The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 6, 2026

COMPARATIVE EVALUATION OF QUANTITATIVE PCR AND HBSAG ELISA FOR DETECTION OF HEPATITIS B VIRUS INFECTION: A CROSS-SECTIONAL STUDY FROM PAKISTAN

Abdullah[*1], Khalid Mehmood[2], Ms. Rabia Butt[3], Attiya Uol[4], Miss iqra[5],

[*1,2,4]*Al-Razi Institute, Lahore*
[3]*HOD of MLT*

DOI: https://doi.org/10.5281/zenodo.21026155

Keywords
Hepatitis B virus; HBsAg; ELISA; quantitative PCR; viral load; occult HBV infection; Pakistan

Article History
Received: 01 May 2026
Accepted: 06 June 2026
Published: 21 June 2026

Corresponding Author: *
Abdullah

Abstract

Background: *Hepatitis B virus (HBV) infection affects over 400 million people globally and remains a major cause of cirrhosis and hepatocellular carcinoma (HCC). Accurate and timely diagnosis is critical for effective management, yet diagnostic capacity varies widely across resource-limited settings.*

Objective: *To compare the diagnostic performance of quantitative PCR (qPCR) and HBsAg enzyme-linked immunosorbent assay (ELISA) for the detection and monitoring of HBV infection in a hospital-based cohort in Pakistan.* ***Methods:*** *In this cross-sectional study, 60 HBV-confirmed patients (35 male, 25 female; mean age 43.85 ± 14.67 years) were enrolled from Govt Teaching Hospital Shahdara, Lahore. Each participant underwent parallel testing by qPCR (HBV DNA viral load) and HBsAg ELISA. Statistical analyses included the Shapiro-Wilk normality test, Spearman rank correlation, and Kruskal-Wallis test (SPSS v25).*

Results: *A highly significant positive correlation was observed between ELISA optical density and HBV viral load (Spearman $r_s = 0.862$, $p < 0.0001$). Median viral load was significantly higher in ELISA-positive patients (33,715 IU/mL) compared to borderline (2,595 IU/mL) and negative (3,046 IU/mL) groups (Kruskal-Wallis $H = 11.269$, $p = 0.004$). Importantly, two patients (3.3%) were ELISA-negative yet had detectable HBV DNA by qPCR (809 and 5,283 IU/mL), indicating occult HBV infection (OBI). No significant differences in viral load were observed by age or sex.*

Conclusion: *ELISA demonstrates strong concordance with quantitative HBV viral load and remains a valuable cost-effective screening tool. However, qPCR is indispensable for detecting OBI and low-viremia cases. A complementary diagnostic approach ELISA for first-line screening and qPCR for confirmation and monitoring is recommended for comprehensive HBV management in Pakistan and similar endemic settings.*

1. INTRODUCTION

Hepatitis B virus (HBV) infection affects approximately 400 million chronic carriers worldwide, placing them at elevated risk for liver cirrhosis, hepatocellular carcinoma (HCC), and liver failure (WHO, 2024; Terrault et al., 2018). Pakistan carries a substantial share of this burden, with HBV prevalence estimated at 2.5–4%; genotype D predominates, a strain associated with elevated rates of occult HBV infection (OBI) owing to S gene mutations that reduce HBsAg antigenicity (Ali et al., 2022; Umar et al., 2019).

Two primary modalities are used for HBV diagnosis: HBsAg ELISA, the WHO-recommended first-line screening test valued for its cost-effectiveness and scalability, and HBV DNA quantification by qPCR. ELISA is limited during the window period of early infection, in immune-escape mutants, and at low viral loads — circumstances that collectively yield false-negative results (Raimondo et al., 2019; Makvandi, 2016).

Objectives

- To compare qPCR and HBsAg ELISA for HBV detection in terms of diagnostic accuracy and laboratory feasibility.
- To evaluate the role of qPCR in detecting occult HBV infection and measuring viral load.
- To assess the ability of HBsAg ELISA to detect HBV in low-viremia cases.

Research Questions

- Does qPCR detect HBV infection more effectively than HBsAg ELISA?
- What proportion of patients are qPCR-positive but HBsAg-negative?
- Can HBsAg ELISA reliably detect HBV infection at low viral loads?

qPCR directly amplifies HBV DNA, enabling detection before seroconversion and in OBI states where HBsAg is undetectable (Terrault et al., 2018). Despite this sensitivity advantage, its high cost and infrastructure requirements restrict routine use in resource-limited settings.

This study systematically compared qPCR and HBsAg ELISA in a Pakistani hospital cohort to quantify diagnostic concordance, characterize OBI cases, and generate evidence-based recommendations for HBV management in resource-constrained settings.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

A cross-sectional study was conducted at Govt Teaching Hospital Shahdara, Lahore, Pakistan, over a three-month period following institutional ethics approval. The facility's molecular diagnostics unit served as the study site.

2.2 Participants

Sixty consecutive HBV-confirmed patients were enrolled via purposive sampling from all age groups presenting with clinical suspicion (jaundice, elevated liver enzymes) or referred for HBV screening.

