



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

HIV-AIDS: An Unsolved Pandemic

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Abstract: HIV i.e. Human Immunodeficiency Virus has been on top for causing various deaths worldwide. Though a complete cure for HIV is not available till date, various vaccines have been invented to control the effectiveness of virus. AIDS i.e. Acquired Immunodeficiency Syndrome is the one of the deadliest diseases caused due to HIV. It infects different cells of the immune system, such as CD4+ T cells (T-helper cells), dendritic cells, and macrophages etc. AIDS affected rates and mortality rates are usually more noticeable in resource-constrained countries than in the developed countries. People die due to AIDS as it remains undetectable for a long time. Thus, a simple and accurate testing measures are required for testing AIDS so as to control its spread and mortality rates. In this paper, we study some of HIV-AIDS detection/diagnosis assays followed by their respective comparison. We also review the treatments currently available to deal with the virus.

Index Terms - Point of care (POC), HIV diagnostics, antiretroviral, viral load, drug resistance, spectrophotometer and electrophoresis.

I. INTRODUCTION






HIV (human immunodeficiency virus) is a virus that attacks our immune system, the body's natural defense system. Without a strong immune system, the body fails to fight off disease. White blood cells play an important role in the immune system as they fight with the diseases. HIV infects and thus destroys certain white blood cells called CD4+ cells. If lost too many CD4+ cells, the body will no longer be able to defend itself against infection. The last and prior stage of HIV infection is AIDS (Acquired Immunodeficiency Syndrome). People with AIDS tend to have a low count of CD4+ cells and thus are easily recipient to get infections or cancers that rarely occur in healthy people. Even without treatment, it takes usually 10 to 12 years for HIV to progress to AIDS. When HIV is diagnosed before turning to AIDS, medicines can lower the damage to immune system. If not detected with AIDS, these medicines can bring the immune system to a healthier state. With treatment, many people with HIV are able to live long with active lives.

Two types of HIV have been identified and characterized from patients infected with the virus:

1. HIV-1
2. HIV-2.

Among these, HIV-1 is the most lethal and pathogenic, and it is assumed to be of type HIV-1 when stated nothing about the type whereas, HIV-2 is only constrained to some areas of Central and Western Africa [1]. The number of people newly infected with HIV was 1.7 million each in 2018 [2] and in 2019. The large number of new infections is mostly due to the lack of knowledge regarding the HIV status of infected individuals that fail to exhibit apparent symptoms but are highly infective. This late diagnosis of virus has been a leading cause for several deaths. Below is a figure that shows the statistics of HIV infections over the year 2019.

Summary of the global HIV epidemic (2019)

	People living with HIV in 2019	People newly infected with HIV in 2019	HIV-related deaths in 2019
 Total	38.0 million [31.6 million – 44.5 million]	1.7 million [1.2 million – 2.2 million]	690 000 [500 000 – 970 000 million]
 Adults	36.2 million [30.2 million – 42.5 million]	1.5 million [1.1 million – 2.0 million]	600 000 [430 000 – 840 000]
 Women	19.2 million [16.4 million – 22.2 million]	790 000 590 000 – 1.1 million]	300 000 [220 000 – 420 000]
 Men	17.0 million [13.8 million – 20.4 million]	870 000 630 000 – 1.2 million]	390 000 [280 000 – 560 000]
 Children (<15 years)	1.8 million [1.3 million – 2.2 million]	150 000 [94 000 – 240 000]	95 000 [61 000 – 150 000]

Source: UNAIDS/WHO estimates



Figure1: Summary of the global HIV epidemic (2019).

HIV can spread from contact with infected blood, semen, or vaginal fluids. Most people get infected by virus by having unprotected sex with someone who has HIV. Another major cause of getting infected by HIV is by sharing drug needles with someone who tested HIV positive. It can also spread from a mother to her baby during pregnancy, birth, or during breastfeeding. HIV doesn't survive well outside the body, thus it can't be spread by casual contacts like kissing or sharing drinking glasses with an infected person.

Its early symptoms can be: fever, sore throat, headache, muscle aches and joint pain, swollen glands and skin rashes. After the early symptoms vanish, an infected person may not have symptoms again for many years. After a period of time, these symptoms reappear and last for a time interval. These symptoms usually are: swollen lymph nodes, extreme tiredness, weight loss, fever and night sweats.

