

**REAL-TIME HUMAN IMMUNODEFICIENCY VIRUS
TEST ANALYZER BASED ON DIGITAL IMAGE PROCESSING**

BY

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Abstract

Many kinds of HIV testing kits have recently become available to be used in determining whether an individual's blood contains HIV virus or not. In the *determine* method of the HIV rapid test procedure, when a drop of blood is introduced at the lower testing region of the test kit, a red horizontal line appears on the test section indicating the presence of the HIV virus. The absence of the red line shows that the blood does not contain the virus. However, a similar red line must appear at the control section of the kit to show that the test procedure is successful and complete. The reading, interpretation and confidentiality of the HIV test result has often been abused by the medics and the counselors. There has been an immense widespread of HIV due to people's ignorance of their status. The spread can be curbed by introducing a human friendly, confidential, automatic and reliable testing system that has been developed by this study. In this study, an electronic system that automatically acquires the red color signals that appear on the test kits has been developed. The system analyses the color signals, processes them, displays and relays the test result to the client. The main objective of the study was to design and develop a real-time HIV test analyzer based on computer aided image processing technique and. An image processing software in a client/server system was created. The system used *Complementary metal-oxide-semiconductor* (CMOS), digital camera to capture the image and the programmed software, developed in C#, processes the captured image and sends the testing results to the display unit. This technique will not only eliminate the human error associated with the use of HIV testing kits, but will also improve the testing productivity in comparison to those achieved by the trained technicians. It will also enhance the confidentiality of the test result hence reducing the stigma associated with the disease and encourage more people to know their HIV status. The system can be installed in our medical facilities and at the Voluntary Counseling and Testing (VCT) to aid the medical personnel in HIV screening and testing. The system has been tested successfully and the testing results have proved that the system is reliable.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV) [1, 2, 3]. HIV infects human cells and uses the energy and nutrients provided by those cells to grow and reproduce.

AIDS is a disease in which the body's immune system breaks down and is unable to fight off infections, known as "opportunistic infections," and other illnesses that take advantage of a weakened immune system.

When a person is infected with HIV, the virus enters the body and lives and multiplies primarily in the white blood cells. These are cells that normally protect us from disease. The hallmark of HIV infection is the progressive loss of a specific type of immune cell called cluster differential type four (CD4) cells. As the virus grows, it damages or kills these and other cells, weakening the immune system and leaving the person vulnerable to various opportunistic infections and other illnesses ranging from pneumonia to cancer.

In some people, the CD4 cells decline and opportunistic infections that signal AIDS develop soon after infection with HIV. However, most people do not develop symptoms for 10 to 12 years, and a few remain symptom-free for much longer. As with most diseases, early medical care can help prolong a person's life.

Many people are unaware that they are infected with HIV [4]. AIDS has since remained one of the world's leading killer diseases. Less than 1% of the sexually active urban population in Africa has been tested, and this proportion is even lower in rural populations. HIV tests are usually performed on venous blood. Tests used for the diagnosis of HIV infection in a particular person require a high degree of both sensitivity and specificity.

One of the major reasons that deter people from undertaking the test is the stigma associated with it. The confidentiality that goes with the test results is paramount and has been abused by the

medics and the counselors. Furthermore several test results have been wrongly interpreted by the medics leading to wrong information.

The HIV blood test is usually done in hospitals and could take some time before the patient knows the result. Other quick HIV test is performed through the use of special kits that produce a certain pattern indicating the status of the blood condition [5, 6]. Such quick tests should be performed by a trained technician and may be conducted in a small clinic or laboratory. The test results can be obtained within a few minutes.

As an example, one of these quick HIV test kits has two bars (red horizontal lines) to indicate the testing results. The kit has a control bar that appears when the testing procedure is conducted correctly. The other bar is called the testing bar, which indicates the testing result. Figure 1.1 shows a sample of an unused test kit. It has two white sections on which the red bars appear after the test. The upper white section is called the control section while the lower white section is called the testing section

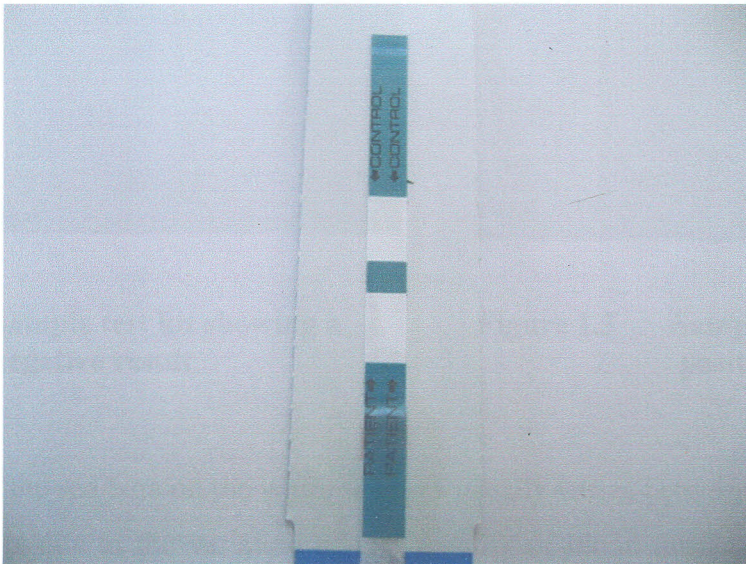


Figure 1.1 Unused test kit for the *determine* method of the rapid test procedure.

The test kit contains cotton wool treated with the testing reagents. When a drop of blood is placed at the bottom of the test kit, red bar pattern will appear in white regions of the testing kit.

The testing bar appears due to the reaction between the reagents that exist in the kit and the blood. The appearance of the red bar on the lower white section (test region) section will indicate that the result of the test is positive and the blood is contaminated with the HIV virus. Otherwise, the test is negative.

However for a conclusive interpretation of the results a similar red bar must appear at the upper white section of the control region. The control region contains blood sensitive reagents. Red bar forms on it when sufficient amount of blood reaches it. The appearance of this bar will indicate that the testing procedure is complete and successful. Otherwise the result is invalid or inconclusive or indeterminate.

Figures 1.2 and 1.3 show sample kits displaying negative and positive results after test procedure.

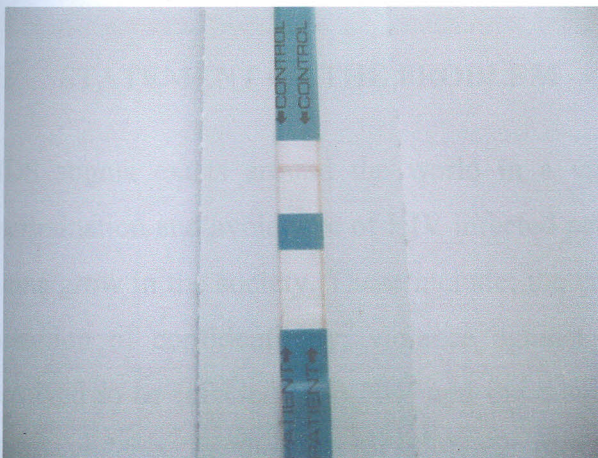


Figure 1.2 Sample test kit showing a negative result

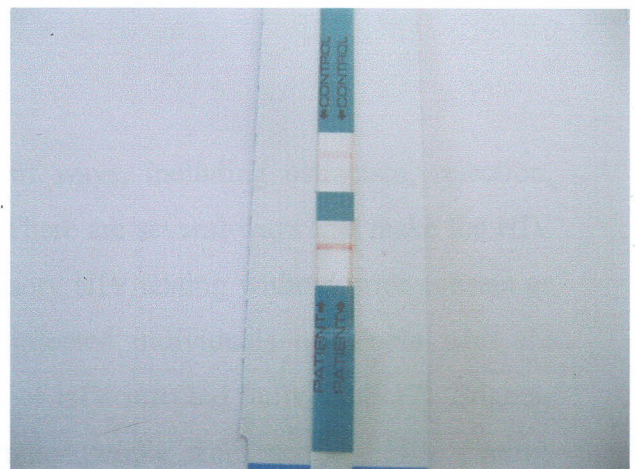


Figure 1.3 Sample test kit showing a positive result

The intensity of the red bars on the white sections usually varies between very high intensity and very low. This is due to the variation of the quantity of blood dropped in each test kit. Such variations in the appearance of the testing bar have made the manual interpretation of the testing result hard in some cases. For example, when the intensity of the bar is so low it could make technicians interpret the results differently. It is important to have very accurate testing because the false positive result will affect the patient psychologically. It can also cause serious harm; a

patient who has received a false negative result may continue to infect other people not knowing that he/she carries the HIV virus.

This research has therefore addressed the need to develop a HIV testing system that is electronic, automatic, confidential, reliable, efficient and user friendly. The system is a developed software program that analyzes, processes and classifies the developed images of HIV test kits as either positive, negative or invalid result.

Today there is a fundamental trend in the automated test and measurement industry that has had a heavy shift towards software-based test and measurement systems. This has called for communication between computers and peripheral devices in order to enhance their control as computer-aided tests and measurements determine the precision and the reliability of laboratory measurements.

1.2 STATEMENT OF THE PROBLEM

AIDS stigma exists around the world in a variety of ways, including ostracism, rejection, discrimination and avoidance of HIV infected people. There are several ways that make the HIV stigma grow in the society. These include; the compulsory HIV testing without prior consent or protection of confidentiality, violence against HIV infected individuals or people who are perceived to be infected with HIV and the quarantine of HIV infected individuals [7]. Stigma-related violence or the fear of violence prevents many people from seeking HIV testing, returning for their results, or securing treatment, possibly turning what could be a manageable chronic illness into a death sentence and perpetuating the spread of HIV [8].

HIV and AIDS affect economic growth by reducing the availability of human capital [9]. Without proper nutrition, health care and medicine that is available in developed countries, large numbers of people suffer and die from AIDS-related complications. Those who are unable to work will also require significant medical care. The forecast is that this will probably cause a collapse of economies and societies in countries with a significant AIDS population. In some heavily infected areas, the epidemic has left behind many orphans cared for by elderly grandparents [10].

1.3 JUSTIFICATION OF THE STUDY

With the much negative devastating impacts of HIV and AIDS on our society, it is of paramount importance to fight the AIDS stigma so as to combat the spread of HIV and AIDS. This will drastically reduce the numerous deaths caused by the menace and will eventually translate into community's economic growth, improved nutrition, greater human resource, reduced orphanage and even poverty reduction. It is therefore the focus of this study to develop an electronic system that can carry out the HIV test and the test result directly accessed or relayed to the client without necessarily passing through a third party. This will greatly protect the confidentiality desired to go along with the test. This, hopefully, will reduce the stigma associated with HIV and AIDS, making more people to know their HIV status. Moreover, producing a self-operated smart system that analyzes the test results will make it possible to perform such testing at remote places where the trained technicians may not be available. The availability of an accurate and cost effective system for HIV testing may allow using such systems by the patients themselves in pharmacies or small clinics without the technicians support. Clearly, this will improve the HIV screening process and hopefully reducing the widespread of HIV.

1.4 OBJECTIVES OF THE STUDY

1.4.1 Main objective

The main objective of this study was to design and develop a software based system for real-time HIV test analysis based on digital image processing techniques.

1.4.2 Specific objective

The specific objective of this study was to develop a software program that analyzes, processes and classifies the developed images of HIV test kits as either positive, negative or invalid result.

1.5 HIV TESTING

1.5.1 Introduction

HIV tests are used to detect the presence of the human immunodeficiency virus in serum, saliva, or urine. Such tests may detect HIV antibodies, antigens, or Ribonucleic Acid (RNA). This virus destroys the body's ability to fight off illness, and is the cause of AIDS.

Based on the criteria used to conduct the HIV test by the government, there can be four HIV testing options. Thus, Anonymous, Mandatory, Voluntary and Routine HIV testing.

Anonymous testing is whereby the testing has only a number attached to the specimen that will be delivered for testing. Items that are confirmed positive will not have the HIV infected individual's name attached to the specimen. Sites that offer this service advertise this testing option.

In the mandatory HIV testing option, one has no choice over whether to be tested or not. Essentially it is a compulsory test that one cannot refuse.

Voluntary testing on the other hand is whereby one has to actively and freely choose to take an HIV test.

Routine testing is an HIV test that is offered to everyone within a certain population (for example, pregnant women, or people within a certain age group) on a routine basis. It is also referred to as 'opt-out' screening, which means the test is automatically performed unless the individual concerned raises an objection and 'opts out'. Both voluntary and routine HIV tests require a person's full written or verbal consent.

In the United States, one emerging standard of care is to screen all patients for HIV in all health care setting [11]. In 2006, Centers for disease Control (CDC) announced an initiative for voluntary, routine testing of all Americans aged 13-64 during health care encounters. If successful the effort was expected to reduce new infections by 30% per year [12].

Because it restricts freedom of choice, and can result in stigmatization and discrimination for anyone who tests positive, mandatory testing is generally seen as an unsuitable way of combating HIV and AIDS. For screening a certain population for HIV, routine opt-out policies are therefore considered to be more effective from both a public health and an ethical point of view.

1.5.2 HIV testing methods

There are a number of tests that are used to find out whether a person is infected with HIV. These include the HIV antibody test, P24 antigen test and Polymerase Chain Reaction (PCR) test. In this work, we will only briefly examine the HIV antibody test since it is the most appropriate test for routine diagnosis of HIV among adults. Antibody tests are inexpensive and are very easy to administer. The test looks for antibodies to the virus in a person's blood. For most people these antibodies take 6 weeks to 3 months to develop. The following are the common types of the HIV Antibody tests used in our medical laboratories.

