PROJECT TITLE: A RAPID PROCEDURE THAT DETECTS P24 ANTIGEN AND HIV-RNA BIOMARKERS SIMULTANEOUSLY TO EARLY DIAGNOSE HIV WITHIN 72 HOURS OF INFECTION

NAME: TAKUWA MERCY

REG NO: 14/U/15111/PS

STUDENT NUMBER: 214012180

DECLARATION

I Takuwa Mercy declare that the work contained in this report has been done by me and has never been presented to this or any other Institution as an academic Requirement.

Signature: 

TAKUWA MERCY
APPROVAL

This Project is submitted in partial fulfillment of the requirements for a Bachelor’s degree in Biomedical Engineering at Makerere University.

Title: A rapid procedure that detects p24 antigen and HIV-RNA biomarkers simultaneously to early diagnose HIV within 72 hours of infection

Presented by

Takuwa Mercy

Supervisor: Dr. Robert Ssekitoleko

Signature: ......................................

Department of physiology

School of Biomedical sciences

College of health sciences

ABSTRACT

Newly infected individuals are transferring the virus prior to knowing their true status. This is a significant barrier to eliminating Human Immune Virus (HIV) even in the current era of antiretroviral therapy. Currently, 36.7 million people globally are living with the Virus. Measures to reduce the virus have been set up by the World Health Organisation however, these have been rendered ineffective due to lack of reliable methods to detect, quantify and characterise cells that harbour low levels of the virus. Currently, diagnosis of HIV infection is based on serology which is possible only 3 months after exposure, this is a drawback since almost half of new HIV infections come from individuals who have just been infected within 3 months. Post-exposure prophylaxis (PEP) is taken within 72 hours of infection for 28 days to avoid becoming HIV-positive, this demands for early detection. Current technologies that detect the virus at an early stage have emerged recently, however, they are highly sophisticated, expensive which makes their deployment in low resource countries difficult. This research project is exploring a new procedure that can accurately detect the virus within the first 72 hours of infection. This will be achieved by detecting both P24 antigen protein and HIV-RNA biomarkers simultaneously which appear in the blood within 24 hours-2 days after HIV exposure using Magnetic sensors. Magnetic iron Oxide-based on Magnetic tunnel junction have been used in accurate detection and identification of biomolecules tagged with magnetic nanoparticles including HIV- DNA and p24 antigen protein. However, these have been detected separately in all the studies conducted. This research is detecting HIV-RNA instead of DNA and detecting both biomarkers labelled with magnetic nanoparticles at the same time using the magnetic sensor. Hence, individuals will know their true status 3 days after exposure and take PEP within 72 hours as recommended.

SPECIFIC AIMS

Human Immunodeficiency Virus (HIV) infection causes an irreversible weakening of the immune system eventually leading to the development of AIDS in the majority of infected persons. The World Health Organization estimates that 36.7 million people are living with HIV globally of whom 2.1 million are newly infected annually [1]. Currently, the annual number of people newly infected with HIV has declined by 20% due to tremendous progress in early diagnosis and treatment [2]. Accurate and early detection of the infection has proved to be one of the best ways to challenge the growing global concern however, Acute HIV infection (AHI) diagnosis is still a major hindrance and identifying individuals that are newly infected with HIV remains problematic [3][4][5][6]. AHI, a period between the acquisition of human immunodeficiency virus (HIV) and the development of HIV-specific antibodies is characterized by high viral replication, hyper-transmission potential and non-specific febrile illness [7][3][8][9]. In newly infected individuals, HIV antigen p24 is usually present in their serum or plasma 7-10 days before the HIV antibody. This nullifies all test procedures which utilise HIV antibodies to determine one’s status. One will always test negative until 3 months when the HIV antibodies are detectable which puts the public at risk [1][8]. For that reason, early detection of HIV allows infected individuals will prevent further transmission allow individuals make more informed decisions such as practising safer sex [5]. Currently, HIV is diagnosed earlier by immunological and molecular techniques [10][11][12]. These are procedures used for early detection of HIV infection during the AHI phase; One is Polymerase Chain Reaction for detecting plasma HIV-RNA [12] a rapid procedure with isolation and detection of 4 hours is highly prone to false positive results due to cross contamination [13]. P24 antigen assay a highly specific procedure compared to serological assays however, it is susceptible to false positive reaction because of interfering and immune complexes [3]. Overall, most of these tests are laboratory-based, take more than 1 hour to obtain results, highly sophisticated, expensive which makes their deployment in low resource settings infeasible [10].

