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Collage of Graduate studies

Assessment of Sensitivity and Specifity Immunochromatograophy Test And ELISA for detecting Human Immunodeficiency Virus Antibodies among Screening patients in Khartoum state

تقدير حساسية وخصوصية الاختبار بواسطة تقنية الكروماتوغرافي الممنع مقارنة بالاليزا لوجود الاجسام المضاده لفيروس نقص المناعه المكتسب(الايدز) لديالفاحصين المتطوعين بولاية الخرطوم

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جامعة السودان للعلوم والتكنولوجيا

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Declaration

I, the signing here-under, declare that I'm the sole author of the (M.Sc.) thesis entitled Assessment of sensitivity and Specifity Immunochromatography test and ELISA for detecting Human immunodeficiency virus Antibodues among screening patient in Khartoum State which is an original intellectual work. Willingly, I assign the copy-right of this work to the College of Graduate Studies (CGS), Sudan University of Science & Technology (SUST). Accordingly, SUST has all the rights to publish this work for scientific purposes.

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إقرار

أنا الموقع أدناه أقر بأنني المؤلف الوحيد لرسالة الماجستير المعنونة تقدير حساسية وخصوصية الاختبار بواسطة تقنية الكروماتوغرافي المناعي ومقارنتها بالاليزا لتحديد الاجسام المضادة لفيروس نقص المناعة المكتسب لدى المرضى المفحوصين في ولاية الخرطوم

وهى منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعه السودان للعلوم والتكنولوجيا، عليه يحق للجامعه نشر هذا العمل للأغراض العلمية .

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DEDICATION

To my beloved parents.

My brother.

My teachers. Spcially:

Prof . Yousif Fadlallah Hamed Elnil

MyAll friends espically Maysara Abd allaha

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Thanks first to ALLAH for helping and blessing me in doing this work, then I would like to express my deepest thanks and gratitude's to my supervisor Prof. Yousif Fadlalah Hamed Elnil for his great efforts , help and patience I received from him during my work and Iam are asking ALLAH to bless him as well as good things he does in his life.

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Abstract

Human Immunodeficiency Virus (HIV) is global and serious problem , with increase in mortality and morbidity worldwide.

This was prospective , descriptive and cross sectional study aimed to assess the level of HIV Ab using the ICT and ELISA for detecting Ab and Ag (p24) .It was conducted among Screening patient in Khartoum state (National Public Health Laboratory) from 1 April to 30 June (2015) on a total of eighty nine (n=89)to compare the sensitivity and specificity of immunochromatographic test and ELISA in detecting HIV Ab .All subject examined in this study from both sex male and females . 14 (15.7 %) of screening patients were positive for HIV Ab by ICT, Where it decrease to 13(14.6%) by ELISA procedure run on the same serum sample .there was one false negative result when tested by ICT . total of previous blood transfusion .only 7 % who had positive for HIV Ab when tested by ELISA .

The highest prevalence was on the age group (21- 40) years and low rate (61 – 80). The result obtained show that the ICT technique has 100% ,98.6 % respectively sensitivity and specificity for detection of HIV Ab .While ELISA revealed 100% for both sensitivity and specificity .Results showed that the ICT used in this study for HIV Ab was less specific compare to ELISA.

Among married screening patients 55/89 (61.8 %) higher than single 34/89(38.2%) positive cases were 7/55(7.9%) among married and 6/34(6.7%) among single .were examined by ELISA .

In those who has previous surgical operation and multi transfusion only 7 % who had positive for HIV when tested by ELISA .

ملخص الدراسه

يعتبر مرض الايدز مشكله عالميه وخطرة وذلك لزيادة الاصابه والاماته في مختلف انحاء العالم ودلك لاعطاء نتائج حقيقيه وموثقه .

تعتبر هذه الدراسه وصفيه مقطعيه انيه هدفت لتقييم فعالية ICT في فحص الاجسام المضادة ELISA للكشف عن الانتجين والاجسام المضاده . واجريت بين مرضي من ولاية الخرطوم ((المعمل القومي للصحه العامه)) في الفترة من1 ابريل وحتي 30 يونيو 2015م .وكان عددهم الكلي 89 وذلك لمقارنة حساسيه ونوعيه ICT في الكشف عن الانتجين الاجسام المضاده للايدز وال ELISA في الكشف عن الانتجين /الجسم المضاد) .

كل المشتركين الذين فحصوا في هذه الدراسه من كلا الجنسين رجال و نساء 89/14 (15.7%) من المرضي المفحوصين اظهروا ايجابيه للاجسام المضاده باستخدام للايدز بينما 89/13 (14.8%) ايجابيون باستخدام طريقة الاليزا والتي اجريت لنفس عينات فصل الدم .

توجد عينه واحدة اظهرت نتيجة ايجابيه كاذبه عندما اختبرت بواسطة ال ICT.

مجموع نقل الدم السابق كانت هناك 7% فقط اظهروا ايجابيه لفيروس نقص المناعه المكنسب عندما فحصوا بالاليزا .

وكان اعلي معدل انتشار في مجموعة العمر 21-40 سنه ومنخفضة في مجموعة العمر 61 - 80 سنة .

وقد اظهرت هذه النتايج ان تقنية ال ICT لها حساسيه ونوعيه 100% و 98.6% بالتتابع وذلك لفحص الاجسم المضاده للايدز بينما الاليزا اظهرت 100% للحساسيه والنوعية معا .

اوضحت النتائج ان استخدام ICT في هذه الدراسه للاجسام المضاده للايدز اقل نوعيه مقارنة بالاليزا.

في اواسط المرضي المتزوجين الذين فحصوا 89/55 (61.8 %) وكانوا اكثر من غير المتزوجين 59/34 (38.2%) .الحالات الموجبه كانت 55/7 (7.9%) في اواسط المتزوجين و34/6 في اواسط غير المتزوجين وذلك عند الفحص بواسطة الاليزا .

وعند الذين اجروا عمليات جراحية سابقة وايضا نقل دم مستمر فقط 7% لديهم ايجابيه للاجسام المضاده للايدز عندما فحصوا بالاليزا .

Content

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List of Abbreviations

Abbreviation	Complete Word
Ab	Antibody
Ag	Antigen
AIDs	Acquired Immunodeficiency Syndrome
CCR5	C C Chemokine receptor 5
CD4	Cluster of Differentiation 4
CDC	Centers for Disease Control and Prevention
CNS	Central Nervous System
CTL	Cyto Toxic –T-Lymphocyte
CXCR4	CX C Chemokine receptor 4
DNA	Deoxy Ribonucleic Acid
EBV	Epstein Bar Virus
ELISA	enzyme linked Immune Sorbent Assay
gag	group-specific antigen
gp	Glycoprotein
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus -1
HIV-2	Human immunodeficiency virus -2
HTLV-lll	Human T Lymphotropic Virus lll
ICT	Immuno Chromatography Test
IFA	Immuno Fluorescence Assay
IFA	Immunofloursent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LAV	Lymphadenopathy Associated Virus
LTR	Long Terminal repeats
mRNA	Ribonucleic Acid messenger-

Nef	Negative regulatory factor
P24	Protein 24
PCR	Polymerase Chain Reaction
pol	Polymerase
RDT	Rapid Diagnostic Test
Rev	Reverse transcriptase
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
SIV	simian immunodeficiency virus
Tat	Trans Activating Protein
Th	T-helper cell
TMB	Tetra Methyl Benzidine
TNF	Tumor Necrosis Factor
Vif	Viral infectivity factor
Vpr	viral protein r
Vpu	viral protein u
Vpx	viral protein X
WB	Western Blot Technique
WHO	World Health Organization

CHAPTER ONE

1Chapter One

1. Introduction

In early 1980 a new epidemic was first noted that we now called the Acquired Immunodeficiency Syndrome. **(**Gladwin and Trattler **,** 2004)

Human immunodeficiency virus is an RNA virus and belongs to the genus lentivirus within the family Retroviridae, so called because viruses in the family possess a reverse transcriptase (RT) enzyme to convert the viral RNA template into DNA, which integrates in the cellular DNA to cause persistent infection. The other virus in the genus lentivirus is simian immunodeficiency virus (SIV), which infects monkeys. There are two known HIV viruses that cause human infection namely HIV- 1 and HIV- 2. Human immunodeficiency virus - 1 is further divided into three groups: 'major' group, M; 'outlier' group, O; and 'new' group, N. Group M has several subtypes (subtypes A to K), (Kurdesia and Wreghitt , 2009).

They are serologically and geographically distinct but have similar epidemiological characteristics. (Heymann, 2004)

The pathogencity of HIV-2 may be lower than that of HIV-1; they have genotypic and phenotypic differences. HIV-2 has lower disease progression and lower rates of mother-to-child transmission, (Heymann, 2004).

The virus destroys all the T-cell lymphocytes. This disables the immune system to defend the body against diseases and tumors. Various infections will be able to develop.. These infection which normally won't cause severe or fatal health problems will eventually cause the death of the HIV patient, (Rombauts, 1997).

In 2013, 5.3 million people were living with HIV worldwide, including 3.3 million children. The global prevalence rate (the percentage of people aged 15 - 49 who are infected) was 0.8 %. There were 2.3 million new HIV infections, including 260 000 children. Approximately 95% are in low and middle-income countries. About 700 infections are in children under 15 years of age. An estimated 5,500 new HIV infections are in adults aged 15 years and older, of whom: almost 47 % are

among women .About 39 % are among young people (15-24) 6 million people died from AIDS-related illnesses. The testing capacity has increased over time; the majority of people with HIV are still unaware that they are infected. (UNAIDS, 2013).

HIV antibody tests that are simple to perform and give results in under 10 minutes are becoming increasingly used in diagnosis and for small scale screening of donor blood. Many of these tests have sensitivities and specificities equal to ELISA and can often be performed more reliably than ELISAs. Simple/rapid assays are designed for testing individual samples can be read visually without the need for a reader, are easy to perform and interpret, reagents are usually supplied ready to use, and most assays have good stability, (Chess brough, 2007).

Enzyme linked Immune Sorbent Assay are used mainly for screening blood in regional blood transfusion centers and testing samples in HIV surveillance work. The micro plate format of the ELISA enables large numbers of samples to be tested cost-effectively. ELISAs are less suitable for use in district laboratories where the number of HIV tests are usually few with less opportunity for batching of tests, power supplies (needed to run the equipment) are less stable and district laboratory staff may not have the training and experience to perform and interpret ELISA and maintain the equipment, particularly ELISA micro plate readers, (Chess brough, 2007)

1.1 Rationale

Human Immunodeficiency Virus infection has resulted in the death of several million people worldwide. Control of the spread of HIV infections is one of the major concerns of the scientific community today, (Vajdy, 2008).

ELISAs are ideal screening tests for HIV antibodies in clinical laboratories that perform large-volume batch testing, they require complex instrumentation and skilled personnel with technical expertise, and typically have a turnaround time of a few days. To overcome these limitations and to encourage more patients to be tested, advances in technology have led to the development of rapid and simpler

methods to screen for HIV antibody. Because these tests are highly sensitive, simple to perform, and provide results in less than 30 minutes, (Steven, 2010)

1.2. Objectives

1.2.1General objective

To assess the rapid test based on an immune chromatography test(ICT) in relation to ELISA in the diagnosis of HIV among screening patients.

1.2.2. Specific objectives

1. To detect HIV antibodies using ICT

2. To confirm HIV antibodies using ELISA

3. To compare the specificity and sensitivity of ICT versus ELISA.

CHAPTER TWO

Chapter Two

2. Literature Review

2.1 Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) is a retrovirus that infects humans.

a. The clinically asymptomatic phase can last 3 to 12 years.

b. It eventually leads to symptoms of disease such as opportunistic infections and other noninfectious diseases that constitute the syndrome known as acquired immune deficiency syndrome (AIDS), (Wright;2013)

AIDS is defined by the Centers for Disease Control and Prevention (CDC) as any person with HIV infection and a CD4 lymphocyte count below 200 cells/μL (or a CD4 count below 14%) or having an AIDS indicator condition, (Wright,2013)

2.2 History of HIV

AIDS was officially recognized for the first time in June ,1981 at the Center for Disease Control ,U.S.A. in previously healthy homosexual men dying with pneumocystis jiroveci pneumonia and candidiasis .Since then ,AIDS has been reported from all continents.The virus causing AIDS was independently identified by a team of French scientist led by Dr.luc Montaginer of Pasteur Institute and American scientist lead by Dr. Robert C Gallo of National Cancer Institute in 1983 - 1984 .The virus has been called by different names LAV i.e Lymphadenopathy Associated Virus by the French and HTLV lll i.e Human T Lymphotropic Virus lll by the American .The International Committee on Nomenclature of Viruses named it the ''Human Immunodeficiency Virus'' and to date two types , HIV-1 and HIV-2 are identified, (NACO, 2007).

2.3 Epidemiology

1. HIV-1. Majority of worldwide cases
    a. Group M represents 90% of human infections
        - *Subtypes*: A, B, C, D, F, G, H, J, and K

b. Groups N, O, P—Rare. West/Central Africa/Cameroon

2. HIV-2. Predominantly in West Africa

a. Lower transmission rates than HIV-1, slower disease progression. (This may be accounted for by lower viral load.), (Wright, 2013). People living with HIV in 2013, there were 35million (33.2 million – 37.2 million).

Since the start of the epidemic, around 78 million (71 million – 87 million) people have become infected with HIV and 39million (35million –43 million) people have died of AIDS-related illnesses, (UN AIDS, 2014).

