entered into a computer. All statistical analyses were performed using STATA (Stata Corporation, College Station, Texas, USA) software. Frequency and proportions of socio-demographic characteristics of the blood

donors were calculated. Multivariate analysis was applied to evaluate the role of potential risk factors for HIV-infection. Odds ratios for specific risk factors; adjusted for age group, sex, occupational group, address and type of donation; were calculated using a logistic regression model. Logistic regression models are used to see the relation between HIV positivity and donor risk factors. A *P*-value of less than 0.05 was considered indicative of statistical significance and 95% CIs were given when appropriate.

The test parameters that can assist in determining the usefulness of a test are sensitivity, specificity, efficiency, and predictive values (Constantine *et al.,* 1992). Sensitivity of a test is the ability of a test to detect truly infected individuals, while specificity is the ability of the test to identify all uninfected individuals correctly. Test efficiency refers to the overall ability of a test to correctly identify all positives and negatives (the absence of false positives and false negatives). Positive predictive value is the percentage of people with a positive test result who actually have the disease and negative predictive value is the percentage of people with a negative test who do not have the disease. Each calculation was made by the following formulae:

- Sensitivity = True positives / (True Positives + False Negatives) * 100
- Specificity = True Negatives / (True Negatives + False Positives) * 100
- Efficiency = (True Positive + True Negative) / (True Positive + False Positive + True Negative + False Negative) * 100
- Positive Predictive Value = True Positives/(True Positives + False Positives)* 100
- Negative Predictive Value = True Negatives/(True Negatives + False Negatives)* 100

Kappa statistical analysis was used to measure the level of agreement between Ag/Ab EIA and HIV-1 DNA PCR assays. This was based on comparing the observed proportion of agreement (A_{obs}) between two readings made by the two different screening assays with the

proportion of agreements (A_{exp}) that would be expected simply by chance (Kirkwood and Sterne, 2003). Hence Kappa (K) was defined as:

$$K = (A_{obs} - A_{exp}) / (1-A_{exp})$$

The possible interpretation of Kappa based on Byrt *et al.* (1993), Lantz and Nebenzahl (1996), and Kirkwood and Sterne (2003) is as follows:

- Poor agreement < 0.20,
- Fair agreement = 0.20 to 0.40,
- Moderate agreement = 0.40 to 0.60,
- Good agreement = 0.60 to 0.80, and
- Excellent agreement = 0.80 to 1.00.

3.5. ETHICAL CONSIDERATION

This study was conducted in accordance with the current Ethical guidelines applicable to the donation of blood at the ERCS-NBTS. All blood donations in all circumstances be voluntary and non-remunerated; no coercion should be brought to bear upon the donor. The donor should provide informed consent to the donation of blood or blood components and to the subsequent (legitimate) use of the blood by the transfusion service. Patients should be informed of the known risks and benefits of blood transfusion and/or alternative therapies and have the right to accept or refuse the procedure. Anonymity between donor and recipient must be ensured except in special situations and the confidentiality of donor information assured. This study was approved by the Biology Department of Addis Ababa University and Research and Ethical Clearance Committee of EHNRI.

4. RESULT

4.1. CHARACTERIZATION OF BLOOD DONORS

A total of 408 blood samples were collected from blood bags of one time donation. The socio-demographic characteristics of the blood donors showed an age range of 17 to 80 years, with the mean age of 29 years and the median age of 28. Out of the 408 donors, 142 (34.8%) were aged between 15 to 24 years; 39.2% were between 25 to 34 years, and 106 (26.0%) in the age group of 35 years and above. The peak age range of donors was 25 to 34 years and more than 94 percent donors were in the range of 15 to 45 years. Among the 408 blood donors, 319 (78.2%) were males; with 1:3.6 female to male sex ratio in the blood donors (Table 1). The ERCS-NBTS had collected a total of 16734 blood units, that is, 4145 (24.8%) were from female and 12589 (75.2%) were from male donors; with 1:3 female to male sex ratio.

Occupational distribution of donors showed that 166 (40.7%) of the blood donors were engaged in private work (self-employed); while 17.9% were student, 15.9% were civil servant and the unemployed accounted for 13.5% of the donors. And 49 (12.0%) of the blood donors were engaged in very diversified occupational types [driver, farmer, NGO, refugee, police (it is excluded from other civil servant and used as one type of occupation by the ERCS-NBTS), house wife, daily labourer, pensioner]. Regarding the residence of blood donors, 68 (16.7%) were from different regions and/or towns outside Addis Ababa, while the rest (83.3%) were from Addis Ababa. In the previous years blood donation was made as replacement, voluntary or with remuneration. However donor remuneration was strictly forbidden in the recent years and remunerated donors were differed automatically. Consequently, only 35 (8.6%) of the 408 donors were voluntary and the majority (91.4%) were replacement donors.

Table 1. Socio-demographic characteristics of blood donors (n = 408), ERCS-NBTS, Addis Ababa, July 2003

Characteristic	Proportion (%)
Age, years (median [IQR])	28 (22-35) *
Age category	
15 – 24	142 (34.8)
25 – 34	160 (39.2)
≥ 35	106 (26.0)
Sex	
Male	319 (78.2)
Female	89 (21.8)
Occupation	
Private work**	166 (40.7)
Student	73 (17.9)
Civil servant	65 (15.9)
Unemployed	55 (13.5)
Others***	49 (12.0)
Residence	
Addis Ababa	340 (83.3)
Outside Addis Ababa	68 (16.7)
Type of blood donation	
Replacement	373 (91.4)
Voluntary	35 (8.6)

* Data given as number
** Self-employed
*** Diversified occupational types (driver, farmer, NGO, refugee, police, house wife, daily labourer, pensioner)

4.2. SERO-STATUS OF THE BLOOD DONORS SCREENED BY Ag/Ab EIA.

The crude prevalence of HIV-infection among the 408 blood donors was around 4.2% (17/408), as screened by Ag/Ab EIA. Table 2 shows the sero-status of the blood donors by their socio-demographic characteristics. From 160 blood donors who were between the age group of 25 to 34 years, 7 (4.4%) were HIV-infected; and of 106 blood donors above 34 years of age only 8 (7.6%) were found to be HIV positive. Donors of the age category 25 to 34 years and 35 years and older were the major groups which contributed about 41.2% (7/17) and 47.1% (8/17) of the prevalence, respectively. Blood donors at the age of 15 to 24 years had the least HIV positivity (1.4%). In this study, no donors above the age of 45 years and below the age of 20 years were HIV positive. Among 319 male blood donors, 16 (5.0%) were HIV-positive, while from 89 female donors only 1(1.1%) was positive (Figure 4). From 319 male blood donors, 89 were randomly selected and the prevalence for HIV-infection was found to be 5.6 percent.

