Open Access

Review Article

HIV Molecular Diagnostics: Recent Developments and Emerging Trends

Zarafshan Amjad, Aroosh Shabbir, Zarafshan Asjad, Kushaf Mohsin

Department of Microbiology, The Institute of Molecular Biology and Biotechnology (IMBB)/University of Lahore, Lahore Pakistan

ABSTRACT

Human immunodeficiency virus is a member of the Retroviridae family's Lentivirus genus. Only humans are infected with HIV and it is passed from one person to the next. It is not spread by insect bites, mosquitoes, bats, or any other kind of animal. The diagnosis of HIV can be done by polymerase chain reaction, HIV qualitative nucleic acid assays, HIV RNA load assays, Antigen rapid test, Point of care testing, Nucleic acid sequence based amplification, HIV genotypic drug resistance testing, Alere q test and viral load assay. Molecular methods such as PCR, real-time PCR, and genotyping are critical for the diagnosis and management of HIV in Pakistan. These techniques allow for their early detection, monitoring of their viral load, and testing for drug resistance, which can guide treatment and surveillance strategies. However, limited access, high costs, and inadequate training prevent their widespread use in Pakistan. Nucleic acid amplification test plays significant role in detection of HIV infection by providing HIV microorganism masses and by antiviral resistance. Modern diagnostic techniques are investigating novel therapeutic approaches and helping to lower the number of HIV diagnoses. HIV diagnosis has significantly improved through such diagnostic methods as western blot, PCR, quick test, Enzyme immunoassay and p24 antigen. Vaccines and antiretroviral medication are both attractive options for preventative and therapeutic regimens, respectively. The development of CRISPR/Cas9 represents a significant advancement in the treatment of HIV. This review discusses molecular approaches for the diagnosis of HIV.

Keywords: Antigen Rapid Test, Human Immunodeficiency Virus, Nucleic Acid Amplification Test Polymerase Chain Reaction, Point of Care Testing, Viral Load.

How to Cite This Article: *Amjad Z, Shabbir A, Asjad Z, Mohsin K. HIV Molecular Diagnostics: Recent Developments and Emerging Trends. Pak Armed Forces Med J 2026; 76(3): 440-446. DOI: https://doi.org/10.51253/pafmj.v76i3.13312*

INTRODUCTION

The human immunodeficiency virus causes infection in humans. Because of Auto immunodeficiency syndrome several people have died, which has been a global public health concern for more than 40 years. HIV-1, a retrovirus that spreads through bodily fluids and secretions, is the cause of AIDS. Over the course of two to ten years, if left untreated, this can result in immunodeficiency and death. Rapid diagnostics and efficient antiretroviral medication were discovered and widely used, which resulted in a significant decrease in death and morbidity as well as an increase in the number of people in need of lifelong viral suppressive therapy.[1]

Human immunodeficiency virus is a member of the Retroviridae family's Lentivirus genus. The HIV virion is spherical and has radius of 100–130 nm. Around 7 to 12 trimeric complexes of viral envelop protein develop into viral envelop, this type of protein can be found from the host cell and it has cellular protein. Envelope is made up of the transmembrane glycoprotein 41, which is necessary for viral fusion, and the exterior glycoprotein 120, this makes viral fusion easier. When immune system responds, CD4 cells, also known as "T-helper cells," alert other cells such the cytotoxic T cell and the B cells to fulfil their roles. During CD4 cell apoptosis by HIV, and as their population declines, gaps appear throughout the immunological repertoire.[3] The American Center for Disease Control originally identified acquired immunological disorder syndrome in 1981, and the human immunodeficiency virus was later identified as the disease's causative agent. The hazards modeled by AIDS are recognized as important intimidations to manhood. Therefore, the first designation of HIV is very important for all experts around the biosphere.[4] Across membrane surfaces HIV is spread by sexual contact, maternal infant exposure and by transcutaneous immunization. CCR5- tropic viruses (R5 viruses) are exclusively spread by all routes. Spreading is monitored through, infectious agent and host indicators of contamination within the blood plasma. Persons having a past of high-risk sexual conduct, sexually transferred illnesses, blood transfusions, or injectable drug usage were at high risk of HIV.[5] In recent years the Expanded HIV Testing Initiative was launched through the Centers for Disease Control and Prevention to increase access to

