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The performance of hepatitis C virus (HCV) antibody point-of-care tests on oral fluid or whole blood and dried blood spot testing for HCV serology and viral load among individuals at higher risk for HCV in South Africa

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Abstract

Background and Aims: To enhance screening and diagnosis in those at-risk of hepatitis C virus (HCV), efficient and improved sampling and testing is required. We investigated the performance of point-of-care (POC) tests and dried blood spots (DBS) for HCV antibody and HCV RNA quantification in individuals at higher risk for HCV (people who use and inject drugs, sex workers and men who have sex with men) in seven South African cities.

Methods: Samples were screened on the OraQuick HCV POC test (471 whole blood and 218 oral fluid); 218 whole blood and DBS paired samples were evaluated on the ARCHITECT HCV antibody (Abbott) and HCV viral load (COBAS Ampliprep/COBAS TaqMan version 2) assays. For HCV RNA quantification, 107 dB were analyzed with and without normalization coefficients.

Results: POC on either whole blood or oral fluid showed an overall sensitivity of 98.5% (95% CI 97.4-99.5), specificity of 98.2% (95% CI 98.8-100) and accuracy of 98.4% (95% CI 96.5-99.3). On the antibody immunoassay, DBS showed a sensitivity of 96.0% (95% CI 93.4-98.6), specificity of 97% (95% CI 94.8-99.3) and accuracy of 96.3% (95% CI 93.8-98.8). A strong correlation (R^2 = 0.90) between viral load measurements for DBS and plasma samples was observed. After normalization, DBS viral load results showed an improved bias from 0.5 to 0.16 log10 IU/mL.

Conclusion: The POC test performed sufficiently well to be used for HCV screening in at-risk populations. DBS for diagnosis and quantification was accurate and should be considered as an alternative sample to test. POC and DBS can help scale up hepatitis services in the country, in light of our elimination goals.

KEYWORDS
dried blood spot, HCV, high risk, point-of-care, screening

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1 | INTRODUCTION

Globally, an estimated 71 million people have chronic hepatitis C, accounting for almost 400 000 annual deaths.[1] Hepatitis C virus (HCV) is efficiently transmitted through blood.[2] Groups of people at high risk of acquiring HCV infection include people who inject/use drugs (PWID/UD), men who have sex with men (MSM), sex workers (SW), prisoners and healthcare workers.[3] In the era of direct acting antivirals (DAAs), short courses of all-oral therapy can be used to cure most cases of HCV infection.[4]

Although data is limited, HCV seroprevalence in PWIDs is around 22% across sub-Saharan Africa[3]; 11% to 55% in Central Africa; 2% to 38% in West Africa and 1% to 65% in Southern and East Africa.[5] In Kenya, HCV prevalence in high risk groups is 7%[6] while in Tanzania, PWID have documented HCV seroprevalences of 28%[7] and 50%[8] and MSM, 14%.[9] In South Africa, a high HCV seroprevalence (55%) and viraemic prevalence (43%) was reported in PWID in three major cities.[10] Screening for HCV among people engaged in criminalized or stigmatized practices can be challenging. Fear and experienced stigmatization by healthcare workers of PWID/UD as well as the inadequacy of services to meet their needs are barriers to HCV screening.[11,12]

Furthermore, HCV diagnosis requires confirmation of antibody positivity by nucleic acid confirmatory testing (qualitative or quantitative).[13-15] In sub-Saharan Africa (SSA), the use of anti-HCV POC is increasing as more research is focused on viral hepatitis.[16,17] However, dried blood spot (DBS) testing and the uptake of anti-HCV POC for diagnostics has been slow as innovative diagnostic methods focused on priority diseases, such as HIV and TB[18] in SSA. Currently, whole blood is still being transported to centralized laboratories for HCV antibody and then confirmatory HCV RNA testing. A cold chain is to be maintained throughout the blood transport process and should reach the testing laboratory within 48 hours.[19] For HCV RNA testing, blood is collected in ethylene-diamine tetra-acetate (EDTA) tubes[20] to avoid hemolysis and maintain RNA integrity,[21,22] for optimal RNA amplification. Moreover, whole blood requires centrifugation for plasma/serum separation before testing. With centralized laboratory testing, inherent and unavoidable delays in process adversely affect linkage to care and treatment initiation.[23]

More affordable options with less invasive sampling methods and time to results, require evaluation, especially in lower-and-middle income countries (LMICs) where HCV prevalence is unknown, populations are hard to reach and centralized laboratory infrastructure varies.[18] POC anti-HCV tests, such as the OraQuick HCV test and SD Bioline HCV have shown high sensitivities and specificities in recent years.[24-26] Of the Khuroo et al[25] meta-analysis, four studies looked at assessing OraQuick to a reference standard assay, with none of the studies from Africa.