Presently, researchers from the Spanish National Research Council (SNRC) are developing a bio sensor which is a rice grain-sized chip combining micro-mechanical silicon structures and gold nanoparticles for detecting P24 antigen [14][15][16]. Fortunately, any individual who has been exposed to the virus can take Post-exposure prophylaxis (PEP) within 72 hours after exposure to reduce the risk of becoming HIV-positive [17][18][19]. Nevertheless, PEP is expensive and few can afford so it requires one to be tested to avoid unnecessary costs [20][21][22]. The goal of this study is to develop a cheaper and rapid test procedure which will be used to test for the virus after two days of exposure. **Aim 1: To quantify P24 antigen and HIV RNA biomarkers in plasma in the first 72 hours of HIV exposure,** this is to determine the exact concentration of biomarkers. Sub aim 1.1 To quantify of p24 antigen after HIV infection in plasma. Sub aim 1.2: To quantify plasma HIV-1 RNA by real-time PCR [23]. This will be crucial in determining the size of magnetic nanoparticles required to mark and label the biomarkers for the sensor to recognise them [24]. **Aim 2: To detect both p24 antigen and HIV-RNA biomarkers using Magneto-resistive (MR) biosensor:** Magnetic iron Oxide (MgO) based on Magnetic tunnel junction have been used in accurate detection and identification of biomolecules tagged with magnetic nanoparticles including both HIV –DNA and P24 antigen [6]. Sub aim 2.1: To detect p24 antigen using Magneto-resistive (MR) biosensor. Sub aim 2.2: To detect HIV-RNA using Magneto-resistive (MR) biosensor. **Sub aim 2.3: To simultaneously detect both HIV RNA and p24 antigen protein.** Basing on the results obtained, this research study is utilising the same to detect P24 antigen and HIV-RNA are the biomarkers. **Aim 3: To evaluate the time taken by the magneto resistive biosensor in detection of both P24 antigen:** This is to ensure the procedure takes a very short time to obtain the results. Due to limitations of the current diagnostic methods, a new rapid and accurate technology for the diagnosis should come into the

practice. HIV continues remains the major cause of death and people die because the symptoms of the disease remain undetectable for a long time after the initial viral infection. Therefore, a simple, accurate and specific method is necessary to detect the infection in early stage to stop the progression of the disease. This research study estimates to reduce transmission levels of HIV infection by 80% and avert 30–85 new infections in resource limited settings.

**RESEARCH STRATEGY**

**SIGNIFICANCE**

The World Health Organization (WHO) estimates that more than 37 million people are living with HIV, of whom 2.1 million are new infections and 95% of these live in developing countries[25][3][8]. Approximately 1.9 million are newly infected with HIV annually and since 2010 there have been no declines in new HIV infections among adults WHO set a global target of reducing HIV transmission by 50% by 2020 & eliminating HIV by 2030. To achieve this target, sensitization of people about the infection has been done but this has not been effective as evident from the increasing new HIV infections and most locations in Africa are not applying the 90-90-90 targets[26]. Furthermore, WHO has advocated for male circumcision and ABC (Abstinence, be faithful and Condom use) among people, however, the high rates of sexually transmitted Infection (STIs)in many populations indicates that condoms are not being used. Male circumcision ten years ago demonstrated a 60% reduction in transmission in most countries, the challenge now is most adult males are not volunteering for circumcision. Scientists have taken a number of approaches to the development of a vaccine for HIV, but the nature of the virus presents significant challenges. HIV infects only humans and chimpanzees. Evaluating vaccine effectiveness in the chimpanzee model is problematic for several reasons. Chimpanzees are scarce, expensive, and do not show signs of disease when infected[27][28]. Development of drugs to treat HIV has failed to progress because the virus has an extraordinarily high mutation rate, such that an infected individual often harbours many variations. This high mutation rate allows HIV to easily evolve resistance to the drugs used to treat it. This has left only one option for people infected with the virus that is to take ARVs in order to suppress the virus. Antiretroviral (ARVs) and their use in combination have reduced mortality and improved the lives of sufferers however, these medications must be taken for life time which is unfavourable to most patients and currently there are limited treatment options for patients with multiclass resistance.