Correspondence: Dr Aroosh Shabbir, Department of Microbiology, IMBB/University of Lahore, Lahore Pakistan

Received: 27 Mar 2025; revision received: 02 Jun 2025; accepted: 03 Jun 2025

HIV testing and reduce number of undiagnosed HIV infections. The CDC currently recommends regular HIV analysis on behalf of people aged 13 to 64 years. Several arguments have been offered that imply senior persons are further prospective toward advance HIV illness as well as have a worse response to therapy.[6]

HIV remains a public illness in Europe. It infects around 34 million individuals globally, 1.2 million of them living in United States. However, the situation is believed that 20% of HIV-positive persons in the United States are uninformed of their infection. HIV primarily affects CD4+ helper T cells that remain important controllers of humoral and cellular immune responses. Therefore, the body is incapable to defend itself in contradiction of opportunistic contaminations because of their death and reduction via contrivances that are not completely implicit. Once HIV a CD4+ T cell that has been activated, it takeovers and modifies the transcriptional and translational apparatus for self-reproduction.[7] The ability to track HIV infection progression using the CD4 cell count before, in recent times, forecast the progression toward AIDS through assessing plasma viral load. The diagnosis of HIV can be done by many molecular serological and immunological techniques. The early determination of infective agents like bacteria and viruses is crucial for clinical point-of-care functions. Enzyme-linked immunosorbent assay, polymerase chain reaction, reverse transcription polymerase chain reaction and distinct biosensor innovations are used to recognize or eliminate viruses.[8] The conventional molecular methods for the diagnosis of human immuno-deficiency virus are polymerase chain reaction and screening method. The advance method for diagnosis of HIV are Viral Load, VL Tests, Genetic Diversity, and point of care viral load testing platforms. The early 1980s saw the first recognition of AIDS. To diagnose human immunodeficiency virus infections, protect blood and blood product supplies, basic serologic assays for HIV antibodies were first created utilizing preparations of culture-derived viral antigens. Numerous serologic tests for rapid testing, high-throughput screening, additional confirmation, epidemiological monitoring, and incidence determination were developed during the course of the following three decades. Measures like improvements in clinical symptoms, VL suppression, and increases in CD4 cell counts can be used to track how well antiretroviral therapy is working for people with HIV. As a comparison of immunological and clinical monitoring markers, VL monitoring is the recommended method because it can accurately indicate ART efficacy. The measurement of VL may be complicated by genetic variability. Most of the primers and probes are used for PCR amplification and hybridization in the VL tests.[9]

METHODOLOGY

Detection of HIV Using Polymerase Chain Reaction (PCR)

HIV can be detect by polymerase chain reaction method. In order to identify viral nucleic acid in patient samples, polymerase chain reaction strengthens the viral nucleic acid. The test remains extremely sensitive and specific; it may detect very minute amounts of viral agents. HIV infection in infants delivered to HIV positive moms may be accurately diagnosed by PCR. Infants carry protective antibodies up to around fifteen months of age, as a result, antibody test is not a trustworthy predictor of infection in the offspring. PCR determines the DNA sequence of viruses and have a capability to identify the existence of virus instead of viral antibody. PCR require only 1 to 3 ml of blood for testing.[10]