New HIV infections have fallen by 38% since 2001.Worldwide, 2.1million (1.9 million – 2.4 million) people became newly infected with HIV in 2013, down from 3.4 million (3.3 million–3.6million) in 2001. New HIV infections among children have declined by 58% since 2001, (UN AIDS, 2014)

Worldwide, 240 00(210 000 – 280 000) children became newly infected with HIV in 2013, down from 580 000(530 000 – 640 000) in 2001,(UN AIDS, 2014).

AIDS-related deaths have fallen by 35% since the peak in 2005. In 2013, 1.5 million (1.4million–1.7million) people died from AIDS-related causes worldwide compared to 2.4 million (2.2 million – 2.6million) in 2005, (UN AIDS, 2014)

In 2010, in resource poor countries, 6.6 million HIV were receiving treatment, representing only 47 percent of the people eligible for treatment.

In 2012, approximately 9.7 million had access to ART in low- and middle-income countries, (UN AIDS, 2014).

2.3.1Epidemiology in Sudan

Sudan is surrounded by several countries that having high rates of AIDS, (FMOH SNAP, 2002) .

The first case of the disease reported in Sudan was in 1986, (Mahfouz, 2007)

In 2002, a survey by Sudan National AIDS Program (SNAP) found the prevalence of HIV infection among general population was 1.6%, (UNHCR, 2013)

The disease is more frequent in the age groups of 15 - 45, and 40% of the population is in this age range .In 2007 alone, the confirmed cases of the virus

reached 290,000. Of these, 4060 (1.4%) infected were adults aged 15 - 49 years old, with women constituting 59% of this age group. The estimated number of infected children less than 15 years was 25,000, and the number of deaths exceeded 25,000. These figures place Sudan at an intermediate HIV and AIDS prevalence of 1.6% Approximately 70% of Sudanese people living in the countryside have insufficient information or knowledge about the risks of HIV/AIDS .Reports of UNAIDS in 2011 showed that the prevalence of AIDS in Sudan after separation of Southern Sudan was around 0.4%), (UNAIDS, 2011).

However, recent research by UNDP's HIV/AIDS suggested that the mode of spread of the disease is continuous and the prevalence may reach 1.2% of the population by the year 2015. (UNDP Sudan, 2012)

2.4 HIV Structure

HIV is an enveloped RNA virus belonging to the lentivirus subgroup of retroviruses, which cause slowly progressing diseases, often with long incubation periods. By possessing the enzyme reverse transcriptase, retroviruses are able to reverse-transcribe RNA to DNA (normally RNA is transcribed from DNA). The DNA genome produced (provirus) becomes integrated in the DNA of the infected cell, ensuring permanent infection and replication of the virus, (Kayser *et al.* , 2005)

Structurally HIV consists of:

– An inner core containing two copies of single stranded RNA, viral enzymes and capsid protein p24 (group specific core antigen which does not vary). (Kayser et *al.*, 2005)

– Double layered lipid envelope, derived from the membrane of the host cell. The envelope contains virus specific glycoprotein's gp120 (protrudes from the surface) and gp41 (embedded in the envelope) .These enable the virus to attach to and infect host cells. The gene that encodes gp120 mutates rapidly, producing many antigenic variants. (Kayser *et al.*, 2005)

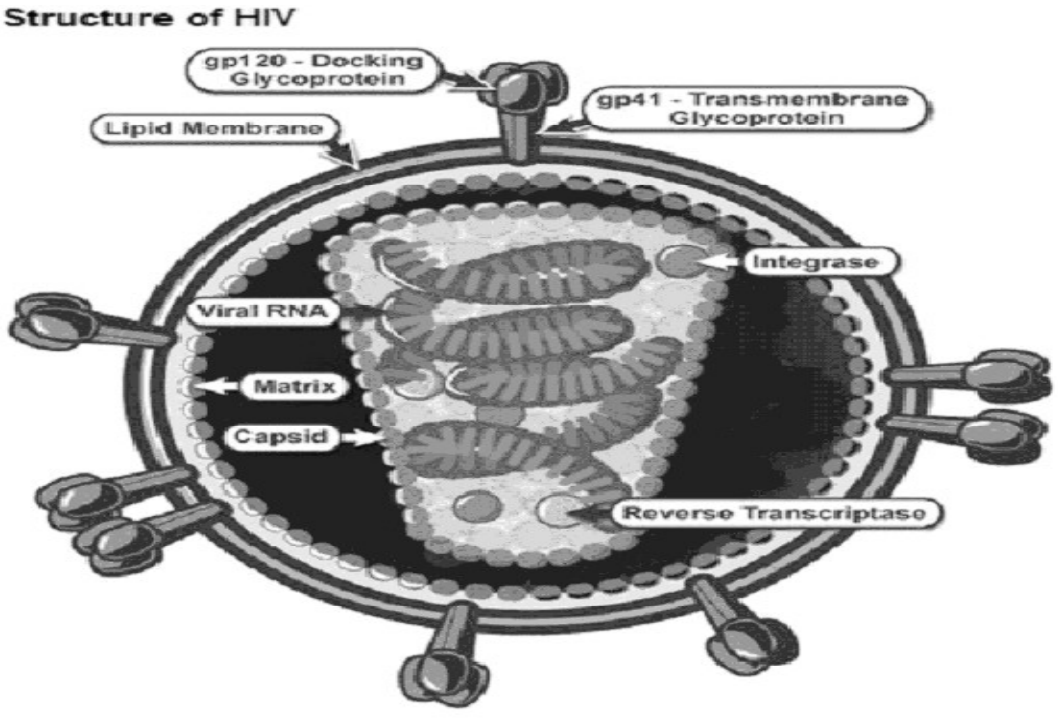

Figure (A) Structure of HIV virus http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/Pages/structure.aspx.

2.4. 1 Non infectious particle

Table (A) Genes not essential to viral replication, (Kayser *et al.*, 2005)

Genes	Function
Vif	makes the virus more infectious
Nef	inhibits or activates viral transcription as required, influences T-cell activation, reduces CD4 expression-
Vpr	Controls rate of replication.
Vpu	only in HIV 2, controls rate of replication
Vpx	only in HIV 1, contributes to viral release, increases CD4 turnover

(Kayser *et al*., 2005)

2.4 .2 HIV genome

Table (B) Show genes essential to viral replication

Genes	Function
Tat	"transactive transcription, enhances the transcription an thus the expression of viral proteins by binding to the TAR (transactivation responsive region) in the LTR
Rev	posttranscriptional activator for splicing and transport of viral mRNA (production of structural proteins)
LTR	Promoter and enhancer elements.

(Kayser *et al*., 2005)

Table (C)structural genes

Gene	Product	Function
Gag	p55	Nucleocapsid, precursor of p18, p24, p15
	p18	Matrix protein
	p24	Capsid protein
Pol		Polymerase region
	p66/51/10	Reverse transcriptase/RNase/protease
	p31	Integrase
Env	gp160	Glycoprotein, precursor of gp120, gp41
	gp120	Surface protein (binds to CD4 molecule of host cell)
	gp41	Trans membrane protein

(Kayser *et al*., 2005)

2.4.3. Human immunodeficiency virus Antigen and Antibodies

Shortly after infection with HIV, initial replication gives rise to increasing concentrations of virus in the blood and the virus becomes seeded widely

throughout the tissues of the body. A decrease in the number of CD4_ T cells is usually associated with the primary infection. Antibodies to the virus core and envelope antigens are produced (IgM first, rapidly followed by IgG). During this time approximately 50% of people develop an acute glandular fever-like illness with some or all of the following symptoms: sore throat, oral ulcers, enlarged lymph glands, fever, malaise, skin rash, and occasionally arthralgia and neurological symptoms. This is called a seroconversion illness because it occurs at a time when the infected person is first making antibodies against HIV. The seroconversion illness usually resolves in 1–3 weeks. Following seroconversion and the presence of detectable antibodies in the serum, a person is described as being seropositive, (Chessbrough , 2007)

2.5 Classification

HIV belong to the Family Reteroviridae and sub family Lentivirinae .Two types are recognized HIV-1 and HIV-2.Both differ in geographical distribution ,Biological and molecular characteristics and extent of transmissibility .These viruses store their genetic information as RNA .RNA must be converted into DNA by a special enzyme reverse transcriptase .HIV-1 has 3 groups , HIV-1 major group (HIV-1–M) ,outlier (HIV-1–O) and (HIV-1–N) .The strain of HIV-1isolated from people in U.S.A and Europe are genetically diverse from strain isolated in Africa and Asia .HIV-1major group can be further classified in to sub types designated A through K excluding I .such sub types have envelope gene sequences that vary by 20% or more between subtypes .The sub types differ in geographical description , Biological Characteristics and major mode of transmission etc. HIV-1 sub type O and N are more distant to all other HIV-1 sub types but less so compared to HIV-2. so these are classified under HIV-1 only and have limited distribution in West Africa .HIV-2 has also been reported from other countries and these also comprises of heterogeneous group of viruses . HIV-2 has been divided into 5 subtypes (A-E) India predominantly has HIV-1 M sub type C. Sub types A and B are less frequent. However western developed countries have HIV-1 M sub types predominant sub

type .Sub type C is usually acquired by Heterosexual contact and subtype B by Heterosexual,(NACO , 2007)

2.6 Stability

HIV is completely in activated($\geq 10^5$units of infectivty) by treatment for 10 minutes at room temperature with any of the following : 10% household bleach , 50% ethanol , 35% isopropanol 1% Noinder p40 0.5% lysol, 0,5 paraformaldehyde , or 0,3 % hydogen peroxide . The virus is also inactivated by extremes of pH (PH 1.0,pH 13.0). The virus is not activated by 2.5% Tween 20 . HIV is readily inactivated in liquid or 10% serum by heating at 56°C for 10 minutes, Lyophilized blood product would need to heated at 68% for 72 hours to ensure inactivation of contaminating virus , (Brooks *et al* ,2010) .

2.7 Transmission

Human Immunodeficiency virus is transmitted principally in three ways: by sexual contact, by blood (through transfusion, blood products, or contaminated needles), or by passage from mother to child. Although homosexual contact remains a major source of HIV within the United States, “heterosexual transmission is the most important means of HIV spread worldwide today .Treatment of blood products and donor screening has essentially eliminated the risk of HIV from contaminated blood products in developed countries, but its spread continues among intravenous drug users who share needles. In developing countries, contaminated blood and contaminated needles remain important means of infection. Thirteen to thirty-five percent of pregnant women infected with HIV will pass the infection on to their babies; transmission occurs in utero, as well as during birth. Breast milk from infected mothers has been shown to contain high levels of the virus also. HIV is not spread by the fecal-oral route; aerosols; insects; or casual contact, such as sharing household items or hugging. The risk to health care workers is primarily from direct inoculation by needle sticks. Although saliva can contain small quantities of the

virus, the virus cannot be spread by kissing, (Holmes , 1998).

2.8 Replication cycle

In general, the replication of HIV follows the typical retroviral cycle .The initial step in the entry of HIV into the cell is the binding of the virion gp120 envelope protein to the CD4 protein on the cell surface. The virion gp120 protein then interacts with a second protein on the cell surface, one of the chemokine receptors. Next, the virion gp41 protein mediates fusion of the viral envelope with the cell membrane, and the virion core containing the nucleocapsid, RNA genome, and reverse transcriptase enters the cytoplasm, (Levinson , 2010).

Chemokine receptors, such as CXCR4 and CCR5 proteins, are required for the entry of HIV into CD4-positive cells. The T cell–tropic strains of HIV bind to CXCR4, whereas the macrophage-tropic strains bind to CCR5. Mutations in the gene encoding CCR5 endow the individual with protection from infection with HIV. People who are homozygote's are completely resistant to infection and heterozygote's progress to disease more slowly. Approximately 1% of people of Western European ancestry have homozygous mutations in this gene and about 10% to 15% are heterozygote's. One of the best characterized mutations is the delta-32 mutation in which 32 base pairs are deleted from the CCR5 gene, (Levinson ,2010)

In the cytoplasm, reverse transcriptase transcribes the genome RNA into double-stranded DNA, which migrates to the nucleus where it integrates into the host cell DNA. The viral DNA can integrate at different sites in the host cell DNA, and multiple copies of viral DNA can integrate. Integration is mediated by a virus-encoded endonuclease (integrase). Viral mRNA is transcribed from the proviral DNA by host cell RNA polymerase (augmented by virus-encoded Tat protein) and translated into several large poly proteins. The Gag and Pol polyproteins are cleaved by the viral-encoded protease, the Env polyprotein is cleaved by a cellular protease, (Levinson , 2010)

The Gag polyprotein is cleaved to form the main core protein (p24), the matrix protein (p17), and several smaller proteins. The Pol polyprotein is cleaved to form the reverse transcriptase, integrase, and protease. The immature virion containing the precursor polyproteins forms in the cytoplasm, and cleavage by the viral protease occurs as the immature virion buds from the cell membrane. It is this cleavage process that results in the mature, infectious virion, (Levinson , 2010)

Note that HIV replication is dependent on cell proteins as well as viral proteins. First there are the cell proteins required during the early events, namely CD4, and the chemokine receptors, CCR5 and CXCR4. Cell proteins, such as actin and tubulin, are involved with the movement of viral DNA into the nucleus. The cell protein cyclin T1 and the viral protein Tat are part of the complex that transcribes viral mRNA. Cell proteins are also involved in the budding process by which the virus exits the cell, (Levinson, 2010).