One of the best remedies to these limitations is Post-exposure prophylaxis (PEP) which are anti-HIV drugs taken within 72 hours [29][30][31][18]after a known or suspected exposure to HIV in order to prevent HIV infection. The medications are very expensive, can cause serious side effects if taken inappropriately[32][33][34]. So this calls for early detection of the virus within 72 hours to avoid unnecessary expenditures by both the government and patient. Following a recent exposure to HIV-1, it takes 2-3 months for the antibody response to reach detectable levels, during which time testing for antibodies to HIV-1, including the use of rapid antibody tests will not indicate the true infection status. This is because HIV infection occurs in 3 phases that is acute HIV infection (window period) and chronic HIV infection. The most dangerous is the Acute HIV infection where it is hard to detect HIV infection and yet some one can still transmit the virus to others[3][35][36][37]. As a result, infected people are transmitting the virus prior to knowing which has contributed to the increased number of newly people. Accurate and early detection of the infection in the window period proves to be one of the best ways to challenge the growing global concern. **However, the best method for early diagnosis remains a challenge especially in developing countries[38][18][39].**

Currently, assays have been developed to early detect HIV and shorten the window period, these include; nucleic acid amplification tests, this captures portions of HIV’s RNA and amplify them in order to facilitate viral detection[40][41][42]. Because of the extremely high sensitivity of nucleic amplification tests, window

periods are short but false positives are common, it is also expensive and require complex instrumentation for quantification of the viral load. Secondly, antigen tests detect the presence of a protein called P24 (capsid) which makes up HIV’s protein shell. Antigen tests are 100% specific but have a low sensitivity of 89% meaning that they produce no false positives. Lastly, Polymerase Chain Reaction for detection of HIV-RNA in plasma has been developed [12][43][43][44] this includes APTIMA. This is a rapid procedure with isolation and detection of 4 hours, it is highly sensitive with a specificity of about 97-98% but highly prone to false positive results due to cross contamination. In summary, most of these tests are laboratory-based, highly sophisticated, expensive and they take a minimum of 4 hours to obtain results, which makes their deployment in low resource settings infeasible [13]. Therefore, there is need for early detection of HIV using a simple, rapid, accurate and affordable test procedure.

AHI/ window period, a period between the acquisition of human immunodeficiency virus and the development of HIV-specific antibodies is characterized by high viral replication, hyper-transmission potential, rapid loss of CD4+ T-cells in both peripheral blood and mucosal lymphoid tissues and non-specific febrile illness. Although individuals in AHI period frequently develop fever, rash, fatigue, or headache within 2-6 weeks after initial exposure, however, they often misdiagnosed at initial presentation or simply missed

![Viral load and antibody levels vary with time after infection. HIV-1 RNA, anti-HIV antibodies, and HIV-1 capsid protein (p24 antigen) are the main viral markers used to detect HIV infection. From figure 1 it is evident that both P24 antigen and HIV RNA begin to increase immediately after infection and these will act as the best biomarkers in this research study. Following 2-7 days after HIV infection, the biomarkers are within a minimum concentration, they increase rapidly within 3 weeks and then drop after within 3 months. Week one to three is the AHI/window period.](https://qph.fs.quoracdn.net/main-qimg)

**Figure 1: shows the changes in viral and antibody levels with time after infection**

Study of individuals with AHI/ window period offers the best opportunity for understanding the HIV transmission event in humans. This is because it is possible to intervene during AHI and limit HIV viral replication and also integrate into a latent pool that renders HIV incurable. People in Acute HIV Infection are transmitting the virus prior to knowing their HIV status. Identifying such individuals remains problematic and this research study addresses the problem.