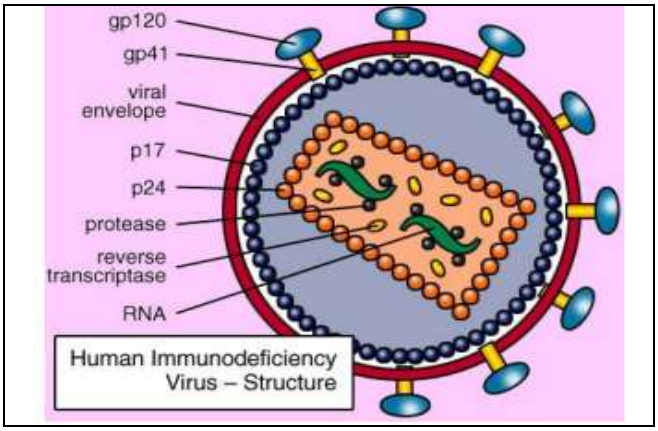

Figure-1: Structure of HIV2

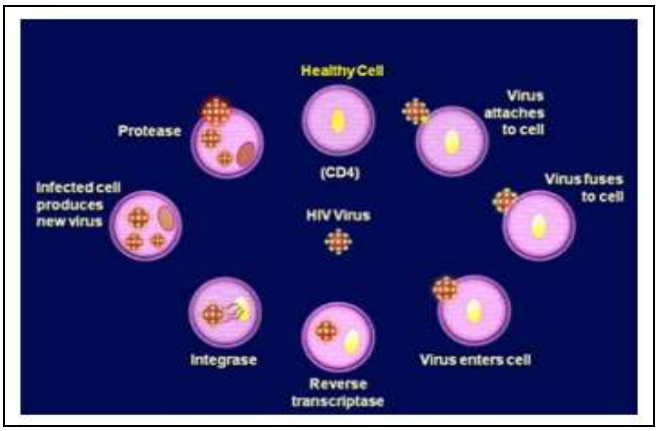

Figure-2: Life cycle of HIV2

HIV Detection Techniques: Molecular and Immunoassay Methods

According to a research, HIV molecular strategies expand and discover HIV specific nucleic acid in vitro by polymerase chain reaction and alternative in vitro enzyme-mediated nucleic acid amplification methods. HIV specific antibodies and antigens in body fluid can be determined by classical and fast immunoassays techniques. Nucleic acid amplification test plays significant roles in detection of HIV. Improper use of nucleic acid amplification test can give false positive results.[11]

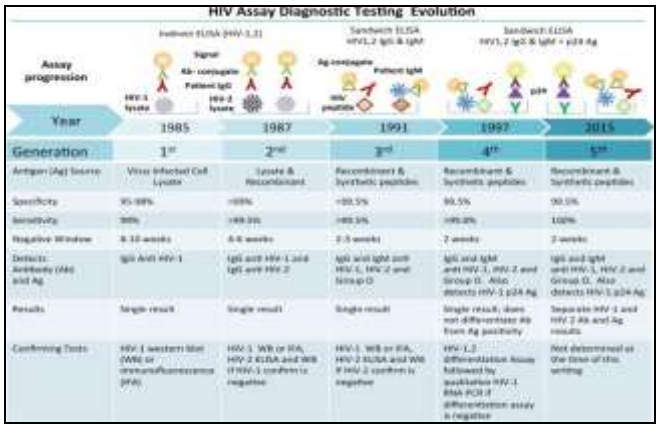

Figure-3: Schematic representation of the 30-year evolution of HIV diagnostic assays[12]

Advanced Methods for HIV Diagnosis and RNA Extraction

HIV can also be diagnosed by some other methods, HIV qualitative nucleic acid assays, HIV RNA load assays, ART (Antigen Rapid Test), HIV genotypic drug resistance testing, Point of care testing, Nucleic acid sequence based amplification, branched chain DNA amplification, and reverse transcription PCR. The Nucleic acid sequence based amplification test uses the boom technique to extract whole RNA from the sample of plasma. This technique produces high-grade RNA that is utilize in different examinations, like HIV genotyping to find point mutations associated to antiretroviral resistance. The method's primary limitations are the time-consuming description of RNA extraction.[13]

HIV Diagnosis and Monitoring: Qualitative Nucleic Acid Assays and Viral Load Testing

