


RESEARCH NOTE

Open Access



# Using dried blood spot for the detection of HBsAg and anti-HCV antibodies in Cameroon

Sebastien Kenmoe<sup>1</sup>, Paul Alain Ngoupo Tagnoukam<sup>1</sup>, Cyprien Kengne Nde<sup>2</sup>, Ghislaine Flore Mella-Tamko<sup>1</sup> and Richard Njouom<sup>1\*</sup> 

## Abstract

**Objective:** Dried blood spots (DBS) offer multiple benefits for collecting, storing and shipping whole blood samples. Our objective was to compare, for the first time in Africa, the performance of DBS with respect to plasma in the detection of Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C Virus (anti-HCV) using Architect, Abbott Diagnostics.

**Results:** DBS had a sensitivity of 99%, a specificity of 100%, a positive predictive value of 99%, a negative predictive value of 100% and a kappa index of 0.99 for the detection of HBsAg. For anti-HCV detection, the sensitivity, specificity, positive predictive value, negative predictive value and kappa index were 99%, 98%, 98%, 99%, and 0.97, respectively. This study confirms that DBS may be a reliable alternative specimen type for HBV and HCV diagnosis.

**Keywords:** Dried blood spot, Diagnosis, Hepatitis B, Hepatitis C, Cameroon

## Introduction

According to the WHO report published in 2017, more than 250 million and about 70 million persons are chronically infected with Hepatitis B virus (HBV) and Hepatitis C virus (HCV), respectively [1]. Sub-Saharan Africa is the region with the highest burden of HBV infection (prevalence > 8%) [2]. Data from a national survey conducted in Cameroon in 2011 showed a prevalence of 12% and 1% of HBV and HCV, respectively in the general population [3]. Diagnosis remains the cornerstone for therapeutic actions as well as for preventive actions for hepatitis viral infection. In low-income countries, the lack of diagnostic infrastructure in rural and peripheral areas represents a major barrier to access to viral hepatitis screening [4]. The detection of viral hepatitis diagnosis is typically based on venous blood. However, dried blood spot (DBS) has been shown to be an effective alternative to serum or plasma in people of all ages [5]. According to WHO guidelines, priority actions for HBV and HCV testing

include the use of DBS in regions that lack infrastructure or expertise for venous blood collection and for vulnerable populations [6]. However, the performance and feasibility of DBS use for the diagnosis Hepatitis C and B have not yet been described in many low-income countries in Sub-Saharan Africa including Cameroon. The present work was designed to evaluate the diagnostic accuracy of DBS for Hepatitis B surface antigen (HBsAg) and HCV antibodies (anti-HCV) detection.

## Main text

### Materials and methods

This cross-sectional study included 400 paired plasma and DBS samples (100 HBsAg positive, 100 anti-HCV positive and 100 HBsAg negative and, 100 anti-HCV negative) collected at the Centre Pasteur of Cameroon (CPC). The samples were obtained from patients received at CPC as part of the routine HBsAg and anti-HCV screening activities and no additional parameter was assessed. Paired plasma and DBS samples were prospectively prepared for all study samples. A 4 mL whole blood was collected from each patient and five spots of 50  $\mu$ L were prepared on Whatman 903 protein saver card (Whatman, GE Healthcare, NJ). The blood samples were

\*Correspondence: njouom@pasteur-yaounde.org

<sup>1</sup> Virology Department, Centre Pasteur du Cameroun, 451 Rue 2005, Yaoundé 2, P.O.Box 1274, Yaounde, Cameroon

Full list of author information is available at the end of the article



further centrifuged for 10 min at 2500 rpm and plasma was immediately tested. For DBS, two dishes were cut and transferred into 1.5 mL tube. Elution of DBS was further performed with 500 µL of PBS (pH 7.2) followed by overnight incubation at +4 °C. The next day, the eluates were subjected to centrifugation at 10,000 rcf for 5 min, and the supernatant was used for HBsAg and anti-HCV screening.

Plasma and DBS were tested using Architect HBsAg Qualitative II and Architect Anti-HCV assays (Abbott Diagnostics, Wiesbaden, Germany), according to the manufacturer’s instructions. Samples with cut-off values (S/Co) < 1 were considered negative and those with S/Co ≥ 1 were considered positive for HBsAg and anti-HCV, as recommended by the manufacturer.