In SSA, there are many remote regions with poor access to laboratories and the use of DBS in these areas can be practical for screening and diagnosis as DBS can be easily transported from distant areas to centralized laboratories.[14] DBS sampling is less invasive (heel-finger-earlobe-prick) compared to venous needle insertion[27] and this can aid in sampling where venous access is challenging. Considering that blood spotting can be less painful than venipuncture, more people may be willing to test. However, collection of proper quality and volume of blood onto spots requires practice and training[27] and it is for this reason that most performance studies opt for precise aliquots of blood by using pipettes to transfer venous whole blood onto filter cards.[28,29] Still, comparative quantitative results on capillary vs venous whole blood for HCV[30] and HIV[31] were reported. Of the 18 studies on DBS for anti-HCV testing[32] and 15 studies on HCV-RNA using DBS,[33] none of the performance studies were from Africa. To address the limited knowledge on POC and DBS in SSA and to help scale up hepatitis services in our country, we have investigated the performance of anti-HCV POC testing and the use of DBS as an alternative sample matrix to that of plasma for HCV antibody and viral load testing.

2 | METHODS AND MATERIALS

2.1 | Study setting

This was a cross-sectional study that was integrated into existing HIV testing, prevention and sexual health services provided by community-based implementing sites for high risk populations. The larger study has been well-described in "Hepatitis C within key populations across seven cities in South Africa study."[34] The parent study conveniently sampled people from high risk population groups from Cape Town (SWs, MSM, PWID/UD), Durban (SWs, PWID/UD), Pietermaritzburg (SWs), Mthatha (SWs), Port Elizabeth (SWs), Johannesburg (MSM) and Pretoria (MSM and PWID/UD). Participants were recruited from mobile and community-based health services targeting people at high risk for, or living with HIV. Participants were tested for HBV surface antigen (HBsAg), HCV (antibody, viral load and genotype) and HIV antibody (unless with documentation of existing HIV infection).

In Cape Town, the city with the proposed largest sample size in the larger study,[34] anti-HCV POC testing was done using oral fluid, and DBS testing. In the other cities, anti-HCV POC testing was done using whole blood, without DBS testing. The selection of testing modalities was based on available funding and human resources.

To avoid repeated blood draws, sufficient whole blood was drawn from all participants for blood related testing that may have been needed for rapid or other confirmatory testing.

2.2 | Sample

In total, 3439 participants (1528 SWs, 746 MSM, 1165 PWID/UD) were enrolled in the larger study between August 2016 and October 2017.[34] To validate the anti-HCV POC results (oral and blood samples) and evaluate the DBS, venous blood samples were sent to the reference laboratory, National Institute for Communicable Diseases (NICD, Johannesburg) for laboratory testing. Furthermore, a subset of samples (blood and DBS) negative for anti-HCV POC were also sent to the laboratory.

Sample size calculations were based on the formula $[DEFF \times Np(1-p)]/[(d2/Z21-\alpha/2 \times (N-1)+p \times (1-p)]$[35] using an 8% prevalence[36] and precision of 4%. For anti-HCV POC, based on a

population of 2450 from all sites except Cape Town (the number of participants expected to be recruited across cities where anti-HCV POC testing would be done on blood), the sample size needed was 110 POC negative samples; equating to every 22nd negative POC test per site (2450/110 = 22).

For the DBS evaluation, using the same formula as above, and based on a population size of 1050 from Cape Town sites (the number of high risk people anticipated to be recruited in the city as part of the larger study), a sample size of 103 was calculated and every 10th negative POC test (1050/103 = 10) was sent to the laboratory.

2.3 | Blood collection, point-of-care testing and DBS preparation

Whole blood specimens were collected once from each participant by venipuncture in EDTA-treated vacutainers. The anti-HCV POC (OraQuick HCV Rapid Antibody Test) was performed on site by trained nurses as per manufacturer's instructions (OraQuick HCV Rapid Antibody Test, OraSure Technologies, Bethlehem, Pennsylvania). Whole blood samples were used on the OraQuick HCV Rapid Antibody Test in all cities except Cape Town. Venipuncture whole blood was collected using the specimen loop provided in the test kit. In Cape Town, oral fluid samples were collected using the sample pad provided in the test kit. The blood or oral fluid sampler was transferred into the developer solution and the test result was read between 20 and 40 minutes as per manufacturer's instructions.