This research study therefore will early detect individuals newly exposed to HIV such that they can take PEP within 72 hours for only 28 days and remain uninfected instead of taking ARVs for lifetime. This will be attained by firstly quantifying the levels of P24 antigen and HIV- RNA in patients suspected to be newly infected within 72 hours. After quantification, **Magnetic biosensors will be used in detecting both biomarkers**[24]. Magnetic bio sensors have been used recently in the p24 detection assay using magnetic nanoparticle[6]. They have also been used in the detection of target DNA labelled magnetic nanoparticles [18]. This research study is basing on the results obtained in these studies to develop a rapid procedure for early detection of HIV. This will provide the following important benefits to the public; it will accurately early detect HIV infection, **this is critical for timely therapy as newly**

**infected individuals will take PEP and prevent HIV infection.** The potential for early initiation of
treatment will allow for preserved immune-system. It will also be useful to improve blood safety by
reducing the antibody negative window period in blood donors in resource limited settings where nucleic
acid testing is not practical or feasible. It will drastically prevent further transmission of the virus. Hence,
this research study estimates to reduce transmission levels of HIV infection by 80% and avert 30–85 new
infections in resource limited settings.

**INNOVATION**

In recent years, it is evidenced that individuals newly infected with HIV are transmitting the virus prior to
knowing their HIV status[45]. This is because such individuals are in the window period where the HIV
antibody tests provide a negative test even when they have the virus. Different approaches to early detect
HIV in the window period have been developed however, most of them are highly sophisticated,
expensive, do not provide confirmatory tests for example p24 antigen test. This makes their deployment
in low resource settings infeasible [8]. Although these procedures detect HIV a bit early, they do not do it
within 72 hours of infection which still makes PEP usage inappropriate due to wrong timing. This research
project is early detecting HIV infection using an accurate, cheaper and rapid procedure, this will be
achieved by detecting both P24 antigen protein and HIV-RNA biomarkers simultaneously using Magnetic
sensors. Magnetic iron Oxide-based on Magnetic tunnel junction have been used in accurate detection
and identification of biomolecules tagged with magnetic nanoparticles including HIV- DNA and p24
antigen protein [18] [19]. However, these have been detected separately in all the studies conducted.
This research is aiming at firstly detecting HIV-RNA instead of DNA and secondly, detecting both
biomarkers labelled with magnetic nanoparticles at the same time using the magnetic sensor. For the
sensor to operate effectively, the concentration of the biomarkers will be determined.

To quantitatively evaluate the sensor response in the presence of magnetic nanoparticles particles, the
relationship between the nanoparticle coverage and the concentration of the target biomarkers that is
HIV- RNA and p24 antigen will be determined. To do this, a known size of probe RNA onto the SiO2
surface will first be immobilised, then hybridized with complementary target RNA at different
concentrations. Carboxyl groups will be used for the immobilization of the detecting antibodies via
covalent bonding. The iron oxide nanoparticles (IONPs) will be characterized under TEM observation
and their magnetic property measurement will be carried out at room temperature using a vibrating
sample magnetometer. After the bio functionalization of the IONPs with detecting antibodies and the MTJ
sensor with capturing antibodies on its sensing area, the detection of target p24 antigen biomolecules
will be carried out through a sandwich immunoassay. The surfaces of all the samples will be examined
by scanning electron microscopy after the bonding of nanoparticles to determine their concentration.
Upon quantifying both biomarkers and sensor, the sensor will be tested in the laboratory to ensure that
the procedure takes a maximum of 10 minutes to obtain the results.