The highest HIV prevalence was in civil servants, of whom 7.7 percent (5/65) were positive, followed by private workers (4.2%). Among students, HIV sero-positivity was 2.7 %, and the least sero-prevalence of HIV infection [1/55 (1.8%)] was recorded from the unemployed group. Of the HIV-positive individuals, 7/17 (41.2%) were private workers and 5/17 (29.4%) were civil servant blood donors.

Large portion of the blood donors, 340 (83.3%) were residing in Addis Ababa, of which 10 (2.9%) were found HIV-infected. The remaining 68 blood donors came from other locations and 7 (10.3%) of them were HIV-positive (1 from Bahir Dar, 1 from Nazireth, 1 from Jimma, 1 from Mojo, 1 from Alemgena, and 2 from Silte Zone). Although donors from other locations have accounted for the higher HIV prevalence, donors from Addis Ababa contributed about 58.8 percent (10/17) for the overall prevalence of HIV-infection. In relation

to the type of blood donation, all HIV-infected blood donors were replacement donors. The prevalence of HIV-1 infection in replacement donors was about 4.6 percent (17/373). Hence, 100 percent of the HIV overall prevalence among blood donors was due to replacement donation.

Table 2. HIV sero-status among 408 blood donors by socio-demographic characteristics, ERCS-NBTS, Addis Ababa, July 2003.

Characteristic	HIV-positive (%)
Age category (years)	
15 – 24	2 (1.4)
25– 34	7 (4.4)
≥ 35	8 (7.6)
Sex	
Male	16 (5.0)
Female	1 (1.1)
Occupation	
Private work*	7 (4.2)
Student	2 (2.7)
Civil servant	5 (7.7)
Unemployed	1 (1.8)
Others**	2 (4.1)
Residence	
Addis Ababa	10 (2.9)
Outside Addis Ababa	7 (10.3)
Type of blood donation	
Replacement	17 (4.6)
Voluntary	0 (0.0)

* Self-employed

** Diversified occupational types (driver, farmer, NGO, refugee, police, house wife, daily labourer, pensioner)

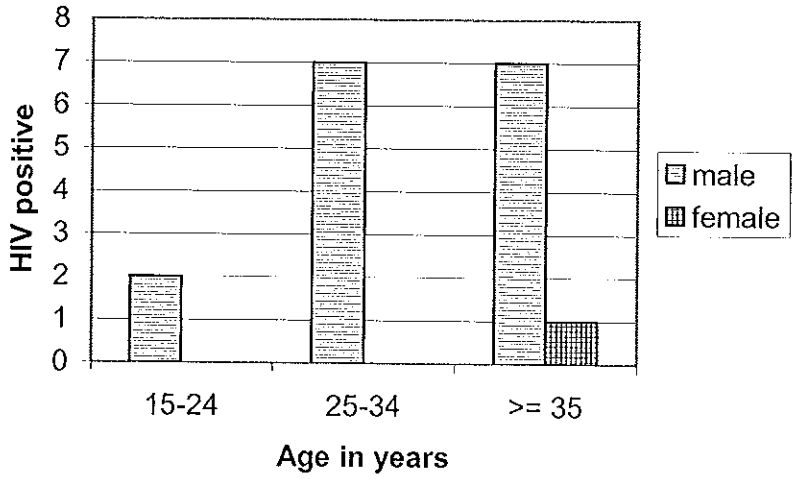


Figure 4. Age and sex distribution of HIV positive blood donors (n = 17), ERCS-NBTS, Addis Ababa, July 2003.

4.3. SOCIO-DEMOGRAPHIC FACTORS AND HIV INFECTION

Socio-demographic variables such as age, sex, occupation, residence, and type of blood donation were considered as the risk factors. Hence, the association of socio-demographic factors with HIV infection among the blood donors was analysed by the logistic regression model (Table 3). The results of this analysis showed that HIV prevalence was lowest among the youngest age group (15-24 years). The age categories from 25 to 34 and 35 years and older had higher HIV prevalence compared to the HIV prevalence of donors aged between 15 to 24 years. Donors aged 35 and above were significantly more likely to be HIV-positive than donors under 25 years (OR, 6.43; 95% CI, 1.06-38.93). On the other hand, blood donors between the ages of 25 and 34 years had no significant risk of HIV-infection as compared to the lower age group donors. Compared with donors from Addis Ababa, those donors from other places had about five-fold risk for HIV-infection (OR, 4.59; 95% CI, 1.52-13.83).

Comparison of the risk of HIV-infection between male and female donors did not show statistically significant difference (P= 0.139). Variables such as donor occupational groups (unemployed, private work, student, civil servant and others) and sex of blood donors as risk factors were not statistically associated with HIV infection.

Table 3. Logistic regression analysis of socio-demographic factors for HIV sero-positivity among 408 blood donors, ERCS-NBTS, Addis Ababa, July 2003.

Characteristics	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age category				
15 – 24	1.0			
25– 34	3.20 (0.65-15.67)	0.151	-	-
≥ 35	5.7 (1.19-27.49)	0.030	6.43 (1.06-38.93)	0.043
Sex				
Female	1.0			
Male	4.65 (0.60-35.53)	0.139	-	-
Occupation				
Unemployed*	1.0			
Private work	2.38 (0.29-19.76)	0.423	-	-
Student	1.52 (0.13-17.22)	0.735	-	-
Civil servant	4.50 (0.51-39.74)	0.176	-	-
Others**	2.30 (0.20-26.16)	0.503	-	-
Residence				
Addis Ababa	1.0			
Outside Addis Ababa	3.79 (1.39-10.33)	0.009	4.59 (1.52-13.85)	0.007

OR = Odds Ratio
CI = Confidence Interval
* Self-employed
** Diversified occupational types (driver, farmer, NGO, refugee, police, house wife, daily labourer, pensioner)

4.4. PCR TESTING FOR HIV-1 DNA

Of 408 blood donations, 17 (4.2%) were positive by Ag/Ab EIA whereas by HIV-1 DNA PCR testing 16 (3.92%) were found positive and 8 samples, which were negative by Ag/Ab EIA, were equivocal (inconclusive results) (Table 4). Among the 16 HIV-1 DNA PCR positive samples, two were negative by Ag/Ab EIA. On the other hand, 3 blood donations repeatedly diagnosed as reactive for HIV-1, by using Ag/Ab EIA, were found to be negative by HIV-1 DNA PCR. Thus, HIV screening of 408 blood units by the two methods showed that both Ag/Ab EIA and HIV-1 DNA PCR assays gave negative results for 381 (93.4%) and positive results for 14 (3.4%) samples. Accordingly, in 395 cases, the results were concordant between the two screening methods (Table 4).

