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Comparison between Molecular Diagnosis of Hepatitis B Virus from Plasma and Dried Blood Spot Sample

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Abstract

Background: In Bangladesh, molecular testing for HBV DNA is largely restricted to tertiary healthcare facilities due to the high cost, technical complexity, and challenges of plasma sample collection, storage, and transport. Dried blood spot (DBS) sampling, obtained through finger-prick collection, offers a minimally invasive, low-cost, and field-friendly alternative for HBV DNA detection in resource-limited settings. **Objective:** This present study was undertaken to detect HBV DNA in DBS and plasma by real-time PCR. **Methodology:** The study was conducted in the Department of Virology at Dhaka Medical College (DMC), Dhaka, Bangladesh, from January 2024 to December 2024 for a period of one year. This cross-sectional study included 80 confirmed HBV-infected patients recruited from the Departments of Hepatology, Medicine, and Gastroenterology at Dhaka Medical College Hospital (DMCH), Bangladesh. Paired plasma and DBS samples were analyzed for HBV DNA using real-time polymerase chain reaction (PCR). Sensitivity, specificity, accuracy, and correlation between plasma and DBS results were calculated. **Results:** DBS demonstrated a sensitivity of 96.1%, specificity of 100%, and accuracy of 96.4% for HBV DNA detection compared with plasma. The mean HBV DNA load was 3.50 $\log_{10}$ IU/mL in plasma and 2.86 $\log_{10}$ IU/mL in DBS, showing a strong positive correlation (R = 0.8062). **Conclusion:** DBS is a valid and practical alternative sample to plasma. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):300-305*]

Keywords: Hepatitis B virus; HBV DNA; dried blood spot; plasma; real-time PCR; molecular diagnosis

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Introduction

Hepatitis B virus (HBV) infection is a major global public health concern and a leading cause of chronic liver disease. Worldwide, an estimated 254 million people are chronically infected, with about 820,000 deaths each year from complications such as cirrhosis, hepatocellular carcinoma (HCC), and liver failure[1-2]. Chronic HBV accounts for nearly half of all HCC cases globally, underscoring its oncogenic potential[3]. The global prevalence of HBV infection is approximately 3.2%, while in

Bangladesh it is higher at around 4.0%, reflecting its endemic nature[4-5].

The World Health Organization (WHO) aims to eliminate viral hepatitis as a public health threat by 2030, targeting ≥90% diagnosis and ≥80% treatment coverage among infected individuals[6]. Despite an effective vaccine, early detection and monitoring remain crucial for timely treatment initiation, transmission prevention, and reduction of HBV-related complications[7-9]. However, in low- and middle-centrifugation, and cold chain storage, making it difficult to implement in peripheral or resource-limited healthcare settings[10-12].

Dried blood spot (DBS) sampling offers a practical alternative to plasma for HBV DNA testing. It involves collecting a few drops of capillary blood on filter paper, which can be dried, stored, and transported at room temperature[13]. DBS sampling is minimally invasive, inexpensive, and well-suited for remote or resource-constrained areas where conventional plasma collection is not feasible[14]. Previous studies have shown that DBS provides comparable sensitivity and specificity to plasma for HBV DNA quantification[15-16].

Adopting DBS for molecular diagnosis could expand HBV testing coverage, improve patient access to care, and facilitate large-scale surveillance in Bangladesh. Evaluating its diagnostic performance compared with plasma is therefore crucial for validating its utility and supporting integration into national HBV control and elimination strategies.

Methodology

Study Settings and Population: This cross-sectional study was conducted in the Department of Virology at Dhaka Medical College (DMC), Dhaka, Bangladesh. Participants were recruited from both inpatient and outpatient units of the Departments of Hepatology, Medicine, and Gastroenterology at Dhaka Medical College and Hospital (DMCH). All molecular analyses were performed in the Department of Virology at DMC, between January 2024 and December 2024. The calculated sample size was approximately 73; including a 10% attrition rate, the final sample size was 80 participants. Purposive sampling was applied. All the confirmed HBsAg-positive individuals were included in this study.