1.5.2.1 ELISA

The *enzyme-linked immunosorbent assay* (ELISA), or *enzyme immunoassay* (EIA), was the first screening test for HIV. It has a high degree of sensitivity as it can detect the smallest amount of HIV in the blood.

In an ELISA test, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens have been attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared "**secondary antibody**" — an antibody that binds to human antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence.

1.5.2.2 Western blot

In the Western blot procedure, cells that may be HIV-infected are opened and the proteins within are placed into a slab of gel, to which an electrical current is applied. Different proteins will move with different velocities in this field, depending on their size, while their electrical charge is leveled by a surfactant called **sodium lauryl sulfate**. Some commercially prepared Western blot test kits contain the HIV proteins already on a cellulose acetate strip. Once the proteins are well-separated, they are transferred to a membrane and the procedure similar the ELISA method discussed above continues.

Tests in which less than the required number of viral bands is detected are reported as indeterminate: a person who has an indeterminate result should be retested, as later tests may be more conclusive. In a generally healthy low-risk population, indeterminate results on Western blot occur on the order of 1 in 5,000 patients [13]. However for those individuals that have had high-risk exposures to individuals where HIV-2 is most prevalent, Western Africa, an inconclusive Western Blot test may prove infection with HIV-2 [14]. HIV-2 is a genetic type two strain of HIV.

1.5.2.3 Rapid or point-of-care tests

Rapid Antibody Tests are qualitative immunoassays intended for use as a point-of-care test to aid in the diagnosis of HIV infection. These tests should be used in appropriate multi-test algorithms designed for statistical validation of rapid HIV test results.

1.5.2.4 Intelligent Diagnostics (iDiagnostics) Rapid HIV Test

This is similar to a home pregnancy test where the patient collects a drop of blood/urine and drops the sample onto a cassette. Results are then read visually from the cassette, usually in 15 minutes time [15]. The accuracy of this test has not been confirmed by the Food and Drug Administration (FDA) and it is not authorized for use in the United States [16].

1.5.2.5 Reveal HIV Test

This is a rapid *in vitro* qualitative test for the detection of antibodies to HIV in whole blood, serum or plasma. Reveal is among the fastest rapid HIV test available and it detects signs of early infection better than some other rapid tests [17]. Reveal HIV is approved in Canada, the United States, Europe, Africa, Asia, and South America [18].

All these testing methods are used in our medical facilities. However, they don't address the issue of confidentiality as required by the Human Rights in the HIV testing. The medic gets to know the test results of a client first before the patient gets the information. This research focuses on a HIV testing system where the client gets the first hand information and the medic comes second.

1.5.3 Advantages of HIV testing

HIV Testing has several advantages. It enables us to monitor our immune system and initiate early treatment that can greatly improve our long term health. Knowing that one is HIV positive may help him/her change social behaviors that would put him/her and others at risk. Women and their partners considering pregnancy can take advantage of treatments that potentially prevent transmission of HIV to the baby.

HIV testing provides an opportunity for infected persons to know that they are infected so that they seek medical treatment that may help improve their own health. For some uninfected women with risks for HIV, the prenatal care period could be an ideal opportunity for HIV prevention and subsequent behavior change to reduce risk for acquiring HIV infection. For those who think they may have been exposed to the virus, having a test and receiving a negative result (which means they are not infected with HIV), can put their mind at rest. If one tests negative, one will feel less anxious after testing and continue living positively.

1.5.4 When to take an HIV test

A person who is infected with HIV will become infectious almost immediately. However, he/she will normally have to wait a while before an HIV test will produce an accurate result. The length

of time a person should wait before taking an HIV test will depend on the type of test they are taking.

In the vast majority of cases, the standard test normally offered at an HIV testing centre is an HIV antibody test. Getting tested before three months have elapsed may result in an unclear test result, as an infected person may not yet have developed antibodies to HIV. These three months are known as the 'window period'. So it is best for one to wait for at least three months after the last time one would be at risk before taking the test. Some test centers may recommend testing again at 6 months, just to be absolutely sure, though in most cases this is not necessary.

1.5.5 HIV test results.

HIV test results will either come out to be **negative**, **positive** or **invalid** and each is interpreted differently. For example, a negative test result means that the person is not infected with HIV. However, it could mean that the person is in the window period. A negative test also may not mean that one is immune to HIV. If he/she continues with unsafe behaviors, he/she is still at risk of contracting HIV.

A positive test result means the person is infected with the HIV virus but does not necessarily mean that he/she has AIDS. A person with HIV is infected for life and he or she can pass the virus to others by having unprotected sex, or by sharing injection needles or equipment which contain fluids from his/her body such as saliva and blood. Those carrying the HIV virus should not donate blood, plasma, semen, body organs, or other tissues because these can be sources of transmission of the virus.

All diagnostic tests have limitations, and sometimes their use may produce erroneous or questionable results. These can be categorized as false positive or false negative. When the result is false positive, the test incorrectly indicates that HIV is present in a non-infected person. When it is false negative, the test incorrectly indicates that HIV is absent in an infected person. Most false negative results are due to the window period [19]. It is therefore imperative to carry out HIV test at the right moment. Invalid result in the *determine* method occurs if the red bar fails to

appear on the control section of the test kit after the test has been performed. In such a case the test must be repeated to get a valid result.

1.5.6 Human rights on HIV testing

The joint United Nations Program on HIV/AIDS (UNAIDS) /World Health Organization (WHO) policy statement on HIV testing states that conditions under which people undergo HIV testing must be anchored in a human rights approach that pays due respect to ethical principles[20]. According to these principles, the conduct of HIV testing of individuals must meet three conditions thus; confidentiality; counseling; and consensus

1.5.6.1 Confidentiality

Considerable controversy exists over the ethical obligations of health care providers to inform the sexual partners of individuals infected with HIV that they are at risk of contracting the virus [21]. Some legal jurisdictions permit such disclosure, while others do not. Most testing sites are now using confidential forms of testing. This allows for monitoring of infected individuals easily, compared to anonymous testing that has a number attached to the positive test results.

1.5.6.2 Counseling

HIV testing must go along with counseling. The client must be pre-counseled so as to prepare him/her for the results psychologically. This eliminates the psychological trauma that can even lead to the breakage of the client. Counseling enables the client to accept his/her situation positively hence having a chance for a prolonged life.

1.5.6.3 Consensus

It is important for the medics to be able to obtain informed consent for HIV testing. The HIV test cannot be performed without the full consent of the client. The client must be willing and must never be coerced to be tested.

CHAPTER TWO

LITERATURE REVIEW

2.1 HIV/AIDS STATISTICS

The Joint United Nations Program on HIV/AIDS (UNAIDS), estimates that there are now 33 million people living with HIV/AIDS worldwide. Since the beginning of the epidemic, AIDS has killed more than 25 million people worldwide.

AIDS ranks with malaria and tuberculosis as one of the top three deadliest infectious diseases among adults and is the fourth leading cause of death worldwide. More than 15 million children have been orphaned by HIV [22].

2.1.1 The global AIDS epidemic

According to the Global AIDS Epidemic Report of 2010, 33.4million people were living with HIV/AIDS in the world at the end of 2009, (see table 2.1 below). Sub-Saharan Africa remains the region most heavily affected by HIV. In 2010, sub-Saharan Africa accounted for 22.5 million (67%) of HIV infections worldwide, 68% of new HIV infections among adults and 91% of new HIV infections among children [23]. The region also accounted for 72% of the world's AIDS-related deaths in 2008 [24, 25]. AIDS has had a devastating effect on individuals, families and communities everywhere the disease has spread [26]. This necessitated the move by the United Nations to come up with the Millennium Development Goal (MDG) 6, whose main agenda is to arrest the spread of HIV and to promote access to treatment [27].

Table 2.1 Global Summary of the AIDS Epidemic, 2010

Regional HIV and AIDS statistics, 2010

	Adults and children living with HIV	Adults and children newly infected with HIV	Percentage adult prevalence (15-49) years	AIDS related deaths among adults and children
SUB SAHARAN AFRICA	22.5 million	1.8 million	5.0	1.3 million
OTHER REGIONS GLOBALLY	9.8 million	0.8 million	0.8	0.5 million
GLOBAL TOTALS	32.3 million	2.6 million	5.8	1.8 million

SOURCE UNAIDS [23]

2.1.2 Global statistics in HIV prevention

The percentage of HIV-positive pregnant women who received treatment to prevent transmission of the virus to their child increased from 33% in 2007 to 45% in 2008. The data in 2010 indicate that fewer than 40% of young people have basic information about HIV and less than 40% of people living with HIV know their status [28, 29]. The number of new HIV infections continues to outstrip the numbers on treatment, for instance for every two people starting treatment, a further five become infected with the virus [30].

2.1.3 Statistics in coverage of HIV testing, care and treatment services in Kenya.

In Kenya, the 2010 Survey indicates that HIV testing uptake has tripled among women aged 15-49 since 2003 and nearly doubled among men. Overall, 36 percent of Kenyans adults aged 15-64 have tested at least once for HIV and received results [31]. Nearly two-thirds of Kenyans report never having been tested for HIV, and are therefore unaware of their status. Testing is particularly low among older Kenyans age 50-64; among this cohort, only 17.5 percent have tested for HIV. The disparity between urban and rural areas is substantial: 50 percent of urban residents have tested for HIV at least once compared to only 30 percent of rural residents [32]. The increase in HIV testing among women is in part due to Prevention of Mother to Child Transmission (PMCT) services and testing in antenatal clinics. Nearly one-third of women who reported having ever tested said they were tested at an antenatal clinic.

Among those who have never been tested for HIV, the most common reason for not testing among both sexes was low perception of risk (61 percent). This underscores the importance of ongoing campaigns to improve knowledge about risk factors for HIV transmission and attitudes toward testing. Sixteen percent have never tested because they did not want to know their test results or were afraid others would know the results. A small but notable proportion of respondents (14 percent) said they were unaware that there was a test for HIV or did not know how to access testing. Five percent cited distance to the nearest known testing site as the major barrier, which may suggest that mobile testing services should be given more consideration. The cost of the test or the lack of access to or availability of treatment were very infrequently cited as barriers to testing (<2 percent) [32].

2.2 HIV TESTING HISTORY

With the onset of the AIDS epidemic, Red Cross became a leader in *HIV testing*. In *March 1985*, the Red Cross began testing all donated blood with the first Food and Drugs Administration (FDA) approved *HIV test* [33]. Until recently, Public Health Service (PHS) had made no recommendations regarding routine testing of recipients of organs, tissues, semen, or donated human milk. However, in response to the *July 18, 1991*, report of the PHS Workgroup on Organ and Tissue Transplantation, Human Resource and Services Administration (HRSA) asked United

Network for Organ Sharing (UNOS) to request that transplant centers implement an interim voluntary HIV-testing policy for organ recipients [34].

In 1996, Rich Rothman, while working on the development novel diagnostic test to identify people with various infectious diseases, joined Kelen to strengthen the HIV testing program and oversee the department's research efforts [35].

On December 1st, 2000 – UNAIDS held an intensive campaigns towards Mass HIV Testing all over the world. Up to date the 1st day of December has remained the world's AIDS day and is celebrated annually. The basic goal of the campaign is to encourage high risk group population to undertake the test. This high risk population includes drug users and their wives, homosexuals, people with Sexually Transmitted Diseases (STD), people living abroad for long time and people who were transfused with unscreened donor blood [36].

The review, published in the November 15, 2002, issue of Morbidity and Mortality Weekly Report, affirms the importance of HIV testing during pregnancy to ensure the mother and child fully benefit from therapies to prevent and treat HIV infection, and emphasizes the need for routine standardized monitoring to better assess prenatal testing rates in the United States [37]

On October 28, 2003, Medical Services of United States announced that it had completed all the necessary testing required for the country of Belize. The results from the testing for Belize were obtained through independent testing in a FDA approved laboratory in the United States. The test results showed that the VScan HIV rapid test kits were 100% accurate in all categories against the Western Blot test. The Western Blot test is considered the gold standard for HIV testing [38].

On April 15, 2004, the Chinese Government put out a press release where they announced free HIV testing for the entire Chinese population. With the testing of its HIV test kit nearing completion and based on the excellent test results obtained during the testing and the fact that the VScan HIV test kit can be used in any environment, does not require refrigeration, uses whole blood and does not need trained medical personnel or medical facilities, the Chinese Government fully adopted the VScan HIV testing for this exercise [39].

In September 2006, the Centers for Disease Control (CDC) released Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. These

new recommendations advised for routine HIV screening of adults, adolescents, and pregnant women and also recommended reducing barriers to HIV testing [40].

On May 30, 2007, the WHO and UNAIDS issued new guidelines regarding HIV testing and counseling in healthcare facilities. In previous guidelines, it was up to the patient to actively seek out HIV testing. In the current guidelines, the provider initiates the HIV testing [41].

On June 27, 2009, the Center for Urban Population Health contributed to The Cream City Medical Society led annual "Take the Test, Take Control" event in conjunction with the National HIV Testing Day as organized by the National Association of People with AIDS (NAPWA) [42].