Table 4. HIV screening results of 408 blood donors by Ag/Ab EIA and HIV-1 DNA PCR, ERCS-NBTS, Addis Ababa, July 2003.

		HIV-1 DNA PCR			
		Positive	Negative	Equivocal	Total
Ag/Ab EIA	Positive	14	3	0	17
	Negative	2	381	8	391
	Total	16	384	8	408

4.5. EVALUATION OF DISCREPANT RESULTS

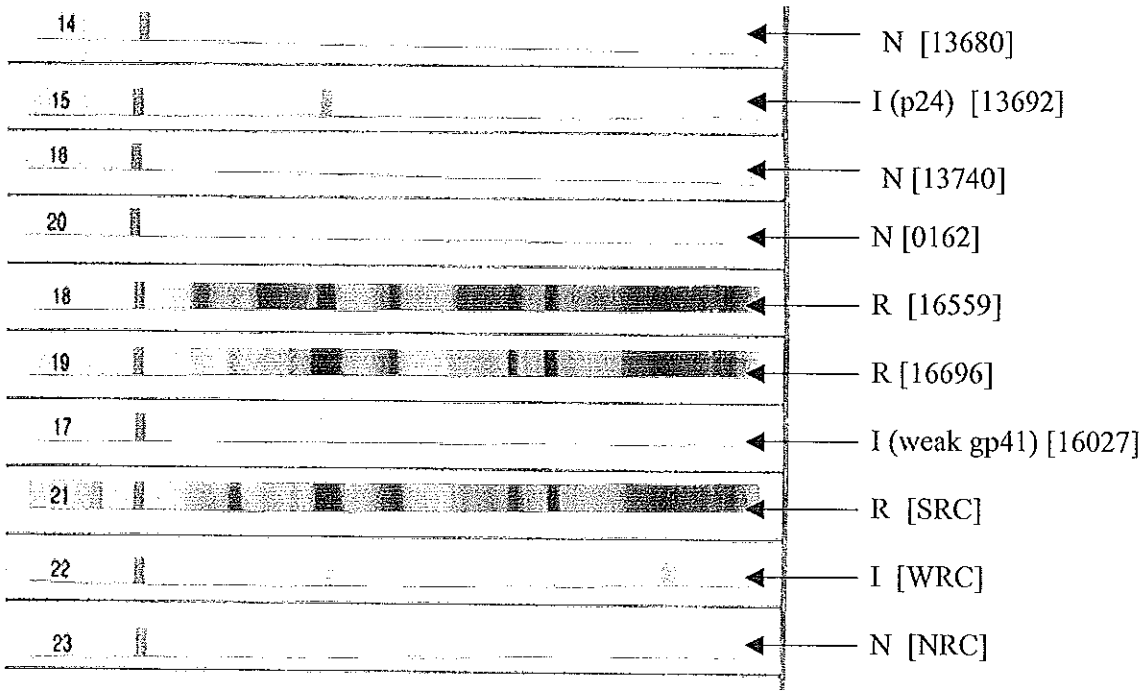
Table 5 provides summary data of supplementary tests of blood units that were discordant between Ag/Ab EIA and HIV-1 DNA PCR.

Table 5. Summary data of supplementary tests on HIV positives and equivocal samples (n=27), ERCS-NBTS, Addis Ababa, July 2003.

No	Sample ID	Ag/Ab EIA	PCR	HIV-serology			HIV RNA assay (*)
				Determine	Ab EIA	Western blot	
1	13740	P	N	N	N	N	< LDL
2	13745	P	P	R	ND	ND	ND
3	13908	P	P	R	ND	ND	ND
4	14347	P	P	R	ND	ND	ND
5	14583	P	P	R	ND	ND	ND
6	15260	P	P	R	ND	ND	ND
7	15493	P	P	R	ND	ND	ND
8	16027	P	N	N	R	I (weak gp41)	< LDL
9	16031	P	P	R	ND	ND	ND
10	16139	P	P	R	ND	ND	ND
11	16559	P	P	R	R	R	P (6100)
12	16688	P	P	R	ND	ND	ND
13	16696	P	P	R	R	R	P (8500)
14	16699	P	P	R	ND	ND	ND
15	16717	P	P	R	ND	ND	ND
16	0161	P	P	R	ND	ND	ND
17	0162	P	N	N	N	N	< LDL
18	13680	N	P	N	N	N	< LDL
19	13692	N	P	N	N	I (p24)	< LDL
20	13684	N	E	N	N	ND	< LDL
21	13685	N	E	N	N	ND	< LDL
22	13686	N	E	N	N	ND	< LDL
23	13737	N	E	N	N	ND	< LDL
24	14582	N	E	N	N	ND	< LDL
25	14585	N	E	N	N	ND	< LDL
26	16337	N	E	N	N	ND	< LDL
27	073	N	E	N	N	ND	< LDL

E = Equivocal for HIV-1 DNA PCR test; I = Indeterminate for Western Blot test; P = Positive
R = Reactive; N = Non-reactive or Negative for HIV-1/2 tests; ND = Not done;
* Number of RNA copies/ml detected; < LDL = Less than the lower detectable limit for NucliSens NASBA

Two blood donations (sample ID 13680 and 13692) were detected as positive by PCR, but were negative by Ag/Ab EIA. Test by using Western blot assay (Figure 5) gave nonreactive result for one specimen (ID 13680) and an indeterminate result for the other blood unit (ID 13692) in which only p24 band was noted. Viral RNA load was assessed in these two samples by using NucliSens NASBA and the results were below low detectable limit (<LDL). On the contrary, 3 blood donations (sample ID13740, 16027 and 0162) were found to be HIV-positive by Ag/Ab EIA, in which PCR gave negative results. And test with third-generation Ab EIA gave an HIV positive result in one blood sample (ID 16027). Western blot assay gave indeterminate results for one specimen (ID 16027) as a weak band for gp41. In the other two blood samples (ID 13740 and 0162) there was no band formation, indicating that both specimens were not infected by HIV. Viral RNA assessment by NucliSens NASBA of the three specimens (ID 16027, 13740 and 0162) showed that all were <LDL. In these three samples the signal value of the wild type is 1 (Appendix IV), that is, there was no HIV RNA amplification in the 3 specimens, proving that they were HIV-negative.



