



Evaluation of Viral Load as an Alternative to Western Blot for Confirming HIV Infection in a Moroccan Context

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ABSTRACT

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Background: In Morocco, the transition from Western Blot (WB) to viral load testing for HIV confirmation aligns with WHO recommendations. This study evaluates the diagnostic performance of PCR-based viral load as an alternative to WB in a Moroccan clinical context.

Methods: A retrospective descriptive analysis was conducted using data from 232 hospitalized patients (175 men, 94 women; median age 42–44 years) at Ibn Rochd University Hospital (April 2021–March 2022). WB and initial pre-therapeutic viral load (quantitative RT-PCR) results were compared. Statistical measures included sensitivity, specificity, positive predictive value (PPV), and Youden Index (YI).

Results: Viral load testing demonstrated 95% sensitivity, 100% specificity, and 100% PPV, with a YI of 95%. Notably, 52.8% of patients exhibited high or very high viral loads (>100,000 copies/mL), reflecting delayed diagnosis. WB, while specific (99.9%), has limitations in turnaround time and early-phase sensitivity.

Conclusion: PCR-based viral load testing offers superior advantages, including rapid results, quantitative assessment, and enhanced sensitivity during early infection. Its high diagnostic accuracy supports its adoption as a confirmatory tool in Morocco, aligning with national goals to improve early HIV diagnosis, patient management, and epidemic control. Study limitations include retrospective design and potential hospital-based selection bias. These findings advocate for policy shifts toward molecular testing to optimize HIV care in resource-limited settings.

KEYWORDS:

HIV, PCR, Western blott, Viral Load.

INTRODUCTION

HIV remains a major public health issue, both globally and nationally. In 2022, it was estimated that 39 million people were living with HIV worldwide, with 1.3 million new infections and 630,000 AIDS-related deaths [1]. In Morocco, although the low prevalence (0.07% among adults), approximately 21,500 people were living with HIV by the end of 2022. Since 2010, the country has seen a significant

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and continuous decrease in new infections, with an estimated 760 new cases in 2022. 72% of them occurred within high-risk populations. Targeting these high-risk groups represent the key role in prevention and screening strategies.[2].

The Moroccan Ministry of Health has set ambitious goals to cover 90% of the HIV infections with antiretroviral therapy (ART) by 2030. The Minister has deployed a numerous measures including the prevention hardening, early screening and fighting social stereotypes [2]. In this context, accurate and early HIV diagnosis plays a crucial role in limiting transmission and improving patient care.

Recently, Morocco decided to follow the recommendations of the World Health Organization (WHO) and to phase out the Western Blot, the traditional reference serological test, in

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favor of viral load testing, a faster molecular approach [3, 4]. This study aims to evaluate PCR-based viral load as an alternative to the Western Blot for confirming HIV infection. The objective is to determine whether viral load testing—with its advantages in speed, accuracy, and quantification—can replace Western Blot while maintaining an equivalent level of diagnostic performance.

PATIENTS AND METHODS

We conducted a retrospective descriptive study based on the data extracted from the Laboratory Information System (LIS) of the Immunology Department at Ibn Rochd University Hospital in Casablanca, covering the period from April 2021 to March 2022. We included all requests for Western Blot (WB) testing for hospitalized patients in the Infectious Diseases Department of the same hospital, along with the corresponding initial pre-therapeutic viral load PCR results. The Western Blot was performed manually using the MP Diagnostics HIV BLOT 2.2© kit. Nitrocellulose strips were coated with partially purified and inactivated HIV-1 antigenic proteins, as well as a synthetic peptide specific to HIV-2. Anti-HIV-1 and anti-HIV-2 antibodies, if present, bind to these proteins and are detected by a labeled secondary antibody.

The viral load was determined using quantitative RT-PCR (TaqMan technology) at the National Institute of Hygiene. This technique is based on reverse transcription of viral RNA into complementary DNA (cDNA), followed by PCR amplification and real-time detection via fluorescence.

HIV-positive patients were then classified based on the viral load—defined as the amount of viral RNA detected in the blood, expressed in copies/mL—according to the following categories:

1. **Undetectable viral load**
 - < 40 copies/mL
2. **Low viral load**
 - 40 – 1,000 copies/mL
3. **Moderate viral load**
 - 1,000 – 100,000 copies/mL
4. **High viral load**
 - >100,000 copies/mL
5. **Very high viral load**
 - >1,000,000 copies/mL

We calculated the sensitivity, specificity, and positive predictive value of the viral load test, as well as the Youden Index (YI), which measures a method's accuracy—in our case, the viral load-. The Youden Index is calculated by the following formula:

$$YI = \text{Sensitivity} + \text{Specificity} - 1$$

A test is considered ineffective if its YI were negative. The closer the index were to 1, the more effective the test is considered to be.

RESULTS

A total of 232 patients were included in the study, 175 were men and 94 were women. The median age was 44 (women) and 42 (men). The viral load results among our patients were distributed as :

- 12.4% having a very high viral load.
- 40.4% having a high viral load.
- 39% having a moderate viral load.

Figure 1 shows the distribution of initial viral load values among our patients.