2.3 IMAGE PROCESSING THEORY

Image processing can be defined as the manipulation of an image for the purpose of either extracting information from the image or producing an alternative representation of the image. There are numerous specific motivations for image processing but many fall into the following categories:

- i) to remove unwanted signal components that are corrupting the image (denoising) and
- (ii) to extract information by rendering it in a more obvious or more useful form.

2.3.1 Digital image processing

Digital image processing refers to processing of a two dimensional picture by a digital computer. A digital image is an array of real or complex numbers represented by a finite number of bits.

An image given in the form of a transparency, slide, photograph or chart is first digitized and stored as a matrix of binary digits in a computer memory. This digitized image can then be processed and/or displayed on a high resolution television monitor.

Digital Image processing has a broad spectrum of applications, such as remote sensing via satellites and other spacecrafts, image transmission and storage for business applications, medical processing, radar, sonar, and acoustic image processing, robotics, and automated inspection of industrial parts. Figure 2.1 summarizes the digital image processing sequence

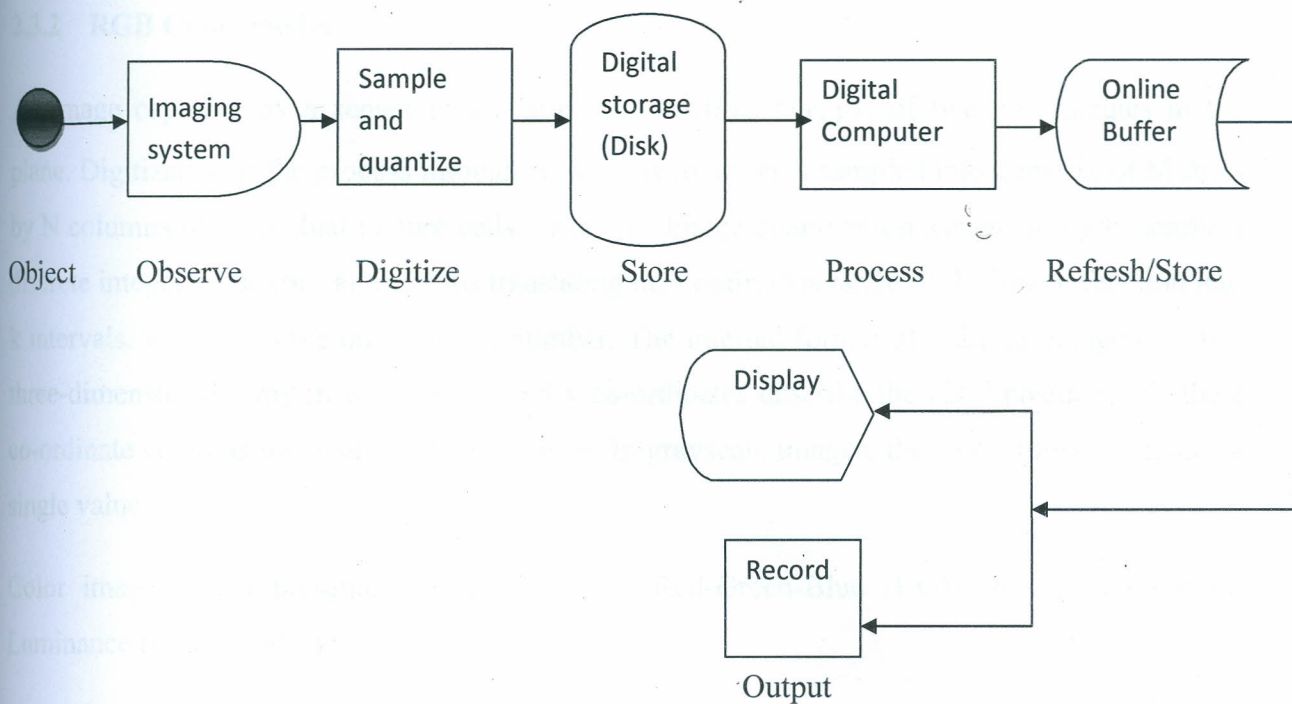


Figure 2.1 A typical Digital Image processing sequence

The image processing techniques include the following processes:

1. Image representation and modeling
2. Image enhancement
3. Image restoration
4. Image analysis
5. Image reconstruction
6. Image data compression.
7. Image Segmentation
8. Edge Detection

In this study, we have used the Image analysis process. Image analysis involves the making of quantitative measurements from an image to produce a description of it simple image analysis techniques include reading a label on a grocery item, sorting different parts on an assembly line or measuring the size and orientation of blood cells in a medical image. We have performed quantitative measurements of the RGB intensities in the control and test regions of the test kit to and declare the test results as positive, negative or invalid.

2.3.2 RGB Color model

An image captured by a sensor is a continuous function, $f(x, y)$, of two co-ordinates in the plane. Digitization is the process through which this function is sampled into a matrix of M rows by N columns of individual picture cells, or pixels. Image quantization assigns to each sample a discrete integer value (or values), thus translating the continuous range of the image function into k intervals, where k is the quantization number. The internal format of a digital image is thus a three-dimensional array in which the x and y co-ordinates describe the pixel position, and the z co-ordinate contains the pixel's intensity value. In grayscale images, the z co-ordinate contains a single value.

Color images are represented using either the Red-Green-Blue (RGB) or Hue-Saturation-Luminance (HSL) models.

The alpha (α) component describes the opacity of an image, with zero representing a clear pixel and 255 representing a fully opaque pixel. This enables an image to be rendered over another image, with some of the underlying image showing through.

Figure 2.2 shows part of a digital image with Red-Green-Blue intensity values superimposed on each pixel.

R:206 G:189 B:202	R:201 G:175 B:197	R:190 G:158 B:188	R:187 G:149 B:191	R:182 G:137 B:189	R:172 G:122 B:173	R:172 G:120 B:179	R:172 G:110 B:168
R:206 G:181 B:200	R:199 G:172 B:190	R:196 G:157 B:182	R:189 G:149 B:177	R:177 G:133 B:188	R:166 G:129 B:183	R:163 G:120 B:172	R:165 G:110 B:166
R:204 G:186 B:219	R:196 G:166 B:193	R:189 G:157 B:199	R:183 G:155 B:186	R:176 G:131 B:185	R:175 G:123 B:175	R:163 G:118 B:172	R:159 G:105 B:176
R:213 G:194 B:218	R:194 G:180 B:206	R:190 G:163 B:199	R:189 G:148 B:192	R:185 G:134 B:196	R:182 G:134 B:191	R:168 G:118 B:179	R:162 G:109 B:167
R:217 G:211 B:207	R:208 G:193 B:214	R:207 G:184 B:205	R:194 G:164 B:207	R:190 G:154 B:195	R:184 G:142 B:188	R:181 G:132 B:175	R:174 G:122 B:173
R:226 G:224 B:216	R:216 G:218 B:201	R:211 G:201 B:203	R:205 G:182 B:199	R:199 G:163 B:195	R:197 G:154 B:188	R:181 G:141 B:193	R:174 G:123 B:182
R:231 G:219 B:219	R:229 G:218 B:219	R:231 G:216 B:212	R:219 G:204 B:212	R:214 G:185 B:194	R:206 G:174 B:214	R:191 G:153 B:194	R:179 G:145 B:196
R:229 G:223 B:218	R:231 G:224 B:216	R:234 G:221 B:219	R:229 G:213 B:214	R:224 G:210 B:212	R:206 G:185 B:203	R:199 G:175 B:205	R:190 G:165 B:186

Figure 2.2 Part of a digital image, enlarged to show individual pixels, with Red-Green-Blue intensity values superimposed on each pixel.

Color conveys a significant amount of information, and is an important component in human visual perception of images. However, historically color has not been used in digital image processing. Traditional use of grayscale images in image processing has been primarily motivated by the greater storage costs for multi-spectral image data, and the greater complexity in processing these images. In grayscale images, each pixel within an image is represented by a single number: the intensity value.

In the RGB color model, each pixel is represented by a three-dimensional vector, (r,g,b) , which gives the pixel's intensity in each of the red, green and blue color components. This representation of all colors as a combination of red, green and blue is consistent with the way colors are perceived by humans. The RGB color model can be thought of as a three dimensional co-ordinatization of color space.

In the RGB color model, $(0,0,0)$ represents black, $(255,255,255)$ represents white, $(255,0,0)$ represents pure red, etc. Figure 2.3 illustrates the RGB color model

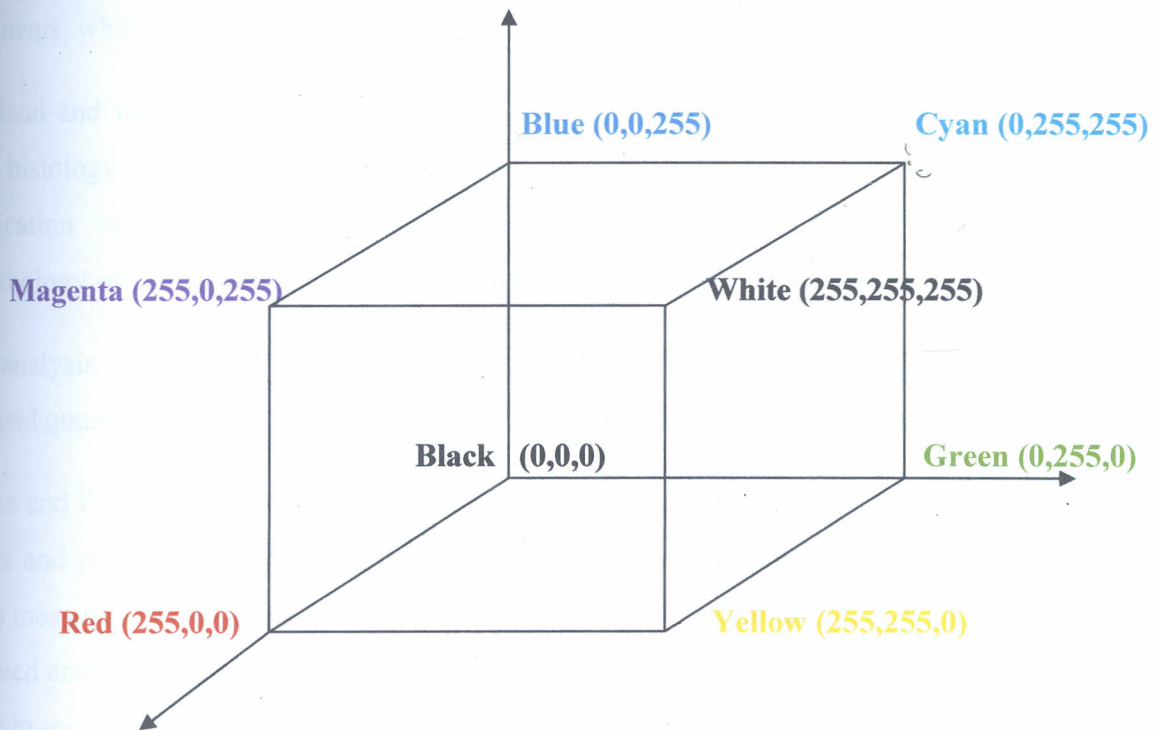


Figure 2.3 RGB color cube

2.4 APPLICATION OF IMAGE PROCESSING TECHNIQUES IN DIAGNOSIS OF DISEASES

Application of image processing and computer vision techniques to biological images has had varying levels of success, and of application, with different medical imaging modalities.

Within the histopathology field, a number of applications of image processing techniques are cited, including the following:

Wurflinger et al. describe the use of eight image measurements, including eccentricity and bending energy, to distinguish between various types of cells [43].

Both Tanaka et al. [44] and Pavlova et al. [45] discuss the applicability of conversion of histology images to the Hue Saturation and Value (HSV) color model to interpret the images. Both groups of authors state that the advantage of the use of this color model is that any

deviations in processing samples with histology stains are isolated in the saturation and value components, while hue remains constant.

Sutherland and Ironside [46] apply morphological operations to isolate areas of granular cells within histology images. The benefits of pairing a color watershed technique with color pixel classification schemes in order to improve results in classifying cellular structures in cytopathology images are examined by Lezoray and Cardot [47] in their paper.

Image analysis work by Sutherland et al. [48] uses thresholding and morphological operations to locate and quantify features of Creutzfeldt-Jakob disease in brain tissue samples.

Poulsen and Pedron [49] expand on their previous published work by experimenting with image analysis and pattern recognition methods that eliminate the need to explicitly segment images prior to measuring parameters and classifying objects. In both their earlier and more recent work, automated analysis of data resembles the manual analysis process by first locating the regions of interest in reduced resolution images, then performing detailed analysis of the selected regions at a higher resolution. Their previous work then segmented the high resolution images into background, cells and cell nuclei, followed by feature measurement and object classification. In their more recent work, the authors explore image processing techniques which do not require explicit image segmentation as an initial step. This classical sequence of image processing steps (image segmentation, feature extraction and object classification) is also applied by Liu et al. [50] in their examination of bone cross sections.