DBS samples were prepared by spotting 50 μL of whole blood from the EDTA tubes onto each of the five circles on a Whatman-903 perforated protein saver card (GE Healthcare, Piscataway, New Jersey). DBS cards were dried and packed in nonpermeable, plastic sealable bags with two desiccant sachets and a humidity indicator card.

2.4 | Blood and DBS transport and receipt

All blood sample tubes and DBS cards were linked with a unique participant identification code and transported to the NICD. Blood tubes and DBS cards were stored at 4-8°C at the sites prior to shipping to the laboratory. For the convenience of transport, blood tubes and DBS were transported in sealed foil-lined cooler boxes with frozen ice-packs. At the laboratory, whole blood from EDTA tubes were centrifuged at 3000 rpm for 10 minutes at room temperature. The plasma was transferred into 1.5 mL microtubes and stored at −70°C until testing. DBS cards were stored at 4 to 8°C until testing.

3 | LABORATORY TESTING

3.1 | Antibody testing

The ARCHITECT HCV antibody assay was performed on the recommended volume of 70 μL plasma using the automated ARCHITECT i1000SR CMIA system (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois). For DBS samples from Cape Town sites, one spot (50 μL) from the DBS card (GE Healthcare, Piscataway, New Jersey) was eluted in 400 μL (Gavin Cloherty, Abbott diagnostics, personal communication) of 1X PBS buffer (Lonza, Belgium) and 0.05% of Tween 20 (Merck, Germany).[37] The DBS were incubated at room temperature with continuous agitation at maximum speed 300 motions per minute for 1 hour. A volume of the DBS eluate (70 μL) was processed on the ARCHITECT i1000SR system following manufacturer's instructions (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois). For plasma and DBS, the signal to cut-off ratios as indicated by the manufacturer, were used.

3.2 | Viral load testing

The COBAS Ampliprep/ COBAS TaqMan version 2 (CAP/CTM) assay (Roche Molecular Systems, Pleasanton, California) was used to measure HCV viral load on plasma. Plasma (700 μL) was vortexed for 10 seconds (Heidolph, Reax Top, Germany) before processing on the CAP/CTM assay. To prevent unequal spread of blood owing to variable hematocrit levels, the whole perforated spot on the DBS card was used. Two DBS spots were eluted in 1.0 mL of sample pre-extraction (Spex) buffer (Roche Molecular Systems, Pleasanton, California). The tubes containing the DBS in buffer were incubated on a dry and cooling heating block Eppendorf Thermomixer R (Sigma, South Africa), at 56°C with continuous shaking for 10 minutes (Roche Molecular Systems, Pleasanton, California).[38] To quantify HCV on DBS, 700 μL of DBS eluate was tested using the CAP/CTM assay (Roche Molecular Systems, Pleasanton, California).

To calculate the normalization coefficient, we had to account for the 50 μL of whole blood that was spotted onto the Whatman 903 card. Two circles of DBS were used for viral load analysis in a volume of 1000 μL of SPEX buffer, providing a total volume of 1100 μL. 50% volume of plasma in DBS was assumed. Thus, 2 × 50 μL of whole blood × 50% (100 μL × 50% = 50 μL of plasma). The normalization coefficient was calculated to be 22. This was obtained by dividing the total volume (whole blood and SPEX buffer) by the whole blood volume on each Wattman 903 card (1100 μL/50 μL = 22).

For plasma and DBS, the threshold value was set at the manufacturer's lower and upper detection limits which corresponded to 15 IU/mL and > 1.00E+08 IU/mL, respectively. On the CAP/CTM assay, any result lower than the lower detection limit (LDL) of 15 IU/mL was reported as LDL and any result where virus was not detected was reported as "target not detected" (TND).

3.3 | Statistical analysis

As per the Standards for Reporting of Diagnostic Accuracy Studies (STARD),[39] the performance of POC and DBS was calculated by sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy (Microsoft Excel 2016, Microsoft, Washington). Accuracy was the measure of agreement between the POC test and the laboratory reference immunoassay and also between the sampling matrices.