The figure below shows how HIV virions bud and release.

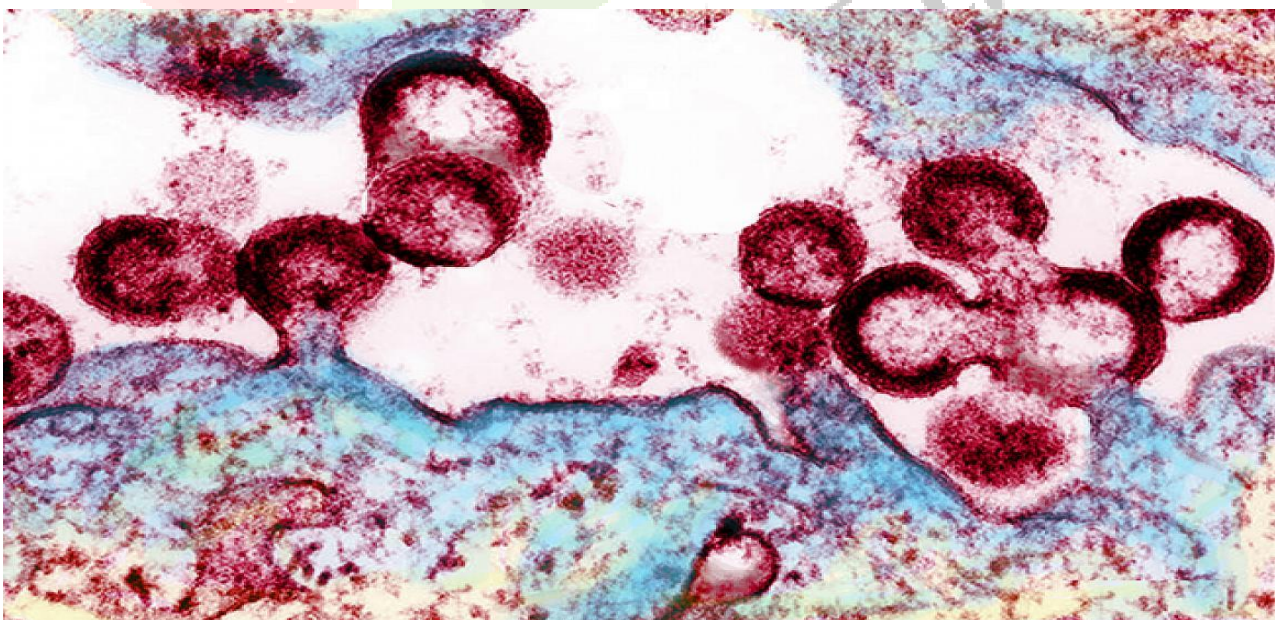


Figure2: Budding and release of HIV virions.

HIV infection can have three stages:

- (i) Acute HIV infection, which has flu like symptoms, rash, muscle pains and is observed for 3–6weeks following the infection,
- (ii) Clinical Latency, which shows mild or no symptoms and
- (iii) AIDS, which is confirmed by count of CD4 cell falling below 200cells/mm³ of blood and/or one or more defining illnesses [1,3, 4].

Prompt HIV diagnosis allows initiating ART i.e. Anti-Retroviral Therapy treatment in the early phase of detecting infection, thus assuring high survival rates in the infected population and thus reducing the spread of virus. ART has been showing to have 96% efficacy in reducing HIV transmission in infected individuals [5, 6]. Out of total 37.9millions of HIV infected patients worldwide, only 23.3million received ART by the end of 2018 [2]. The World Health Organization (WHO) has an attempt to reach the ambitious goal to end the AIDS epidemic by 2030. It has also established several guidelines to initiate ART for all the infected patients having a CD4 count <350cells/mm³; due to which, more than 15 million infected people were provided with ART since 2010 [2, 6, 7]. Current conventional techniques have their limitations, i.e. they cannot detect virologic failure, are time consuming, require qualified staff, and specialized facilities. Thus, new assays are needed which will be fast, inexpensive, do not require specialized facilities and can be utilized in the point of care (POC) settings as needed. So, here in we discuss the available assays promoting viral spread measurements and compare them with based on some points.