The goals of applying image processing techniques in the histopathology field range from cell counting and cell type classification, to deriving quantitative measurements of disease features from images, and automatically determining whether a disease is present within analyzed samples. Commercial products which apply image processing technology to automatically assess histologic and cytologic samples exist in a few areas where extremely high volumes are processed, such as analysis of blood samples, for example. An extensive literature search has revealed only one paper which discusses the application of image processing techniques to detect pulmonary disease. In this case, Sutton et al. [51] used texture properties to detect lung abnormalities in chest

CHAPTER THREE

METHODOLOGY

3.1 Basis of the study

In this study, the image results of the HIV test kits based on the *determine* method of the rapid test procedure were captured, processed, analyzed and automatically displayed on the computer screen. In the *Determine* method a chemically treated test kit is used. The kit comprises of a capillary cotton wool presoaked with the testing reagents and chemicals as in the ELISA procedure and taped on a hard paper strip. The strip has two white sections at the middle, the lower and the upper sections. The lower white section is the test section where the color change for the test result will appear. If a red color lining appears in this section then the result is that the client is HIV positive. However if there is no color change (remaining white) then the result is that the client is HIV negative. The upper white section of the strip is the control section where a color change MUST appear to indicate that the test is correctly performed and complete. The absence of the red color on this section invalidates the result.

A drop of the serum or whole blood is introduced at the lower end and rises by capillary action towards the treated white sections. The red color linings on the white section(s) will appear indicating the test results.

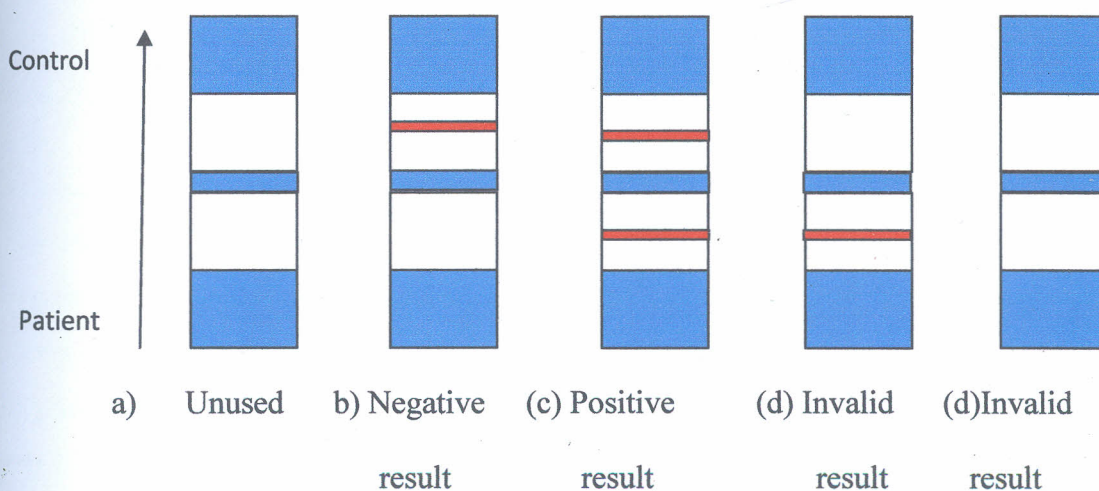


Figure 3.1 HIV Test kits before and after the test with possible color changes

The figures 3.1 a, b, c and d show the test kits in different stages; before and after the tests, and their corresponding test results. The arrow shows the direction of flow of the blood sample under the test.

Fig 3.1(a) shows an unused test kit ready to be used to perform the test. The two sections are still white and clear.

Fig 3.1(b) shows a HIV negative result. The lower section has no color change while the red color lining on control section indicates that the test is perfect and complete.

Fig 3.1(c) shows a HIV positive result. The lower section has a red color change while the upper color change at the control indicates a perfectly done test.

Fig 3.1(d) shows an indeterminate result. The lower red lining shows a positive result but the red lining at the control section is missing to confirm the validity of the test.

Fig 3.1(e) also shows another indeterminate result. There is no color change at the lower section, an indication of a negative result. However, there is no mark at the control section to confirm the validity of the test.

For the indeterminate results (d) and (e), the test should be repeated to confirm the results.

3.2 IMAGE PROCESSING SOFTWARE

A software program that identifies the presence or absence of the red color linings was developed. This software, written in C sharp (C#) programming language, could process the images and display the results on the computer screen. The software is implemented in the Client-server technology.

3.2.1 Client-Server architecture

The HIV Test Analyzer system was designed to comprise of both the client and server applications (client-server architecture). It also has a central database that is currently implemented in Microsoft (MS) Access.

A client/server architecture involves some software and hardware setup where the client makes a request for some form of service from the server. The server hosts a service that it “serves” to client request. The client-server model of computing is a distributed application structure that partitions tasks or workloads between the providers of a resource or service, called servers, and service requesters, called clients [52]. Often clients and servers communicate over a computer network on separate hardware, but both client and server may reside in the same system. A server machine is a host that is running one or more server programs which share their resources with clients. A client does not share any of its resources, but requests a server's content or service function. Clients therefore initiate communication sessions with servers which await (*listen* for) incoming requests.

An example of a client server system is an automated teller machine (ATM) network. Customers typically use ATMs as clients to interface to a server that manages all of the accounts for a bank. This server may in turn work with servers of other banks (such as when withdrawing money at a bank at which the user does not have an account). The ATMs provide a user interface and the servers provide services, such as checking on account balances and transferring money between accounts [52].

To provide access to servers not running on the same machine as the client, middleware is usually used. Middleware serves as the networking between the components of a client-server

system; it must be run on both the client and the server. It provides everything required to get a request from a client to a server and to get the server's response back to the client. Middleware often facilitates communication between different types of computer systems. This communication provides cross-platform client-server computing and allows many types of clients to access the same data [52].

In this study the client software makes requests to the server for user validation and test results processing. They are both separate but installed in the same computer.

3.2.1.1 Server system

The server system handles all the client requests. It also provides the environment where clients are taken through the preliminaries such as counseling, general data capture, and ID generation. All these are done before clients head to the client station for test procedures. The server is also used to perform all the system configurations such as imaging details, among others. The analyzer was also designed in such a way that through the server, one is also able to browse test results history based on some parameters specified by the user. These test parameters can be in terms of age, gender, occupation and place of residence. Figure 3.2 shows the main server window of the Server System. On it the new client can initialize the testing procedure by checking on the client menu and checking again on the new client menu.

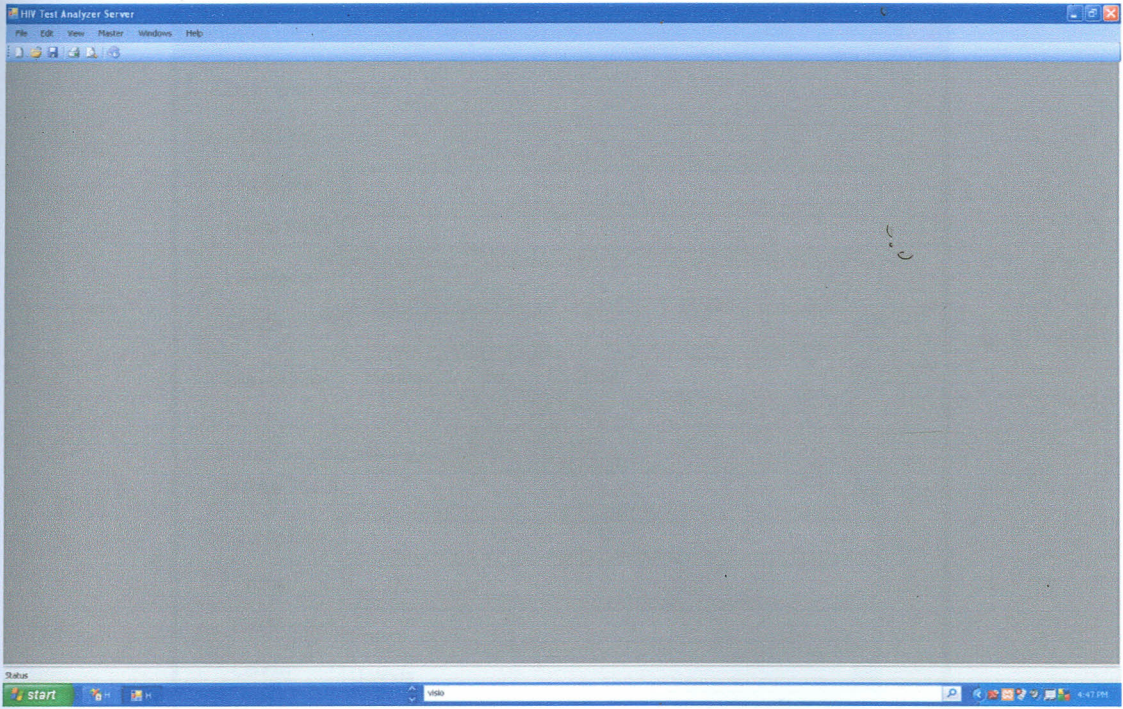


Figure 3.2 Main Server Window

Upon checking on the new client menu, the screen dump shown in figure 3.3 will appear prompting the client to enter his personal details, contacts and miscellaneous information.

The screenshot shows a software window titled "Client" with a blue header bar. The window contains two main sections of form fields:

- Client Personal Details:**
 - Test ID: Text input field.
 - First Name: Text input field.
 - Middle Name: Text input field.
 - Last Name: Text input field.
 - Gender: Dropdown menu.
 - Date of Birth: Calendar icon showing "Monday July 05, 2010".
- Contact and Miscellaneous:**
 - Marital Status: Dropdown menu.
 - Occupation: Text input field.
 - Phone: Text input field with a format of "() - -".
 - Box Number: Text input field.
 - Country: Dropdown menu showing "Uganda".
 - City: Dropdown menu.

At the bottom of the window are two buttons: "Save" and "Close".

Figure 3.3 Capturing details of test client

The server system program was developed in such a manner that the clients name (First name, Middle name and Last name) are optional. The client can proceed with other details and perform the test procedure without them. The other details like gender, date of birth, marital status, country and city must be provided. These will help the centre user to do the statistical analysis on HIV prevalence as per the given parameters. The contact address and the phone number should also be provided for easy communication with the client.

Once these details are captured as per the program, the client will save the details and close the registration exercise. Upon the closure of the registration exercise, the analyzer will generate a unique Identification (ID) number at the Test ID column and assign it to the client. This acts as the Personal Information Number (PIN) that the client will use at the client test terminal system to perform the test procedure.

The screen dump shown in the figure 3.4 shows a successfully captured data with the client's ID assigned at the Test ID column. The system will automatically inform the client of the successful saving of the records and his unique ID generated.

The screenshot displays a software window titled "Client" with two main sections: "Client Personal Details" and "Contact and Miscellaneous".

Client Personal Details:

- Test ID: 9
- First Name: JOHN
- Middle Name: EDWARD
- Last Name: OCHIENG
- Gender: Male
- Date of Birth: Saturday, June 23, 1979

Contact and Miscellaneous:

- Marital Status: Divorced
- Occupation: TEACHER
- Phone: (0722) 768-999
- Box Number: 23451
- Country: Kenya
- City: Nakuru

Buttons at the bottom of the form are "Save" and "Close".

A "Record Saved" dialog box is overlaid on the bottom right, containing the text: "Client records saved successfully. Client Test ID is 9" and an "OK" button.

Figure 3.4 Successfully captured data of the clients records with a generated Client Test ID

3.2.1.1.1 Database Objects in the server system

As can be seen in the screen dump shown in figure 3.5, the database objects include; Clients, Countries /Cities, settings, TempTestIDs, Test Records. These are discussed in details below.

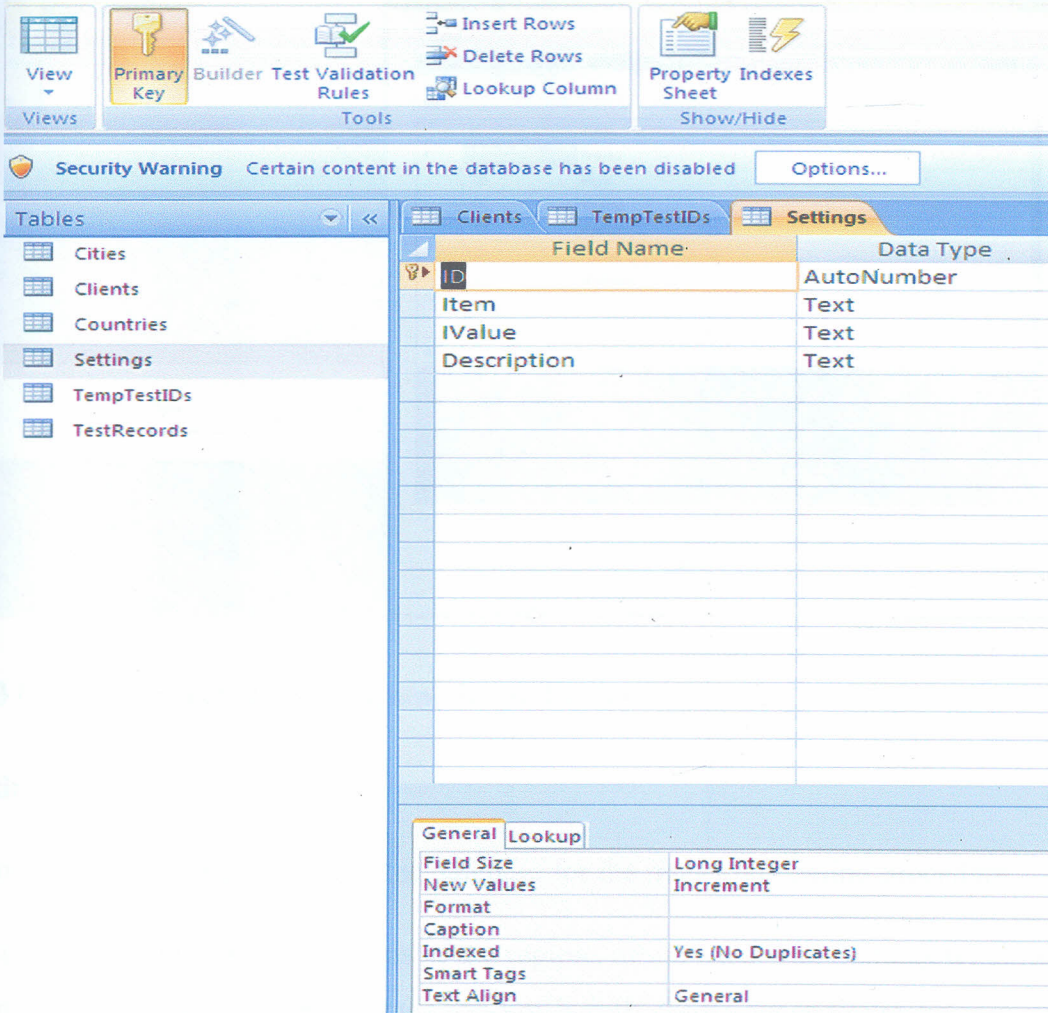


Figure 3.5 Database Objects in the server system

a) Countries/Cities

This, as illustrated in figure 3.6, contains the list of the countries and their corresponding cities that the clients come from or reside.

