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Short Communication



Detection of HIV-1 RNA by RT-qPCR Using GeneXpert Technology

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Abstract

Real-time PCR is a molecular biology technique that combines nucleic acid amplification and detection. This technique is ideal for monitoring HIV infection treatment. The objective of this study is to detect HIV-1 RNA using GeneXpert technology, specifically the Xpert HIV-1 Viral Load test which can detect and quantify HIV-1 RNA from groups M, N and O across a range of 40 to 10,000,000 copies/ml. We collected and tested 30 patient samples: 25 adults on treatment and regularly monitored, and 5 children born to HIV-positive parents. 1 ml of plasma was pipetted and transferred into the sample chamber of the cartridge. The test was run on the GeneXpert system according to the specified timeline in the instructions. The Xpert HIV-1 Viral Load test showed excellent performance, with results either negative (undetectable viral RNA) for 26.6% of patients, or positive (viral RNA at 40 copies/ml or between 40-106 copies/ml) for 20% and 53.4% of patients respectively. The Xpert HIV-1 Viral Load offers a rapid (<90 min) alternative to centralized testing, with sensitivity suitable for therapeutic monitoring, particularly in remote areas. These results demonstrate the effectiveness of antiretroviral treatment in HIV-positive patients, showing cases with undetectable viral RNA along with some cases of first-line treatment failure.

Keywords: HIV-1 viral load, Genexpert, Molecular diagnostics

Introduction

AIDS is now considered a pandemic that has caused approximately 40.1 million deaths since its emergence (UNAIDS, 2021). Significant progress has been made, and today antiretroviral treatments can combat HIV, consequently delaying AIDS onset and reducing mortality and morbidity. The international community has committed to ending the epidemic by 2030 (UN Press, 2021).

Achieving this goal depends on HIV programs' ability to provide treatment to all people living with HIV and maintain them on treatment with controlled viral load. In this context, diagnosis plays a central role. Today, measuring HIV-1 RNA concentration in blood plasma (viral load) using nucleic acid-based molecular diagnostic tests has been established as the standard of care for assessing prognosis and antiretroviral

treatment response in HIV-positive patients (Désiré et al., 2001; World Health Organization, 2020; Sène et al., 2022). Viral load levels strongly predict disease progression for both HIV-1 and HIV-2 (Ba et al., 2019), and alone or combined with CD4 T-cell counts, have significant prognostic value. It is therefore essential to strengthen testing strategies, including expanded population screening and targeted repeat testing for high-risk groups.

HIV testing has undeniable individual benefits (improved health and life expectancy) and collective benefits by potentially impacting epidemic dynamics, since antiretroviral treatment significantly reduces transmission risk at individual level. The need for rapid, flexible HIV testing that can accommodate various throughput requirements and random access for urgent samples is more critical than ever, as every

patient deserves better care. Our use of GeneXpert technology aligns with this approach.

Material & Matheos

Sampling

This study was conducted at Diamniadio Children's Hospital with a cohort of 30 patients from Diamniadio district and hospital inpatients. This included 25 adults on antiretroviral treatment with regular follow-up, and 5 children born to HIV-positive parents. Sampling occurred between mid-July and December 2022. Blood was collected in EDTA tubes (dipotassium ethylenediaminetetraacetic acid) and transported to the laboratory. After centrifugation at 2500 rpm, samples were stored at -80°C.

HIV-1 Viral Load Detection

ml of plasma was pipetted into the sample chamber of the cartridge. The test was initiated on the GeneXpert system according to the manufacturer's instructions. This test detects and quantifies HIV-1 from groups M, N and O. The automated Xpert protocol includes: nucleic acid extraction and purification, primer and probe binding to the 3' end of the 5' LTR region (Long Terminal Repeat), RT-qPCR targeting the highly conserved pol gene region, the process takes 90 minutes with quantification across 40-10,000,000 copies/ml.

Result

Viral RNA was undetectable in 26.6% of patients. Viral RNA was below 40 copies/ml in 20% of patients and between 40-10⁶ copies/ml in 53.4% of patients. All pediatric cases showed detectable viremia.

Table 1. Result distribution by RT-qPCR

Viral Load (copies/ml)	Number	Percentage (%)
Undetectable	8	26.6
<40	6	20
40-10 ⁶	16	53.4

Discussion

Over six months, we detected and quantified viral load in 22 patients including all 5 children, while 8 showed undetectable viral load. Viral load quantity depends on multiple factors: newly infected individuals tested early show low initial viral load, untreated chronic infection leads to high viral load. Children of treated HIV-positive parents may have undetectable viral load if parents adhere to treatment, but may become HIV-positive without maternal treatment. Regularly treated patients should achieve undetectable viral load long-term, while untreated HIV-positive patients typically maintain high viral load (Expert Consensus: viral load and HIV transmission risk). However, deviations from this pattern occur due to: missed medical appointments, irregular medication adherence, emerging antiretroviral resistance. These factors can lead to treatment failure or unexpected viral load fluctuations.

Molecular diagnosis by RT-qPCR provides reliable, rapid results to clinicians, enabling effective treatment management and viral evolution monitoring to assess treatment efficacy. To achieve UNAIDS' 95-95-95 targets by 2025, studies have evaluated Xpert HIV-1 Viral Load performance with convincing results (Sacks et al., 2019; Sène et al., 2022; Ehret et al., 2022). The quantitative GeneXpert HIV-1 test shows 97-100% sensitivity, specificity and accuracy (Bwana et al., 2021; Mukendi et al., 2021).

This is a test that detects and quantifies HIV-1 RNA from groups M, N and O (Gueudin M et al., 2016), though it may show limitations for certain variants such as HIV-2.

Conclusion

The GeneXpert rapid, accurate HIV-1 RNA technology is widely used globally and proves particularly valuable in remote areas for viral load quantification. With its strong performance characteristics, sensitivity, and rapid processing time, it serves as a powerful diagnostic and surveillance tool in the HIV/AIDS pandemic. However, its deployment must be accompanied by training programs to prevent pre-analytical errors.

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