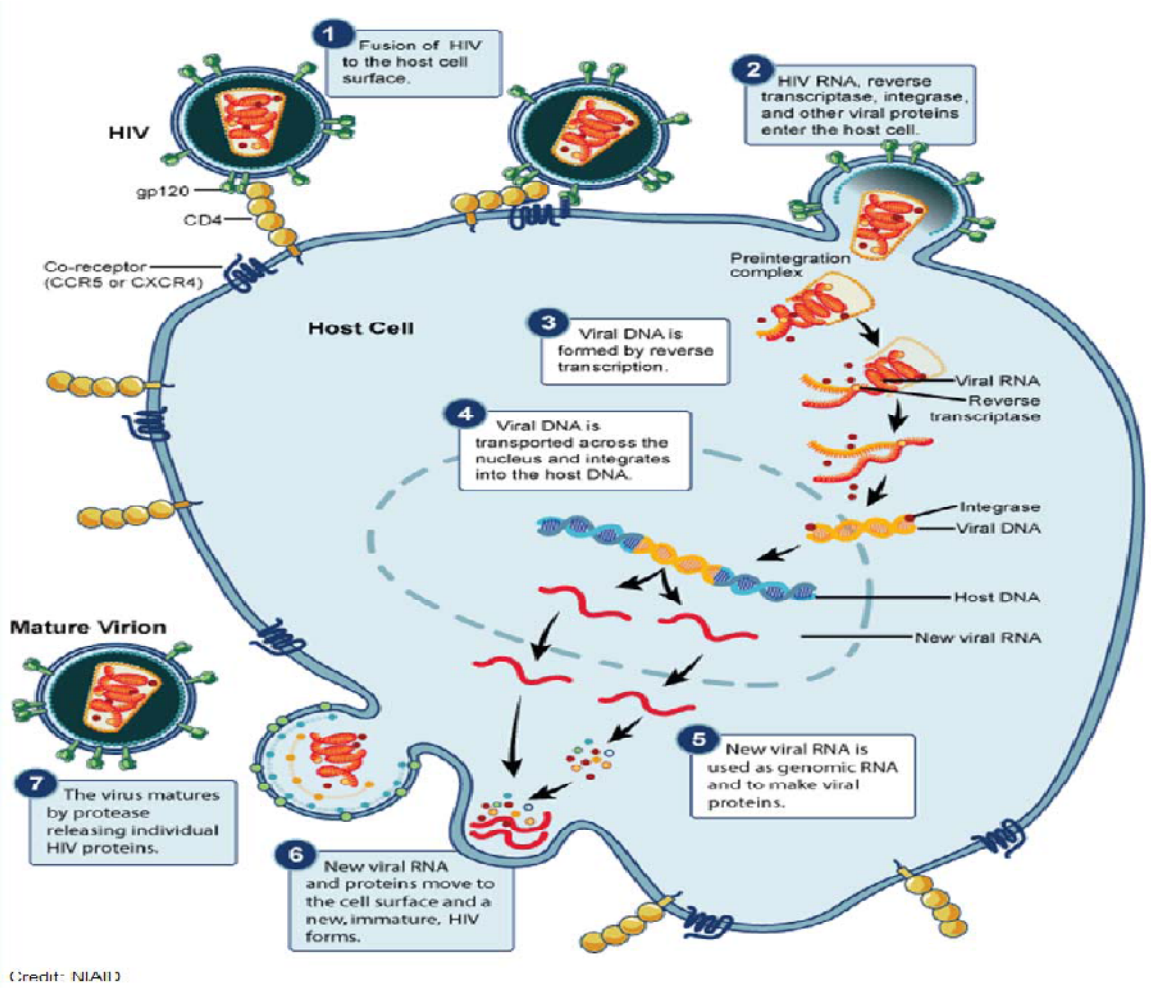

Figure (B) Diagram of HIV replication cycle

http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/Pages/hivReplicationCycle.aspx

2.9 Pathogenesis & Immune Responses

characterized because the infection typically is not diagnosed until weeks or months after exposure to the virus. However, the emerging picture is that these infections typically begin when the virus penetrates the rectal or vaginal mucosa and infects helper T cells which lie below these protective surfaces. The virus uses these cells' biosynthetic machinery to make many more copies of itself, and the newly made viruses then infect other cells. So in the early stages of infection, the virus multiplies relatively unchecked while the innate system gives it its best shot, and the

adaptive system is being mobilized. After a week or so, the adaptive system starts to kick in, and virus specific B cells, helper T cells, and CTLs are activated, proliferate, and begin to do their thing. Consequently, during this early, "acute" phase of a viral infection, there is a dramatic rise in the number of viruses in the body (the "viral load") as the virus multiplies in infected cells. This is followed by a marked decrease in the viral load as virus-specific CTLs go to work. With many viruses (e.g., smallpox), the end result of the acute phase of a viral infection is "sterilization": The immune system destroys all the invading viruses, and memory B and T cells are produced to protect against a subsequent infection by the same virus. In contrast, HIV-1 infection always leads to a "chronic" phase that can last for ten or more years. During the chronic phase of infection, viral loads decrease to low levels compared with those reached . during the height of the acute phase, but the number of virus-specific CTLs and Th cells remains high – a sign that the immune system is still trying hard to defeat the virus . However, as the chronic phase progresses, the total number of Th cells slowly decreases, because these cells are killed as a consequence of the viral infection. Eventually there are not enough Th cells left to provide the help needed by virus-specific CTLs. When this happens, the number of these CTLs also begins to decline, and the viral load increases – because there are too few CTLs left to cope with newly infected cells . In the end, the immune defenses are overwhelmed, and the resulting profound state of immunosuppression "opportunistic" infections can be lethal to an AIDS patient whose immune system has been destroyed leaves the patient open to unchecked infections by pathogens that normally would not be the slightest problem for a person with an intact immune system. Sadly, these "opportunistic" infections can be lethal to an AIDS patient whose immune system has been destroyed, (Sompayrac ,2012)

2.10 Clinical finding & Stages

2.10.1Acute phase

Also called the acute HIV syndrome, is the period of viremia characterized by nonspecific symptoms of infection. It develops in 50% to 70% of infected adults typically 3 to 6 weeks after infection. There is a spike of plasma virus and a modest reduction in CD4+ T cell counts, but the number of blood CD4+ T cells often returns to normal. In many patients, however, the infection is occult and there are no symptoms, **(**Abbas,2012) **.**

2.10.2 Chronic phase

May last for many years. During this time, the virus is contained within lymphoid tissues, and the loss of CD4+ T cells is corrected by replenishment from progenitors. Patients are asymptomatic or suffer from minor infections. Within 2 to 6 months after infection, the concentration of plasma virus stabilizes at a particular set-point, which differs among patients. The level of the viral set-point and the number of blood CD4+ T cells are clinically useful of the progression of disease. As the disease progresses, patients become susceptible to other infections, and immune responses to these infections may stimulate HIV production and accelerate the destruction of lymphoid tissues. As discussed earlier, HIV gene transcription can be enhanced by stimuli that activate T cells, such as antigens and a variety of cytokines. Cytokines, such as TNF, which are produced by the innate immune system in response to microbial infections, are particularly effective in boosting HIV production. Thus, as the immune system attempts to eradicate other microbes, it brings about its own destruction by HIV,**(**Abbas,2012)

2.10.3 AIDS phase

when the blood CD4+ T cell count drops below 200 cells/mm3**.** HIV viremia may climb dramatically as viral replication in reservoirs other than T cells accelerates unchecked. Patients with AIDS suffer from combinations of opportunistic infections, neoplasms, cachexia (HIV wasting syndrome), kidney

failure (HIV nephropathy) and CNS degeneration (AIDS encephalopathy) .Because CD4+ helper T cells are essential for both cell-mediated and humoral immune responses to various microbes, the loss of these lymphocytes is the main reason that patients with AIDS become susceptible to many different types of infections. Furthermore, many of the tumors that arise in patients with AIDS have a viral etiology, and their prevalence in the setting of AIDS reflects an inability of the HIV-infected patient to mount an effective immune response against oncogenic viruses. Cachexia is often seen in patients with chronic inflammatory diseases and may result from effects of inflammatory cytokines (such as TNF) on appetite and metabolism. The CNS disease in AIDS may be due to neuronal damage by the virus or by shed viral proteins such as gp120 and Tat, as well as the effects of cytokines elaborated by infected microglial cells. Many of these devastating consequences of HIV infection, including opportunistic infections and tumors, have been significantly reduced by highly active antiretroviral therapy.**(**Abbas,2012)

Table (c) Clinical Features of HIV Infection **.**

Acute HIV disease	Fever, headaches, sore throat with pharyngitis ,generalized lymphadenopathy, rashes
Clinical latency period	Declining blood CD4+ T cell count

Acquired Immunodeficiency Virus (AIDS)	**Opportunistic infections** **Protozoa** (Toxoplasma, Cryptosporidium) **Bacteria** (Mycobacterium avium, Nocardia, Salmonella) **Fungi** (Candida, Cryptococcus neoformans, Coccidioidesimmitis ,Histoplasmacapsulatum, Pneumocystis) **Viruses** (cytomegalovirus, herpes simplex, varicella-zoster) **Tumors** Lymphomas (including EBV-associated B cell lymphomas) Kaposi's sarcoma Cervical carcinoma ,Encephalopathy and Wasting syndrome .

(Abbas,2012)

2.11 Laboratory diagnosis of HIV

Humman Immunodeficiency Virus infections are routinely detected by serology (antibodies or viral antigen). The circulating virus count (viral load) is determined by means of quantitative RT-PCR. The AIDS diagnosis is a clinical procedure that presupposes positive confirmation of HIV infection. (Kayser, 2005)

Infection with HIV is diagnosed by the detection of HIV-specific antibodies in plasma or serum. These antibodies appear a few weeks after infection, shortly before or after the symptoms of the acute retroviral syndrome. the delay to the appearance of antibodies can be determined: about 5% of patients seroconvert within 7 days, 50% within 20 days, and more than 95% within 90 days. Therefore, a period exists (called the "window period") during which, although the patient is infected, antibodies cannot be detected in the plasma. For a few days, the HIV-

specific p24 antigen is detectable alone, without antibodies .Therefore; screening tests now combine the detection of antigen and antibody. Gene amplification tests [polymerase chain reaction (PCR), as well as other hybridization techniques] for the detection of viral genomes should not be used for early diagnosis. They are much more expensive than the antibody tests. Antibody tests remain positive for the life time of HIV-infected people, except possibly in very rare cases when treatment was started before sero conversion, **(**Southwick , 2007).

2.11.1 Screening tests

-Rapid tests.

-ELISA (Enzyme-linked immune sorbent assay)

2.11.2 Supplemental or confirmatory

-Immune fluorescent assay (IFA)

-Western blot

2.11.3 Other tests

-p24 antigen

-Plasma/ serum viral load

- PCR

2.11.4 Alternative to classical tests

-Oral fluid (saliva) HIV tests

-Urine tests (Seth , 2003)

2.12. Antigen detection

HIV virus tests are available but are very expensive. Antigen tests mainly detect HIV core antigen p24 which is found as long as it is in excess of p24 antibody. This is typically in the early stage of HIV infection (4–7 days prior to sero conversion).In most patients, HIV antigen re -appears in the late stages of HIV disease when the level of anti-p24 antibody falls. Nucleic acid tests have also been developed which enable minute amounts of viral material to be detected. Virus detection tests are mainly performed in specialist virology laboratories. They are used to diagnose HIV infection in newborn infants and to monitor viral load in

patients receiving anti-retroviral therapy. Virus testing of donor blood aimed at reducing the 'window' period is not recommended,(NYSDOH ,2014).

2.13 Antibody detection

Serological tests for HIV antibody have many important purposes. These tests have played a critical role in screening the donor blood supply to prevent transmission of the virus through blood transfusions or administration of blood products. In addition, serological tests are typically used in the initial diagnosis of HIV infection, because most individuals will develop antibody to the virus within 1 to 2 months after exposure. Serological tests are also used in epidemiology studies to provide health officials with information about the extent of the infection in high-risk populations; these groups can then be targeted for counseling, treatment, vaccine trials, and their medical or social concerns can be addressed. HIV antibody tests can be divided into two groups:

(1)screening tests, whose goal is to detect all infected persons,

(2) confirmatory tests, performed on samples giving a positive result on a screening test, to differentiate true positive from false-positive results. The standard screening method for HIV antibody has been the ELISA, and the standard confirmatory test is the Western blot. In addition to the standard tests, rapid tests have been developed that can detect HIV antibody within minutes, making them an attractive alternative to the ELISA in certain situations. Modifications of these tests, which use saliva or urine or are available as home test kits, have also been developed, (Constantine and Zink , 2005)

2.13.1 Rapid test (ICT)

While ELISA are ideal screening tests for HIV antibodies in clinical laboratories that perform large-volume batch testing, they require complex instrumentation and skilled personnel with technical expertise, and typically have a turnaround time of a few days. To overcome these limitations and to encourage more patients to be tested, advances in technology have led to the development of

rapid and simpler methods to screen for HIV antibody. Because these tests are highly sensitive, simple to perform, and provide results in less than 30 minutes, they are used throughout the world. Rapid tests are ideal for use in resource-limited settings in developing nations and in situations in which fast notification of test results is desired. For example, rapid results are important in guiding decisions to begin prophylactic therapy with antiretroviral drugs following occupational exposures, as this therapy appears to be most effective when administered in the first few hours following exposure. Other situations in which rapid tests are very beneficial include testing women whose HIV status is unknown during labor and delivery, and testing patients in sexually transmitted disease clinics or emergency departments who are unlikely to make a return visit for their test results, (Abbas ,2012)

2.13.2 Enzyme-Linked Immunosorbent Assays (ELISA)

ELISAs have been used for screening procedures for HIV, because they are easy to perform, can be adapted to test a large number of samples, and are highly sensitive and specific. Several manufacturers have developed commercial kits that are useful in screening blood products and in diagnosing and monitoring patients. Over the years, technical advances have been made in these ELISA, resulting in improved sensitivity and specificity , (Fauci and Lane ,2008) .