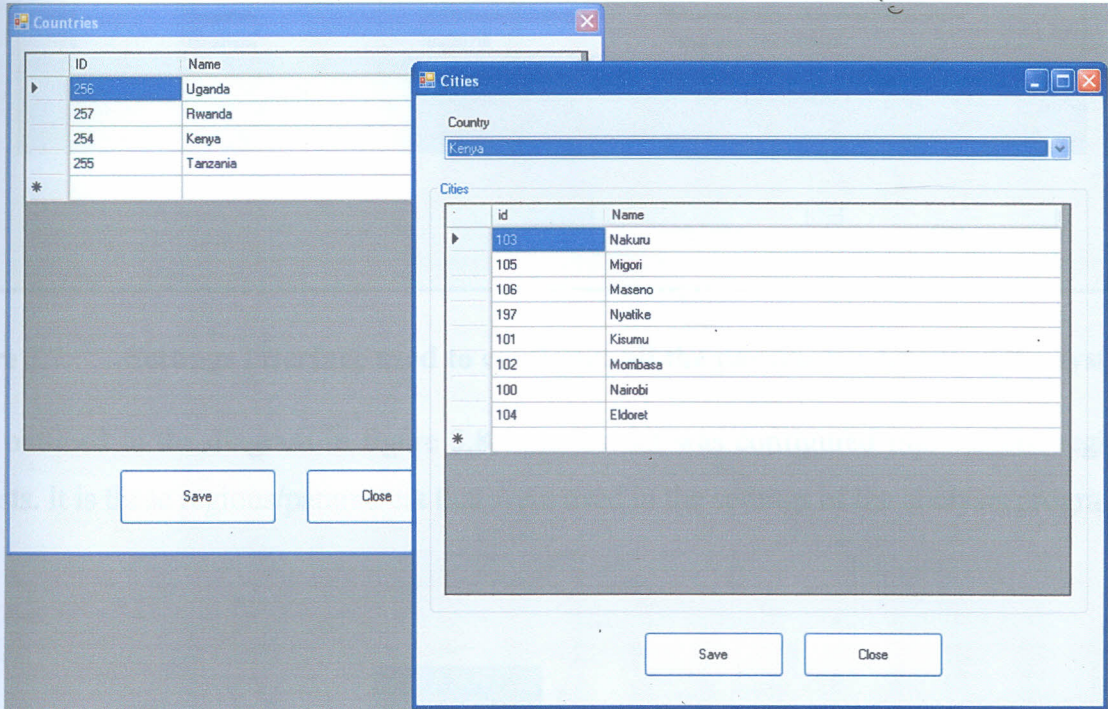


Figure 3.6 The screen dump of countries and cities.

b) Settings

The following settings were stored in the database for the image processing procedure.

The system was to analyze the sections of the regions of interests. The Settings window appears as shown in the dump below. The settings window is fully illustrated in figure 3.7.

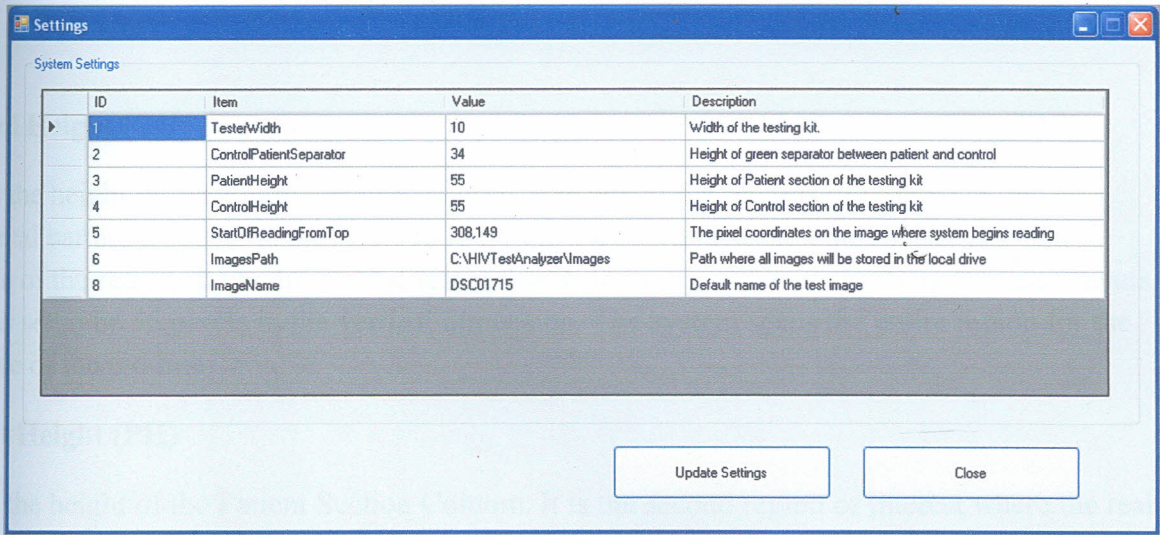


Figure 3.7 Settings interface used to configure all the parameter details of the system

As is outlined in the diagram in figure 3.8, the test kit was configured into various regions of interests. It is these regions/parameters that were used in the settings of the analysis program.

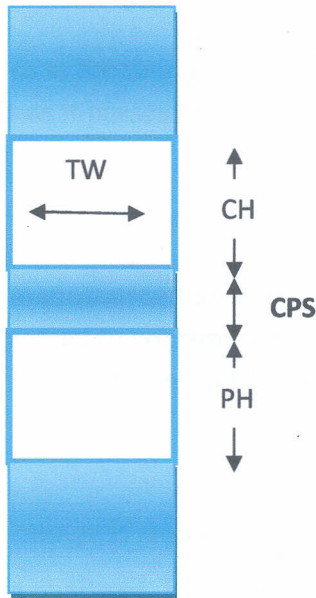


Figure 3.8 Setting configurations of the test kit

Control Height (CH)

This is the height of the control section column. It is one of the regions of interest. A red horizontal bar must appear at its centre to mark the success and completion of the test. The absence of the red mark invalidates the test and calls for a repeat. From the image measurements, CH was set to be 55 pixels in the vertical dimension. The system scans the entire region for the presence of the red line.

Patient Height (PH)

This is the height of the Patient Section Column. It is the second region of interest where the real test of virus presence takes place. A red horizontal bar is expected to appear at its centre if the blood contains HIV virus. The absence of the red bar shows that the blood is HIV Virus free.

From the image measurements it was set to be 55 pixels in the vertical dimension.

Control-Patient Separator (CPS)

This is the region that separates the CH and PH. The program is set in such a way that it skips this region during the search of the red line. It was found to be 34 pixels wide.

So the search of the red line commences at the top end of the Control height and goes 55 pixels down the kit. It then halts for 34 pixels as it passes the CPS and resumes at the top end of the patient height for another 55 pixels down the kit.

Tester Width (TW)

Since the line is horizontal, the program must ascertain that the red mark identified is continuous horizontally. The program was therefore set to confirm that the red line is continuous by 10 pixels across the two regions of interests. The tester width was therefore set to be 10 pixels.

Image path

This gives the path where the captured image is stored in the computer. The system will go to this file path to identify the image to be analyzed. In the system all the images were saved in drive C.

Image name

This is the name the picture grabber (camera) automatically assigns the image taken. The image name is unique for each image captured.

StartOfReadingFromTop

This setting parameter instructs the analyzer the pixel co-ordinate that the scanning must begin so as to scan the regions of interests as per the preset control height, patient height, and the control-patient separator. It is given in coordinate form. To get this pixel coordinate, the image is opened in MS studio by paint. The scanner will read the point where the regions of interest begin in pixel coordinate form.

In the study, the startOfReadingFromTop was taken as the topmost left corner (origin) of the upper white section at the control section.

b) Test records

This can be used to perform rapid data analysis based on the test data. One can perform a search and do a categorization of the data using a number of parameters. For instance, after a daylong of continuous testing by the machine, one can perform statistical analysis to get the number and details of the tested clients in terms of location, age, occupation, sex etc.

One can know how many clients tested positive, coming from a particular city and of a particular age and sex. Several analyses can be done in terms of the most vulnerable age and sex, risky occupations and much more. The screen dump in figure 3.9 is pretty much intuitive.

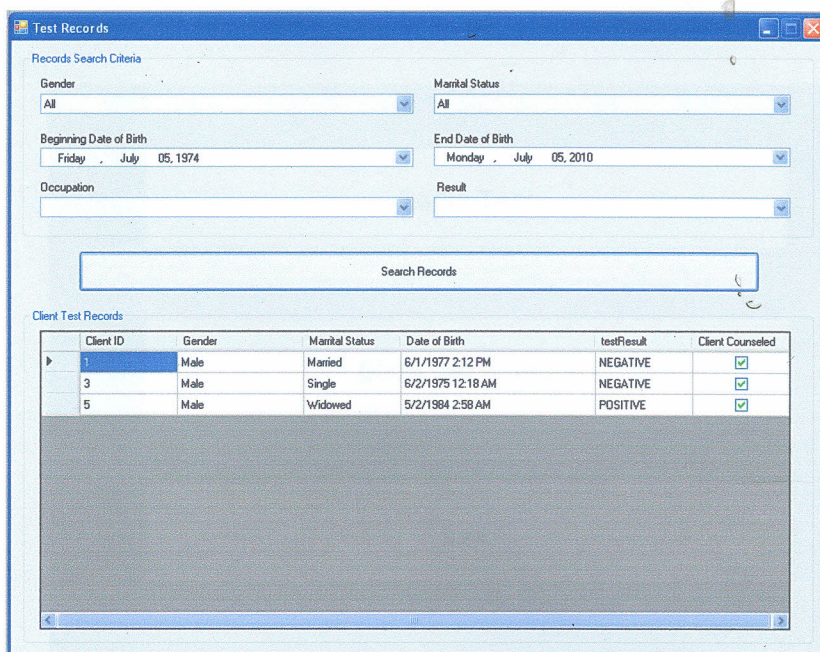


Figure 3.9 Test data analysis screen

3.2.1.2 Client System

Through the client system, the analyzer was designed such that clients (users/customers) are able to perform the actual HIV test procedure. It presents a user interface similar to the one shown in figure 3.14. Here users get to interact with the Analyzer. User can enter test ID, get instructions on how to go about test, and then perform the actual test. Before a user can go through the actual HIV Test, he/she must enter the ID generated and assigned to him/her by the server after a successful counseling process. User can also be able to request for his/her test results. The client system offers a four steps process.

Step 1. Beginning the Test

The client initiates the testing process by clicking on the BEGIN TEST bar, from the main client point of test terminal shown in figure 3.10.

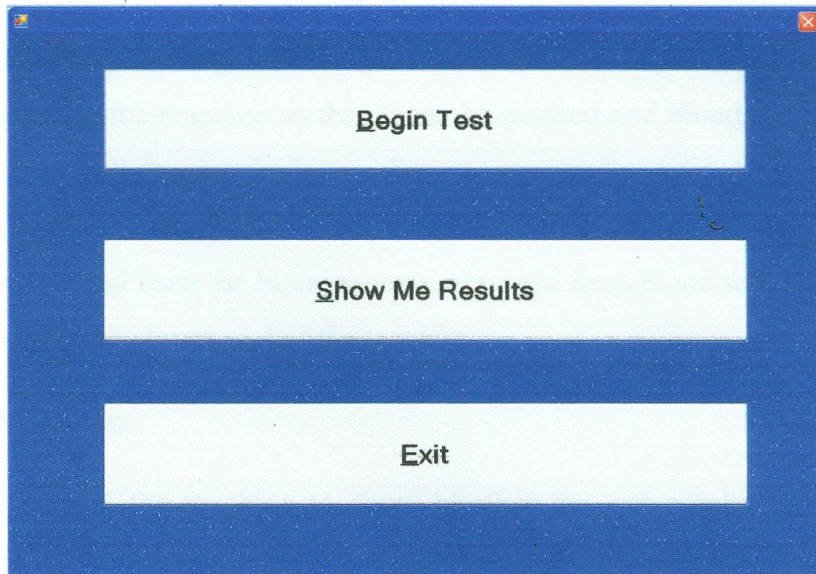


Figure 3.10 Client point of test terminal

Step 2. Entry of personal number ID

The system will request the client to enter his/her personal Identification number (ID) generated while at the server system. The screen dump shown in figure 3.11 prompts the client to enter his/her tests ID.