This is very different from existing assays which only detect one biomarker but not both at the same time.
The reason for choosing Magnetic nanoparticles is that when used in labelling molecules, it amplifies the
signal making it easier to detect the biomarkers even at lower concentrations[7]. Magnetic tunnel junction
(MTJ) sensors are ideal sensors for bio detection due to their low cost, high sensitivity, and lab-on-chip
compatibility. They also offer a higher MR ratio such as, 604% at room temperature and therefore
potentially higher sensitivity at low magnetic field. With this the research will be simplified and have high
chances of being implemented. The time taken by the magneto resistive biosensor in detection of both
P24 antigen. This is to ensure the procedure takes a very short time to obtain the results. During the

process of biomarker detection, a clock timer will be started to measure the time taken by the magnetic sensor to detect both biomarkers. This highly sensitive HIV-1 RNA and p24 antigen procedure will be useful in reducing the antibody negative window period in newly infected individuals. Individuals will take PEP at the right time therefore reducing their chances of becoming HIV positive[4]. This will further reduce unnecessary expenses incurred by the government as PEP is very expensive and not everyone can achieve.

**APPROACH**

The test procedure will detect the p24 antigen a viral capsid protein and HIV-1 RNA which are detectable in blood earlier than HIV antibodies during infection. After acquisition of HIV, a positive antigen test does not confirm that a sample is infectious and specimens that test reactive in the antigen test must be confirmed using a more specific method [20]. Presence of HIV-1 RNA confirms a positive test hence very specific, however, currently a Polymerase Chain reaction which is an expensive procedure is done to obtain a specificity of about 97-98%. It is the reason this research combines the early detection of both P24 and HIV-RNA biomarkers in a single procedure to ensure the test is accurate yet affordable.

**Aim 1: To quantify P24 antigen and HIV RNA levels in plasma in the first 72 hours of HIV exposure**[46][47]: This is to determine the concentration of these biomarkers in the plasma. This will be crucial in determining the exact size of magnetic nanoparticles required to mark the biomarkers for the sensor to recognise them. Syringes will be used to obtain patient blood and centrifuge for separation of plasma from whole blood, quadruple for blood collection with CPDA-1 or additive solution as a preservative. Human subjects will be used (blood will be obtained twice weekly from known seroconverting individuals)

**ETHICAL CONSIDERATIONS.** All study participants will be enrolled after completing an IRB-142 approved informed consent process. HIV counselling and testing will be offered throughout the study.

**Sub aim 1.1 To quantify of p24 antigen after HIV infection in plasma.** The focus will be on plasma rather than serum because a comparison of paired serum and plasma samples from 245 adult HIV-1-infected individuals of all stages of chronic infection was performed and it showed that plasma contains more p24 antigen than serum[24].

**Proposed experiment:** Whole blood will be obtained and centrifuged to obtain plasma from HIV-1-infected patients. The detectable amount of p24 antigens in plasma is in the pg/ml range. To determine the concentration levels of p24 antigen in plasma, a known volume of plasma from individuals infected with HIV-1 before seroconversion will be obtained from centrifugation, a known volume of plasma from individuals who have tested negative consecutively for 6 months will also be obtained and this will act as the control. HIV-1 p24 antigen standard will be diluted to prepare a series of six standards of varying concentrations. Concentrations will vary between 0.0 and 125 pg/ml. A standard curve will be generated from which optical density values of the unknown specimens are interpolated to determine their concentration. The standard curve will be constructed using a linear graph and plotting the concentration of the HIV-p24 antigen standard (pg/ml) on the X-axis versus the mean optical densities for each standard on the Y-axis. Each standard will be added in duplicate wells, and at least 5 controls will be included (3

negatives and 2 positives). If the value of the unknown sample is higher than the value of the highest standard, the sample will be diluted in normal human plasma and the whole process will be repeated.