The first-generation of ELISAs was developed based on a solid-phase, indirect-assay system that detected antibodies to only HIV-1. In these tests, HIV antibodies in patient serum were detected after binding to a solid support coated with viral lysate antigens from HIV-1 cultured in human T-cell lines, followed by adding an enzyme-labeled conjugate and substrate. These first-generation assays may lead to false positive results caused by reactions with HLA antigens or other components and they were unable to detect antibodies to HIV-2. The second-generation ELISA These assays demonstrated improved specificity and sensitivity overall and were able to detect antibodies to both HIV-1 and HIV-2. However,

decreased sensitivity resulted when samples containing antibodies to certain subtypes of HIV that lacked the limited antigens used in the assays were tested . Third-generation assays use the sandwich technique, based on the ability of antibody to bind with more than one antigen. In this method, antibodies bind to recombinant HIV-1 and HIV-2 proteins coated onto the solid phase. After washing, enzyme-labeled HIV-1 and HIV-2 antigens are added and bind to the already bound HIV-specific patient antibodies. After substrate is added, color development is proportional to the amount of antibody in the test sample. This format improved sensitivity by simultaneously detecting HIV antibodies of different immunoglobulin classes, including IgM. Enhancements of this method have increased sensitivity further by detecting low affinity antibodies and antibodies to group O HIV-1 and the more common group M. These enhancements resulted in a diagnostic sensitivity 100 % and diagnostic specificity 99.9 %.Most recently ,fourth-generation assays have been developed that can simultaneously detect HIV-1 antibodies, HIV-2 antibodies, and p24 antigen. False-positive results may also occur in HIV-antibody ELISA tests. These can result from several factors, including heat inactivation of serum prior to testing, repeated freezing/thawing of specimens, presence of auto reactive antibodies, history of multiple pregnancies, severe hepatic disease, passive immunoglobulin administration and recent exposure to certain vaccines , (Schappert, J *et al* ,2006 and Yeom, J S *et a l*,2006).

2.14 Confirmatory Western Blot

Western blot (WB) and immunoblot (IB) tests are the most widely used supplemental or confirmatory tests for detection of anti-HIV antibodies

. Both tests, are highly specific, but in comparison with screening tests more laborious and costly. Their high specificity is mainly based on the fact that they allow determination of the reactivity of anti- HIV antibodies with particular HIV proteins. In WB, electrophoretically separated natural HIV proteins derived from whole virus lysates are transferred (blotted) to a solid membrane. HIV viral proteins on WB membrane may contain contaminating human cell proteins. In

contrast, recombinant or synthetic HIV proteins mechanically applied onto the solid membrane are used in the IB test. HIV proteins on IB membrane, however, do not contain contaminating human cell proteins and are highly specific. Briefly, in both WB and IB patient's serum is incubated with a nitrocellulose membrane strip containing separated different HIV proteins. Antibodies directed against particular HIV proteins are identified with enzyme- labeled anti-human IgG, similarly to the EIA method the presence of anti-HIV antibodies against at least one HIV protein derived from each *env*, *gag* and *pol* regions is required for anti-HIV positivity. The absence of reactivity against any of HIV proteins on membrane is considered as a negative WB result. WB results that do not meet the criteria for a positive result are considered indeterminate. (Yilmaz ,2001)

2.15 Indirect Immunofluorescence Assay

The indirect immunofluorescence assay (IFA) is another confirmatory assay. This test is generally simple to perform but the results are analyzed microscopically and require expertise for interpretation.(NYSDOH ,2014)

2.16 Poly merase Chain Reaction

Two kinds of polymerase chain reaction methods have been developed to detect HIV nucleic acid: the reverse-transcriptase polymerase chain reaction (RT-PCR) and more recently, real-time PCR. A commercial RT-PCR was the first assay for quantitative measurement of circulating HIV nucleic acid. The basic principle of this test is to amplify a DNA sequence that is complementary to a portion of the HIV RNA genome**,(Griffith, 2007)**

2.17 HIV RNA

It is therefore important to use both a plasma HIV RNA assay and an antibody test to establish the diagnosis. Low levels of virus (a commonly cited cutoff is <5000 copies/mL) may be indicative of a false-positive result and should not be considered diagnostic of primary HIV infection. Standard antibody testing should be repeated in 3 to 6 weeks. Methods used to measure plasma HIV RNA include

conventional and real-time reverse transcriptase (RT)-PCR, bDNA, and NASBA. Plasma HIV RNA levels during seroconversion do not appear significantly different in patients who have acute symptoms versus those who are asymptomatic.(NYSDOH ,2014)

CHAPTER THREE

Chapter Three

3. Materials and Methods

3.1Study design

This was a prospective, descriptive and cross sectional study

3.2. Study area

This study was conducted in National Public Health Laboratory Khartoum state.

3.3. Study population

Screening patients attending virology lab at in National Public Health Laboratory.

3.4. Study duration

Study was carried out during 3month period from 1 April to 30 June 2015

3.5. Ethical consideration

Permission to carry out the study was obtained from the Collage of Graduate Studies , Sudan University of Science and Technology .All screening patients examined were informed for the purpose of the study before collection of the of the samples and the verbal consent was taken from them .

3.6. Sample size

A total of eighty nine subjects (n=89) were enrolled in this study.

$$\mathbf{n}=\frac{(\boldsymbol{Z}^2\times\mathbf{P}\ (\mathbf{1}-\mathbf{P}))}{\mathbf{e}^2}$$

Z= standard normal distribution corresponding to desired confidence level

(1.96 for 95% confidence level)

e=desired population

p= expected true proportion

3.7. Data collection

Personal and clinical data were collected from by direct interviewing questionnaire from each subject (Appendix)

3.8. Sample collection

Under a septic condition after wearing the gloves, alcohol antiseptic (70% ethanol) was used to clean the skin .Venous blood (3mls) was obtained from screening patients. Samples were collected by vein puncture and haemlysis was avoided. Serum was collected into the collection tubes (did not contains anticoagulant) and left to settle for 30 minutes in the rack for blood coagulation and then was centrifuged at 3000 rpm for 5minutes to get serum specimen supernatant .If serum specimen were not immediately tested they were kept at -20 ºC till used.

3.9. Laboratory methods

3.9.1. Immunochromatography test (ICT)

3.9.1.1Principle of the test

BioTracer HIV 1\2 O Rapid card test is based on the principle of Immunochromatography in vitro test for the qualitative determination of antibody against HIV. When the sample is added to sample pad, it moves to the conjugate pad and re suspend HIV antigen conjugated gold complex that is dried on the conjugate pad .The mixture moves along the membrane by capillary action and react with HIV antigen that is immobilized the test reaction zone .If antibody against HIV is present enough in the sample, a colored band in the test reaction zone appeared .If there is no antibody against HIV or not sufficient in the sample, the area will remain

colorless. The sample continues to move to the control reaction zone and form red or purple color, indicating the test is working properly and the result is valid.

3.9.1.2. Storage and stability

BioTracer HIV 1\2O Rapid card should be stored at 1-30 ºC (34- 86 F). The test device is sensitive to humidity as well as heat. Perform the test immediately after removing the device from the device pouch .Do not uses it beyond the expiration date, 24 month from manufacturing.

3.9.1.3. Procedure of the test

The test devices and specimens allowed to room temperature before removed from the pouch prior to use.

Step1 The test devised was removed from the sealed pouch, and placed on a clean and flat surface .

Step2 10µl of serum was transferred in to the sample well(s) and 4 drops (100 µl) of sample diluent was added in to the sample wells.

Step3 As the test work was begun; purple (red) color was seen moved across the result window in the center of test devised

Step4 At 5 -20 minutes the test result was interpreted and the test result was not interpreted after 20 minutes.

3.9.1.4. Interpretation of the results

A color band will appear at the control reaction zone (C) of the result window to show that the test is working properly.

A color band of the test reaction zone (1and 2) indicate the positive test result .

Negative:

The presence of only one band of the control reaction zone (C) indicates a negative result

Positive:

In addition to band in the control reaction zone (C), any other band appear in the test reaction zone (1and/or 2), indicated the specimen contain HIV 1/ 2 O antibodies

Invalid:

If no band appear in the result window after performing the test, the result is considered invalid and re- tested the sample with a new device.

3.9.2. Detection of HIV by capture Enzyme Linked Immunosorbant Assay (ELISA)

3.9.2.1. Principle of ELISA (appendix)

Murex HIV Ag/Ab Combination is based on micro wells coated with a synthetic peptide representing an immunodominant region of HIV-1 (O),recombinant protein derived from the envelope proteins of HIV-1 and HIV-2 and an HIV pol protein and monoclonal antibodies raised against p24 of HIV-1. Then conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labeled with horseradish peroxidase. Test specimens and control sera are incubated in the wells and reactive HIV core and/or antibodies to HIV in the sample or control sera bind to the antibodies and/or antigens on the micro well; sample and any excess antibodies are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV core and/or specific antibody already bound to the reagents on the well. Samples not containing reactive core antigen or specific antibody will not cause the conjugate to bind to the well.Unbound conjugate is washed away and a solution containing 3, 3', 5, 5'tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound conjugate

develop a blue green color which is converted to an orange color which read at 450nm after the reaction has been stopped with sulphuric acid.

3.9.2.2. Storage and stability of ELISA

All components must be stored at 2 to 8°C, unless otherwise stated, under which condition they will retain activity until the expiry date of the kit.

3.9.2.3. Procedure of the test

Step 1 The conjugate was Reconstituted and mixed, the substrate solution and Wash Fluid was prepared.

Step 2 The number of wells required for the test only used.
Touching the tops or bottoms of the wells were avoided.
Step 3 25 µl of Sample diluent was added to each well.
Step 4 100 µl of samples or 100 µl controls was added to the corresponding wells.
For each plate the first column of wells was used for the assay controls. The controls added to the designated wells after the samples were dispensed .100 µl of the negative Control into each of three wells A1to C1 was Pipetted and 100 µl of the p24, anti-HIV-1 and HIV-2 positive Controls into wells D1 to F1 respectively.
White background was used to aid visualization of sample addition.
Step 5 The wells were covered with the lid and incubated for 60 minutes at 37°C.
Step 6 At the ended of the incubation time the plate was washed 3times with washing machine .
Step 7 Immediately after the plate was washed, 100 µl of conjugate were added to each well.
Step 8 The wells were covered with the lid and incubated for 30 minutes at 37°C.
Step 9 The plate was washed washed 3times with washing machine at the end of the incubation time.
Step 10 Immediately after the plate was washed, 100 µl of substrate Solution were added to each well.

Step 11 The wells was covered with the lid and incubated for 30 minutes at 37°C. Kept away from direct sunlight. A blue green color was developed in wells containing reactive samples.

Step 12 50 µl of stop solution were added (0.5M to 2M sulphuric acid) to each well.

3.9.2.4. Reading of the result

Within 15 minutes the absorbance was read at 450 nm, using 620 nm to 690nm as the reference wavelength if available.

The instrument was blanked on air (no plate in the carriage)

3.9.2.5. Calculation of cut –off value

The Cut-off value was calculated by adding 0.150 to the mean of the Negative Control replicate

Cut Off value (C.V) = N.C+0.150

3.5.9.2.6. Quality control range

Results of an assay are valid if the following criteria for the Controls are met:

Negative Control

The mean absorbance is less than 0.15.

Positive Controls

The absorbance of each of the Positive Controls is more than 0.8 above the mean absorbance of the Negative Control.

Assays which do not meet these criteria should be repeated

3.5.9.2.7. Interpretation of the result

Non-reactive Results

Samples giving an absorbance less than the cut-off value are considered negative in the assay.

Reactive Results

Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay

3.9.2.8. Performance characteristic

3.9.2.8.1. Diagnosis of specificity

Diagnosis of specificity was calculated as follows:

$$\text{Specificity} = \frac{\text{true}\,(-\text{ve})}{\text{true}\,(-\text{ve}) + \text{false}\,(+\text{ve})} \times 100$$

3.9.2.8.2. Diagnosis of sensitivity

Diagnosis of sensitivity was calculated as follows:

$$\text{Sensitivity} = \frac{\text{true}\,(+\text{ve})}{\text{true}\,(+\text{ve}) + \text{false}\,(-\text{ve})} \times 100$$

3.9.2.8.3 Positive Predictive Value

Diagnosis of Positive Predictive Value was calculated as follows:

$$\text{Positive Predictive Value} \quad \frac{\text{true}\,(+\text{ve})}{\text{true}\,(+\text{ve}) + \text{False}\,(+\text{ve})} \times 100$$

3.9.2.8.4 Negative Predictive Value

Diagnosis of Negative Predictive Value was calculated as follows:

$$\text{Negative Predictive Value} \quad \frac{\text{true}\,(-\text{ve})}{\text{true}\,(-\text{ve}) + \text{False}\,(-\text{ve})} \times 100$$

3.10. Data analysis

Statistical package of social science (SPSS version 21.0). Computer soft ware was used for data analysis .Significant level were set at ($P<0.05$).