N=Non-reactive; R=Reactive; I=Indeterminate; SRC=Strong-reactive Control; WRC=Weak-reactive Control; NRC=Non-reactive Control

Figure 5. Western blot analysis of 3 Ag/Ab EIA positive, 2 HIV-1 DNA PCR positive and 2 wrongly reported samples, ERCS-NBTS, Addis Ababa, July 2003.

Similarly, viral RNA assessment by using NucliSens NASBA of the 8 HIV-1 DNA PCR equivocal samples (ID 13684, 13685, 13686, 13737, 14582, 14585, 16337 and 073), that were negative by Ag/Ab EIA, showed that they were all <LDL. Thus, since there was no HIV RNA amplification in these samples, all the eight samples were considered as HIV-negative.

The two blood donations (ID 16559 and ID 16696) that have been reported as HIV-negative in the ERCS-NBTS screening were found HIV-positive using all other screening tests. However, with proofreading of test results in ERCS-NBTS, both samples were confirmed to be positives, but reporting error.

Among the 408 blood donations as screened by various gold standards (HIV-1 DNA PCR and HIV RNA assay), 394 blood units were negative and 14 positive for HIV-1 infection. Therefore, the prevalence of HIV-1 infection in blood donors is 3.4 percent.

4.6. TEST PERFORMANCE AND EFFICIENCY.

Taking the confirmatory results obtained by HIV-1 DNA PCR and NucliSens NASBA (14 positives and 394 negatives) as a reference, test parameters of Ag/Ab EIA such as sensitivity (100%), specificity (99.2%), test efficiency (99.3%), positive predictive value (82.4%) and negative predictive value (100 %) were calculated. Measure of the level of agreement (Kappa statistical analysis) was 84.8 percent and it indicted an excellent agreement between Ag/Ab EIA and HIV-1 DNA PCR assays. Thus, this study showed that no blood sample was found to be false negative for HIV infection by the AG/Ab EIA.

5. DISCUSSION

The first description of transfusion-associated HIV infection occurred in the United States in late 1982 and early 1983 (Ammann *et al.,* 1983). After the introduction of HIV-antibody testing in 1985 in the USA, only about 5 cases of transfusion-associated HIV infection per year were reported during the subsequent five years, as compared with reports of 714 cases in 1984 (Selik *et al.,* 1993). The prevalence of HIV infection among the blood donors in the United States was 0.09 percent in 1984-1985 (Busch *et al.,* 1990), 0.018 percent in 1989 (Alter *et al.,* 1990), and 0.008 percent in 1991-1995 (Kleinman *et al.,* 1998).

The prevalence of HIV-infection among blood donors in different countries shows various rates. In a study conducted in Sao Paulo, Brazil, in 1992 to 1993, 0.28 percent of the blood donors were HIV-infected (Carvalho *et al.,* 1996). The prevalence of HIV-infection among blood donors in Bangkok, Thailand, in 1990 to 1993 was 0.9 percent (Kitayaporn *et al.,* 1996). In Abidjan, Côte d'Ivoire, in 1991 the overall HIV prevalence was 11.0 percent of the first time donors and 2.1 percent in repeat donors (Savarit *et al.,* 1992). In Harare, Zimbabwe, the prevalence was 8.8 percent (McFarland *et al.,* 1997). In Mbarara, Uganda, in 1994 the prevalence was 3.9 percent (Rukundo *et al.,* 1997) and in Kenya in 1994, the prevalence of HIV among blood donors was 6.4 percent (Moore *et al.,* 2001). In Addis Ababa, HIV prevalence in blood donors had increased from 2.3 percent to 6.2 percent between 1987 and 1991 (Zewdie *et al.,* 1992). A progressive increase was reported as 2.3 percent in 1988, 3.1 percent in 1989, and 5.1 percent in 1990 by the same authors. Serial prevalence data from blood donors in Addis Ababa from 1995 to 1999 showed an increase from the baseline 2.3 percent for 1987 to 9.1 percent in 1995, followed by a decline to 6.4 percent in 1999 (Kebede *et al,* 2000), to 5.0 percent in 2000 and 4.4 percent in 2001 (personal communication with ERCS-NBTS).

The 3.4 percent prevalence of HIV infection in blood donors in Addis Ababa, obtained in the present study is lower than the previous reports (5.0% in 2000 and 4.4 % in 2001) of ERCS-NBTS in Addis Ababa. The reason for the declining trend of HIV-prevalence among blood donors could be the avoidance of paid blood donors and implementation of better donor selection criteria. The current lower HIV prevalence rate in the general population in Addis Ababa, as indicated by Antenatal Care-based surveillance (Tsegaye *et al.,* 2002) might also have reflected to the lower HIV prevalence among blood donors. Although the prevalence rate among blood donors determined in this study looks lower than that reported elsewhere in certain African counties (Savarit *et al.,* 1992; Rukundo *et al.,* 1997; McFarland *et al.,* 1997; Moore *et al.,* 2001), it is still very high compared to the prevalence in the developed countries. The reasons for the higher prevalence rate could be that most donations are made from replacement donors from a general population that has a relatively high prevalence of HIV-infection.

In this study the prevalence of HIV-infection in male blood donors (5.0 %) was much higher than in females (1.12 %). From this observation, it is not possible to reason out why the prevalence of HIV-infection in males is higher. However, similar observations are reported by Zewdie *et al.* (1992) where the prevalence was roughly twice that of females. Sentijens *et al.* (2002) also reported a rate of prevalence in males (6.2%) slightly higher than in females (5.1%). In contrast to this, although fewer females than males volunteered to donate blood, a relatively higher prevalence among females (6.1 %) than males (4.7 %) was also reported (Rukundo *et al.,* 1997). Suggesting that additional factors are in play.