Sample Collection: Capillary blood (~50 µL; 1–2 drops) was obtained via finger prick using sterile lancets and applied onto Whatman 903™ Protein Saver Cards (GE Healthcare, USA). DBS cards were air-dried at room temperature for 4–6 hours, labeled with participant ID and date, and stored in zip-lock bags with desiccants until processing. Venous blood (5 mL) was collected into EDTA tubes. Plasma was separated by centrifugation at 3000 × g for 10 minutes and stored at –20°C until molecular analysis.

Processing of Dried Blood Spots (DBS) and Plasma: Discs measuring 6 mm in diameter were excised from dried blood spots (DBS) and eluted in 750 µL of phosphate-buffered saline (PBS). The eluate was centrifuged at 10,000 × g for 2 minutes, after which the supernatant was collected for DNA extraction. Plasma samples obtained from venous blood were processed in parallel using the same downstream procedures.

HBV DNA Extraction and Quantification: HBV DNA was extracted using the QIA amp DSP Virus Spin Kit (QIAGEN, Germany) according to the manufacturer's instructions. Samples were lysed, treated with protease, washed through Mine lute columns (buffers AW1 and AW2), and eluted in 20–150 µL AVE buffer. Extracted DNA was stored at –20°C until analysis. Quantitative real-time PCR was performed using the Quant Studio 3 Real-Time PCR System (Thermo Fisher Scientific, USA) with the ARGEN NoeDx HBV qPCR Kit (ARGEN Biotech, Germany). Each 20 µL reaction consisted of 14 µL Reaction Mix 1 (RM1), 1 µL Reaction Mix 2 (RM2), and 5 µL extracted DNA. Amplification and fluorescence detection were performed at 60°C using FAM and HEX channels. Viral load was expressed in $\log_{10}$ IU/mL.

Statistical Analysis: Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY). Quantitative variables were expressed as mean ± standard deviation (SD), and categorical variables as frequency (%). Diagnostic performance of DBS compared with plasma was calculated as sensitivity (%)"=TP/(TP+FN) ×100, specificity (%)"=TN/(TN+FP) ×100, accuracy (%)"=(TP+TN)/(TP+TN+FP+FN) ×100. A p-value < 0.05 was considered statistically significant.

Ethical Consideration: Ethical approval was obtained from the Ethical Review Committee of Dhaka Medical College (ERC-DMC/ECC/2024/151) on 9 June 2024. Written informed consent was obtained from all participants prior to enrollment. Confidentiality of all participants was rigorously protected, and they

retained the right to withdraw from the study at any stage without consequence.

Results

A total of 80 participants were tested for HBV DNA using both plasma and DBS samples. Table 1 shows the results of the HBV DNA in real-time PCR for 80 participants using both DBS and plasma samples. Among the DBS samples DNA were detected in 71(88.8%) samples, while 9(11.2%) were undetected. In plasma samples, 74(92.5%) were detected, and 6(7.5%) were undetected.

Table 1: Results of HBV DNA test in real-time PCR using DBS and plasma samples

Sample	Detected	Undetected
DBS	71(88.8%)	9(11.2%)
Plasma	74(92.5%)	6(7.5%)

Plasma testing detected 74 positive and 6 negative samples. DBS correctly identified 71 of the 74 plasma-positive samples, resulting in 71 true positives and 3 false negatives. Among the plasma-negative samples, DBS correctly classified all 6 as negative, with no false-positive results observed. The sensitivity of DBS was **96.1%** (95% CI: 88.6–99.2), while the specificity was **100%** (95% CI: 54.1–100.0). The positive predictive value (PPV) was **100.0%** (95% CI: 94.9–100.0), indicating that all DBS-positive results were confirmed by plasma testing. The negative predictive value (NPV) was **66.7%** (95% CI: 29.9–92.5). Overall, DBS demonstrated an accuracy of **96.4%** (95% CI: 89.4–99.2), showing excellent agreement with plasma testing and supporting its utility as a reliable alternative specimen type for diagnostic testing (Table 2).