HIV qualitative nucleic acid assays are applied in three regions, diagnosis of severe infection, assertion of plasma, as well as immediate detection in newborns. Measures like improvements in clinical symptoms, Viral load suppression, and increases in CD4 cell counts can be used to track how well antiretroviral therapy is working for people with HIV. VL testing was strongly advised for use in monitoring patients on antiretroviral therapy and for confirming failure in treatment in the 2013 world health organization consolidated guidelines on the use of antiretroviral medications for treating and preventing human immunodeficiency virus. Virally suppressed and nonsuppressed patients can be effectively managed using diversified service delivery models when viral load monitoring of a patient's response to therapy is used. It is a useful marker for evaluating compliance to antiretroviral therapy.[14]

HIV Detection Using Nucleic Acid Amplification Tests (NATs)

By intensifying viral RNA, the first biomarker that nucleic acid testing may identify if a real time polymerase chain reaction is performed. NAT, including molecular polymerase chain reaction and transcription-mediated amplification testing, has historically provided high sensitivity and specificity to detect human immunodeficiency virus. By intensifying proviral deoxyribonucleic acid that is incorporated into the host cell's genome later in the infection process, can also identify HIV infections. Qualitative PCR or Transcription mediated amplification is a suitable test for people who have not produced enough antibodies to produce reactive results with antibody-based tests, since NAT detects infection at an early stage of human immunodeficiency virus. Infants (less than 18 months old) with maternal antibodies may also be diagnosed using qualitative PCR or Transcription mediated amplification. Prior to or during antiretroviral medication (ART), quantitative NAT is used for monitoring in people with HIV. Rapid HIV tests that are then integrated into other healthcare and community settings have additionally promoted the use of NAT. However, NAT is linked to high expenses, intricate sample pretreatment, and the requirement for specialist training, in addition to the requirement for advanced testing equipment. As a result, a number of further exciting developments have been created to broaden access to high-quality diagnostics globally.[15]

Early HIV Detection Using Molecular Technologies

Although the detection of early human immunodeficiency virus would be beneficial in all socioeconomic contexts, there has not been enough incentive to look for methods to identify adult patients

who have recently contracted the virus because patients rarely ever visit medical facilities during the acute stage of infection. Molecular technologies may be useful in clinical and public health settings since antibody-based tests used for HIV self-testing and HIV facility-based testing are unable to identify human immunodeficiency virus infection in this early stage. However, there is a lack of evidence supporting these technologies from larger-scale research and trials, which is necessary to comprehend how molecular technologies can speed up early diagnosis during this crucial acute phase.[16]

Viral Load Testing and Genetic Diversity: Challenges with PCR Amplification

A research was performed to find a relationship between genetic diversity and VL tests. The measurement of VL may be complicated by genetic variability. Primers and probes developed from the conserved sections of the HIV genome are used in PCR amplification and hybridization, which forms the basis of most VL assays. However, a primer's VL may be underestimated by more than 100 times if it has one or two nucleotide changes at the 3 end. Subtype B viruses served as the primary basis for the development of earlier VL test iterations. With non-B subtypes, these tests frequently failed.[17]

Improved Viral Load Assays Using Conserved HIV Genetic Regions

Primers and probes that target several conserved areas of human immunodeficiency virus group M subtypes and circulating recombinant versions have enhanced the majority of the assays now in use. The communities of assay inventors, doctors, and laboratory professionals must be cautious in order to promptly identify abnormal VL results, as genetic variability can have a substantial impact on these determinations. The results of various VL test kits should be compared by laboratories in order to determine which ones accurately quantify and correspond with strains that are currently in circulation in the entire country. Additionally, it is advised that a single test be employed for the duration of the patient's VL monitoring.[18]

HIV Testing and Monitoring Using Dried Blood Spot (DBS) Specimens

HIV testing can also be done by use of dried blood spot specimen. As an alternative to whole plasma and blood, for the diagnosis and track HIV positive patients the dried blood spot can be used. DBS specimens may be transported at room temperature and are simple to gather, prepare, and package. DBS specimens are frequently utilized in programs for HIV antibody testing, HIV drug resistance monitoring, surveillance, VL testing, early infant diagnosis and proficiency testing. They have been tested and demonstrated results similar to those with plasma. There are alternatives for usage in resource limited settings since many economically accessible filter paper types, as Ahlstrom 226, Munktell TFN and Whatman 903, have been evaluated and proven appropriate for the assortment of dried blood spot samples for early infant diagnosis.[19]