The reason for less number of female blood donors in the ERCS-NBTS could be due to under-weight and anemia problems in females. According to the ERCS-NBTS donor deferral criteria is set for weight and anemia, that is, if the donor is below 45 kg of weight and if

hematocrit (Hct) is below 36 % (females) or 41 % (males) the donor will be automatically deferred. Although there are various reasons for anemia, the major cause is malnutrition (Collins, 1993). The nutritional status of girls and women in sub-Saharan Africa is much poorer than the males (Leslie *et al.,* 1997). This is mainly due to the limited access to food resources caused by natural and human-made disasters, lack of control over inputs and resource allocation at the household level, traditional feeding practices and other customs that limit women's consumption of certain energy- or nutrient-rich foods, and the energy demands of heavy physical labour. According to Looker *et al* (1997), females are more likely than men to have anemia because of the loss of blood each month through menstruation. Approximately 10 percent of females of childbearing age experience heavy menstrual blood loss (≥ 80 ml/month), which is an important risk factor for anemia (Frith-Terhune, *et al.,* 2000). Most females are usually found to be in the underweight category and hence could not fulfill the weight criteria set by the blood bank, as a prescreening donor selection method. The other possible reason for less number of female donors could be fear for donation and mostly males are requested by the family to manage such things.

The difference in HIV infection among different age group donors reflects the fact that individuals remain HIV seropositive for a long period of time. Donors in their early twenties may have an increased risk of acquiring HIV infection due to a higher level of risky behaviour compared to donors in their thirties and older age. However, donors in thirties have a higher prevalence of HIV because many acquired their infections 5 to 10 years earlier. It was also indicated that no donors above the age of 45 and below 20 years were HIV-positive. This could be justified by the fact that young donors were almost all males. The mean age at which people become infected has been estimated to be 15 to 24 years for females, and 25 to 34 years for males (MOH, 2002). This may be because males start to practice sexual intercourse at relatively later age than females (Fontanet *et al.,* 1998). Further evidence to this

is the much higher number of females infected between 15 to 19 years than the number of males in the same age group (MOH, 2002). According to Fontanet *et al.,* (1998), female appeared to become more infected at younger age than male mainly due to earlier initiation of sexual activity and exposure to older sexual partners that were already infected. One explanation for non-infected older blood donors could be that older ones who might have progressive HIV-infection could be deferred at the pre-screening stage due to evident clinical signs (Daar, 1998; Kahn and Walker, 1998; Ward, 1999).

All HIV-infected blood donors were replacement donors, and they solely contributed to the current prevalence rate. A study conducted in Uganda also indicated that replacement donors had higher prevalence rate (8.3 %) as compared with the voluntary donors (2.8 %) (Rukundo *et al.,* 1997). The recruitment of replacement donors (usually a family member or a friend) in greater proportion may result in higher HIV prevalence in donated blood, since such donors could have concealed high-risk behaviours and would not normally donate for altruistic reasons. Such persons might agree to donate blood only as a personal favour to the patient himself or to the family of patients obliged to replace blood that will be or has been transfused.

Several variables considered as risk factors for HIV infection have become standard information (Appendix III) and are used in the blood bank as donor exclusion criteria. Thus, only age and residence of donors, that are specific to this population, had significant association with HIV infection in donors. Older age (35 years and above) in blood donors was significantly associated with HIV infection, reflecting the fact that individuals remain HIV positive for a long period of time. Another likely explanation could also be a prolonged and increased sexual activity with age. The significant association of being outside Addis Ababa and HIV infection in the blood donors could be due to the relatively higher prevalence of HIV

infection in some of those areas. According to MOH (2002), the percentage of pregnant women testing HIV-positive in the Sentinel Surveillance was 15.6 percent in Addis Ababa, 23.4 percent in Bahir Dar, 18.7 percent in Nazireth, 11.6 percent in Soddo and 8.6 percent in Jimma. Travelling from place to place and risky sexual behaviour could also contribute for the higher prevalence of HIV-infection in donors who were from outside Addis Ababa. However, it needs more time and data to clearly conclude the difference in HIV prevalence among donors with respect to their residency.

Current methods of donor risk factor screening and laboratory testing are highly effective in minimizing the risk of transfusion-acquired HIV infection. The combined HIV antigen and antibody assay offer the advantage of early detection of HIV infection via p24 antigen detection. Vironostika HIV Uni-Form II Ag/Ab (Organon Teknica) is one of the eight commercially available combination assays, and currently used by ERCS-NBTS for screening of HIV-infection in blood donors since 2001. HIV infection status in 408 blood donors was detected by this Ag/Ab EIA and 17 donors were repeatedly reactive. Out of the 17 repeatedly reactive samples, 3 were confirmed to be negative by HIV-1 DNA PCR, NucliSens NASBA and Western blot (in which one was indeterminate). Hence, these 3 samples represent false positive results.

One reason for the false positivity could be sample mis-labelling. False positive results that could be due to sample labelling error or due to cross-contamination were reported by Sabino *et al.* (1994). It should also be noted that the false-positivity rate of the Ag/Ab EIA (0.7%) in this study is supported by other reports in which the rate of false-positive results obtained varied from 0.3 to 0.8 percent (Gürtler *et al.,* 1998) and 0.5 percent by using another fourth-generation assay, VIDA HIV DUO Ultra (Saville *et al.,* 2001). The other most probable reason for false-positive results could be due to non-specific reactive antibodies. Out of these

three false positive samples, one was found reactive by Ab EIA. False positive results from non-specific reaction between antibodies due to infection with unknown but related retroviruses have been reported (Gürtler *et al.,* 1998; Ward, 1999). False positivity may also result from the properties of the test itself (Ward, 1999). The virus for the ELISA test is cultivated in human cells, and a certain amount of cellular debris can remain in the viral fragments when they are isolated. This debris can attract non-HIV human antibodies; when the latter accumulate on the assay plastic well surface, they can bind the enzyme-linked antibodies and reaction will be observed and be interpreted as HIV positive.

In this study, out of the 16 HIV-1 DNA PCR reactive samples, only 2 samples were confirmed to be negative for HIV-1 RNA and Western blotting (one was indeterminate). In addition, the 8 equivocal samples on HIV-1 DNA PCR were also confirmed to be negative by HIV-1 RNA assay. Such discrepancies have been reported by others in which 1 of 20 samples (Brown *et al.,* 1997) and 2 of 38 samples (Kleinman *et al.,* 1998) showed false-positive HIV DNA PCR results. There are also other reasons for the false positivity in HIV-1 DNA PCR assays. That is, the HIV-1 primers tend to generate non-specific amplification products at a variable rate, which was significantly reduced after the introduction of the hot-start technology with AmpliTaq Gold in January 1998 (Roth *et al.,* 1999). The most probable reason for contamination was the high sensitivity of the DNA PCR and the high viral titres of some samples that were more likely involved in unintended spilling of the virus (Roth *et al.,* 1999).

