Dry blood spots as a practical alternative for confirming hepatitis E

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Introduction

Outbreaks of Hepatitis E (HEV) genotypes 1 and 2 frequently occur in communities with inadequate access to clean water, sanitation, and laboratory infrastructure. **Diagnosing HEV typically relies on detecting the virus in blood using PCR or identifying IgM antibodies via ELISA.** Historical infections are detected through measuring IgG antibodies with ELISA. **These tests require well-equipped laboratories that are rarely available in settings with hepatitis E outbreaks**. However, transporting blood samples to reference labs presents challenges due to cold chain requirements and handling constraints. Limited evidence suggests that dried blood spots (DBS) could serve as a practical alternative for storing and transporting HEV samples.

Methods

We collected paired blood and DBS samples from suspected HEV cases at the MSF hospital in Bentiu, South Sudan. DBS were stored at ambient temperature for six months in South Sudan and 24 months in Geneva. Serum was stored at -20°C in Bentiu, -80°C in Juba and transported on dry ice to Geneva for testing.

ELISA: Wantai HEV-IgM/IgG ELISA kits (Wantai BioPharm, Beijing, China) were used to detect **IgM or IgG antibodies** in patient's serum and DBS.

RNA Extraction: RNA was extracted from serum samples and DBS using the NucliSens easyMAG instrument (BioMérieux, Marcy-l'Étoile, France), according to manufacturer's instruction.

RT-qPCR: The ampliCube HEV 2.0 Quant real-time quantitative PCR (Mikrogen Diagnotik, Neuried, Germany) system is used to **detect HEV RNA** on a CFX96 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA), with an internal control and quantification by comparing Ct values to a standard curve.

Results

- **83 of 100 PCR-positive serum samples were also positive on DBS**. Among the 17 DBS-negative samples, 15 had high serum Ct values (>30), indicating lower viral loads.
- **53 of 60 (88.3%) anti-IgM ELISA serum-positive samples were also positive on DBS**, while all serum-negative samples remained negative on DBS.
- **57 of 60 (95%) anti-IgG ELISA serum-positive samples were also positive on DBS**. All serum-negative samples remained negative on DBS, except for one case classified as borderline.

Biomarker Comparisons

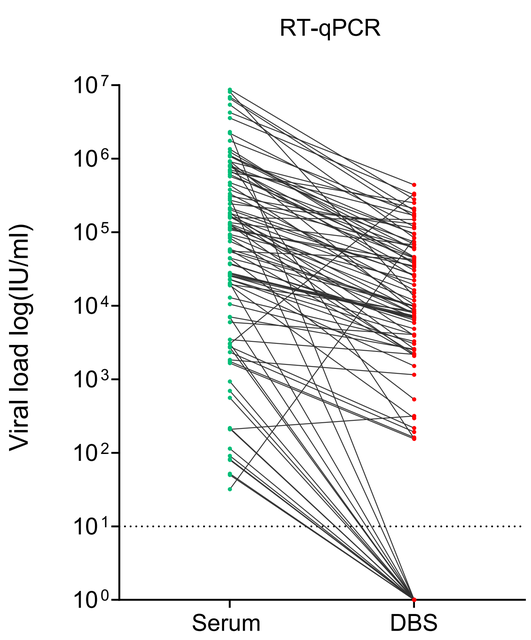

Figure 1: Viral load quantification in serum vs DBS

For serum samples, 200 µL was used for RNA extraction, whereas for DBS, two spots (equivalent to ~ 30 µL of blood) were utilized. Viral loads were then quantified by RT-qPCR for HEV.

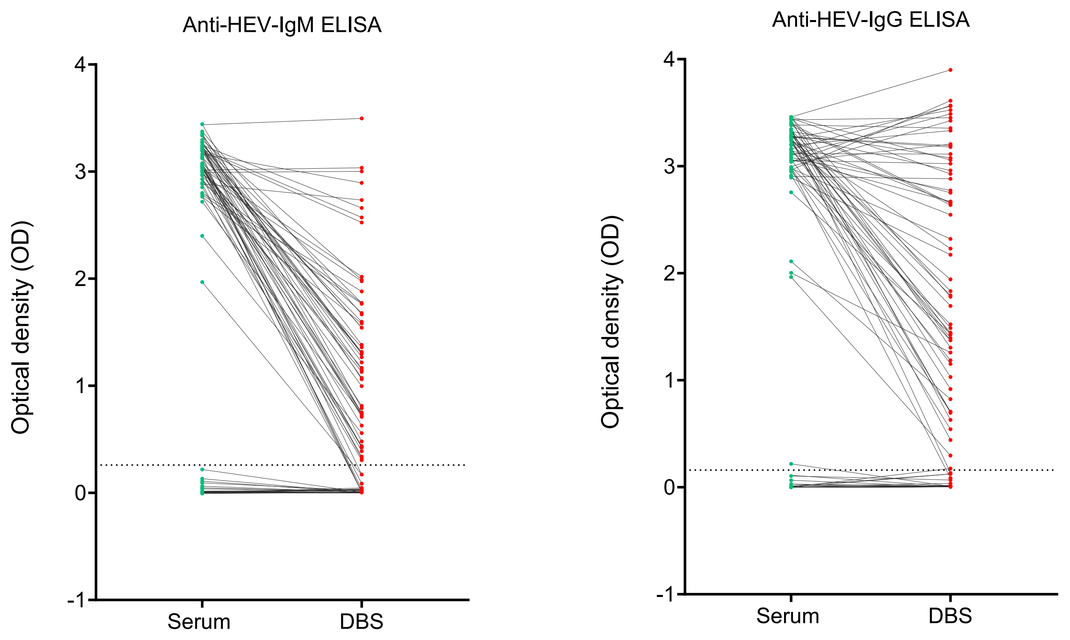

Figure 2: HEV-IgM (left) and HEV-IgG (right) ELISA in serum vs DBS

Wantai HEV-IgM/IgG ELISA kits were used the same way for serum and DBS samples following the manufacturer's instruction

Conclusions

DBS represent a promising alternative to serum for detecting HEV RNA or antibodies, particularly in resource-limited settings where traditional laboratory infrastructure is unavailable. While DBS-based PCR and ELISA tests showed slightly reduced sensitivity compared to serum-based testing, they maintained high concordance, especially in samples with higher viral loads or antibody concentrations.

"DBS offers a practical alternative to serum for HEV antibody or RNA detection.

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