Figures were performed by using Microsoft Office and excel soft ware program.

CHAPTER FOUR

Chapter Four

4. Results

A total of eighty nine (n=89), volunteers were tested for HIV Abs .The age grouped into three groups 20 to 80(20 – 40) , (41- 60) and (61 – 80) with a mean age of 50 ± SD 12.17 years , 45males and 44females .The seroprevelance of HIV revealed from ICT test 14/89,(15.7 %),where it was 13/89, (14.6 %) when tested by ICT and ELISA procedures.

4.1 Distribution of screening patients according to gender

Table (4.1) and Fig (4.1) show that the percentage of male in screening patients 45/89 (50.6)% higher than female 44/89 (49.4)% .positive cases of HIV infection was 8/45 (9)% among male and 5/44, (5.6)% among female ,when examined by ELISA .

Table 4.1:Male and Female HIV positive and Negative when tested by ELISA

Gender	ELISA		Total
	Positive	Negative	
Male	8 (9.0)%	37 (41.6)%	45 (50.6)%
Female	5 (5.6)%	39 (43.8)%	44(49.4)%
Total	13(14.6)%	76 (85.4)%	89(100.0)%

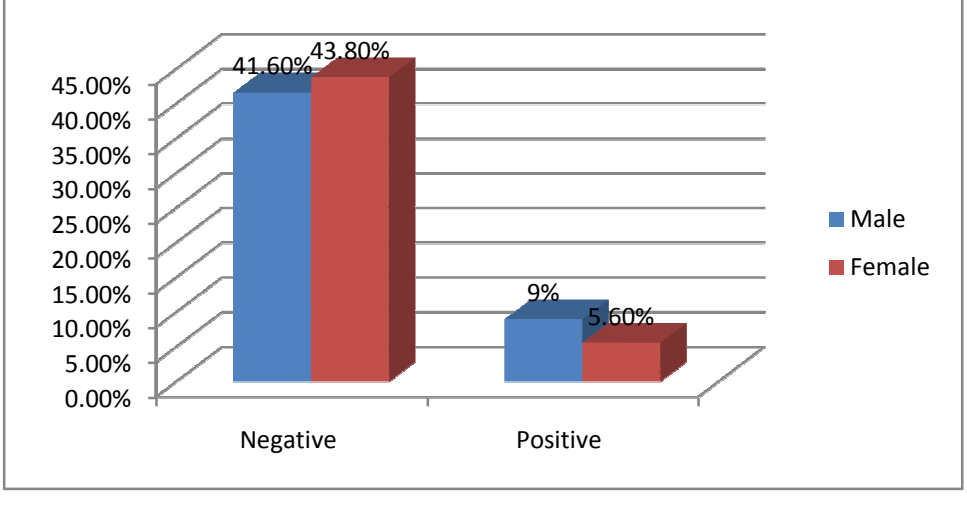

Fig 4.1:Male and Female HIV positive and Negative when tested by ELISA

Table 4.2:Male and Female HIV positive and Negative when tested by ICT

Gender	ICT		Total
	Positive	Negative	
Male	7 (7.9)%	34 (38.2)%	41 (46.1)%
Female	7 (7.9)%	41 (46.1)%	48(53.9)%
Total	14(15.7)%	75 (84.3)%	89(100.0)%

4.2 Distribution of screening patients according to marital status

Table (4.2) revealed that the percentage of married screening patients 55/89 (61.8)% higher than single 34/89(38.2)% .positive cases of HIV infection was 7/55(7.9)% among married and 6/34(6.7) % among single ,were examined by ICT and ELISA .

Table 4.2:Single and Married HIV positive and Negative by ICT and ELISA

Marital Status	ELISA and ICT		Total
	Positive	Negative	
Married	7(7.9)%	48(53.9)%	55(61.8)%
Single	6(6.7)%	28(31.5)%	34(38.2)%
Total	13(14.6)%	76(85.4)%	89(100.0)%

4.3 Distribution of screening patients according to Age groups

Table (4.3) and Fig (4.2) show that out of eighty nine screening patients included in this study 50 (56.2) % were among (20 – 40)years, 32(36)% among (41- 60) year and 7(7.9) % among (61 – 80)

Table 4.3:Age groups HIV positive and Negative by ICT and ELISA

Age	ELISA and ICT		Total
	Positive	Negative	
(20-40)	8(9.0%)	42(47.2%)	50(56.2%)
(41-60)	4(4.5%)	28(31.5%)	32(36.0%)
(61-80)	2(2.2%)	5(5.6%)	7(7.9%)
Total	14(15.7)%	75 (84.3)%	89(100.0)%

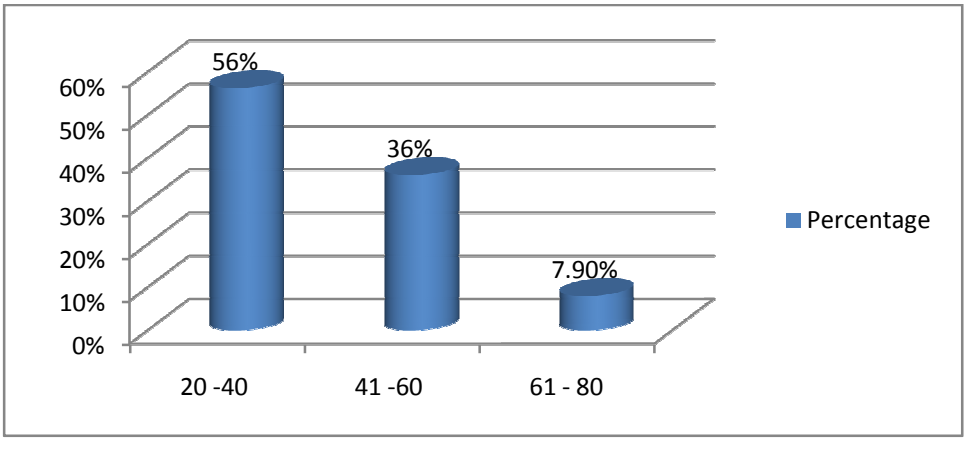

Fig 4.2:Age groups distribution

4.4 The effect of previous blood transfusion on HIV infection

The Result summarized in table (4.4) demonstrated that total of previous blood transfusion .only 7 % who had positive for HIV when tested by ICT and ELISA.

Table 4.4:previous blood Transfusion HIV positive and Negative ICT and ELISA

Blood Transfusion	ELISA and ICT		Total
	Positive	Negative	
Yes	7(7.9)%	34(37.1)%	41(46.1)%
No	6(6.7)%	42(48.3)%	48(55.1)%
Total	13(14.6)%	76(85.4)%	89(100.0)%

4.5 The effect of previous surgical operation on HIV infection

The Result summarized in Table (4.4) demonstrated that total of previous surgical operation**.** Only 7 % who had positive for HIV when tested by ICT and ELISA.

Table 4.5:previous Surgical operation HIV positive and Negative ICT and ELISA

Surgical operation	ELISA and ICT		Total
	Positive	Negative	
Yes	7(7.9)%	33(37.1)%	40(44.9)%
No	6(6.7)%	43(48.3)%	49(55.1)%
Total	13(14.6)%	76(85.4)%	89(100.0)%

4.6 ICT and ELISA Result

Table (4.5) and Fig (4.3) revealed that the same screening patient when tested by ICT showed 14(15.7%) were positive HIV Ab and by ELISA showed 13(14.6%) were positive HIV Ag /Ab .

Table 4.6:ICT and ELISA HIV positive and Negative

ELISA Result	ICT Result		Total
	Positive	Negative	
Positive	12(13.5%)	1(1.1%)	13(14.6%)
Negative	2(2.2%)	74(83.1%)	76(85.4%)
Total	14(15.7%)	75(84.3%)	89(100%)

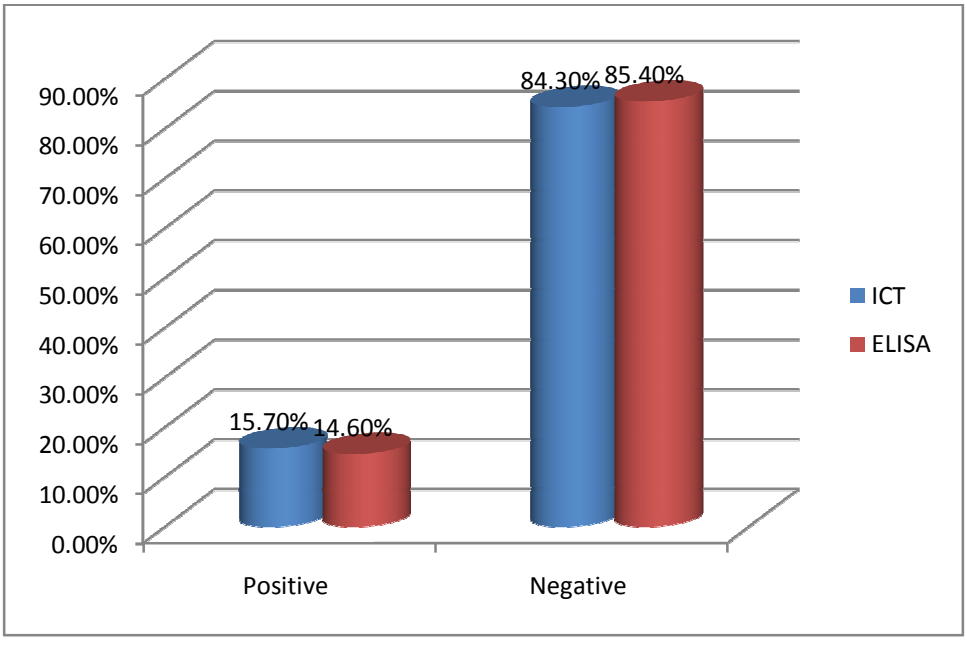

Fig 4.3:ICT and ELISA HIV positive and Negative

4.7. Table of Sensitivity and specificity of ICT and ELISA test

	ICT		ELISA	
ELISA	Disease Present	Disease absent	Disease Present	Disease Absent
Test positive	14	0	13	0
Test negative	0	75	0	76

Sensitivity and specificity of ICT

$$\text{Sensitivity} = \frac{\text{true}(+\text{ve})}{\text{true}(+\text{ve}) + \text{False}(-\text{ve})} \times 100 = \frac{13}{13+0} \times 100 = 100\%$$

$$\text{Specificity} = \frac{\text{true}(-\text{ve})}{\text{true}(-\text{ve}) + \text{False}(+\text{ve})} \times 100 = \frac{75}{75+1} \times 100 = 98.6\%$$

Sensitivity and specificity of ELISA

$$\text{Sensitivity} = \frac{\text{true}(+\text{ve})}{\text{true}(+\text{ve}) + \text{False}(-\text{ve})} \times 100 \quad \frac{13}{13+0} \times 100 = 100\%$$

$$\text{Specificity} = \frac{\text{true}(-\text{ve})}{\text{true}(-\text{ve}) + \text{False}(+\text{ve})} \times 100 \quad \frac{76}{76+0} \times 100 = 100\%$$

4.8 Predictive Value Of ICT and ELISA

ICT Positive Predictive Value

$$\frac{\text{true}(+\text{ve})}{\text{true}(+\text{ve}) + \text{False}(+\text{ve})} \times 100 = \frac{13}{13+1} = \frac{13}{14} \times 100 = 92.8\%$$

ICT Negative Predictive Value

$$\frac{\text{true}(-\text{ve})}{\text{true}(-\text{ve}) + \text{False}(-\text{ve})} \times 100 = \frac{76}{76+0} \times 100 = 100\%$$

ELISA Positive Predictive Value

$$\frac{\text{true (+ve)}}{\text{true (+ve)+ False (+ve)}} \times 100 = \frac{13}{13+0} = \frac{13}{13} \times 100 = 100\%$$

ELISA Negative Predictive Value

$$\frac{\text{true (}-\text{ve)}}{\text{true (}-\text{ve)+ False (}-\text{ve)}} \times 100 = \frac{76}{76+0} \times 100 = 100\%$$

CHAPTER FIVE

Chapter Five

5. 1 Discussion

Early and accurate diagnosis of human immunodeficiency virus (HIV) infection is essential for timely identification of patients needing antiretroviral therapy and for instituting HIV prevention strategies. The primary methodology for HIV testing has shifted from enzyme linked immune sorbent assay (ELISA) to rapid diagnostic tests RDTs ICT as example, in recent years

A recent study has also pointed out the role of variation in specificity of HIV RDTs over time and geographic location as a possible cause of higher than previously encountered false positive HIV results .

This study done to assessment the Immunochromatography test which detect (Ab) and ELISA fourth generation which detect (Ab/Ag p24) of HIV in screening patients.

In this study was found that It showed that ICT and ELISA had 100% sensitivity to HIV antibody but , however the specificity of was ICT was 98.6%where as that obtained by ELISA was 100% These results agree with these study obtained by (Iqbal *et a*l., 2012) and (Meda *et al.* ,1999) in India . However , the result are not in agreement with (Mehra et *al.*, 2014) .

According to the different age groups , group 1(20-40) is the most group have HIV disease 8(9.0%) patients agree to Study in Indian done by (kurapati *et al* ,2012) may be due partner sexual and homosexual .