**Sub aim 1.2: To quantify plasma HIV-1 RNA by real-time PCR [23]**

**Proposed experiment:** Viral particles will be pelleted from blood plasma by centrifugation at 23,000rpm for 1 hour at 4°C, lysed and then extracted with isopropanol (600 µl) and ethanol (1 ml of a 70%, vol/vol, solution). The overall specimen preparation schema will follow the manufacturer's procedure for the Roche Monitor HIV-1 RNA US-RT-PCR assay, with the exception that the pellet was suspended in 50 µl rather than 100 µl of lysis buffer. Real-time PCR will be done on each specimen using a master mixture composed of manganese acetate buffer with and sample will be added to each reaction tube. The tube will be sealed and placed in the reaction plate and then into the TaqMan PCR machine. The PCR program thermocycler conditions will be set at 60°C for 30 min for reverse transcription, 95°C for 5 min for denaturation, and 42 cycles of amplification at 95°C for 20 s, 52°C for 20 s, and 60°C for 1 min. All primers will have high-performance liquid chromatography purified and purchased from Invitrogen (Frederick, MD), and all probes will be high-performance liquid chromatography purified and purchased from Applied bio systems. The standard curve for the assay will be prepared from Armored RNA HIV-1 of subtype in TSM buffer. The quantity of HIV RNA will be determined by measuring the A_{260} and confirmed using the Roche Monitor HIV-1 RNA assay.

**Expected outcome:** Since PCR provides the amount of HIV-1 RNA present, then concentrations for both biomarkers will be obtained.

**Aim 2: To detect both p24 antigen and HIV-RNA biomarkers using Magneto-resistive (MR) biosensor[6]**

Magnetic iron Oxide (MgO) based on Magnetic tunnel junction have been used in accurate detection and identification of biomolecules tagged with magnetic nanoparticles including both HIV –DNA and P24 antigen separately in different studies.

**Sub aim 2.1: To detect p24 antigen using Magneto-resistive (MR) biosensor**

**Proposed Experiment:** For the magnetic detection of p24 antigen, both of the iron oxide nanoparticles (IONPs) and (Magnetic tunnel junction) MTJ sensors will be surface biologically functionalized. Carboxyl groups will be used for the immobilization of the detecting antibodies via covalent bonding.

The iron oxide nanoparticles (IONPs) will be characterized under TEM observation and their magnetic property measurement will be carried out at room temperature using a vibrating sample magnetometer. After the bio functionalization of the IONPs with detecting antibodies and the MTJ sensor with capturing antibodies on its sensing area, the detection of target p24 antigen biomolecules will be carried out through a sandwich immunoassay configuration as shown in figure 2 below.

Sub aim 2.2: To detect HIV-RNA using Magneto-resistive (MR) biosensor

In order to detect the existence of target RNA molecules, the MTJ sensor arrays will similarly be first biologically treated so as to be able to capture the functionalized nanoparticles. The sensor surface will be passivated with a 100-nm-thick SiO2 layer and then spin coated with polyethylenimine in chloroform solution (about 70 nm in thickness), followed by a 1 min rinse in ethanol. The probe RNA solution (a mixture of 20 µl 49base single RNA strands and 80 µl phosphate buffered saline, with a pH of 10) will be introduced by micropipette spotting, followed by incubation in a humid atmosphere at room temperature for 2 hours. The sample will be then irradiated with 50 mJ of UV light for 30 seconds to increase the hybridization efficiency. Pre hybridization will be done by incubation in 1M NaCl and 2.5% polyethylene glycol (pH 5.0) at 55 °C for 1 h to deactivate unbound epoxy groups. The target RNA solution (containing 20 µl of complementary RNA with biotin at the 3’ end, 50 µl of hybridization buffer, and 30 µl of de-ionized water) will be introduced and incubated at 42 °C for 1.5 hours, followed by several washing steps to wash away unbound RNA. The sample surface will be then neutralized in 1% bovine serum albumin (BSA) solution for 45 minutes[48].

A solution containing the nanoparticles (NPs) will be introduced to the sensor array via a micropipette. After the NPs have had a chance to bond to the functionalized sensor surfaces, unbound NPs will be washed away with a phosphate buffer solution buffer solution. The MTJ sensor array will then be mounted on an open dual-inline socket with a flat surface, so that magnetic nanoparticles are easily delivered to the sensor surface through a micropipette, without the need for microfluidics [19].