Table 3 presents the viral load detected by Real-time PCR in plasma and DBS samples. The viral load in IU/ml has been converted to $\log_{10}$IU/ml. The mean viral load in plasma was 3.50±0.88 $\log_{10}$IU/mL and in DBS samples it was 2.86±0.77 $\log_{10}$ IU/mL with a difference of 0.7 $\log_{10}$ IU/ml (95% confidence interval [CI]: 0.55–0.85, $p < 0.0001$). The median viral load was 3.35 $\log_{10}$IU/mL in plasma and 2.73 $\log_{10}$ IU/mL in DBS. Additionally, the viral load range varied significantly, with plasma values ranging from 2.37 to $\log_{10}$ 6.82 IU/mL, while DBS values ranged from 1.35 to 4.47 $\log_{10}$IU/mL.

Table 3: HBV DNA Viral Load Determined by Real-Time PCR Test (Log_{10}IU/ml)

Parameters	Plasma Viral Load	DBS Viral Load	P value
Mean±SD	3.50±0.88	2.86±0.77	0.0001
Median	3.35	2.73	
Range	2.37- 6.82	1.35- 4.47	

SD=Standard deviation; p- value was obtained by student's t test

Correlation and Agreement Analysis: Pearson's correlation analysis revealed a strong positive correlation between plasma and DBS viral loads ($R = 0.8062$, $p < 0.001$), indicating that DBS reliably reflects plasma viral load (Figure I).

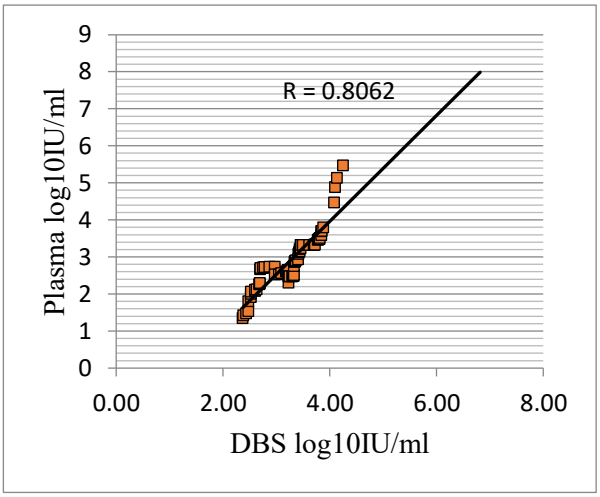

Figure I: Pearson's Correlation Coefficient of HBV DNA Levels in DBS and Plasma Samples

Table 2: Diagnostic Validity of DBS Sample in Real-Time PCR

DBS	Plasma		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	Sensitivity (95% CI)	Accuracy (95% CI)
	Detected (n=74)	Undetected (n=2)					
Detected	71(TP)	0(FP)	96.10% (88.6-99.2)	100% (54.1-100.0)	100.0% (94.9-100.0)	66.7% (29.9-92.5)	96.39% (89.4-99.2)
Undetected	3(FN)	6(TN)					
Total	**74**	**6**					

TP= True positive; TN = True negative; FP= False positive FN = False negative

Discussion

Hepatitis B virus (HBV) infection remains a major global health challenge, affecting over 250 million individuals worldwide and contributing substantially to liver-related morbidity and mortality[6,17-19]. Chronic infection can progress to cirrhosis, hepatocellular carcinoma, and liver failure, highlighting the critical importance of early detection and timely intervention to reduce disease progression and improve clinical outcomes[20].