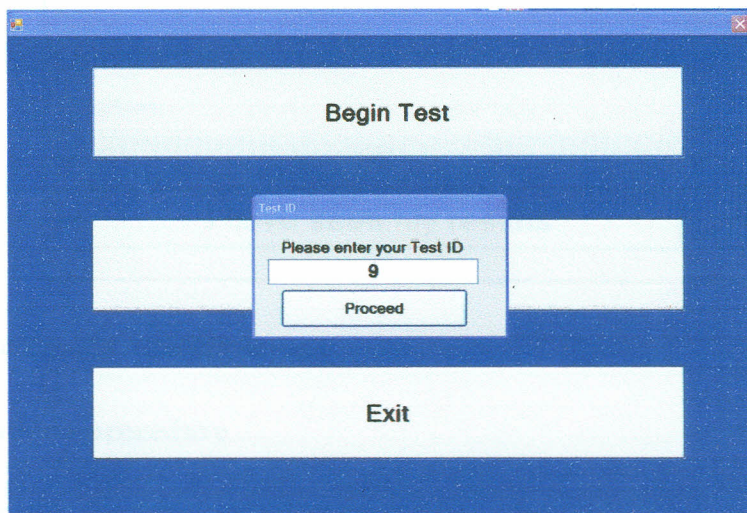


Figure 3.11 Beginning Test; user required to enter test ID

Once the ID is entered and positively validated, the human client is prompted to place his/her finger at a specific point on the machine so that it can be pricked and blood drawn from it into the test kits. The machine will process the image that was captured corresponding to the entered ID. This is automatic and takes less than a second for the process to be complete. During our pretrial tests, samples of blood from the blood sachets, obtained from blood screening laboratory, were physically dropped on the lower end of the test kits.

Step 3. Request of the results.

The client will now request the machine to show him/her the test results. This is done by checking on Central white bar written show me results. Once the client has clicked the show me results bar, the results will be automatically displayed on the screen. One of the four possible results that will be displayed for every test session is shown in figure 3.12.

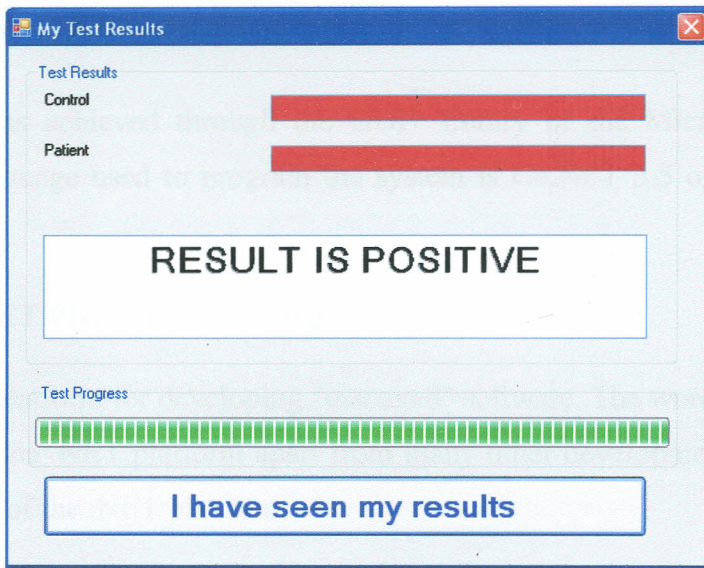


Figure 3.12 Test results viewing screen

Step 4. Closing the test procedure

Once the client has seen the results, he/she simply closes the procedure by clicking the toolbar written “**I have seen my results**”. Once this is checked, the machine will prompt a message

written **“Thank you, we are happy to serve you”**. He then quits and proceeds to the doctor and the counselor for post-test counseling. Figure 3.13 shows the exiting screen.

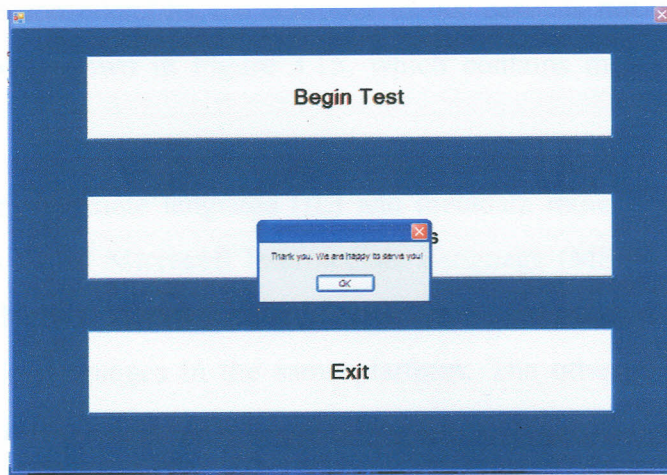


Figure 3.13 Test procedure closing window.

3.2.2 Image Processing and Analysis

Image processing was achieved through the GDI+ library of the Microsoft .NET Platform Technology. The language used to program the system is C#.NET 3.5 of Visual Studio .NET 2008.

3.2.2.1 Microsoft .NET Platform Technology

Microsoft .NET is a platform for developing "managed" software. The word managed is key here — a concept setting the .NET platform apart from many other development environments. It is an integral capability of the .NET platform.

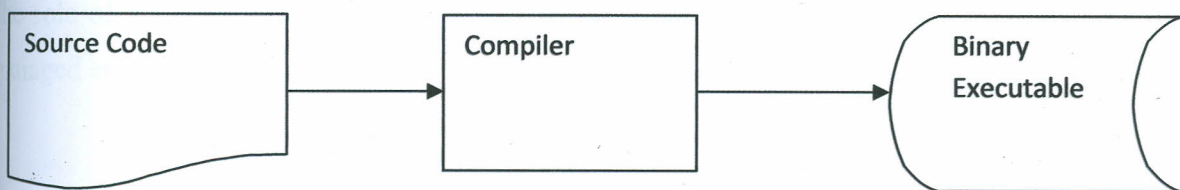


Figure 3.14 Traditional compilation.

In the traditional compilation process (Figure 3.14), the executable file is binary and can be executed by the operating system immediately. However, in the managed environment of .NET, the file produced by the compiler (the C# compiler in our case) is not an executable binary. Instead, it is an assembly, shown in Figure 3.15, which contains metadata and intermediate language code.

An assembly contains intermediate language (IL) and metadata rather than binary code. This intermediate language is called Microsoft Intermediate Language (MSIL), which is commonly referred to as IL. IL is a high-level, component-based assembly language. IL supports a common type system and multiple languages in the same platform. The other part of an assembly is metadata, which is extra information about the code being used in the assembly.

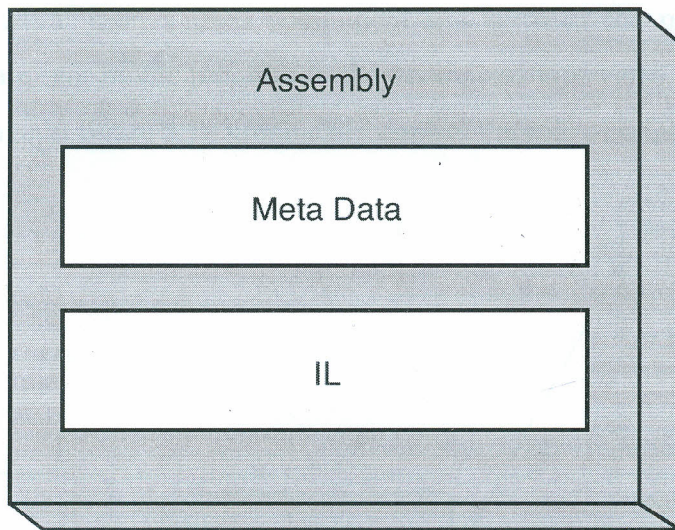


Figure 3.15 The contents of an assembly

The role of an assembly is to be a unit of deployment, execution, identity, and security in the managed environment.

3.2.2.2 GDI+ Library

GDI stands for Graphical Device Interface. Windows GDI+ is the subsystem of the Windows XP operating system or Windows Server 2003 that is responsible for displaying information on screens and printers. GDI+ is an API that is exposed through a set of C++ classes.

GDI+ is a library that provides an interface that allows programmers to write Windows and Web graphics applications that interact with graphical devices such as printers, monitors, or files.

All graphical user interface (GUI) applications interact with a hardware device (a monitor, printer, or scanner), that can represent the data in a human-readable form. However, there is no direct communication between a program and a device; otherwise, you would have to write user-interface code for each and every device with which your program interacts.

To avoid this monumental task, a third component sits between the program and device. It converts and passes data sent by the program to the device and vice versa. This component is the GDI+ library. The image in figure 3.16 graphically depicts the role of GDI

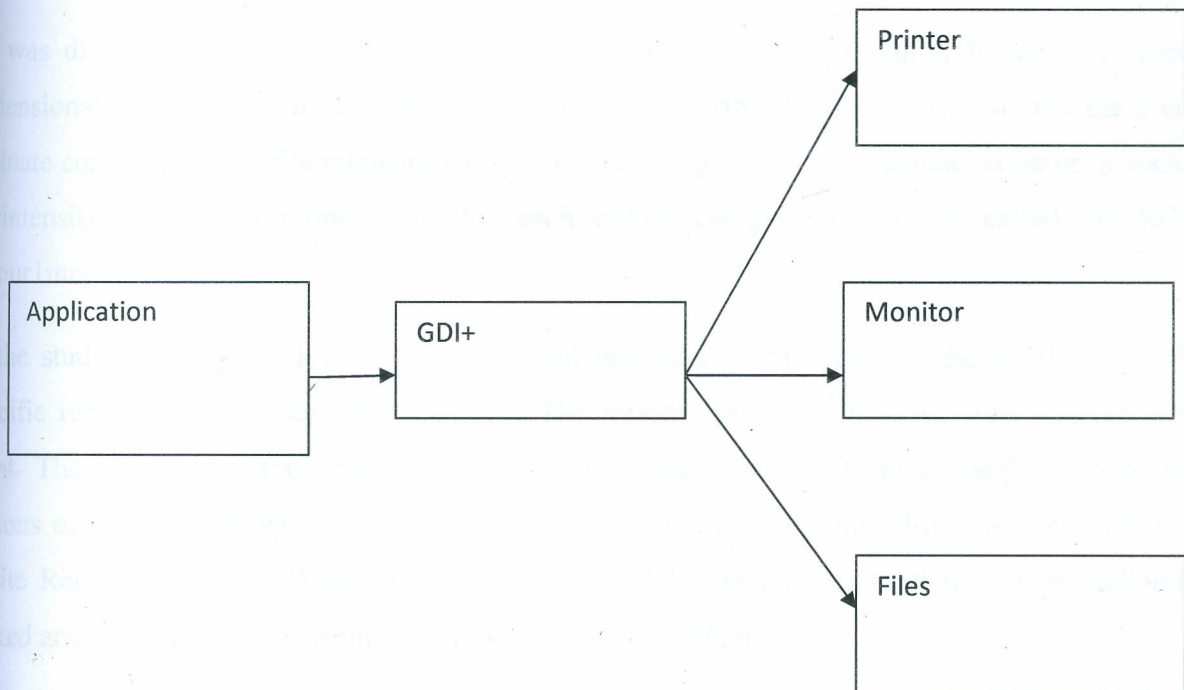


Figure 3.16 Graphical representation of the role of GDI+

GDI+ allows application programmers to display information on a screen or printer without having to be concerned about the details of a particular display device. The application programmer makes calls to methods provided by GDI+ classes and those methods in turn make the appropriate calls to specific device drivers. GDI+ insulates the application from the graphics hardware, and it is this insulation that allows developers to create device-independent applications.

In processing the image samples below, as well as the ones that will be captured at the HIV Test Terminals, GDI+ helped us achieve the image processing needs. It enabled us to display the results of the processed images on our computer screen.

3.2.2.3 Investigating the RGB composition of the ROIs

The image processing involves identifying the areas/sections of interest i.e. the control and the patient areas. The program scans these two regions looking for particular colorations. Depending on the color status of the 2 sections, a reading may be positive, negative or invalid. The flow chart shown at the appendix 1 shows the scanning procedure.

As was discussed earlier in chapter two, the internal format of a digital image is a three-dimensional array in which the x and y co-ordinates describe the pixel position, and the z co-ordinate contains the pixel's intensity value in colour images, the z co-ordinate contains a vector of intensity values, with one value for each colour component. This is called the RGB colour model.

In the study a sample of kits were picked and the color components in the RGB model for specific regions of interests were recorded. The images, saved in drive C, were opened with paint. The show color tool was then used to investigate the RGB color composition of the regions of interests (ROI). Four arbitrary points on the white regions, White Region1 (WR1), White Region 2 (WR2), White Region3 (WR3) and White Region 4 (WR4) were randomly picked and their RGB color composition were recorded and analyzed.

Similarly, four arbitrary points on the red line that appeared after the test procedure were also picked and the analysis of their RGB color composition made. These regions were named Red

Region1 (RR1), Red Region2 (RR2), Red Region3 (RR3) and Red Region4 (RR4) respectively. The analyses were done on 4 images of randomly picked used test kits.