The study revealed that the percentage of married screening patients 55/89 (61.8)% higher than single 34/89(38.2)% .positive cases of HIV infection was 7/55(7.9)% among married and 6/34(6.7) % among single agree to Study in Indian done by (kurapati *et al* ,2012) showed of a total 319of blood donors were HIV positive , 145 were married and 155were single so the disease among single higher than the married people. Regard to positive result between

gender also this previous study show 244 were male and 75 female were agreement compare to my study the percentage of male in screening patients 45/89 (50.6)% higher than female 44/89 (49.4)% .positive cases of HIV infection was 8/45 (9)% among male and 5/44, (5.6)% among female .The study showed that gender may be one factor in reactive positive result of HIV by both techniques , this is agree with (kurapati *et al* ,2012) study ,marital status is another risk factor especially in single group due to freedom in adult group .

Previous blood transfusion is another and important risk factor for HIV infection in this study also agree with Study done by (kurapati *et al* ,2012).

5.2Conclusion

The prevalence of HIV in screening patients in Khartoum state was 14.6%.

It was conducted that ELISA was more sensitive than ICT .but both tests had 100% specificity.

In this study, it was concluded that ICT was sensitive enough but not specific to confirm the HIV status of a screening patients.

5.3. Recommendation

1. More studies are required, using large sample size from different hospitals to acquire more accurate result.

2. ELISA technique could be used as screening test to detect presence of HIV Ab and HIV Ag /Ab, and confirmed by Western Blot Technique and PCR to detect HIV –Ag to compare the sensitivity and specificity.

3. Employing more confirmatory test e.g RT- PCR to compare between the sensitivity and specificity.

4.General surveillance through mass screening to identify those with infection occult .

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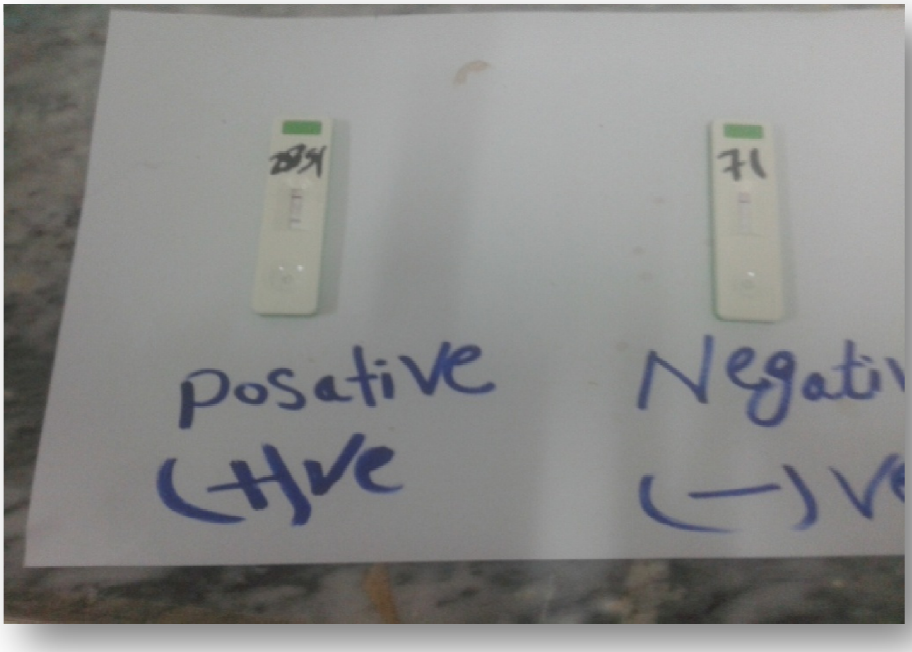
Appendix (1) Immunochromatography Rpid device image

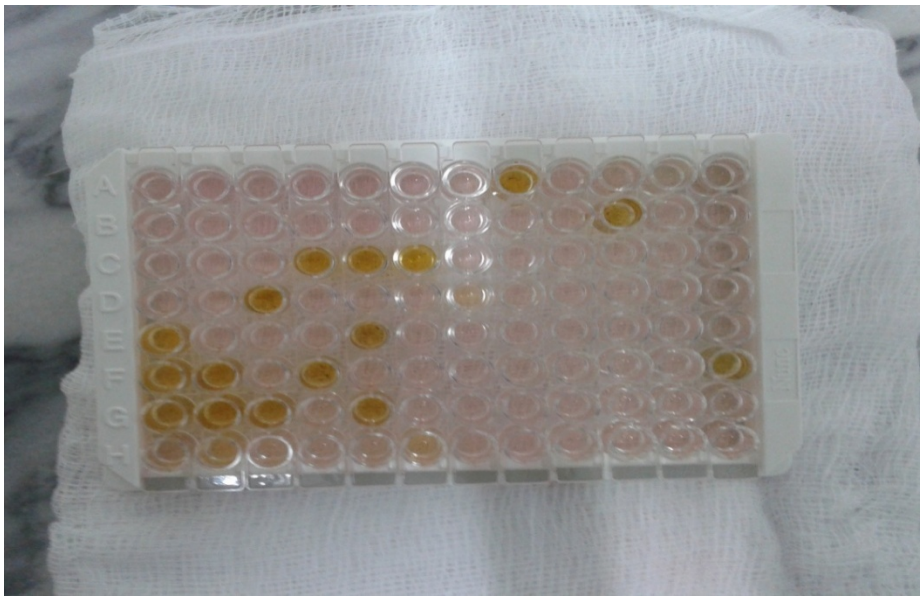


Appendix (2) Immunochromatograph Result

Appendix (3**) ELISA KIT image**

Appendix **(4) ELISA washer**

Appendix **(6) ELISA Reader**



Appendix **(6) ELISA Result**

Appendix

SUDAN University of Science and Technology

Collage of graduate studies

Microbiology Department

Questionnaire

Serological detection of HIV infection among HIV screening patients

Date…/…/2015 **questionnaire NO……**

Name of pt……..

Sex:

Male ☐ **Female** ☐

Marital status :

Single ☐ **married** ☐

Age :

…….

Yes or No :

Previous blood transfusion

YES ☐ **NO** ☐

Previous surgical operation

YES ☐ **NO** ☐

Test required : **HIV.**

Result:

ELISA……. **ICT……**

BioTracer™ HIV 1/2/O Rapid Card

IVD For in vitro diagnostic use only

※ Please read the Instructions for use carefully.

INTENDED USE

The **BioTracer™ HIV 1/2/O Rapid Card** is a chromatographic immunoassay for the qualitative detection of antibodies against HIV 1/2/O in serum, plasma or whole blood.

EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is a causative agent of Acquired Immune Deficiency Syndrome(AIDS) and is mainly transmitted by sexual contact. AIDS has been widespread over the world these days since HIV was discovered from Africa at first on 1981. There is 2 types of HIV, that is, HIV 1 and HIV 2. And then it is important to detect antibody against the HIV 1/2/O type for diagnosing whether the virus is infected or not. Because HIV is not an endogenous retrovirus in humans, the presence of antibodies against HIV is acceptable evidence of infection or prior exposure to this virus. It is now known that HIV is an enveloped virus that contains a positive strand RNA.

PRINCIPLE OF THE PROCEDURE

BioTracer™ HIV 1/2/O Rapid Card is based on the principle of an immunochromatography *in vitro* test for the qualitative determination of antibody against HIV. When the sample is added to sample pad, it moves to the conjugate pad and resuspends HIV antigen-conjugated gold complex that is dried on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with HIV antigen that is immobilized the test reaction zone. If antibody against HIV is present enough in the sample, a colored band in the test reaction zone is appeared. If there is no antibody against HIV or not sufficient in the sample, the area will remain colorless. The sample continues to move to the control reaction zone and forms a red or purple color, indicating the test is working properly and the result is valid.

COMPOSITION

1. Test devices
2. Instructions for use
3. Sample diluent

SPECIMEN COLLECTION AND PREPARATION

1. Whole blood specimen collection
   1) Whole blood is collected in syringe or evacuated tube containing the anticoagulant.
   2) Whole blood specimens should be tested immediately after collection. In the case of storing at 2~8°C, it should be tested within 24 hours.
2. Plasma / Serum specimen collection
   1) Plasma or serum specimens should be tested immediately after collection.
   2) Do not leave the specimens at room temperature for prolonged period. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.

※ Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

TEST PROCEDURE

Allow the test devices and specimens to room temperature before removal from the pouch prior to use.

1. Remove the test device from the sealed pouch, and place it on a clean and flat surface.
2. Transfer **10ul of serum, plasma or 20ul of whole blood** into the sample well(S). And then add 4drops(100ul) of sample diluent into the sample well(S). On treating the whole blood, it should be transfered that sample and sample diluent into the sample well(S) almost simultaneously, otherwise, it may cause clogging of the sample well(S).
3. As the test begins to work, you will see purple(red) color move across the result window in the center of the test device.
4. **Interpret the test result at 5~20 minutes.** Do not interpret the test result after 20 minutes.

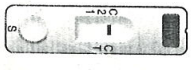
※ Caution: Perform the test immediately after removing the test device from the foil pouch.

READING AND INTERPRETATION OF RESULT

1. A color band will appear at the control reaction zone(C) of the result window to show that the test is working properly.
2. A color band of the test reaction zone(1 and 2) indicates the test result.

NEGATIVE:

The presence of only one band of the control reaction zone(C) indicates a negative result.



POSITIVE :

In addition to band in the control reaction zone(C), any other band will appear in the test reaction zone(1 and/or 2). This indicates that the specimen contains HIV1/2/O Antibodies.

INVALID :

If no band appears in the result window after performing the test, the result is considered invalid. Retest the sample with a new device.

FOLLOW-UP ACTION

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

LIMITATIONS OF THE METHOD

A negative result does not rule out infection by HIV because the antibodies to HIV may be absent in window period or may not be present in sufficient quantity to be detected at early stage of infection. The samples considered positive should be tested again with ELISA, western blot or other confirmatory test for final judgment.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

A study was performed using 755 positive and negative serum specimens. Each specimen was assayed with the **BioTracer™ HIV 1/2/O Rapid Card** and a commercially available HIV test (ELISA) according to the respective instructions for use.

The results are summarized in the following tables.

		BioTracer™ HIV 1/2/O Rapid Card		Total
		Positive	Negative	
ELISA	Positive	425	0	425
	Negative	1	329	330
Total		426	329	755

The sensitivity is more than 99.9 % (425/425) and the specificity is 99.7 % (329/330).

2. Reproducibility

1) Within run performance test was determined by one analyst with ten devices each of 3 different lots for 4 different positive specimens. There was no variation within the test devices of each lot and between three different lots.

2) Between run performance test was determined by three analyst with 3 different lots for 4 different positive specimens. There was no variation between different analyst.

WARNING AND PRECAUTIONS

1. For professional in vitro diagnostic use only.
2. Do not eat or smoke while handling specimens.
3. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4. Avoid splashing or aerosol formation.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. Decontaminate and dispose all specimens, reaction kits and potentially contaminated materials, and they should be discarded in a biohazard container.
7. Do not use the test kit if the packing is damaged.

STORAGE AND SHELF-LIFE

BioTracer™ HIV 1/2/O Rapid Card should be stored at 1-30°C (34-86°F). The test device is sensitive to humidity as well as to heat. Perform the test immediately after removing the test device from the foil pouch. Do not use it beyond the expiration date, 24months from manufacturing.

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Date issued : 2014. 10

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(주)바이오포커스

2. Instructions for use

DiaSorin The Diagnostic Specialist

en

REF 7G79-09 / 11
GE41/42

Revised September, 2014

Murex HIV Ag/Ab Combination

Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies

The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection.

Customer Service
For additional product information, please contact your local customer service organization.

This instructions for use must be read carefully prior to use. The instructions for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

IVD

Key to symbols used

REF	List Number	IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number	2°C 8°C	Store at 2-8°C
	Expiration Date		CAUTION: Consult accompanying documents
	Manufacturer		Consult instructions for use

* See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

INTENDED USE

Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies.

The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection.

SUMMARY AND EXPLANATION OF THE TEST

Two types of human immunodeficiency virus, HIV-1 and HIV-2, have been described and implicated as causative of the Acquired Immunodeficiency Syndrome (AIDS). Both are retroviruses which are transmitted by exposure to certain infected body fluids, primarily blood and genital secretions, and by transplacental passage. Infection by HIV-1 has been reported worldwide; HIV-2 infection has been reported as occurring mainly in West African and some European countries[1].

The two types of virus show substantial antigenic cross reactivity in their gag and pol proteins, but the envelope glycoproteins are less cross reactive.

It is necessary for screening purposes to use epitopes from the envelope proteins of both viruses in addition to major cross reacting gag or pol proteins to ensure detection of antibodies against both types of virus at all stages following infection[2,3]. Variants of HIV-1, classified together as group O, have been identified in samples from Cameroon and Europe[4,5]. Group O is highly divergent from the originally known subtypes of HIV-1 (together classified as group M). Specific epitopes from the envelope region of this virus can be used to detect antibody to group O in infected individuals; reliance on cross reactions to the known subtypes of HIV is not satisfactory[6]. The earliest specific antibody response following infection by HIV may be of immunoglobulin M (IgM) followed by a response in immunoglobulin G (IgG)[7]. Maximum sensitivity for detection of anti-HIV seroconversion is achieved by assays which respond to both IgM and IgG whilst HIV core antigen is typically detectable during a short period prior to antibody seroconversion.