SAMBA Semi-Q Platform for HIV Viral Load Monitoring Using Isothermal Amplification

Diagnostic for the Real World manufactures the SAMBA, Semi Q platform, a semi quantitative assay that amplifies HIV nucleic acid from an input volume of plasma and captures amplified HIV targets onto the solid phase of a dipstick using a proprietary isothermic method. Within 90 minutes, the signal is amplified and a visible blue band is produced using sensing devices tagged with several hapten moieties and a colored antihapten detection conjugate. When the tested material VL is less or more than 1,000 copies/ml, respectively. It produces a qualitative result to assess the success or failure of ART. It has been demonstrated to identify circulating recombinant forms, groups N and O, and all main HIV-1 group M subtypes. The SAMBA's accuracies were 99%, 96.9%, and 95% respectively.[20]

SAMBA HIV-1 Semi-Quantitative Test for Viral Load Monitoring

Subsequent examination of the Malawian and Ugandan data revealed that a 1,000 copies/ml SAMBA cutoff might differentiate between patients who were suppressed and those who were not. Additionally, there was 98% concordance between the simple amplification-based assay results from 150 samples from Ukraine and the Abbott Real-time assay results. Crucially, the samples were examined on-site in labs at and medical institutions in Malawi and Uganda, and the results were available the same day as the test. The device also incorporates a miniature leukodepletion column that can remove almost 99.9% of the WBC from 100 microliters of venous whole blood, among other features.[21]

HIV Quantification Using Roche Cobas Liat Platform with Real-Time PCR

Roche Cobas Liat platform can be used for quantification of HIV. The Liat HIV quantitative

plasma assay extracts RNA from 150 microliters of plasma using a pencil-sized, self-contained Liat tube and magnetic beads. An actuator motor to regulate every stage of the viral load detection procedure processed the tube, and it contains reagents for RNA extraction and real-time polymerase chain reaction quantification in several sections. An internal armored HIV RNA is also used in this experiment to aid in the precise VL computation. To detect HIV-1 group M, group O, and HIV-2, the dynamic detection range is s 57 to 1.6 × 106 with a turnaround time of 30 to 35 minutes.[22]

Comparison of Liat HIV Quantitative Plasma Assay with VL Testing

When compared to the reference Roche CAP CTM v 2.0 and Abbott Real-time human immunodeficiency virus assays, the results of the Liat HIV quantitative plasma assay demonstrated good performance. At a threshold of 1,000 copies/ml, the Liat assay's clinical sensitivity was 100% and its specificity was 88.2% when compared to the Roche platform. The quantitative output of the Liat assay is one benefit over SAMBA Semi Q. This device features Ethernet and wireless capabilities and is fully compatible with Health Level-7 communication protocols. Liat tubes currently have a six-month shelf life and must be transported and stored via cold chain transportation.[23]

HIV Viral Load Quantification Using Alere q Platform

The Alere q assay produces data in one hour using microarray-based technology. It extracts Ribonucleic acid from 0.5ml to 1ml of plasma using a cartridge that contains chaotropic salts, heat, and mechanical disruption. This method determines VL by using amplification and real-time fluorescence. The microarray has internal controls to detect any errors in addition to target areas for calculating VL and control spots for positive and negative hybridization. There is no requirement for cold-chain storage, because the analyzer only processes one specimen at a time and its throughput is minimal.[24]

DISCUSSION

Hans *et al.*,[24] performed a study that is similar with the study Niemz *et al.*,[25] who observed that blood is frequently screened for transfusion-transmitted illnesses using molecular methods called nucleic acid tests (NATs). They are primarily carried out in centralized labs with highly qualified personnel and cutting-edge equipment. Standard point of care setting is unable to meet the extraction processes and DNA/RNA extraction requirements of the majority of NATs. NATs have a great sensitivity and accuracy in detecting and measuring viral DNA/RNA. Nucleic acid test-based viral load measuring techniques avoid the sero-negative window period when testing for an AIDS infection. The detection time for HIV has been condensed to 2.93 days. Sample collection, sample processing, data analysis, interpretation, and waste disposal are all necessary for the implementation of NAT-based point of care systems. The testing would be pointless without carrying out the necessary processes, which frequently affects rural and underdeveloped locations where there is a clear lack of technological capabilities and qualified staff.[25,26]