The results obtained were recorded and analyzed. An image processing software program was developed based on this RGB analysis report.

3.3 SYSTEM FLOW CHART

The program was designed in a systematic work flow. First the system scans the control region (the upper ROI) to find out if a red line exists. If there is no red line found at this control region, it automatically displays an invalid result and begins a new cycle of the process. However, if a red line is identified, it proceeds to perform a similar scan at the test region, the second ROI at the lower section. If a red line is identified in this second section, a positive result is declared. If there is no red line identified then a negative result is declared. The system then ends the process after the declaration of the results. A schematic flow chart diagram is represented at the Appendix 1.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RGB composition results analysis

As was discussed in chapter 3, the RGB colour model analysis was done on the red and white regions after the test procedure and the results obtained and recorded.

Sample regions of interests were randomly picked on four image samples. For each image sample, four regions were randomly picked on the white region and named White Region1 (WR1), White Region 2 (WR2), White Region 3 (WR3) and White Region 4 (WR4) respectively. Their RGB color components were read and recorded. Similarly two other points on the red line that appeared on the images were also randomly picked, namely Red Region1 (RR1), Red Region 2 (RR2) Red Region3 (RR3) and Red Region 4 (RR4) respectively. These Red Regions and the White Regions formed our regions of interests (ROIs). Their RGB colour components were also read and recorded. Several statistical analyses were then done on the obtained data and the conditions to declare a region as RED was reached at.

The following statistical analyses were done for each sample image.

i) Red Dominance

This is how the red color dominates over other colors. It is calculated as the difference in color composition between Red and Green (R-G) & the difference between Red and Blue (R-B)

ii) Color percentage composition.

This is the percentage composition of each color in each region of interest.

iii) Color Sum.

This is the sum of all the color composition in each region i.e $R+G+B$.

All these parameters together with the RGB compositions in both regions were averaged and mean parameter values were obtained.

The analyzed results were as displayed in tables 4.1 to 4.4 as follows.

Table 4.1 RGB composition results and analysis at the ROIs of sample 1. (The unit of RGB intensity values is candela)

COLORS	Regions of interest									
	WR1	WR 2	WR 3	WR 4	AVG WR	RR1	RR2	RR 3	RR 4	AVG RR
RED	183	167	170	176	174.00	179	158	170	165	168.00
GREEN	176	162	164	168	167.50	162	124	140	143	142.25
BLUE	170	156	158	166	162.50	152	114	136	127	132.25
	RED DOMINANCE									
R - G	7	5	6	8	6.50	17	34	30	22	25.75
R - B	13	11	12	13	12.25	27	44	34	38	35.75
	PERCENTAGE COMPOSITION									
RED	34.59	34.43	34.55	34.51	34.52	36.31	39.90	38.12	37.93	38.07
GREEN	33.27	33.40	33.33	32.94	33.24	31.31	33.65	31.39	32.87	32.31
BLUE	32.14	32.17	32.11	32.55	32.24	30.83	28.79	30.49	29.20	29.83
	COLOR SUM									

Table 4.2 RGB composition results and analysis at the ROIs of sample 2. (The unit of RGB intensity values is in candela)

COLORS	Region of interest									
	WR1	WR 2	WR 3	WR 4	AVG WR	RR1	RR2	RR 3	RR 4	AVG RR
RED	181	175	179	182	179.25	180	160	178	170	172.00
GREEN	177	172	176	178	175.75	161	131	153	150	148.75
BLUE	168	165	167	171	167.75	155	117	148	132	138.00
	RED DOMINANCE									
R - G	4	3	3	4	3.50	19	29	25	20	23.25
R - B	13	10	12	11	11.50	25	43	30	38	34.00
	PERCENTAGE COMPOSITION									
RED	34.41	34.18	34.29	34.27	34.29	36.29	39.22	37.16	37.61	37.57
GREEN	33.59	32.46	33.72	33.52	33.32	32.11	33.80	31.94	33.19	32.76
BLUE	31.94	32.23	31.99	32.20	32.09	31.25	28.68	30.90	29.20	30.01
	COLOR SUM									
R+G+B	526	512	522	531	522.75	496	408	479	452	458.75

Table 4.3 RGB composition results and analysis at the ROIs of sample 3. (The unit of RGB intensity values is candela)

COLORS	Region of interest									
	WR1	WR 2	WR 3	WR 4	AVG WR	RR1	RR2	RR 3	RR 4	AVG RR
RED	139	190	175	181	171.25	164	163	160	165	163.00
GREEN	149	187	171	176	170.75	146	144	140	147	144.25
BLUE	140	172	165	169	161.50	122	127	122	125	124.00
	RED DOMINANCE									
R - G	-10	3	4	5	0.50	19	19	20	18	19.00
R - B	-1	18	10	12	9.75	42	36	38	40	39.00
	PERCENTAGE COMPOSITION									
RED	32.48	34.61	34.25	34.41	33.94	37.96	37.56	37.91	37.76	37.80
GREEN	34.81	34.06	33.46	33.46	33.95	33.80	33.18	33.18	33.64	33.45
BLUE	32.71	31.33	32.29	32.12	32.11	28.24	29.26	28.91	28.60	28.75
	COLOR SUM									
R+G+B	529	485	511	526	512.75	493	396	422	437	437

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Table 4.4 RGB composition results and analysis at the ROIs of sample 4. (The unit of RGB intensity values is candela)

COLORS	Region of interest									
	WR1	WR 2	WR 3	WR 4	AVG WR	RR1	RR2	RR 3	RR 4	AVG RR
RED	175	177	176	178	176.5	157	160	161	159	159.25
GREEN	171	174	174	174	173.25	143	142	148	140	143.25
BLUE	159	165	165	168	164.25	130	128	132	129	129.75
RED DOMINANCE										
R - G	4	3	2	4	3.25	14	18	13	19	16
R - B	16	12	11	10	12.25	27	32	29	30	29.5
PERCENTAGE COMPOSITION										
RED	34.65	34.30	34.17	34.23	34.34	36.51	37.20	36.51	37.86	37.02
GREEN	33.86	33.72	33.79	33.46	33.71	33.26	33.02	33.56	33.33	33.29
BLUE	31.49	31.98	32.04	32.31	31.96	30.23	29.77	29.93	30.71	30.16
COLOR SUM										
R+G+B	526	512	515	520	518.25	496	430	441	420	446.75

The dimensional SI unit of the EGB intensity values is Lux. Lux is used in photometry as a measure of the intensity of light that hits or passes through a surface. The following consistent observations were made from the analyzed data. The interest was focused in the red region. Thus;

For all the red regions

- i) The mean dominance of red over green is at least 16. i.e $R-G \geq 16$
- ii) The mean dominance of red over blue is at least 29.5, i.e $R-B \geq 29.5$
- iii) The mean percentage composition of the color Red in the red region is at least 37.02% with at least 3.5% dominance over the rest.
- iv) The mean color sum in all the red regions was found to be at most 458.75,

$$\text{i.e } R+G+B \leq 458.75$$

Further experimental investigations revealed that when these conditions were adjusted in such a way that $R-G \geq 10$, $R-B \geq 20$ and Red Percentage Composition ≥ 36 , then more accurate results were achieved. A software program was therefore written using these three conditions. More than 100 samples were fed into the system and accurate and successful results were obtained. This confirmed the reliability of the system.

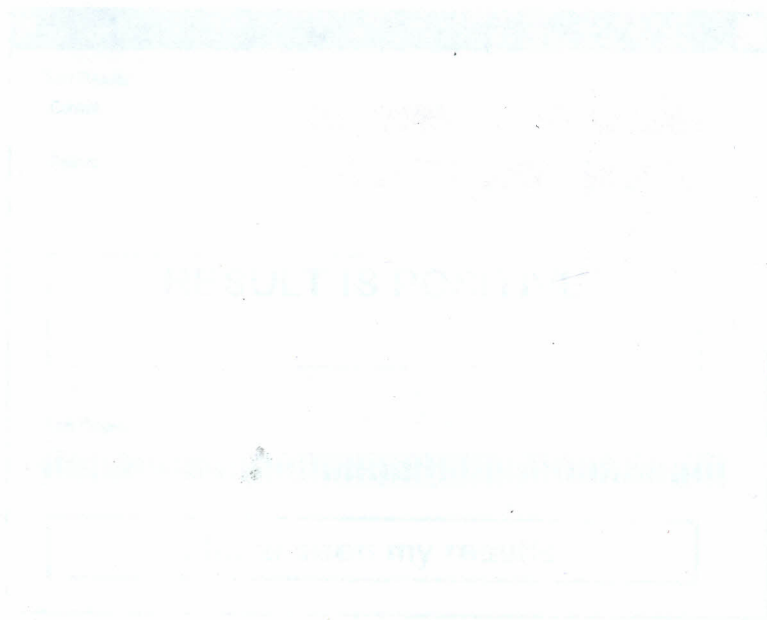
4.2 The image processing software program

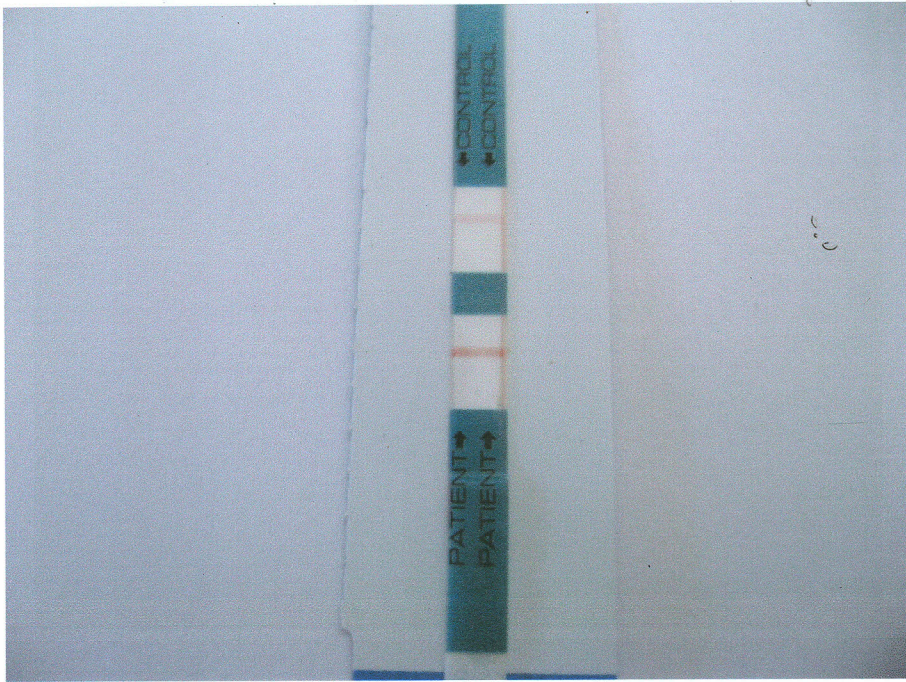
Having set the conditions to a region as red the part of the program code was written in such a way that that if $R-B > 10$ and $R-G > 20$ and the percentage of red color $> 36\%$ then the system is to declare the region as red. Or else no red found. The investigation of the red region was done in the order that the system scans through the Control region first, searching for red, followed by the test region. The results from both the regions are combined and used to declare whether the test result is positive, negative or invalid. A sample of the program code for the complete HIV test analyzer system is contained in the appendix 2.

4.3 Display of test results on the screen by the system analyzer

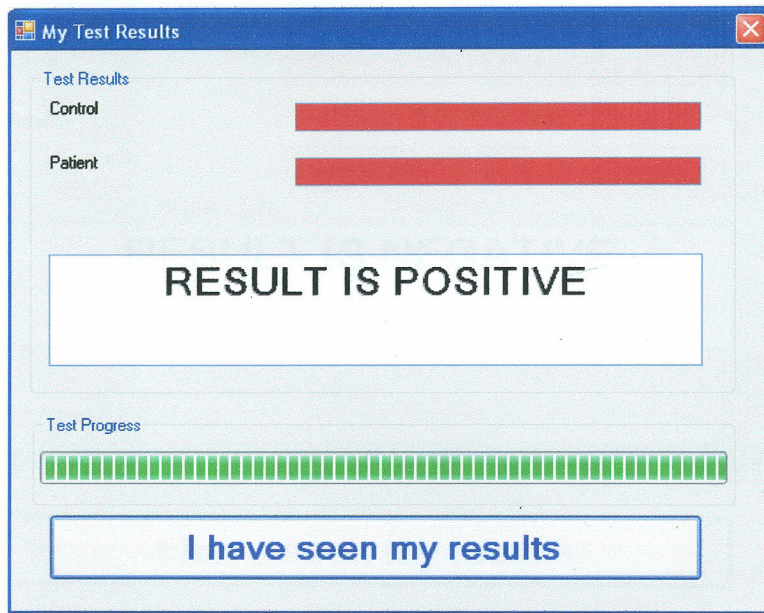
Sample images were set into the program at the server end. This was done by picking an image then subjecting it into the program processing with a client's details. The following results were obtained as displayed on the screen. The displayed results were found to be consistent with the manually read results.

Figures 4.2 to 4.5 show the consistency between the manually read results from test kits and the corresponding displayed results from the system.