This study evaluated the utility of dried blood spot (DBS) samples as an alternative to plasma for molecular detection and quantification of HBV DNA. DBS offers several advantages, including minimal invasiveness, reduced blood volume requirements, simplified collection by non-specialized personnel, and elimination of cold-chain logistics, making it particularly suitable for resource-limited and peripheral health settings[21].

Our results demonstrate that DBS exhibits high concordance with plasma for HBV DNA detection, which are consistent with previous studies reporting 95.0% to 98.0% sensitivity and 100.0% specificity of DBS for HBV DNA detection[15,22-24], confirming its reliability as a practical alternative to plasma for routine molecular testing.

Quantitative analysis showed that HBV DNA viral loads were slightly lower in DBS compared to plasma. The average viral load measured in plasma was 3.50 ± 0.88 log10 IU/mL, whereas DBS samples yielded 2.86 ± 0.77 log10 IU/mL, reflecting a mean difference of 0.64 log10 IU/mL. In DBS, viral loads ranged from 1.35 to 4.47 log10 IU/mL, while plasma values spanned from 2.37 to 6.82 log10 IU/mL. This variation aligns with previous findings and is largely explained by the smaller blood volume inherent to DBS sampling and differences in DNA extraction efficiency[15,25-27].

Although some differences were observed, DBS viral loads showed a strong correlation with plasma measurements and Bland-Altman analysis confirmed good agreement between the two sample types. This supports the use of DBS as a reliable tool for viral load monitoring in both clinical and epidemiological settings[30]. Nonetheless, the slightly lower limit of detection limit of DBS then plasma makes it less sensitive for identifying very low-level viremia or occult HBV infections. Sensitivity may be enhanced by increasing the number of blood spots or refining extraction protocols, but plasma remains the preferred sample type for detecting low-level infections[25,27-29].

The practical advantages of DBS are substantial. Finger-prick capillary blood collection is minimally invasive, safer, and more acceptable to patients, particularly children. DBS samples can be stored and transported without refrigeration, reduce biohazard risk, and facilitate decentralized testing, allowing peripheral centers to send samples to central laboratories[26,30].

In conclusion, this study demonstrates that DBS is a sensitive, specific, and feasible alternative to plasma. Implementation of DBS-based molecular testing can expand diagnostic coverage, support early diagnosis, and enable monitoring of antiviral therapy in resource-limited settings. Adoption of this approach could significantly strengthen HBV control strategies, particularly in low- and middle-income countries such as Bangladesh.

There are some limitation of the study. Due to financial constraints, only 80 samples were able to be investigated. The test result may be impacted by the filter paper's (DBS card) properties and drying time.

Conclusion

This study demonstrated that dried blood spot (DBS) samples showed high diagnostic validity for HBV DNA detection compared with plasma. A strong positive correlation was observed between the HBV DNA levels measured in the DBS and plasma samples. Although the HBV DNA viral load values obtained from DBS were slightly lower than those obtained from plasma samples, DBS reliably detected HBV DNA in most participants. These findings indicate that DBS can be used as a feasible alternative specimen for HBV molecular testing, particularly in resource-limited and peripheral healthcare settings where plasma collection, storage, and transportation are challenging. Further studies with larger sample sizes are recommended to optimize DBS-based molecular testing and evaluate its role in routine hepatitis B virus (HBV) viral load monitoring.

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Conflict of Interest
None

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None

Authors' contributions
Concept – Jahan N, Leema NS; Design – Jahan N, Leema NS; Supervision – Banu SS; Resource – Jahan N, Leema NS; Materials – Leema NS; Data Collection and/or Processing – Jahan N, Leema NS; Analysis and/or Interpretation -Tuhin TI, Ahmed N, Bhuiyan AH, Banu SS; Writing – , Tuhin TI, Ahmed N, Bhuiyan AH

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations. Use of AI for Writing Assistance: ChatGPT used for Grammar checking.

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