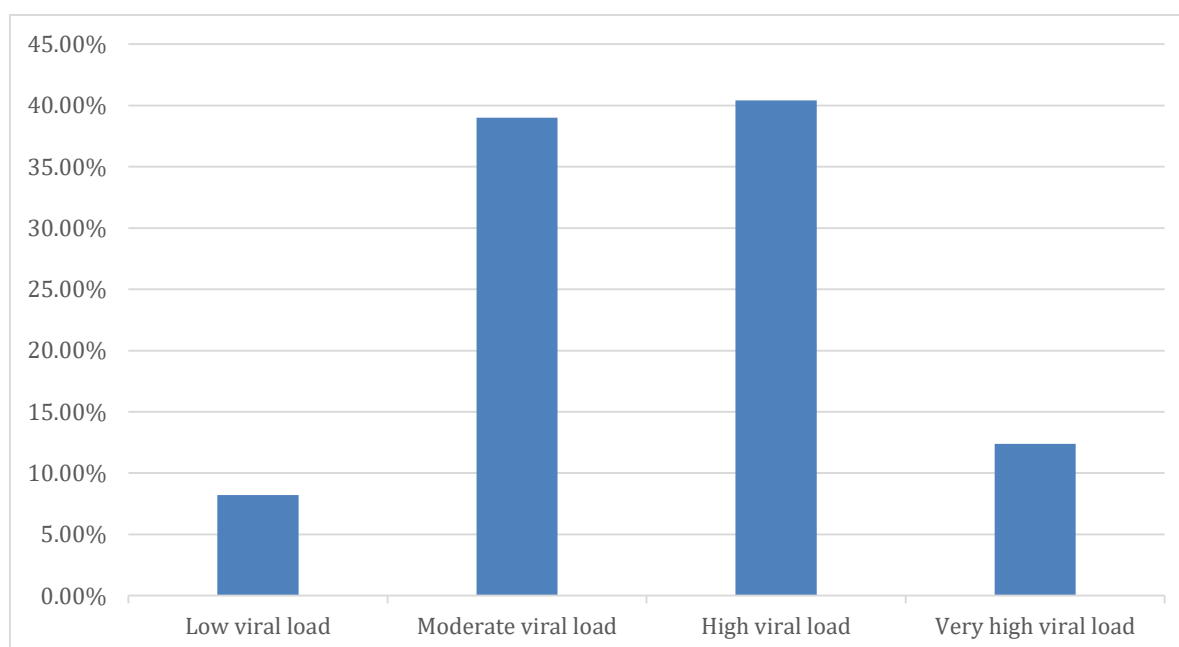


Figure 1. Initial Viral Load Values distribution Among Patients

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The viral load test had a 95 % sensitivity, a 100 % specificity, and a 100 % positive predictive value. The Youden Index was then 95% (Table 1).

Table 1: Statistical Results

Parameter	Value
Sensibility	95%
Specificity	100%
Positive Predictive Value (PPV)	100%
Youden Index	95%

DISCUSSION

Our study shows that PCR-based viral load testing demonstrates high sensitivity (95%) and perfect specificity (100%) for confirming HIV infection.

While the Western Blot has historically been used as the reference test for HIV confirmation, it shows a sensitivity of 97–99% for established infections. However, its sensitivity is lower during the acute phase of infection [5,6]. Its specificity is approximately 99.9%, making it a highly reliable test for ruling out false positives [5]. Nevertheless, it presents significant limitations, particularly in terms of technical complexity, delayed turnaround time, and ambiguity in interpretation (e.g., inconclusive or indeterminate bands).

Using viral load testing as an alternative to Western Blot offers several advantages:

- **Faster diagnosis:** Turnaround time is significantly reduced, allowing for earlier patient management.
- **Quantification of infection:** Unlike the Western Blot, which provides only a qualitative result, viral load testing allows assessment of the infection's dynamics and helps guide antiretroviral treatment initiation.
- **Improved sensitivity during early infection:** In the first few weeks after infection, the viral load is often detectable before the appearance of antibodies targeted by the Western Blot, reducing the risk of false negatives.

Our results also confirm that the majority of the included patients have a high or very high viral load (52.8%), and that the average patient age is significantly high. These findings reflect a delayed diagnosis. This delay underscores the importance of earlier screening and faster diagnostic confirmation to improve patient care and to limit viral transmission.

However, our study has certain limitations, including its retrospective nature and the fact that it is based on hospital data, which may introduce selection bias. Furthermore, although the specificity of viral load was 100% in our study, rare cases of transient detectable viral load in seronegative patients may occur, notably due to laboratory contamination or nonspecific reactions.

CONCLUSION

Phasing out the Western Blot in favor of viral load testing for confirming HIV infection represents a significant advancement in HIV diagnosis in Morocco. Our results demonstrate that PCR offers faster, more reliable, and more informative confirmation, with excellent sensitivity and perfect specificity. Integrating viral load testing as a confirmatory tool could thus enable earlier diagnosis, improved patient management, and better control of the epidemic in Morocco.

Conflict of Interest

The authors declare no conflicts of interest.

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