The internal control included in the HIV-1 DNA PCR assay is critical to achieving a high degree of confidence in negative values. The internal control controls for sample recovery, impurities in extracted samples that may inhibit the amplification reaction, and technical errors during the testing process. A negative result for the internal control indicates that the

test result may be falsely negative, and such a sample should be retested (Abravaya *et al.,* 2000). A positive result for the internal control validates the test result and ensures that a negative result of the sample is truly negative (Rosenstraus *et al.,* 1998). The inclusion of an internal control in each reaction mixture controls sample inhibition that ensures all the negative specimens to be truly negative.

To control for possible errors in a PCR testing, a second aliquot from the same donation or from a subsequent donation of the same donor should be tested in an independent PCR run (Kleinman *et al.,* 1998). Therefore, since follow-up sample collection from donors was not possible in this study, possible errors in PCR testing cannot be ruled out.

Extremely sensitive HIV screening assays, that have a potential for being less specific, lead to false positive interpretations. Independent supplemental tests of high specificity are therefore necessary to further check the presence of antibodies to HIV-1 and/or HIV-2. Western blot assay is the most widely accepted confirmatory assay for the detection of antibodies to HIV, although indeterminate results in uninfected individuals are frequent (Benenson *et al.,* 1989, Meless *et al.,* 2002). A specimen is interpreted as positive for HIV-1 antibodies according to the American Red Cross criteria, requiring the presence of at least three bands, one from each gene product group of *gag, pol* and *env*. The criteria for a negative Western blot interpretation specify no bands. However, those specimens with neither of the positive nor the negative criteria are labelled as indeterminate results. In this study, 2 samples displayed reactivity to only one protein on the Western blot test; hence both were levelled as indeterminate results. The probability of getting such indeterminate results was also reported by Meless *et al.* (2002), where 30.4 percent reactivities to only p24 antigen and 15.2 percent reactivities to only gp41. However, these two specimens were confirmed to be negative for HIV RNA.

Plasma HIV-1 RNA can be detected in virtually all HIV-1-infected individuals although its concentration can vary widely depending on the stage of infection. Sabin *et al.* (2000) indicated that in most cases levels of HIV-1 RNA started to gradually increase soon after the time of infection, provided that drugs are not employed. During primary HIV-1 infection, when there are numerous susceptible target cells without a countervailing host immune response, concentrations of plasma HIV-1 RNA can exceed 10^7 copies/ml (Piatak *et al.*, 1993). Viral set point at the time of seroconversion for Ethiopian is 10^4 RNA copies/ml (Rinke de Wit, *et al.*, 2002). More than 90 percent of virus comes from recently infected cells rather than from long-lived chronically infected cells or from latently infected cells recently activated to produce virus (Ho *et al.*, 1995). Thus, the virus present in the plasma of an infected person at any time are most likely produced by CD4 cells infected within the previous day (Feinberg, 1996). Hence, in this study, NucliSens NASBA was used to confirm HIV-infection and resolve discrepancy. This is also true where plasma RNA detection system has been used to confirm Ag/Ab EIA reactivity and resolve indeterminate antibody test results (Brown *et al.*, 1997; Kleinman *et al.*, 1998). Good recovery of viral RNA has been reported from serum and plasma stored at –70 °C to –80 °C for 6 months to 1 year (Coombs *et al.*, 1993) and Sabin *et al.* (2000) were successful in recovering viral RNA from samples that have been stored at –40 °C for up to 17 years. In our study, no constraint in the recovery of viral RNA or failurity in amplification was seen, notwithstanding the fact that HIV RNA assays have a limit of detection for HIV RNA (Ling *et al.*, 2000; Weber *et al.*, 2002).

A central issue in evaluating a test is its validity, or ability to differentiate accurately between those who have the disease and those who do not. These are measured by sensitivity and specificity, respectively. However, sensitivity and specificity are not fixed properties of a given test, but vary with the conditions under which the test is carried out. In this study, the sensitivity and specificity of Ag/Ab EIA is 100 percent and 99.2 percent, respectively. 100

percent sensitivity indicates that this screening test has picked the entire infected blood in the donation, without a false negative result; while 99.2 percent specificity indicated that this assay could not perfectly detect the true negatives as negative, since it has three false positive results. In this study, no blood unit was found to be false negative as confirmed by nucleic acid testing. However, it is comparable with other studies using the same brand of kit where sensitivity and specificity ranged between 98 to 99.9 percent and 98 to 100 percent, respectively (Gürtler *et al.,* 1998; Tros and Lann, 1999), while using other fourth-generation assay (VIDAS HIV DUO Ultra) demonstrated 100 percent sensitivity and 99.5 percent specificity (Saville *et al.,* 2001). The test efficiency of Ag/Ab EIA in this study was 99.3 percent, indicating that this assay has an excellent efficiency for screening blood donations and minimizing the risk of transfusion transmission of HIV.

In interpreting the results of a test, it is necessary to know the ability of the test to predict the presence or absence of the disease. Hence, in positive tests the positive predictive value helps to know the likelihood that the person tested truly has the disease/infection; but if the test is negative, the negative predictive value would help to know with what degree of confidence the disease can be ruled out (Galen, 1986). Accordingly, the positive and negative predictive values of Ag/Ab EIA, in this study, are 82.4 percent and 100 percent respectively. Positive predictive value is dependent on the prevalence rate of the disease in the population being screened or on specificity of the screening assay (Galen, 1986). However, the positive predictive value in this study indicated that few of those blood donors who tested positive actually do not have infection. Samples with false positive results request more resources for confirmatory repeat tests.

It is very crucial for a blood-screening assay to pick up all infected blood donation and helps to minimize the risk of HIV-1 transmission through transfusion. The extent of reliability or

reproducibility of Ag/Ab EIA is assessed by measuring the test agreement between Ag/Ab EIA and HIV-1 DNA PCR. The Kappa statistic (84.8 %) indicated that there is an excellent agreement between the two assays proving that utilization of Ag/Ab EIA assay is a reliable blood screening method.