Lakshmi *et al.*,[27] performed a study that, the polymerase chain reaction method has been used to identify HIV and it is an in vitro method to amplify DNA, but there is another study by Sathyanarayana *et al.*,[28] in which they described that the amount of DNA produced by PCR increases exponentially by 2n, where n is the number of cycles completed. Denaturing, annealing, and extending phases make up each cycle. A thermocycler is necessary to finish all three steps because they demand different temperatures. Thermocyclers are frequently costly and need skilled workers, who are infrequent in environments with limited resources.[27,28]

To overcome the shortcomings of PCR there is a study by Fakruddin *et al.,* in which they described that recent years have seen the development of isothermal-based assays to address the shortcomings of PCR assay.[29] By the study of Oliveira *et al.,* there is no need for a thermocycler in isothermal assays, which are sequence-specific amplification procedures. This simplifies the procedure and speeds it up (by about 15 to 60 minutes) compared to PCR-based assays. Recombinase polymerase amplification and loop mediated isothermal amplification are two types of isothermal amplification assays that are presently being researched for HIV viral load assessments. Amplification can be carried out at a single temperature due to the isothermal nature of these tests. The cost of point of care based isothermal devices will decrease due to the elimination of the need for a thermocycler, and point of care -based molecular testing would be possible.[30]

Pakistan uses a variety of HIV diagnosis techniques. The Enzyme-Linked Immunosorbent Assay is a widely used screening method for HIV

antibody detection due to its affordability and convenience of use. Quick HIV Testing Rapid detection is provided by kits like as Capillus and SD Bioline, which are frequently used in a three-test protocol to confirm infection. In order to confirm positive results from initial screening, the Western Blot is used as a confirmatory test. Due to its sensitivity and specificity, PCR (Polymerase Chain Reaction) is used for HIV testing and for patient follow-up on antiretroviral medication. The detection window is shortened by Fourth-Generation Rapid Tests, which additionally identify HIV antibodies and p24 antigens.[31]

RECOMMENDATIONS

Future studies should aim to design low-cost HIV diagnostics. Point-of-care and rapid testing is crucial, and the world should gain access to such technologies. It will enhance HIV diagnosis, treatment, and outcomes for patients. Monitoring drug resistance and transmission will be enhanced further, ultimately leading to global control and prevention of HIV. The effective implementation would need governments, researchers, and healthcare provider's collaboration and investment.

CONCLUSION

Molecular methods have revolutionized the way we diagnose and treat human immunodeficiency virus. They enable us to detect it early, monitor the disease, and detect drug resistance. Methods such as polymerase chain reaction have increased the accuracy and speed of tests. Although challenges remain, new technologies are also making tests more available and dependable globally. All these advances are important in combating HIV and enhancing patient care.

Conflict of Interest: None.
Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZA & AS: Data acquisition, data analysis, critical review, approval of the final version to be published.