The results obtained with plasma were used as reference to assess sensitivity, specificity, positive predictive value, and negative predictive value. To obtain the best sensitivity and specificity, we estimated the optimal threshold value based on the sensitivity and specificity curves with respect to threshold values. The concordance between the results obtained from analysis of plasma and DBS was evaluated using the kappa index. P-values < 0.05 was considered statistically significant. Statistical analyses were performed using R software version 3.4.1.

**Results**

Considering the plasma results as a reference, all the 100 HBV positive samples were confirmed for HBsAg testing using DBS. Fourteen HBsAg negative plasma samples were weakly positive (mean ratio 1.2 ± 0.07) on DBS. These samples were re-tested as recommended by the manufacturer, and 13 of them became negative. The first results were therefore considered as false positives and, only 1 false positive result was finally obtained with DBS. The sensitivity, specificity, positive predictive value and negative predictive value were 100%, 99%, 99% and 100% respectively (Table 1). Moreover, an excellent agreement (kappa = 0.99) was obtained between plasma and DBS results.

The results of the 100 anti-HCV negative plasma samples were similar on DBS. Twenty-three of the 100 anti-HCV positive plasma samples were negative on DBS. These 23 false negatives were tested a second time and the same results were obtained.

A new cut-off value of 0.73 was set for HCV results based on the sensitivity and specificity curves with respect to threshold values (Fig. 1). Five false negative results were maintained after adjusting the S/Co value from 1 to 0.73. Thus, a specificity of 98%, a sensitivity of 99%, a positive predictive value of 98% and a negative predictive value of 99% were obtained for anti-HCV detection using DBS. The Kappa index (0.97) shows an excellent agreement between the results obtained with plasma and DBS samples.

**Discussion**

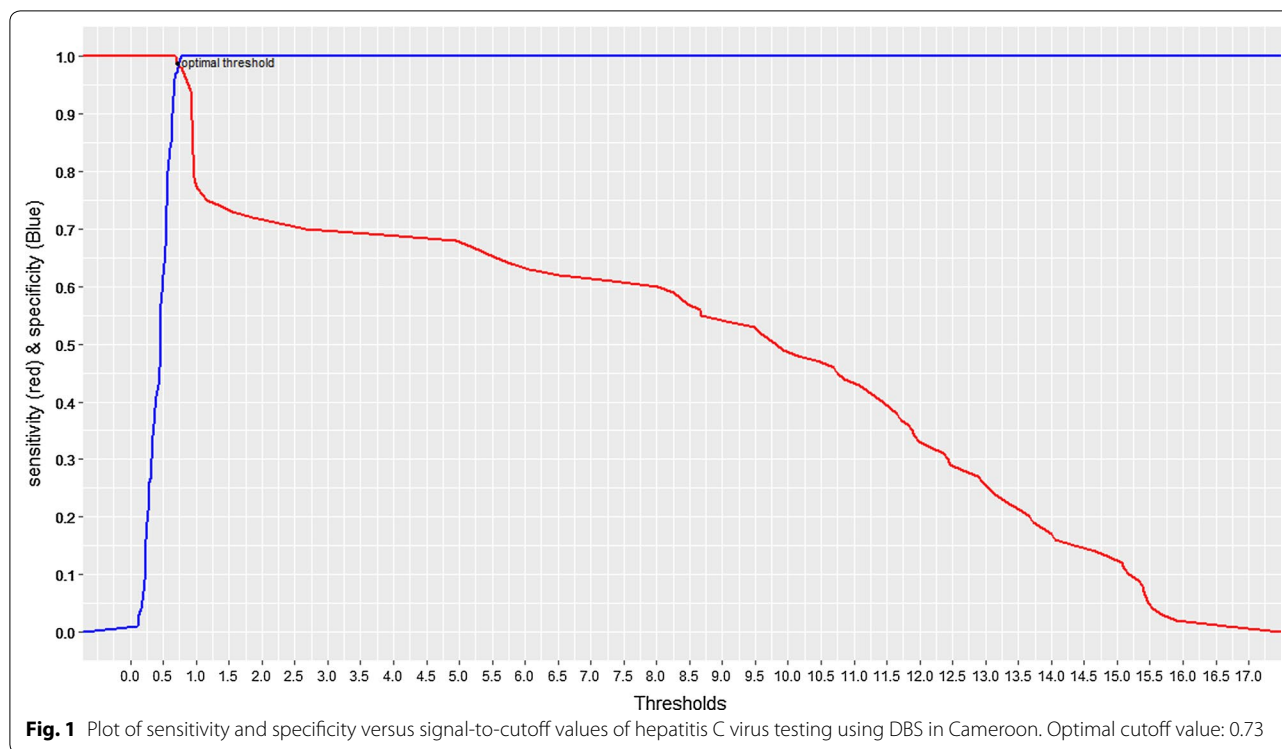
In this work, we evaluated the performance HBV and HCV screening using DBS. Similar to previous study using the same method (Architect, Abbott diagnostics), for the detection of HBsAg, a perfect sensitivity (100%) and a good specificity (99%) were obtained with the DBS, and the kappa index (0.99) also indicated excellent agreement between plasma and DBS [6–8]. Although we applied a lower blood volume (50 µL) on DBS than Ross et al. (100 µL) and Mössner et al. (75 µL), used a lower elution solution (500 µL vs 1000 µL), and used a venous blood compared to the capillary blood used by Mössner et al. Our results reinforce the good performance and confirm the robustness of the use of DBS in HBV screening [7, 8]. However, one should be cautious because Forbi et al. obtained a low sensitivity of 78.6% and a low specificity of 88.6% for detection of anti-HCV on DBS when using a lower blood volume (25 µL) and a variable threshold value depending on the negative control in a study in Nigeria [9]. This variability of the results according to the studies thus justifies the performance evaluation of the DBS in the diagnosis of HBV or HCV for the implementation of the useful recommendations.