The risk of transmitting HIV by transfusion of screened blood could be due to the use of donations made during the window period, when a recently infected donor is infectious but detectable HIV antibodies have not yet developed (Coutlée *et al*, 1994). Hence there is a residual risk, because of the prolonged window period and because some donors with recent infections may not report or may not perceive the high risk of the behaviour that resulted in their exposure. Since this study is a cross sectional survey, it was not possible to investigate incidence rate; and hence estimation of risk of HIV transmission through transfusion was not possible.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Methods of blood donor risk factor screening and laboratory testing are highly effective in minimizing the risk of transfusion-acquired HIV infection. As far as safe blood donation is concerned, false-positivity does not cause any risky problem in transfusion, but it requires more cost on repeat testing and uninfected blood units would be needlessly discarded.

The current HIV-1 prevalence rate in the blood donors in Addis Ababa ERCS-NBTS indicated a declining trend as compared to the previous reports. Only replacement donors were found to be HIV-infected and the recruitment of replacement donors has contributed to the noted prevalence of HIV among blood donors. Replacement donors may be falsifying answers to the interviewer, and thus eliminating one of the screening safeguards. Additionally, higher age groups, civil servants and donors residing outside Addis Ababa have high HIV prevalence. Though prevalence increases with age groups, donors with the age of less than 20 and greater than 45 years are found negative for HIV. Hence, donors at the age of 17 to 20 years are relatively safe for blood donation.

Among the socio-demographic factors, only age groups of 35 years and above and residence outside Addis Ababa are found to be strongly significant for HIV-infection of blood donors. As age increases in blood donors an individual would remain HIV positive for some long time from an infection that may have been contracted 5 to 10 years earlier. The reason for the strong association with residence out of Addis Ababa may be due to the relatively higher prevalence rate of HIV-infection in those places, travelling form place to place, and risky behaviour of the donors.

The test parameters such as sensitivity, specificity, efficiency and predictive values of HIV screening method in ERCS-NBTS highlighted a good screening efficiency of the fourth-generation assay used. And yet the calculated Kappa statistics indicated an excellent screening agreement with that of HIV-1 DNA PCR. In this study, no blood unit was found to be donated in the seroconversion window period. Thus, in spite of all efforts, the ideal of a zero-risk blood supply is still not achievable. However, with declining rates of HIV infection among blood donors and improved testing procedures, the likelihood of transfusion transmission of HIV can be reduced. Screening tests for other concurrent infectious agents might further reduce the risk of HIV transmission through transfusion.

Since this study was a cross-sectional survey, no conclusion can be drawn on the incidence of HIV-infection in blood donors and estimation of risk of transfusion transmission of HIV-1 was not possible. Thus, the limitations of this study are lack of follow up samples and incidence of HIV among one time donation could not be measured. Due to lack of follow-up samples, those PCR positive and equivocal samples could not be retested by the same HIV-1 DNA PCR assay. Hence, HIV RNA assay was used to confirm a definitive diagnosis of such samples.

Blood donors, recipients and other community members ask about the safety of blood supply; that is, whether the donated blood is free of HIV-infection. Therefore, this study has provided good information to alleviate such types of worries among the general population.

6.2. RECOMMENDATIONS

Modern medical care, including surgery and medical treatment for many diseases, is not possible without the use of blood and blood products. Shortage of blood products means that someone may not get prompt and adequate medical care. In spite of screening of blood against HIV antibodies, there may be some, though small it may be, risk of HIV transmission from screened blood. It is important to regularly review measures to protect the quality of blood supply against infectious risks to ensure that the screening procedures are adequate and effective.

Even if the declining trend of HIV-prevalence in donors is an indicator of safe blood supply, any risk of HIV transmission through blood must be avoided as far as possible. A multi-pronged approach is needed to improve the safety of the blood supply. This includes stringent donor selection criteria, improved pre-donation interviews designed to exclude donors at risk of transmitting infectious diseases, the use of appropriate technology for screening infectious agents and education of both donors and the public on the need for safe blood. Replacement donors should be minimized or excluded from blood donation, while emphasis should be given on recruitments of voluntary and safe blood donors. Recruiting, educating and retaining safe blood donors are important components of blood safety efforts. Donors with high-risk behaviour should be educated and encouraged for self-exclusion before donation.

The diagnosis of HIV infection among blood donors is made based on the detection of antigen/antibodies of HIV. This involves a 2-stage repeated testing to confirm the infection, especially when the first test is found to be reactive. However, errors in the reporting of screening results can happen due to personal mistakes. Before the distribution of blood units for transfusion, such error in result reporting should be strictly checked and controlled by the quality control section of the blood bank.

For this type of studies, follow-up samples must be available as there is no absolutely perfect screening assay. This study was conducted in Addis Ababa and the performance of HIV screening in ERCS-NBTS appears to be excellent. However, the situation at the ERCS-NBTS in Addis Ababa is not typical of all locations in Ethiopia. Therefore, similar studies should be done in other parts of Ethiopia to assess the quality/safety of the blood donations. Further studies on HIV incidence rate among blood donors, proper estimation of risk of HIV transmission through transfusion, and prevalence of HIV infection associated with transfusion of blood and blood products are essential for the proper monitoring of the safety of the blood supply. Studies on other transfusion transmittable infectious diseases, especially viral hepatitis should also be undertaken. Why less number of female comes to donate blood, the higher prevalence of HIV-infection in male blood donors and donors from other places need further investigation. The currently used Ag/AB EIA should be validated with WHO collaborating Centers.

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8. APPENDICES

APPENDIX I. Test protocol of Vironostika HIV Uni-Form II Ag/Ab (BIOMÉRIEUX, The Netherlands).

1. Fit the strip holder with the required number of microelisa strips.
2. Pipet 100 µl specimen diluent into all wells, including control wells.
3. Pipet 50 µl sample or control into assigned wells. Include three negative controls, one anti-HIV-1 positive control and HIV-1 antigen positive controls in each strip holder.
4. Agitate the strips using a microshaker (speed approx. 900 rpm for 15 seconds). Incubate the strips at 37 ±2 °C for 60 ±5 minutes.
5. Wash and soak each well six times with phosphate buffer.
6. Pipet 100 µl TMB substrate into each well.
7. Incubate the strips at 15 to 30 °C for 30 ±2 minutes.
8. Stop the reaction by adding 100 µl mol/l sulphuric acid to each well. Thoroughly mix by tapping the side of the strip holder. Plates should be read within 15 Minutes.
9. Read the absorbance of the solution in each well using ELISA reader at 450nm and 620nm as a reference.