ZA & KM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Bekker LG, Beyrer C, Mgodi N, Lewin SR, Delany-Moretlwe S, Taiwo B, et al. HIV infection. Nat Rev dis primers 2023; 9(1): 42. https://doi.org/10.1038/s41572-023-00452-3
2. Kapila A, Chaudhary S, Sharma RB, Vashist H, Sisodia SS, Gupta A. A review on: HIV aids. Indian J Pharm Bio Res 2016; 4(3): 69-73. https://doi.org/10.30750/ijpbr.4.3.9
3. Oh DY, Fong L. Cytotoxic CD4+ T cells in cancer: Expanding the immune effector toolbox. Immunity 2021; 54(12): 2701-2711. https://doi.org/10.1016/j.immuni.2021.11.015
4. Owen SM. Human immunodeficiency virus. Manual of Molecular and Clinical Laboratory Immunology. 2024: 658-667.
5. Prabhu SR, van Wagoner N. Human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS): an overview. In Sexually transmissible Oral diseases. Springer 2023: 51-71. https://doi.org/10.1002/9781119826781.ch5
6. Delaney KP, Dinenno EA. HIV testing strategies for health departments to end the epidemic in the US. Am J Prevent Med 2021; 61(5): S6-15. https://doi.org/10.1016/j.amepre.2021.06.002
7. Patel P, Raizes E, Broyles LN. Human immunodeficiency virus infection. In: Ryan ET, Hill DR, Solomon T, Aronson NE, Endy TP, editors. Hunter's Tropical Medicine and Emerging Infectious Diseases. 10th ed. Philadelphia: Elsevier; 2020: 232-66. https://doi.org/10.1016/B978-0-323-55512-8.00031-4
8. Nsanzimana S, Rwibasira GN, Malamba SS, Musengimana G, Kayirangwa E, Jonnalagadda S, et al. HIV incidence and prevalence among adults aged 15-64 years in Rwanda: Results from the Rwanda Population-based HIV Impact Assessment (RPHIA) and District-level Modeling, 2019. Int J Infect Dis 2022; 116: 245-254. https://doi.org/10.1016/j.ijid.2022.01.032
9. Hull IT, Kline EC, Gulati GK, Kotnik JH, Panpradist N, Shah KG, et al. Isothermal amplification with a target-mimicking internal control and quantitative lateral flow readout for rapid HIV viral load testing in low-resource settings. Anal Chem 2021; 94(2): 1011-1021. https://doi.org/10.1021/acs.analchem.1c03960
10. Basoulis D, Mastrogianni E, Voutsinas PM, Psichogiou M. HIV and COVID-19 co-infection: Epidemiology, clinical characteristics, and treatment. Viruses 2023; 15(2): 577. https://doi.org/10.3390/v15020577
11. Kang T, Lu J, Yu T, Long Y, Liu G. Advances in nucleic acid amplification techniques (NAATs): COVID-19 point-of-care diagnostics as an example. Biosens Bioelectron 2022; 206: 114109. https://doi.org/10.1016/j.bios.2022.114109
12. Alexander TS. Human immunodeficiency virus diagnostic testing: 30 years of evolution. Clin Vaccine Immunol 2016; 23(4): 249-253. https://doi.org/10.1128/cvi.00053-16
13. Drain PK, Dorward J, Bender a, Lillis L, Marinucci F, Sacks J, et al. Point-of-care HIV viral load testing: an essential tool for a sustainable global HIV/AIDS response. Clin Microbiol Rev 2019; 32(3): e00097-18. https://doi.org/10.1128/cmr.00097-18
14. Pham MD, Nguyen HV, Anderson D, Crowe S, Luchters S. Viral load monitoring for people living with HIV in the era of test and treat: progress made and challenges ahead–a systematic review. BMC Public Health 2022; 22(1): 1203. https://doi.org/10.1186/s12889-022-13504-2
15. Maggiolo F, Bandera A, Bonora S, Borderi M, Calcagno A, Cattelan A, et al. enhancing care for people living with HIV: current and future monitoring approaches. Expert Review Anti infect Ther 2021; 19(4): 443-456. https://doi.org/10.1080/14787210.2021.1823217
16. Liu Q, Jin X, Cheng J, Zhou H, Zhang Y, Dai Y. Advances in the application of molecular diagnostic techniques for the detection of infectious disease pathogens. Mol Med Rep 2023; 27(5): 104. https://doi.org/10.3892/mmr.2023.12991