II. HIV DETECTION/DIAGNOSIS TECHNIQUES/ASSAYS:

The HIV/AIDS was clinically discovered in United States when more than half of the infected were dead [9, 10]. As the disease remains undetectable for long time, it cannot be cured when needed, thus patients die. Rapid detection of disease minimizes illness, disability, and therefore death and economic losses. The authentic determination of viral and infected burden is necessary to know the HIV infection, progression of disease and to estimate the efficacy of regimens and vaccines [11], thus reducing the death rate. Below are some of the assays for diagnosis of HIV-AIDS.

2.1 ELISA

Enzyme-linked Immunosorbent Assay i.e. ELISA is most commonly used for detecting HIV due to its simple methodology. It detects if an antibody against HIV is present in the blood sample. The enzyme is conjugated to an antibody (secondary) which further reacts with colorless substrate to give a colored reaction product, which is measured by a spectrophotometer. Colored solution absorbs transmitted light, directly proportional to the amount of antibody present in the serum. In ELISA, patient serum is diluted to 400-fold and is applied to an ELISA plate on which HIV antigens are already attached. If the virus is present (positive sample), the antibodies get attached to the HIV antigen present in the ELISA plate. A secondary antibody linked with the enzyme is then applied into the plate which further binds to the primary antibody. Thereafter, an enzyme specific substrate is applied that causes change in color or fluorescence.

2.2 PCR

Polymerase Chain Reaction i.e. PCR is one of the most powerful techniques for an amplification of specific DNA sequence. The PCR based technique has revolutionized the detection of infectious diseases. Techniques like agarose gel electrophoresis or Real-time-PCR (RT-PCR) are used to detect PCR amplicon. In RT-PCR, fluorescence labelled dye (SYBR green) is used for detection of DNA/RNA, which binds to double stranded DNA and as number of PCR cycles increases, intensity of fluorescence also increases.

2.3 WESTERN BLOT

Western Blot is a type of an immunoblot performed, which characterizes each viral protein. When every viral protein is arranged according to its molecular weight, a nitrocellulose membrane/ strip accommodates each protein after performing polyacrylamide gel electrophoresis. Patient's serum when is treated with this nitrocellulose strip produces a color band as a result of reaction of patient's serum antibody with specific viral antigen. Due to the presence antihuman alkaline phosphates labeled IgG conjugate and color developing solution, color band is observed. Colors are detected, and results are assessed by number of bands and guidelines provided by manufacturers. Patient's sample must at least positively react with one core band and with one envelop band so as to be positive[12].

2.4 HIV SELF TEST

Home HIV test kit technique was first discovered in 1988 for HIV-1 in pregnant women [12]. Test can be performed on a drop of blood sample by pricking finger on a small strip of the filter paper. The blood sample when dried on the filter paper for many days is used for diagnosis of HIV antibodies as well as viral load testing. Then blood sample is extracted from dried blood spot, which is further sent to the laboratories where it can be detected by ELISA, Western blot and some other detection methods. These home test kits are particularly beneficial in rural areas where diagnostic centers are out of reach.