Murex HIV *Ag/Ab Combination* is designed to detect reactive HIV core antigen in addition to IgG, IgM and IgA to the envelope glycoproteins and the cross reacting pol proteins of HIV-1 and HIV-2. Consequently potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE

Murex HIV Ag/Ab Combination is based on microwells coated with synthetic peptide representing immunodominant regions of HIV-1 (O) and HIV-2, recombinant protein derived from the envelope regions of HIV-1 and HIV-2 and HIV pol protein, together with monoclonal antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horseradish peroxidase.

Test specimens and control sera are incubated in the wells and reactive HIV core and/or antibodies to HIV in the sample or control sera bind to the antibodies and/or antigens on the microwell; sample and any excess antibodies are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV core and/or specific antibody already bound to the reagents on the well. Samples not containing reactive core antigen or specific antibody will not cause the Conjugate to bind to the well.

Unbound Conjugate is washed away and a solution containing 3,3',5,5'-tetramethyl benzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue green colour which is converted to an orange colour which may be read at 450nm after the reaction has been stopped with sulphuric acid.

REAGENTS

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions.**

All components must be stored at 2 to 8°C, unless otherwise stated, under which condition they will retain activity until the expiry date of the kit.

COATED WELLS

1. Coated Wells

One plate (7G79-09) or five plates (7Gr9-11) of 96 microwells coated with HIV antigens and monoclonal antibodies.

Allow the wells to reach room temperature (18 to 30°C) before removal from the bag.

Place unused wells in the sealable storage bag provided and return to 2 to 8°C.

SAMPLE DIL

2. Sample Diluent

One bottle containing 8 ml (7Gr9-09) or 48 ml (7G79-11) of a green/brown buffer solution, bovine and murine protein, detergent and saponin. Contains 0.06% ProClin® 300 preservative.

CONJUGATE

3. Conjugate

One bottle (7G79-09) or three bottles (7Gr9-11) containing 11 ml of HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.

CONJUGATE DIL

4. Conjugate Diluent

One bottle (7G79-09) or three bottles (7Gr9-11) containing 22ml of a yellow solution consisting of buffer, bovine protein, saponin and detergent, sufficient to reconstitute one bottle of Conjugate. Contains 0.1% ProClin® 300 preservative.

Reconstitution of Conjugate

Tap the bottle of Conjugate gently on the bench to remove any material adhering to the rubber stopper. Pour the whole contents of the bottle of conjugate diluent into the bottle of conjugate, recap the latter and mix by gentle inversion. Allow to rehydrate for at least 30 minutes with occasional swirling. The reconstituted conjugate will be red in colour. Reconstituted conjugates may be returned to and pooled in the plastic conjugate diluent bottles if required.

After reconstitution the Conjugate may be stored at 2 to 8°C for up to four weeks.

CONTROL 1 +

5. Anti-HIV-1 Positive Control

One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.06% Bronidox® preservative.

CONTROL 2 +

6. Anti-HIV-2 Positive Control

One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.06% Bronidox® preservative.

CONTROL p24 +

7. HIV-1 p24 Positive Control

One bottle containing 1.7 ml of p24 (recombinant antigen) in a buffer containing bovine protein. Contains 0.06% Bronidox® preservative.

CONTROL −

8. Negative Control

Two bottles containing 2.6 ml of normal human serum diluted in a bovine protein buffer. Contains 0.05% Bronidox® preservative.

SUBSTRATE DIL

9. Substrate Diluent

One bottle containing 35 ml of a colourless solution of tri-sodium citrate and hydrogen peroxide.

SUBSTRATE CONC

10. Substrate Concentrate

One bottle containing 35 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and stabilisers in an orange solution.

Substrate Solution

To prepare the Substrate Solution add a volume of colourless Substrate Diluent to an equal volume of orange Substrate Concentrate in either a clean glass or plastic vessel.

It is important that this order of addition is followed and that any pipettes and glassware used to prepare Substrate Solution are clean. Alternatively, the Substrate Solution may be made by pouring the entire contents of the bottle of Substrate Diluent into the bottle of Substrate Concentrate. One bottle of Substrate Solution provides sufficient reagent for at least five plates - see **Table 1:**

Table 1

Volume of Substrate Concentrate and Substrate Diluent Required

Number of Wells											Number of Plates			
8	16	24	32	40	48	56	64	72	80	96	1	2	3	4
Substrate Concentrate (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22
Substrate Diluent (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22

Additional reagent may be required for use with automated systems. Keep away from sunlight. The Substrate Solution should be pale yellow; if it is green before being used it should be discarded and fresh Substrate Solution prepared.

The prepared Substrate Solution from this kit may be used interchangeably with that from all other Murex kits which use orange coloured Substrate Concentrate. Ensure that the Substrate Solution is prepared from the Substrate Diluent and Substrate Concentrate provided together.

The prepared Substrate Solution is stable refrigerated (2 to 8°C) or at 15 to 25°C for up to two days but it must be discarded if crystals have formed.

WASH FLUID

11. Wash Fluid

One (7G79-09) or two (7G79-11) bottles containing 125 ml of 20 times working strength Glycine/Borate Wash Fluid. Contains 0.2% Bronidox® preservative.

Add one volume of Wash Fluid Concentrate to 19 volumes of distilled or deionised water to give the required volume or dilute the entire contents of one bottle of Wash Fluid to a final volume of 2500 ml. Crystals may be observed in the Wash Fluid Concentrate but these crystals will dissolve when the Wash Fluid is diluted to working strength. When diluted the Wash Fluid contains 0.01% Bronidox® preservative.

The Wash Fluid from this kit may be used interchangeably with the Glycine/Borate Wash Fluid from any other Murex kit.

Store the working strength Wash Fluid at 18 to 30°C in a closed vessel under which conditions it will retain activity for one month.

NOTE: The Wash Fluid may develop a yellow colour on storage. This will have no effect on the performance of the assay providing the Wash Fluid is fully aspirated from the wells.

NOTE: Although the Substrate Solution and Wash Fluid are interchangeable, they must not be used beyond the expiry date printed on the component labels.

WARNINGS AND PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

Low levels of fibrin precipitate may be observed in the Kit Controls and product performance is not affected by this. This is a product of certain serum batches used to manufacture the controls.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains components of human origin.

The human sera used for manufacture have been screened and found reactive or non-reactive for analytes as shown in **Table 2** below.

Table 2

Component:	Reactive for	Non-reactive for
Negative Control	N/A	HBsAg, antibodies to HCV, HIV-1 and HIV-2
Positive Control 1	antibodies to HIV-1	HBsAg
Positive Control 2	antibodies to HIV-2	HBsAg

Additionally human sera used for positive controls are also tested for antibodies to HCV and may be reactive.

All reactive serum used has been inactivated prior to use in reagent preparation. However, all material of human origin should be considered as potentially infectious and it is recommended that this kit and test specimens be handled using established good laboratory practice.

Pursuant to EC Regulation 1272/2008 (CLP) hazardous reagents are classified and labeled as follows:

Reagents:	CONJUGATE DIL	SAMPLE DIL	CONJUGATE *
Classification:	Skin sens. 1 H317		
Signal Word:	Warning		
Symbols / Pictograms:			
Hazard Statements:	H317 May cause an allergic skin reaction.		
Precautionary Statements:	P280 Wear protective gloves/protective clothing/ eye protection/face protection. P363 Wash contaminated clothing before reuse. P333+P313 If skin irritation or rash occurs: Get medical advice / attention.		
Contains:	Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).		

* The reconstituted Conjugate contains 0.1% ProClin® 300 which is classified hazardous per EC Regulation 1272/2008.

Reagents:	SUBSTRATE CONC
Classification:	Eye Irrit. 2 H319
Signal Word:	Warning
Symbols / Pictograms:	
Hazard Statements:	H319 Causes serious eye irritation
Precautionary Statements:	P264 Wash hands thoroughly after handling P280 Wear protective gloves/protective clothing/ eye protection/face protection. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Pursuant to EC Regulation 1272/2008 [CLP] WASH FLUID is labeled as EUH210, safety data sheets available on request.

For additional information see Safety Data Sheets available on www.diasorin.com

1. Potentially contaminated materials should be disposed of safely according to local requirements.
2. Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is continued[3]. Sodium hypochlorite should not be used on acid containing spills unless the spill area is first wiped dry.
   Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.
3. Neutralised acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.
4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
5. If any of the reagents come into contact with the skin or eyes wash the area extensively with water.
6. Sulphuric acid required for the Stop Solution and hydrochloric acid used for washing glassware are corrosive and should be handled with appropriate care. If either come into contact with the skin or eyes, wash thoroughly with water.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
2. Do not modify the **Test Procedure** or substitute reagents from other manufacturers or other lots unless the reagent is stipulated as interchangeable. Do not reduce any of the recommended incubation times.
3. Allow all reagents and samples to come to +18 to 30°C before use. Immediately after use return reagents to the recommended storage temperature.
4. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and then rinsed with distilled water or high quality deionised water.
5. Avoid the use of self-defrosting freezers for the storage of reagents and samples.
6. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
7. Do not allow wells to become dry during the assay procedure.
8. Do not cross-contaminate reagents. Dedicate a pipette for use with the Substrate Solution of Murex assays. A pipette should also be dedicated for use with the Conjugate.
9. The Sample Diluent in this assay has the potential to cause false positive results in anti hepatitis B surface antigen (anti-HBs) assays if reagent cross contamination occurs.
   If running Murex HIV Ag/Ab Combination in conjunction with an anti-HBs assay on a fixed tip instrument ensure that the possibility of cross contamination is excluded during the validation process.
10. Do not touch or splash the rim of the well with Conjugate. Do not blow out from micropipettes; reverse pipetting is recommended wherever possible.
11. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
12. Do not contaminate microwells with dust from disposable gloves.
13. When using fully automated processors
    i) It is not necessary to use plate lids and tap dry the wells.
    ii) Do not allow system fluids to contaminate samples or reagents.
    iii) The possibility of cross contamination between assays needs to be excluded when validating assays on fully automated processors.
14. Ensure the assay is run within the temperature limits defined in the assay protocol.
15. Do not use CO_2 incubators.
16. Do not store the Stop Solution in a shallow dish or return it to a stock bottle after use.
17. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on instrumentation.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION

Serum, EDTA plasma or citrate plasma samples may be used. Ensure that the serum samples are fully clotted. Remove any visible particulate matter from the sample by centrifugation. If samples are prepared using liquid anti-coagulants e.g. citrate plasma, the dilution effect should be considered.

SPECIMEN TRANSPORT AND STORAGE

Store samples at 2 to 8°C. Samples not required for assay within 72 hours should be removed from the clot or cell pellet and stored frozen (-15°C or colder). Avoid multiple freeze-thaw cycles. After thawing ensure samples are thoroughly mixed before testing.

PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. **Stop Solution (0.5M to 2M Sulphuric Acid)**, e.g. add between 3.0 ml (for 0.5M) and 11 ml (for 2.0M) of analytical grade concentrated sulphuric acid (18M) to about 80 ml of distilled or deionised water and then make up to 100 ml with more water. Alternatively, the following reagent can be used: 1N Sulphuric Acid (Code N0164 - 10 vial pack and N0165 - 1 vial pack).
2. **Freshly distilled or high quality deionised water** is required for dilution of Wash Fluid, for preparation of the Stop Solution and for use in conjunction with automated washers.
3. **Micropipettes and Multichannel micropipettes** of appropriate volume.
4. **Incubator** capable of maintaining the temperature limits defined in the assay protocol.
5. **Moulded Heating Block** (Code 9F09-02). For use in laboratory incubators. The moulded heating block should ideally be kept in the incubator used. If this is not possible it must be placed in the incubator at least four hours before beginning the assay.
6. **Instrumentation**
   a) Automated microplate strip washer.
   b) Microplate reader.
   or
   c) Fully automated microplate processor.
   All instruments must be validated before use.
   Please contact your representative for details of recommended systems, software protocols for instrumentation and validation procedures.
7. **Disposable Reagent Troughs.** (Code 9F24-01).
8. **Sodium hypochlorite** for decontamination. (Refer to **Health and Safety** Information)
9. **Sodium hydroxide solution** (0.1M). (Refer to **Analytical Precautions**)

TEST PROCEDURE

Please read **Analytical Precautions** carefully before performing the test.

Addition of the various components of the assay to the wells may be confirmed visually by examining the plate for the following colours:

Sample Diluent is green/brown in colour. On addition of Sample or Control the colour will change to blue/green. The colour change will vary from sample to sample but some change should always be visible. The addition of sample or control may be confirmed using a microplate reader at 570 nm or 620 nm with a reference of 690 nm.

Reconstituted Conjugate is red in colour. The addition of Conjugate may be confirmed using a microplate reader at 490 nm with a reference of 690 nm.

Substrate Solution is initially pale yellow with any reactive wells becoming blue green. On addition of Stop Solution the blue green colour of the reactives will change to orange, whilst the negatives will change to pink. The addition of Substrate Solutions may be confirmed using a microplate reader at 450 nm (no reference).