2.3 qPCR (HBV DNA Quantification)

HBV DNA was extracted from serum using the QIAamp Viral DNA Mini Kit (Qiagen, Germany). Quantitative real-time PCR was performed using the Bosphore® Ultra HBV Quantitation Kit (Anatolia Geneworks, Turkey) on the StepOne™ Real-Time PCR System (Applied Biosystems, USA). Cycling conditions: initial denaturation at 95°C for 15 minutes, followed by 45 cycles of 95°C/15s, 55°C/40s, and 72°C/20s. Viral load was expressed in IU/mL.

3. RESULTS

3.1 Demographic and Clinical Profile

Table 3.1

Demographic and Clinical Characteristics of Study Participants (N = 60)

Characteristic	Overall (N=60)
Age (years), Mean ± SD	43.85 ± 14.67
Age Range (years)	18–72
HBV Viral Load, Median (IQR)	30,166 (8,733–81,265)
ELISA Value, Median (IQR)	2.1 (1.4–3.8)
ELISA Positive, n (%)	55 (91.7)
ELISA Borderline, n (%)	3 (5.0)

Characteristic	Overall (N=60)
ELISA Negative, n (%)	2 (3.3)

IQR = Interquartile Range; SD = Standard Deviation.

3.2 Correlation Between study variables

Variables	r_s	*P*
ELISA Value and HBV Viral Load	.862	< .001
Age and HBV Viral Load	−.082	.535
Age and ELISA Value	−.011	.936

rs = Spearman's rank correlation coefficient. *** denotes p < 0.001.

Spearman correlation revealed a very strong positive association between ELISA optical density and HBV DNA viral load (r_r = 0.862, p < 0.0001), confirming that higher OD readings reliably reflect higher viral replication levels. Neither age nor sex showed a significant correlation with viral load or ELISA value (all p > 0.05).

3.3 HBV Viral Load by ELISA Category

ELISA Category	*n*	*M* Viral Load (IU/mL)	*SD*	*Mdn*
Positive (> 1.1)	55	315,919.31	1,047,765.48	33,715
Borderline (0.9–1.1)	3	3,539.33	2,127.01	2,595
Negative (< 0.9)	2	3,046.00	3,163.60	3,046

qPCR and ELISA detect fundamentally different targets: qPCR amplifies HBV DNA directly, while ELISA detects the HBsAg surface protein. Their detection rates therefore diverge based on infection phase, viral load level, and clinical context, explaining cases where the two assays disagree.

Table 3.4
Shapiro-Wilk Test for Normality of Continuous Variables

Variable	*N*	*M*	*SD*	*W*	*p*
HBV Viral Load (IU/mL)	60	289,871	1,006,165	0.295	< .001
Age (years)	60	43.85	14.67	0.959	.040
ELISA Value	60	3.97	5.73	0.489	< .001

Note: M = mean; *SD* = standard deviation; *W* = Shapiro–Wilk statistic. A significant *p* value ($p < .05$)

4. DISCUSSION

The central finding of this study — a very strong positive correlation between ELISA optical density and HBV viral load (r_r = 0.862, p < 0.0001) — affirms the utility of ELISA as a reliable surrogate for viral replication status in high-viremia patients, consistent with WHO guidelines positioning HBsAg ELISA as the primary first-line screening tool in endemic regions (Chevaliez & Pawlotsky, 2018; EASL, 2023).

The significant difference in viral load across ELISA categories ($p = 0.004$) reinforces its value as a broad marker of viral replication. However, ELISA cannot reliably detect low-level viremia (< 1,000–2,000 IU/mL) or immune-escape mutants. qPCR, with commercial detection limits as low as 6.25 IU/mL, is indispensable for OBI detection, treatment monitoring, and drug resistance surveillance (Wang et al., 2012).

These findings reinforce current expert consensus: ELISA for cost-efficient first-line population screening; qPCR as the gold standard for confirmation, staging, and therapeutic monitoring. For patients with unexplained liver disease, persistent clinical suspicion despite negative serology, immunosuppression, or HIV co-infection, qPCR is mandatory irrespective of ELISA result.

Limitations include a single-centre design, a modest sample ($n = 60$), and absence of genotype, liver enzyme, and treatment data. Larger, multi-site longitudinal studies are needed to confirm and extend these findings.

5. CONCLUSION

HBsAg ELISA and qPCR are complementary, not interchangeable, tools for HBV diagnosis. ELISA provides cost-effective population screening with strong concordance to viral load; qPCR is indispensable for OBI detection, low-viremia cases, and treatment guidance. In endemic settings like Pakistan, a sequential algorithm — ELISA for primary screening followed by reflexive qPCR in indicated cases — delivers the optimal balance of sensitivity, cost-efficiency, and clinical impact.

Limitation and Recommendation

- The study was conducted at a single center with a relatively small sample size ($N = 60$), limiting the generalizability of the findings.
- HBV genotype, liver function markers, and treatment history were not assessed, which may have influenced diagnostic performance
- HBsAg ELISA should continue to be used as the primary screening tool for HBV infection in resource-limited settings.
- qPCR testing should be performed in clinically suspected cases with negative ELISA results, particularly among high-risk individuals and patients with unexplained liver disease.

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