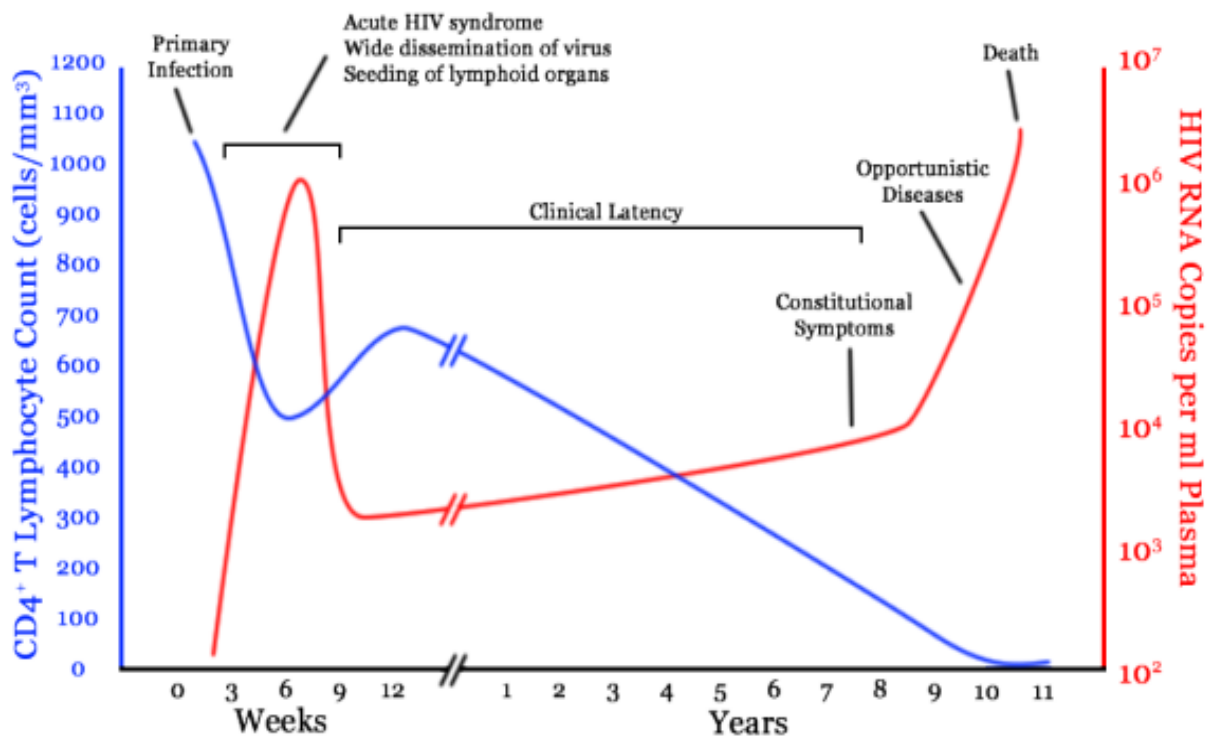


Figure3: CD4+ T cells count vs. HIV RNA copies per ml plasma.

2.5 NUCLEIC AND NON-NUCLEIC ACID AMPLIFICATION ASSAYS

2.5.1 Nucleic acid amplification assays

Nucleic Acid Tests i.e. NAT's are the molecular techniques often used for blood screening for transfusion transmitted infections. Viral DNA/RNA's are detected and quantified by these NAT's with high sensitivity and accuracy. During the testing of an AIDS infection, NAT based viral load measurement assays bypass the sero-negative window period [1] and thus have reduced the ability to detect HIV to 2.93days [47]. For NAT-based POC technologies to be implemented, NAT-based POC devices should have sample collections, sample processing, testing, data analysis and interpretation, and waste disposal [1].

2.5.2 Non-Nucleic acid Amplification assays

These are the cheapest and most readable technologies available for HIV detection. These comprise several serological tests such as Antigen-antibody (Ag/Ab) based assays that can be used in a POC setting [1].

2.6 PAPER AND FLEXIBLE MATERIAL BASED ASSAYS

The main constituent of paper is cellulose fiber, and this can be highly attractive for certain applications as it allows liquid to penetrate within its hydrophilic fiber matrix without the need of an active pump or external source. It has paper based materials for the development of a paper based HIV viral load assay and a paper based rapid CD4 counting device. Magnetic beads with biotinylated capture antibodies are utilized to capture HIV from serum and/or saliva [21, 22]. These are later washed to eliminate conductive media. The captured HIV is then lysed in a low conductive medium, thus releasing ions and biomolecules that modify the electrical conductivity of the medium. It causes a decrease in its overall electrical impedance, thereby increasing the conductivity of medium. The change in the conductivity is measured using impedance spectroscopy at different frequencies. Currently, electrical and optical sensing methods are being used to diagnose different diseases including HIV [23].

Below is a figure showing paper device connected to an android phone and laptop.