Expected outcome: HIV-1 RNA will be detected by the determined size of Fe3O4 nanoparticles using arrays of magnetic tunnel junction sensors with oriented MgO barrier layers.

Sub aim 2.3: To simultaneously detect both HIV RNA and p24 antigen protein: To ensure that both biomarkers are detected at the same time. The quantity obtained for both biomarkers will determine the size of magnetic nanoparticles to label both biomarkers. A direct current biasing field of 500e will be applied perpendicular to the sensing axis to reduce the hysteresis of the MTJ sensor array. This is to make the sensor response more linear and to increase the field sensitivity. A slowly varying magnetic field will be applied along the sensing axis to measure the sensor’s MR transfer curves before and after coating with magnetic nanoparticles. This applied field also serves to magnetize the superparamagnetic NPs bonded to the sensor array. As the applied field strength is varied, the NPs bonded to the sensor have an effective dipole moment which is proportional to this field strength. Each dipole moment creates a small added field component (H dipole) at the sensor, which means that it will experience an effective field strength which is slightly smaller than the external applied field, H effect=H applied −H dipole, which generates a detectable signal on the MTJ sensor arrays[49][50][51]. Because the particles are superparamagnetic and because the applied fields are quite uniform, the NPs do not agglomerate or drift during the experiment and with this both p24 antigen and HIV-1 RNA will be detected.

Aim 3: To evaluate the time taken by the magneto resistive biosensor in detection of both P24 antigen: This is to ensure the procedure takes a very short time to obtain the results. During the process of biomarker detection, a clock timer will be started to measure the time taken by the magnetic sensor to detect both biomarkers. The expected time is a maximum of 10 minutes since we are aiming at developing a rapid procedure. In case it is discovered that the time is not within the expected range, further modification of the sensor will be reconsidered. After all these have been considered, a rapid procedure which will detect HIV within 72 hours of infection will be developed.

**APPROACH TIME LINE**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obtain IRB and safety clearance</td>
<td>30-May 07-Sep</td>
</tr>
<tr>
<td>Survey different health facilities</td>
<td>16-Dec</td>
</tr>
<tr>
<td>Acquisition of materials and equipment</td>
<td>26-Mar</td>
</tr>
<tr>
<td>Sample Collection</td>
<td>04-Jul</td>
</tr>
<tr>
<td>Sample storage</td>
<td>12-Oct</td>
</tr>
<tr>
<td>AIM 1: Quantification of P24 antigen and HIV-RNA</td>
<td></td>
</tr>
<tr>
<td>Analysis of results</td>
<td></td>
</tr>
<tr>
<td>Aim 2: Detection of both biomarkers</td>
<td></td>
</tr>
<tr>
<td>Analysis of results</td>
<td></td>
</tr>
<tr>
<td>Publication of results</td>
<td></td>
</tr>
<tr>
<td>Aim 3: Evaluate time taken by magnetic sensor</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 3: shows Gantt chart showing the different activities that will be carried out*

**INVESTIGATORS**

**Principle investigators**

**Prof. Moses Joloba**

He is the current Chair of the Department of Medical Microbiology, Makerere University College of Health Sciences. He completed his bachelor’s in medicine and Surgery at Makerere University and did his Master of Science in Microbiology. He did his PhD in Molecular Microbiology 2003 at Case Western

Reserve University, USA. He established the Molecular Biology laboratory in the Department of Medical Microbiology focused on TB and HIV related research and in 2006. This laboratory will play a greater role in providing an environment for quantification of the HIV-RNA biomarker. He is also the current director of Supranational Reference Laboratory Uganda and the current head of laboratories and heads the appointments and promotions committee of the department. He is also working with the MAPRONANO project which is an Africa centre of excellence in materials product development and nanotechnology at college of engineering in Makerere University.

Dr. Eng. Robert Ssekitoleko

He is a biomedical engineering lecturer at Makerere University, College of health Sciences department of Physiology. He is dedicated to building the biomedical engineering field to ensure cheaper but quality technologies are developed for low resource settings. He has a master’s degree in Biomedical engineering. He pursued a doctor of Engineering degree in Biomedical engineering from university of Strathclyde. Eng. Robert has won grants from NIH for different research areas. He is currently working on advancing the biomedical engineering field to solve HIV related challenges.