**Table 1 Sensitivity, specificity, positive predictive value, negative predictive value, and kappa index for the detection of HBsAg and anti-HCV on DBS compared to plasma**

	HBsAg			Anti-HCV		
	Value	95% CI	p-value	Value	95% CI	p-value
Se	100	[96.3–100]	1	99	[94.5–99.9]	<0.01
Sp	99	[94.5–99.9]	<0.01	98	[92.9–99.7]	<0.01
PPV	99.0	[94.6–99.9]	<0.01	98	[93.0–99.7]	<0.01
NPV	100	[96.3–100]	1	99	[94.5–99.9]	<0.01
Kappa index	0.99	[0.96–1]	–	0.97	[0.93–1]	–

Se, Sp, PPV, and NPV values for anti-HCV were obtained with adjusted cut-off value of 0.73

Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value



For the anti-HCV test, we obtained 23 false negatives and 100% specificity by applying the threshold value recommended by the manufacturer of the kit (1). As reported previously [10], our data showed that all these 23 false negative samples had significantly lower mean titers than those obtained on plasma (DBS  $0.5 \pm 0.2$  vs plasma  $3.4 \pm 1.9$ ). Compared to previous studies that had better sensitivities, the low sensitivity in the detection of HCV in this study could be explained by the low blood volume deposited on DBS [7, 8]. Other authors have indeed suggested that low blood volumes ( $< 50 \mu\text{L}$ ) could significantly reduce the detection sensitivity of HCV [11, 12].

After adjusting to the best cut-off value as proposed by other authors for the detection of anti-HCV on DBS [10, 12–14], we obtain 98% specificity and 99% sensitivity, which is consistent with previous studies [7, 8]. Therefore our results suggest that a cut-off value of 0.73 could be used for testing anti-HCV on DBS in our context.

The low availability of specialized laboratories in remote areas is one of the major barriers to large scale testing for HBV and HCV in Africa. DBS could therefore be an alternative, simple and less expensive method to facilitate access to HBV and HCV screening especially for people living in peripheral regions. In this context, it is essential to assess the stability of DBS samples in different shipment temperatures and durations. However, Mohamed et al. have previously reported that there was

no significant variation in the HBsAg DBS positive samples stored at room temperature between 1 and 14 days, indicating that DBS could have a good stability at room temperature for a long period [15].

In summary, DBS is a useful, alternative, and simple method in HBV and HCV screening. Using DBS will greatly improve accessibility to HBV and HCV screening and to treatment for populations living in remote areas with limited infrastructure for HBV and HCV diagnosis.

### Limitations

Although the present study is the first in Africa to evaluate the performance of DBS with the widely used method for hepatitis B and hepatitis C diagnosis (Architect, Abbott Diagnostics), we did not assess the impact of transport and storage conditions on DBS.

### Authors' contributions

NR designed the study; GFMT carried out laboratory analyses; KS, PATN and CKN, reviewed the data, conducted statistical analyses and interpreted the results. KS wrote the first draft of the paper; all authors critically reviewed. All authors read and approved the final manuscript.