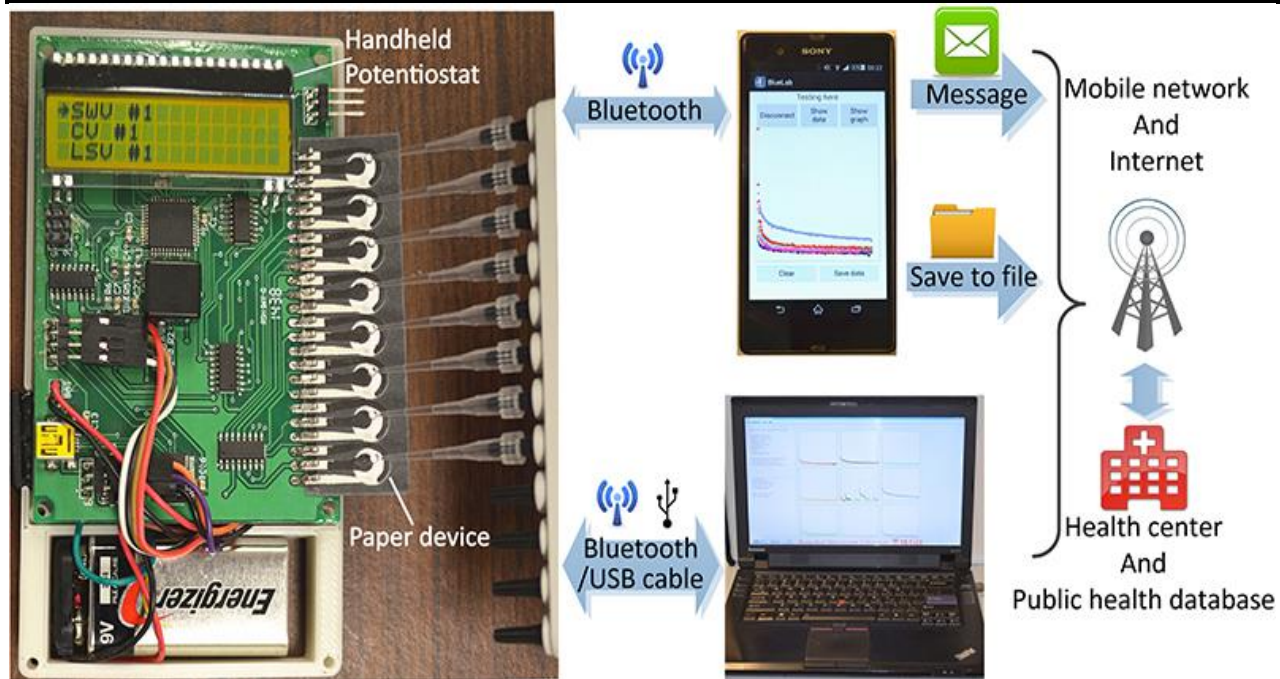


Figure4: Paper device connected to phone/laptop over internet.

Below is a table showing respective pros and cons of various assays discussed above.

Assays	Pros	Cons
ELISA	Can screen large number of samples. Provides sensitivity > 99%. Easy, cost-effective.	Can have high rate of false positive results.
PCR	Rapid. Provide higher sensitivity and specificity.	Can have high rate of false positive results.
Western Blot	Provides high specificity. Defines antibodies for respective HIV proteins.	Expensive. Needs expertize to maintain.
HIV Self -Test	Convenient. Small amount of blood sample is needed for testing.	Expensive. Less confidential.
Nucleic Acid Amplification	Accurate. Highly specific.	Needs expertize to maintain. Samples may get contaminated during laboratory work.
Non-Nucleic Acid Amplification	Cheaper. Most readable technologies are used.	
Paper based assay	Simple, cheaper, portable. Supports mass productivity, simple fabrication and disposability.	Sometimes provide less specificity and accuracy.

Table2.1: Comparison of various diagnostic assays.

III. TREATMENT:

Over the past several years, ART has significantly contributed for HIV treatment. From few years, the development of various inhibitors for HIV has revolutionized the HIV treatment [14]. These medicines do not cure HIV infection, but they do make it a manageable chronic condition. They also reduce the risk of spreading the virus to others. Initially, the drugs which were given as a treatment were falling under monotherapy but later on combination therapies were introduced involving the combination of at least three drugs. This treatment therapy was known as Highly Active Antiretroviral Therapy (HAART) [15]. This therapy represses viral replication and reduces the viral load below the level of detection (50 RNA copies/ml), which can be measured by elevated level of CD4+ T-lymphocyte. There are several different types of HIV/AIDS medicines. Some block or change the enzymes that HIV needs to replicate itself. This enzymes prevent HIV growth, which then significantly reduces the amount of HIV infected cells in the body. Following are the classes of these drugs:

- 3.1 Nucleoside Reverse Transcriptase Inhibitors (NRTI's) block the reverse transcriptase enzyme.
- 3.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's) bind to and later change the reverse transcriptase enzyme.
- 3.3 Integrase Inhibitors block the integrase enzyme and

3.4 Protease Inhibitors (PI's) block the protease enzyme.

3.1 Nucleoside Reverse Transcriptase Inhibitors (NRTI's)

These are the first class of drug sanctioned by FDA [24]. Nucleoside reverse transcriptase inhibitors (NRTIs), also known as Nucleoside analogs ("nukes") target RT enzyme of HIV. These inhibitors act as an alternative substrate to compete with normal cellular nucleosides [27]. Deoxyribose sugar of these nucleoside analogs lacks hydroxyl group at 3' position, which inhibits the formation of phosphodiester bond between incoming 5' nucleoside triphosphates and NRTIs. This terminates the growth of DNA chain of virus which can occur during DNA-dependent DNA synthesis or RNA-dependent DNA [24]. These analogs are also considered as pro-drugs [25].

3.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's)

Non-Nucleoside Reverse Transcriptase inhibitors attack HIV RT enzyme along with nucleoside analog and were first described in 1990 [27]. Proximal to active site, HIV RT enzyme is inhibited by the formation of hydrophobic pocket and binding of NNRTIs to RT. Reduction in polymerase activity and spatial conformational changes in substrate-binding site take place upon binding of NNRTIs to RT enzyme [28]. These NNRTIs are noncompetitive inhibitors and are very specific in their actions [26].

3.3 Integrase Inhibitors

These inhibitors prevent the enzymes from interacting with divalent cations (Mg²⁺) and correct positioning of viral DNA, as they bind themselves near to the active site of enzyme [24]. The retroviral enzyme Integrase initiates and catalyzes a two-step reaction known as integration process. The process involves strand transfer and 3' processing through coordination of divalent ions (Mn²⁺ or Mg²⁺) provided by three amino acids [29]. After reverse transcription, integrase cleaves conserved dinucleotides at 3' end of double stranded DNA. As its result, overhangs of dinucleotides on both ends of genome are produced. In strand transfer reaction, the bounded integrase at 3' end of DNA translocates the viral double stranded DNA into nucleus where it catalyzes the incorporation of viral DNA into host genome. Only those compounds are considered to be effective for HIV treatment that can prevent the strand transfer reaction and are called integrase strand transfer inhibitors (INSTIs). They are considered as effective as they bind to the enzymes already attached with viral DNA [26]. Mutations cause resistance to INSTIs are nearly always present within the active site of integrase enzyme close to three amino acids that help to coordinate with vital magnesium cofactors.

3.4 Protease Inhibitors (PI's)

These inhibitors target the protease enzyme of HIV-1. Protease enzymes catalyze the processing of gag-pol polyprotein precursor and virion gag and are vital for viral maturation [26]. In this, active site of enzymes is targeted i.e. primary resistance mutation in most of these PIs occurs adjacent to these active sites of enzymes. It then causes changes in amino acid, which affects viral replicative fitness. Besides mutation in protease gene, alterations in some important cleavage sites also offer resistance to PIs [24, 31].

IV. CONCLUSION AND FUTURE DIRECTIONS

Since the HIV is diagnosed and treated, the world still suffers from its deadly consequences as not any exact treatment is found till date. Its effect remains vulnerable as it remains undetectable for a period of time. Early symptoms cannot directly claim to be AIDS, but as time progresses and symptoms become more drastic, HIV infection becomes difficult to handle. ART has significantly contributed for altering the HIV global epidemiology. Later on combination therapy HAART was introduced having the potential to reduce the mortality and illness related to HIV-1 infection. Various diagnostic techniques are developed for early detection of HIV infection. These assays can vary as the region changes, i.e. rural areas where high-end technologies can be hardly found, HIV can remain untreated. Thus, irrespective of any region boundary, rapid, inexpensive and simple-to-use HIV-1 diagnostic techniques are urgently needed for early diagnosis that should work under resource-limited settings. Several point of care testing technologies are available and are under the development for the measurement of CD4 cell count and HIV viral load that have promised to further extend access to HIV monitoring tests [13].

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