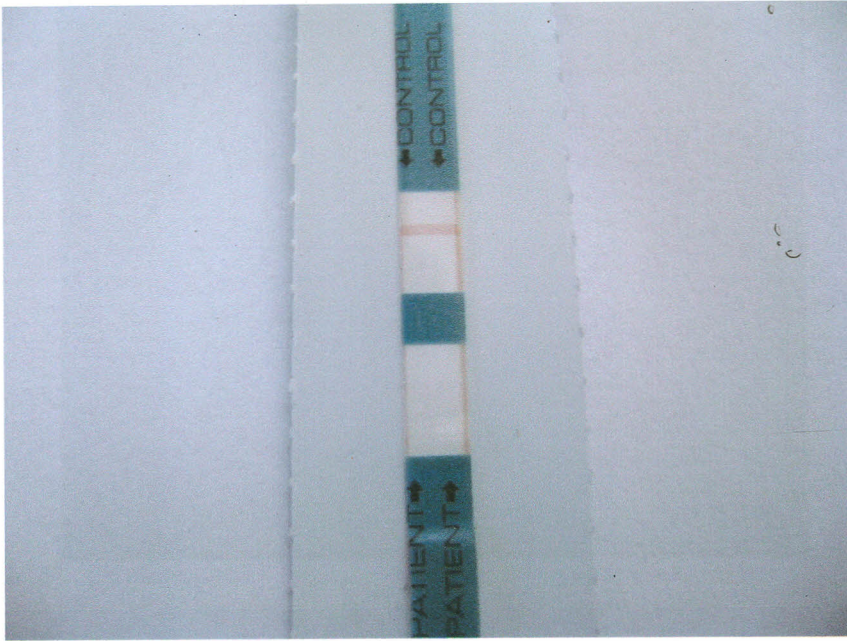


a)

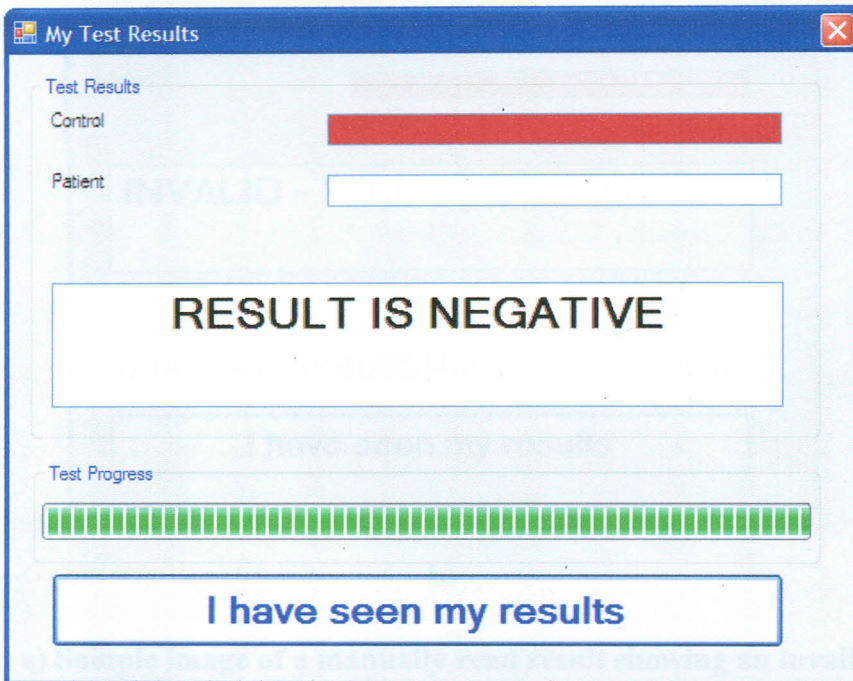


b)

Figure 4.2 a) Sample image of a manually read result showing a HIV positive result
b) Computer screen result from the analyzer showing positive result.

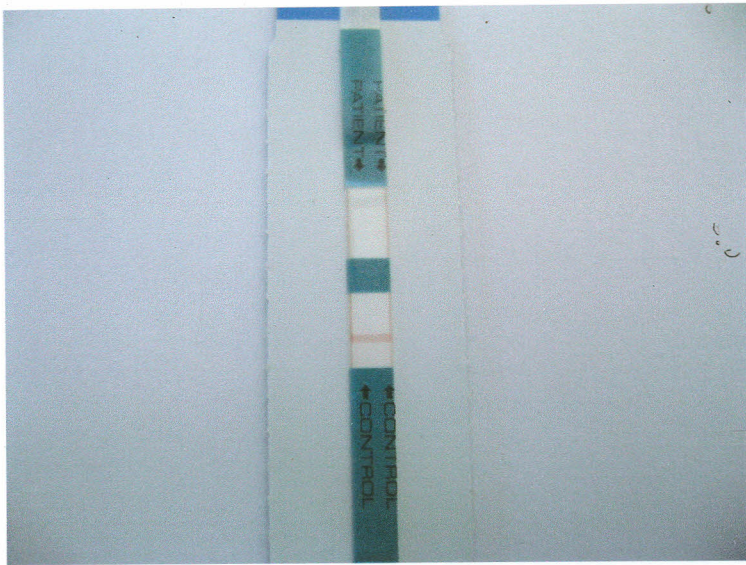


a)

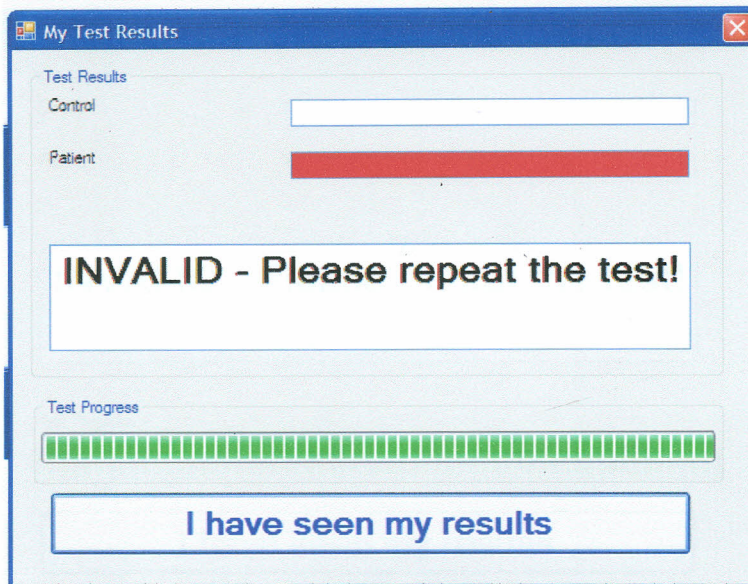


b)

Figure 4.3 a) Sample image of a manually read result showing a HIV negative result
b) Computer screen result from the analyzer showing a negative result

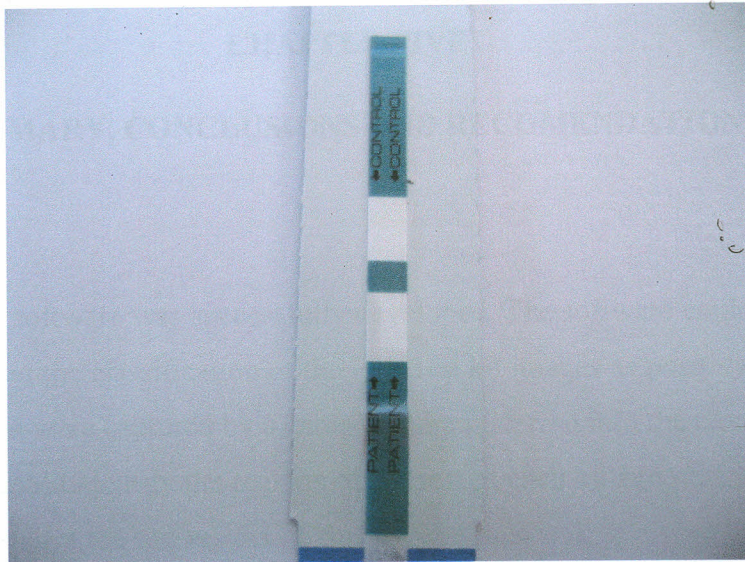


a)

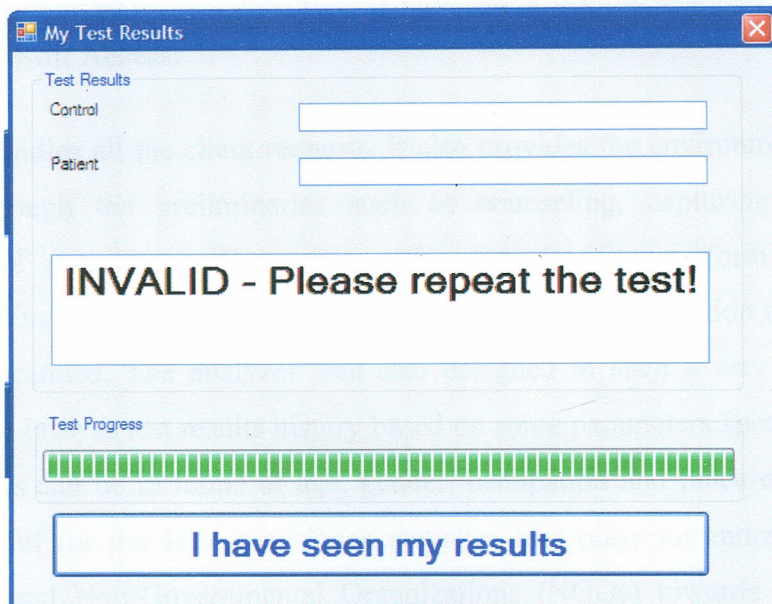


b)

Figure 4.4 a) Sample image of a manually read result showing an invalid result, case 1
b) Computer screen result from the analyzer showing an invalid result, case 1



a)



b)

Figure 4.5 a) Sample image of a manually read result showing an invalid result, case 2

b) Computer screen result from the analyzer showing an invalid result, case 2.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMENDATIONS

5.1 Summary

The image processing software was successfully developed. The software could perform the analysis, processing and the classification of the HIV test kit images as positive, negative or invalid. Sample images were captured by a camera and transferred into the computer data base. The analyzer system could identify the regions of interest of each kit and performs the RGB color analysis then displays the test results on the screen.

The HIV Test Analyzer system was successfully designed to comprise of both the client and server applications (client-server architecture). It also has a central database that is currently implemented in Microsoft Access.

The server system handles all the client requests. It also provides the environment where clients could be taken through the preliminaries such as counseling, capturing his/her general particulars, and PIN generation. The server was also used to perform all the system configurations including setting the image details as per the RGB composition and the regions of interests that were scanned. The analyzer was also designed in such a way that through the server, we are able to browse test results history based on some parameters specified by the user. These test parameters can be in terms of age, gender, occupation and place of residence. This will prove very useful for the HIV prevalence statistics that plays an enormous role by the Ministry of Health and Non-Governmental Organizations (NGOs) towards the fight against HIV/AIDS.

Through the client system, the analyzer was designed such that clients (users/customers) were able to go through the actual HIV test. It is here where the actual HIV test result is displayed after a successful test procedure. User can enter test ID, get instructions on how to go about test, and then perform the actual test. To initiate the testing procedure at the server end, the client must enter the PIN generated and assigned to him/her by the server system.

The client/server model used in the system therefore chiefly assisted in the general image processing techniques and final display of the results in the display unit of the Analyzer system.

5.2 Conclusion

A lot of work has been done and research carried out in an attempt to carry out HIV test efficiently and more effectively. Most testing methods have been developed and are in use in the health sector. Unfortunately, none of these studies has been able to satisfactorily address the issue of the accurate reading and interpretation, confidentiality, automation and speed.

It is highly hoped that this technique will not only eliminate the human error associated with the use of HIV testing kits, but will also improve the testing productivity in comparison to those achieved by the trained technicians. It will also enhance the confidentiality of the test result hence reducing the stigma associated with the disease and motivate more people to know their HIV status.

5.3 Recommendations

Having undergone successful pre-trial tests, we highly recommend that the Government of Kenya, through the ministry of Health, implements the application of the system in the current HIV testing procedures. The system can be installed in our clinics at the test terminals and at the Voluntary Counselling and testing (VCTs) units. The NGOs focusing on the fight against the deadly scourge are also encouraged to adopt the same system.

The System has got some limitations that need to be addressed during implementation. It requires computer literacy hence there is need to induct members of the society in the basic computer

5.4 Suggestions for further research

Although we have fully developed the HIV System analyzer model using software based image processing technique there is still room for further development of the system.

We suggest the use of the laboratory Virtual Instruments Environment Workbench (LabVIEW) program to develop the processing software instead of the wordy C# language that was used.

Developed by National Instruments (NI), LabVIEW is a programming language that depicts program code graphically rather than textually [53]. One major benefit of using LabVIEW programming rather than text based languages like the C# is that the program codes are written by simply connecting icons. In addition, graphical programming offers the performance and flexibility of text-based programming environment that conceals many programming intricacies such as memory allocation and syntax. LabVIEW language will therefore conserve much space and also ease the implementation and multitasking techniques [54]. For the purposes of this research, we had earlier proposed to use LabVIEW language, but upon the development of the system, we lacked the LabVIEW kit that would aid in the development. The C# programming language therefore remained our last resort.

We also suggest another insight into the study whereby several samples can be analysed at ago as in the ELISA method.

It is also recommended that other laboratory medical tests for other diseases that involve colour change be implemented in the similar manner.

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