SEMI AUTOMATED PROCESSING

Step 1	**Reconstitute** and **mix** the **Conjugate**, prepare the **Substrate Solution** and **Wash Fluid.**	
Step 2	Use only the **number** of **wells** required for the test. Avoid touching the tops or bottoms of the wells	
Step 3	**Add** 25 µl of **Sample Diluent** to each well.	25 µl
Step 4	**Add** 100 µl of **Samples** or 100 µl **Controls** to the wells. For each plate use the first column of wells for the assay Controls. Add the Controls to the designated wells after dispensing the samples. Pipette 100 µl of the Negative Control into each of three wells A1 to C1 and 100 µl of the p24, anti-HIV-1 and HIV-2 Positive Controls into wells D1 to F1 respectively. Use of a white background will aid visualisation of sample addition.	100 µl
Step 5	Cover the wells with the lid and **incubate** for 60 minutes at 37°C ±1°C.	60 mins
Step 6	At the end of the incubation time **wash the plate** as described under **Wash Procedures.**	
Step 7	Immediately after washing the plate, **add** 100 µl of **Conjugate** to each well.	100 µl
Step 8	Cover the wells with the lid and **incubate** for 30 minutes at 37°C ±1°C.	30 mins
Step 9	At the end of the incubation time **wash the plate** as described under **Wash Procedures.**	
Step 10	Immediately after washing the plate, **add** 100 µl of **Substrate Solution** to each well.	100 µl
Step 11	Cover the wells with a lid and **incubate** for 30 minutes at 37°C ±1°C. Keep away from direct sunlight. A blue green colour should develop in wells containing reactive samples.	30 mins
Step 12	**Add** 50 µl of **Stop Solution** (0.5M to 2M sulphuric acid) to each well.	50 µl
Step 13	Within 15 minutes **read** the absorbance at 450 nm using 620 nm to 690 nm as the reference wavelength if available. **Blank** the instrument on air (no plate in the carriage).	A_{450}

WASH PROCEDURES

Protocols for recommended washers and procedures for verifying washers and analysers can be obtained from your representative. The following protocol is recommended:

a) **Protocol for automated stripwasher**

Perform 5 wash cycles using working strength Wash Fluid. Ensure, where possible, that:

(i) Flow-through washing with a volume of 500 µl/well is used with instrumentation supplied by DiaSorin. When using other instrumentation for which this is not possible, ensure that the well is completely filled.

(ii) The dispense height is set to completely fill the well, with a slight positive meniscus, without causing an overflow.

(iii) The time taken to complete one aspirate/wash/soak cycle is approximately 30 seconds.

(iv) Ensure that no liquid is left in the well (by use of a double aspirate step in the final cycle where possible).

(v) After washing is completed, invert the plate and tap out any residual Wash Fluid onto absorbant paper.

NOTE: Do not allow the wells to become dry during the assay procedure.

Washers must be rinsed with distilled or deionised water at the end of the test to avoid blockage and corrosion.

FULLY AUTOMATED PROCESSORS

Contact your representative for details of currently available validated protocols. For instrumentation without established validated protocols, the following guidelines are recommended:

1. Do not programme times shorter than specified in the procedure.
2. For each incubation at 37°C, programmed times may be increased by up to 5 minutes.
3. Wells containing Sample Diluent may be left for up to 60 minutes at 18-30°C prior to the addition of Sample and for up to 60 minutes after the addition of samples or Controls before starting step 5 in the assay protocol.
4. Ensure all **Analytical Precautions** are followed.

Protocols written following these guidelines must be fully validated prior to use according to local procedures.

RESULTS

CALCULATION OF RESULTS

Each plate must be considered separately when calculating and interpreting results of the assay.

Approved software may be used for calculation and interpretation of results.

Negative Control

Calculate the mean absorbance of the Negative Controls.

Example:

Well 1	= 0.084, Well 2	= 0.096, Well 3	=	0.070
		Total	=	0.249
Mean Negative Control			=	0.249/3
			=	0.080

If one of the Negative Control Wells has an absorbance more than 0.15 O.D. above the mean of all three, discard that value and calculate the new Negative Control mean from two remaining replicates.

Cut-off value

Calculate the Cut-off value by adding 0.150 to the mean of the Negative Control replicates (see above).

Mean Negative Control	=	0.080			
Cut-Off value	=	0.080	+ 0.150	=	0.230

QUALITY CONTROL

Results of an assay are valid if the following criteria for the controls are met:

Negative Control

The mean absorbance is less than 0.15

Positive Controls

The absorbance of each of the Positive Controls is more than 0.8 above the mean absorbance of the Negative Control.

Assays which do not meet these criteria should be repeated.

In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

INTERPRETATION OF RESULTS

Non-reactive Results

Samples giving an absorbance less than the Cut-off value are considered negative in the assay.

Reactive Results

Samples giving an absorbance equal to or greater than the Cut-off value are considered initially reactive in the assay (see **Limitations of the Procedure**).

Unless local procedures state otherwise, such samples **must** be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate retests are considered repeatedly reactive in Murex HIV Ag/Ab Combination and are presumed to contain reactive HIV core antigen and/or antibodies to HIV-1 or HIV-2. Such samples **must** be further investigated and the results of this assay considered with any other clinical and/or assay information. Samples that are non-reactive in both wells on retest are considered non-reactive for HIV core antigen and HIV antibodies.

No sample addition

Absorbance values significantly higher than the Negative Control may be obtained in wells where the sample has been omitted but all the reagents have been added.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Murex HIV Ag/Ab Combination has been determined by testing samples from random blood donors, patients with AIDS diagnosed according to CDC criteria, patients with AIDS Related Complex (ARC), other patients with known antibody to HIV-1 (including group O), patients with confirmed HIV-2 infection and patients at risk of HIV infection or in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.

Diagnostic Sensitivity

A total of 497 specimens from patients with confirmed HIV-1 infection were tested and found to be reactive with Murex HIV Ag/Ab Combination. The specimens were taken from patients at various stages of HIV infection and included 24 specimens from patients with HIV-1 subtype O infection and a further 109 specimens from patients infected with HIV-1 subtypes other than subtype B.

In addition a total of 100 specimens from patients with confirmed HIV-2 infection were also tested with Murex HIV Ag/Ab Combination and found to be reactive.

The diagnostic sensitivity of Murex HIV Ag/Ab Combination on this population of specimens is therefore estimated to be 100% (597/597) with a lower 95% confidence limit of 99.38% (693/697) by the binomial distribution.

A total of 26 commercial HIV-1 seroconversion panels were tested with Murex HIV Ag/Ab Combination. Using the presence of both core (p24) and an envelope (gp120/160) band on Western blot as the reference criteria, Murex HIV Ag/Ab Combination detected antibody to HIV earlier or in the same sample as Western blot in all of the panels.

Diagnostic Specificity

The Murex HIV Ag/Ab Combination assay demonstrated a specificity of ≥99.5% in a study where specimens from a European blood donor population were tested. A total of 9,290 routine donor plasma specimens were screened with Murex HIV Ag/Ab Combination at three European blood transfusion centres. The results are summarised in Table 3. In the study, 99.77% (9269/9290) of specimens were non-reactive and 0.23% (21/9290) were repeatedly reactive. One of the repeatedly reactive specimens was weakly positive with the Murex HIV Antigen mAb (8E77). None of the remaining 20 specimens were confirmed as positive for the presence of HIV-1 antigen or antibody to HIV-1 or HIV-2.

The specificity of Murex HIV Ag/Ab Combination on presumed negative European blood donors is estimated to be 99.78% (9269/9289) with 95% confidence limits of 99.67% (9258/9289) to 99.87% (9277/9289) by the binomial distribution.*

A total of 267 specimens from patients with conditions unrelated to HIV infection were also tested with Murex HIV Ag/Ab Combination. These included specimens from pregnant women and patients suffering with autoimmune disease and other acute viral infections. A total of five specimens were reactive with Murex HIV Ag/Ab, four were reactive with two other commercially available screening assays. In Western blot studies four produced indeterminate results and one was negative.

In addition, 38 lipaemic, icteric and haemolysed specimens were also tested and found to be non-reactive.

The overall diagnostic specificity of Murex HIV Ag/Ab Combination on confirmed negative specimens during this performance evaluation is estimated to be 99.78% (9569/9590) with 95% confidence limits of 99.67% (9558/9590) to 99.86% (9577/9590) by the binomial distribution.*

*Representative performance data are shown. Results obtained at individual laboratories and with different populations may vary.

Assay Reproducibility

The reproducibility of Murex HIV Ag/Ab Combination was assessed by testing two of the assay controls and four quality assurance panel members as ten replicates on four separate occasions. The results from the testing are summarised in **Table 4.**

Table 3
Reactivity of Murex HIV Ag/Ab Combination with presumed negative specimens from routine European blood donors

Centre	Number of presumed negative specimens tested	Number of repeatedly reactive specimens
A	3086	6[a] (0.19%)
B	2803	9 (0.32%)
C	3382	6 (0.18%)
TOTAL	9290	21 (0.23%)

[a] Includes one specimen which was weakly positive in Murex HIV Antigen mAb (8E77)

Table 4
Murex HIV Ag/Ab Combination - Assay Reproducibility

Specimen	Number of Assays	Number of Replicates	Mean Absorbance/ Cut-off ratio	Intra-assay %CV	Inter-assay %CV
Negative Control	4	10	0.256	8.7	11.3
HIV-1 Positive Control	4	10	8.287	4.3	4.7
QA01	4	10	3.672	4.6	7.3
QA02	4	10	4.696	5.5	12.9
QA03	4	10	3.006	3.9	4.2
QA04	4	10	1.663	6.8	9.2

Sensitivity on AFSSAPS HIV Ag Standard

Sensitivity of Murex HIV Ag/Ab Combination on the AFSSAPS HIV Ag standard was determined at three testing centres.

Table 5
Sensitivity on AFSSAPS HIV Ag standard

Centre	Sensitivity HIV Ag pg/ml
1	31
2	28
3	25
Mean	28

The data shown in Table 5 was obtained during this testing but may not be exactly reproducible on other testing occasions.

LIMITATIONS OF THE PROCEDURE

1. The **Test Procedure** and **Interpretation of Results** must be followed.
2. This test has only been evaluated for use with individual (unpooled) serum, EDTA plasma or citrate plasma samples.
3. A negative result with an antigen/antibody detection test does not preclude the possibility of infection with HIV.
4. A positive result with Murex HIV Ag/Ab Combination should be confirmed by at least one other test.
5. Non-repeatable reactive results may be obtained with any EIA procedure.

   The most common sources of error are:

   a) Imprecise delivery of Sample, Conjugate or Substrate into the wells.

   b) Contamination of Substrate with Conjugate.

   c) Contamination with conjugates from other assays.

   d) Blocked or partially blocked washer probes.

   e) Insufficient aspiration leaving a small volume of Wash Fluid in the wells.

   f) Failure to ensure that the bottom surface of the wells is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before a plate is read.

   g) Failure to read at the correct wavelength (450 nm) or use of an incorrect reference wavelength (not 620 nm to 690 nm).
6. The use of highly haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
7. This test has not been evaluated for use with samples from cadavers.

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September, 2014

National Health laboratory

Department of Immunology and Sensitivity

Murex HIV Ag/Ab combination

ELISA Result of HIVSCRNING

Kit Lot No: 14020 Technologist: Sayed Mutasim

Exp. Date: 9/2015 Date: 25/5/2015

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.099	0.099	0.098	0.081	0.123	0.137	0.126	1.997	0.110	0.132	0.110	0.128
B	0.100	0.118	0.101	0.100	0.092	0.092	0.090	0.092	0.092	1.476	0.102	0.098
C	0.113	0.091	0.090	1.990	2.159	2.093	0.084	0.075	0.089	0.086	0.107	0.098
D	0.141	0.102	2.450	0.085	0.085	0.086	0.267	0.115	0.090	0.107	0.114	0.109
E	1.259	0.121	0.122	0.094	2.040	0.087	0.089	0.83	0.088	0.088	0.098	0.097
F	1.094	2.241	0.118	2.231	0.104	0.087	0.081	0.109	0.086	0.095	0.095	0.990
G	1.005	2.602	1.962	0.094	2.201	0.085	0.089	0.084	0.100	0.093	0.093	0.098
H	0.111	0.370	0.123	0.103	0.121	0.400	0.118	0.101	0.103	0.117	0.112	0.103

National Health laboratory

Department of Immunology and Sensitivity

BioTrace HIV Rapid Test

I C T Result of HIVSCRNING

Kit Lot No: 225/2 Technologist: Sayed Mutasim

Exp. Date: 15/6/2016 Date: 25/5/2015

1	2	3	4	5	6	7	8	(9)	10	11	12
13	14	15	16	17	18	19	20	21	22	(23)	24
25	26	27	28	(29)	(30)	(31)	32	33	34	35	36
37	38	39	(40)	41	42	43	44	45	46	47	48
49	50	51	52	53	(54)	55	56	57	58	59	60
61	62	(63)	64	(65)	66	67	68	69	70	71	(72)
73	74	(75)	(76)	77	(78)	79	80	81	82	83	84
85	86	87	88	89							

National Health laboratory

Department of Immunology and Sensitivity

Murex Ag/Ab combination

ELISA Result of HIVSCRNING

Kit Lot No: 14020 Technologist: Sayed Mutasim

Exp. Date: 9/2015 Date: 25/5/2015

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B	2	10	18	26	34	42	(50)	58	66	74	82
B	N.c	3	11	19	27	35	43	51	59	(67)	75	83
C	N.c	4	12	(20)	(28)	(36)	44	52	60	68	76	84
D	N.c	5	(13)	21	29	37	45	53	61	69	77	85
E	P.c	6	14	22	(30)	38	46	54	62	70	78	86
F	P.c	(7)	15	(23)	31	39	47	55	63	71	79	(87)
G	P.c	(8)	(16)	24	(32)	40	48	56	64	72	80	88
H	1	9	17	25	33	41	49	57	65	73	81	89