Test validity: A test run is valid if

The absorbance of the negative control (NC) is less than 0.250; and the absorbance of the anti-HIV-1 positive control minus the mean of negative controls is greater or equal to 0.600.

Cut-off value:

Cut-off value = mean value of NC + 0.100

Interpretation:

A test sample is reactive if sample absorbance is ≥ cut-off value.

A test sample is non-reactive if sample absorbance is < cut-off value.

APPENDIX II. ERCS-NBTS Enrollment Form.

ETHIOPIAN RED CROSS SOCIETY
NATIONAL BLOOD TRANSFUSION SERVICE
ENROLLMENT FORM

Name .....

Age ..... Sex .....

Occupation .....

Reg. No. .....

City: .....

Woreda..... Keb. ..... H. No. .....

Tel: Res. .....

Office .....

P. O. Box .....

Date	Pack No.	Wt.	B/P	Hct.	Vol.	Screened by	ABO	Rh.	Compon-ent Req.	Type of Donation	Remark

APPENDIX III. ERCS-NBTS Donor Screening Questionnaire Form.

በደም ለጋሹ የሚሞሉ ጥያቄዎች
እንዱን ምልክት ያድርጉ
እምንታ ከሆነ (✓) አፍራሽ ከሆነ (X)

TO BE FILLED BY BLOOD BANK STAFF

ጥያቄዎች	✓.X	Questions	Y. N
የማያቋርጥ ሳል አለብዎት?	✓	Are you healthy?	
ያለጠ ዕጢ አለብዎት		Is your work Hazardous?	
የሌሊት ላብና ትኩሳት አለብዎት?		History of any chronic disease?	✓
የቆየ የተቅማጥ በሽታ አለብዎት?	✓	Sexually transmitted diseases?	✓
የወባ በሽታ አለብዎት?		Multiple sexual Partners?	✓
የወፍ (የጉበት) በሽታ አለብዎት?	✓	Blood transfusions?	✓
የሚጥል በሽታ አለብዎት?		Dlubetis?	
ተደጋጋሚ ነስር አለብዎት?		Hypertension?	
የቆዳ በሽታ አለብዎት?	✓	Ulcers?	
ባለፉት 6 ወራት		Heart Diseases?	
የቀዶ ሕክምና እድርገዋል?	✓	Kidney Diseases?	
ክትባት ተከትበዋል?	✓	Tuberculosis?	✓
ጆሮዎን ተበሰተዋል ወይንስ ተነቅሰዋል	✓	Asthma?	
ባለፈው ሳምንት		Others?	? ✓
መድኃኒት (አስፕሪን) ወስደዋል?		Sister's Comments Acceptance/ Deferal causes	
ጥርስዎን ተነቅለዋል?	✓	Accept for donation	
ጉንፋን ይዞዎት ነበር?			
ለሴቶች ብቻ		Defer Temporarily (duration)	
ነፍሰጡር ነዎት?	✓		
ባለፉት 6 ወራት ነፍሰጡር ነበሩ?	✓	Reject Permanently	
ጡት ያጠባሉ?			
በእኔም ሆነ በደም ተቀባዩ ላይ ሊያደርስ የሚችለውን ጉዳት በመገንዘብ ስለ ጤንነቴ ትክክለኛውን መረጃ በመስጠት ደም መለገሴን እረጋግጣለሁ ።		Form Inspected and Signed	
የለጋሹ ፊርማ		(Name)	

በንግድ ማተሚያ ድርጅት ታተመ

Appendix IV. NucliSens NASBA test results and interpretation.

Sample ID	Qualif.	Result	Det.	Log (Q-in)	Signal
13680	Valid	< LDL	WT		1
			Qa	5.100	1588785
			Qb	4.120	167449
			Qc	3.620	60854
13692	Valid	< LDL	WT		1
			Qa	5.100	1401717
			Qb	4.120	170674
			Qc	3.620	97616
13740	Valid	< LDL	WT		1
			Qa	5.100	1507190
			Qb	4.120	195690
			Qc	3.620	47887
16027	Valid	< LDL	WT		1
			Qa	5.100	1606162
			Qb	4.120	191534
			Qc	3.620	46408
16559	Valid	6100cp/ml	WT		17320
			Qa	5.100	1495284
			Qb	4.120	202069
			Qc	3.620	56513
16696	Valid	8500cp/ml	WT		15164
			Qa	5.100	1271380
			Qb	4.120	127231
			Qc	3.620	26146
0162	Valid	< LDL	WT		1
			Qa	5.100	1502907
			Qb	4.120	202473
			Qc	3.620	99936
13684	Valid	< LDL	WT		1
			Qa	5.100	1947822
			Qb	4.120	373984
			Qc	3.620	106546
13685	Valid	< LDL	WT		1
			Qa	5.100	1769542
			Qb	4.120	265914
			Qc	3.620	70113
13686	Valid	< LDL	WT		1
			Qa	5.100	1827024
			Qb	4.120	273908
			Qc	3.620	88481
13737	Valid	< LDL	WT		1
			Qa	5.100	1940277
			Qb	4.120	314087
			Qc	3.620	85956
14582	Valid	< LDL	WT		1
			Qa	5.100	872098
			Qb	4.120	84050
			Qc	3.620	22269

Appendix IV. Continued

Sample ID	Qualif.	Result	Det.	Log (Q-in)	Signal
14585	Valid	< LDL	WT		1
			Qa	5.100	1583726
			Qb	4.120	156790
			Qc	3.620	43023
16337	Valid	< LDL	WT		1
			Qa	5.100	1395381
			Qb	4.120	192468
			Qc	3.620	87934
073	Valid	< LDL	WT		1
			Qa	5.100	1421125
			Qb	4.120	171145
			Qc	3.620	87956

Interpretation: < LDL means below the detectable limit. The interpretation of < LDL result in any test run of NucliSens NASBA can be either the sample is negative for HIV infection provided that the signal value of the WT is 1 or the sample is positive if the signal value of the WT is 1-80. That means if the signal value of the WT is 1, there is no viral particle in the sample to be amplified and detected, and hence the sample is negative for HIV infection. When the signal value of the WT lies between 1-80, which indicated the presence of viral particles that can be amplified (hence the sample is infected with HIV) but the NucliSens Reader would give as < LDL, this is because of the lower detection limit of the NucliSens Reader is 80 RNA copies/ml.