(b). Co- Investigators

Mr. Gerald Mboowa

He is a Human Genetics and genomics research fellow at Genomics Laboratory. Department of molecular biology and immunology. He holds a bachelor’s degree in Biotechnology and masters of science in immunology and clinical microbiology from Makerere University. He also holds a CAfGEN fellowship in Human Genetics. He is currently doing research in microbial genomics and immunology of infectious diseases specifically AIDS.

Takuwa Mercy

She has a bachelor’s degree in Biomedical engineering at Makerere University. She has obtained a short course training in Bioinformatics and gene sequencing. She is dedicated to researching about infectious diseases especially HIV and TB. She has participated in different innovation competitions and her projects have always qualified to the final stages. She is passionate about biomedical engineering especially in the field of molecular biology and bioinformatics research. With supervision from Doctor. Robert Ssekitoleko, she is persuaded that this work if funded will be a great breakthrough in reducing the prevalence of HIV in low resource countries like Uganda.

RESOURCES AND ENVIRONMENT

The molecular laboratory at Makerere University headed by the main principle investigator of this project Prof. Moses Joloba at College of health sciences is well equipped with different equipment and highly trained staff. This will be utilised to access the PCR and related equipment necessary for biomarker quantification. The Uganda Virus Research Institute (UVRI/MRI) is a research body under Uganda ministry of health responsible for carrying out scientific research concerning communicable diseases. Fortunately, UVRI has collaborated with department of microbiology at Makerere University and established an immunology laboratory that is being equipped with modern facilities to conduct advanced immunological studies on infectious diseases mainly TB, HIV/AIDS and Malaria. Currently the laboratory has biosafety cabinets, ELIZA instruments, Fridges and freezers, micro centrifuges, vortex machine, microscopes, biohazard flow hoods and among others.

Detection of biomarkers calls for nanotechnology components. The project will utilize the collaborators of MAPRONANO centre of excellence facilities at Makerere University that is Micro/Nanoscale Fluid Transport Laboratory (MNFTL). All nanotechnology related components will be purchased from the

Nanotechnology laboratory called Micro/Nanoscale Fluid Transport Laboratory (MNFTL) directed by Prof. Constantine M. Megaridis. These include; magnetic iron oxide nanoparticles, magnetic sensor, anti-p24 antibodies among others.

**BUDGET**

<table>
<thead>
<tr>
<th>Principle Investigator</th>
<th>Professor Joloba Moses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BUDGET FOR DIRECT COSTS</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PERSONNEL (Applicant organisation only)</strong></td>
<td><strong>FROM JANUARY 2021 TO JANUARY 2023</strong></td>
</tr>
<tr>
<td>NAME</td>
<td>ROLE</td>
</tr>
<tr>
<td>Professor Joloba Moses</td>
<td>Principle investigator</td>
</tr>
<tr>
<td>Eng.Dr. Robert Ssekitoleko</td>
<td>Principle investigator</td>
</tr>
<tr>
<td>Gerald Mboowa</td>
<td>Co-investigator</td>
</tr>
<tr>
<td>Takuwa Mercy</td>
<td>Co-investigator</td>
</tr>
</tbody>
</table>

Consultation costs: Consultation at the initial stage from Prof. Constantine M. Megaridis is a foreign expert on nanotechnology who has extensive knowledge about the field.

- Equipment: PCR machine and the illumina sequencing machine and nanotechnology equipment ($24,000$)
- Supplies: Includes all the laboratory supplies and necessary literature ($23,000$)
- Travel: Local travels to facilities and laboratories, international travel where necessary ($20,000$)
- Miscellaneous: Bulk printing costs and software ($4,000$)

**GRAND TOTAL FOR DIRECT COSTS** ($212,500$

*Table 1: shows the proposed Budget*
REFERENCES