### Author details

<sup>1</sup> Virology Department, Centre Pasteur du Cameroun, 451 Rue 2005, Yaoundé 2, P.O.Box 1274, Yaounde, Cameroon. <sup>2</sup> National AIDS Control Committee, Yaounde, Cameroon.

### Acknowledgements

None.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

As a National Reference Laboratory in Cameroon, CPC is a Ministry of Public health facility, which has as role the improvement of pathogen detection through the validation of simple diagnostic tools. Therefore, ethical approval was not required for this study.

**Funding**

We thank the Agence Nationale de Recherche sur le SIDA et les Hépatites virales (ANRS) for the financial support (ANRS No. 12289).

**Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 19 October 2018 Accepted: 14 November 2018

Published online: 16 November 2018

**References**

1. WHO. Global hepatitis report, 2017. Geneva: World Health Organization; 2017. <http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/>. Accessed 13 Mar 2018.
2. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine*. 2012;30:2212–9.
3. Njouom R, Tejiokem M, Texier G, Fontanet A. Prevalence of hepatitis B, hepatitis C and hepatitis D virus infections in Cameroon: results from a national population based survey (The ANRS 12289 project). *J Viral Hepat*. 2015;22:1–2.
4. Easterbrook P, Johnson C, Figueroa C, Baggaley R. HIV and hepatitis testing: global progress, challenges, and future directions. *AIDS Rev*. 2016;18:3–14.
5. Lange B, Cohn J, Roberts T, Camp J, Chaffour J, Gummedi N, et al. Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): two systematic reviews and meta-analyses. *BMC Infect Dis*. 2017;17(Suppl):1. <https://doi.org/10.1186/s12879-017-2777-y>.
6. Brown BS, Klapper PE, Guiver M. Development of diagnostic serological and molecular screening from dried blood spots for HCV, HIV, HBV and syphilis. *J Clin Virol*. 2009;44:527–8.
7. Mössner BK, Staugaard B, Jensen J, Lillevang ST, Christensen PB, Holm DK. Dried blood spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life. *World J Gastroenterol*. 2016;22:7604–12.
8. Ross RS, Stambouli O, Grüner N, Marcus U, Cai W, Zhang W, et al. Detection of infections with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analyses of dried blood spots—performance characteristics of the ARCHITECT system and two commercial assays for nucleic acid amplification. *Virology*. 2013;54:1072.
9. Forbi JC, Obago JO, Gyar SD, Pam CR, Pennap GR, Agwalé SM. Application of dried blood spot in the sero-diagnosis of hepatitis B infection (HBV) in an HBV hyper-endemic nation. *Ann Afr Med*. 2010;9:44.
10. Lee CE, Sri Ponnampalavanar S, Syed Omar SF, Mahadeva S, Ong LY, Kamarulzaman A. Evaluation of the dried blood spot (DBS) collection method as a tool for detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian tertiary referral hospital. *Ann Acad Med Singapore*. 2011;40:448–53.
11. Villar LM, de Oliveira JC, Cruz HM, Yoshida CFT, Lampe E, Lewis-Ximenez LL. Assessment of dried blood spot samples as a simple method for detection of hepatitis B virus markers. *J Med Virol*. 2011;83:1522–9.
12. Soulier A, Poiteau L, Rosa I, Hézode C, Roudot-Thoraval F, Pawlotsky J-M, et al. Dried blood spots: a tool to ensure broad access to Hepatitis C screening, diagnosis, and treatment monitoring. *J Infect Dis*. 2016;213:1087–95.
13. Tuillon E, Mondain A-M, Meroueh F, Ottomani L, Picot M-C, Nagot N, et al. Dried blood spot for hepatitis C virus serology and molecular testing. *Hepatology*. 2010;51:752–8.
14. Chevaliez S, Soulier A, Poiteau L, Pawlotsky J-M. P765 dried blood spots (DBS), a promising tool for large-scale Hepatitis C screening, diagnosis and treatment monitoring. *J Hepatol*. 2014;60:S325–6.
15. Mohamed S, Raimondo A, Pénaranda G, Camus C, Ouzan D, Ravet S, et al. Dried blood spot sampling for Hepatitis B virus serology and molecular testing. *PLoS ONE*. 2013. <https://doi.org/10.1371/journal.pone.0061077>.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