17. Dong T, Wang M, Liu J, Ma P, Pang S, Liu W, et al. Diagnostics and analysis of SARS-CoV-2: current status, recent advances, challenges and perspectives. Chem Sci 2023; 14(23): 6149-6206. https://doi.org/10.1039/D2SC06665C
18. Fosah Tayong GE, Vuchas C, Mbuh NN, Suiteng UN, Mbuli C, Soh KC, et al. Implementation of pooled testing to increase access to routine viral load monitoring for people living with HIV on antiretroviral therapy. Sci Rep 2025; 15(1): 14713. https://doi.org/10.1038/s41598-025-92709-y
19. Vojnov L, Carmona S, Zeh C, Markby J, Boeras D, Prescott MR, et al. The performance of using dried blood spot specimens for HIV-1 viral load testing: a systematic review and meta-analysis. PLoS Med 2022; 19(8): e1004076. https://doi.org/10.1371/journal.pmed.1004076
20. Brook G, Stepchenkova T, Ali IM, Chipuka S, Goel N, and Lee H. Study to evaluate the performance of a point-of-care whole-blood HIV viral load test (SAMBA II HIV-1 semi-Q whole blood). J Clin Microbiol 2021; 59(3): e02555-20 https://doi.org/10.1128/jcm.02555-20
21. Gueguen M, Nicholas S, Poulet E, Schramm B, Szumilin E, Wolters L, et al. Implementation and operational feasibility of SAMBA I HIV-1 semi-quantitative viral load testing at the point-of-care in rural settings in Malawi and Uganda. Trop Med Int Health 2021; 26(2): 184-194. https://doi.org/10.1371/journal.pone.0281279
22. Hans L, Marins EG, Simon CO, Magubane D, Seiverth B, Carmona S. Classification of HIV-1 virological treatment failure using the Roche cobas plasma separation card on cobas 8800 compared to dried blood spots on Abbott Real-time HIV-1. J Clin Virol 2021; 140: 104839. https://doi.org/10.1016/j.jcv.2021.104839
23. Mampa TM. Off-Label Evaluation of Alternative Specimen Types: Cobas® Plasma Separation Card for HIV Viral Load and Dried Blood Spots for COVID-19 Serology Testing.
24. Manjate A, Nilsson C, Axelsson M, Lindroth S, Sirbu D, Sacarlal J, et al. Laboratory-based evaluation of the 4th-generation AlereTM HIV Combo rapid point-of-care test. Plos one 2024; 19(2): e0298912. https://doi.org/10.1371/journal.pone.0298912
25. Hans R, Marwaha N. Nucleic acid testing-benefits and constraints. Asian J Transfus Sci 2014; 8(1): 2-3. https://doi.org/10.4103/0973-6247.126679
26. Niemz A, Ferguson TM, Boyle DS. Point-of-care nucleic acid testing for infectious diseases. Trends Biotechnol 2011; 29(5): 240-250. https://doi.org/10.1016/j.tibtech.2011.01.007
27. Lakshmi V, Sudha T, Rakhi D, Anilkumar G, Dandona L. Application of polymerase chain reaction to detect HIV-1 DNA in pools of dried blood spots. Indian J Microbiol 2011; 51(2): 147-152. https://doi.org/10.1007/s12088-011-0135-0
28. Sathyanarayana SH, Wainman LM. Laboratory approaches in molecular pathology: the polymerase chain reaction. In Diagnostic molecular pathology. 3rd ed. Academic Press. 2024; p: 13-25. https://doi.org/10.1016/B978-0-12-822824-1.00041-9
29. Fakruddin M, Mannan KS, Chowdhury A, Mazumdar RM, Hossain MN, Islam S, et al. Nucleic acid amplification: Alternative methods of polymerase chain reaction. J Pharm Bio allied Sci 2013; 5(4): 245-252. https://doi.org/10.4103/0975-7406.120066
30. Oliveira BB, Veigas B, Baptista PV. Isothermal amplification of nucleic acids: The race for the next "gold standard". Front Sensors 2021; 2: 752600. https://doi.org/10.3389/fsens.2021.752600
31. Saz J, Dalmau-Bueno A, Meulbroek M, Pujol F, Coll J, Herraiz-Tomey Á, et al. Use of fourth-generation rapid combined antigen and antibody diagnostic tests for the detection of acute HIV infection in a community centre for men who have sex with men, between 2016 and 2019. PLoS One 2021; 16(7): e0255065. https://doi.org/10.1371/journal.pone.0255065