DBS viral load results were compared to the plasma (reference sample type) viral load results. Descriptive statistics using Microsoft Excel 2016 (Microsoft, Washington) were reported as the means, medians and interquartile ranges, with the minimum and maximum ranges for plasma and DBS viral load, respectively. All quantitative analyses were performed in log10-transformed values in international unit/ml (IU/mL). A normalization coefficient was calculated and applied to the raw DBS results before analyses to account for volume difference and sample type in the DBS (Ed Marins, Roche Molecular systems, California, personal communication).[40]

Sensitivity of DBS on the CAP/CTM assay was calculated as the number of true positives (viral load ≥15 IU/mL) for DBS in relation to plasma samples that had viral load ≥15 IU/mL. Specificity was calculated as the number of true negatives (virus not detected) on both DBS and plasma samples.

The linear regression analysis was performed to determine the correlation of DBS and plasma viral loads. The Bland-Altman analysis was performed to determine the agreement between the DBS and plasma, using the mean difference between the plasma and DBS viral load values and the average viral load between plasma and DBS. All tests of statistical significance were two-sided with an alpha value of .05.

3.4 | Ethics

The study protocol for the larger study was approved by the University of Witwatersrand Human Research Ethics Committee (WITS HREC, M160510), Human Research Ethics Committee of the University of Cape Town (ref: 004/2016) and relevant provincial research ethics committees. For this nested study, ethics approval was received from WITS HREC (M170698). All participants provided written consent to participate in the study. No remuneration was provided. The data analyses for the patient group were performed on de-identified data.

4 | RESULTS

4.1 | POC results

Of the 714 blood sample received at the laboratory, 16 were deemed ineligible, due to repeat recruitment or no records of POC. Of the 698 received samples that were eligible for testing, an additional eight samples hemolysis during transport and one DBS sample without a blood pair were excluded from the analyses (Figure 1). Of the total of 689 samples analyzed, anti-HCV POC was performed on 471 whole blood samples and 218 on oral fluid samples (Figure 1). PWID/UDs accounted for 523, MSM for 70 and SWs for 96 (Table 1). A total of 516 participants were positive by anti-HCV POC positive. The highest POC positivity was recorded for PWID/UDs (*N* = 494, Table 1). In total, 147 participants had HCV and HIV co-infection as detected on POC and of this, PWID/UDs accounted for 138 (Table 1).

4.2 | Anti-HCV POC compared to the reference laboratory immunoassay

4.2.1 | Whole blood POC

Of the 471 tested on whole blood POC, 370 were found to be positive (Table 2). Four false-negative results were found, of which one sample had co-infection with HIV. Viral load was detected on three of four samples and ranged from 4 to 6 log10 IU/mL. All three samples were HCV genotype 1a. The three samples were received from sites in different provinces.

4.2.2 | Oral fluid POC

Of the 218 oral fluid samples tested by POC, 146 were positive (Table 2), with PWID/UDs accounting for 130 (Table 1). Of the four false-negative results, none were HIV infected. Viral load was detected on two of the four samples, with 5 and 7 $\log_{10}$ IU/mL, respectively. One sample was genotype 1a, the other 3a. Of the four samples, two were tested by the same field operator and all samples were from the same site. There was no significant association with serum HCV antibody titer (as measured by signal/cutoff [S/CO] values) of false-negative result on either whole blood or oral fluid POC compared to the true positives (11.5 S/CO vs 14.0 S/CO).

When POC results on either whole blood or oral fluid were compared to plasma antibody results, the accuracy was 98.4% (95% CI 96.5-99.3) (Table 2). Both sample types (whole blood and oral fluid) on POC testing performed comparably, with high kappa values of 0.96 (Table 2).

4.3 | Anti-HCV DBS compared to plasma on the reference laboratory immunoassay

A total of 218 paired DBS and whole blood samples were tested for HCV antibody. An accuracy of 96.3% (95%CI 93.8-98.8), sensitivity of 96% (95%CI 93.4-98.6) and specificity of 97% (95%CI 94.8-99.3) was calculated (Table 3). There were six false-negatives on DBS serology. Of these six samples, two plasma pairs had detectable virus (viral load of 5 and 7 log10 IU/mL, respectively); two plasma pairs were TND; one plasma pair had an LDL, and one had insufficient plasma for viral load testing.

4.3.1 | HCV viral load DBS compared to plasma on reference quantitative assay

Of the 144 antibody positive concordant pairs, 142 were tested on the CAP/CTM assay (Figure 1). Two samples had insufficient plasma for additional testing. A total of 107 samples were positive with a plasma viral load of ≥15 IU/mL and were compared to DBS of ≥15 IU/mL (Table 4). There were six DBS samples that produced discordant results (Table 4), with two LDL and four TND. All six samples had plasma viral load of ≤100 IU/mL (2 log10 IU/mL). Sensitivity and

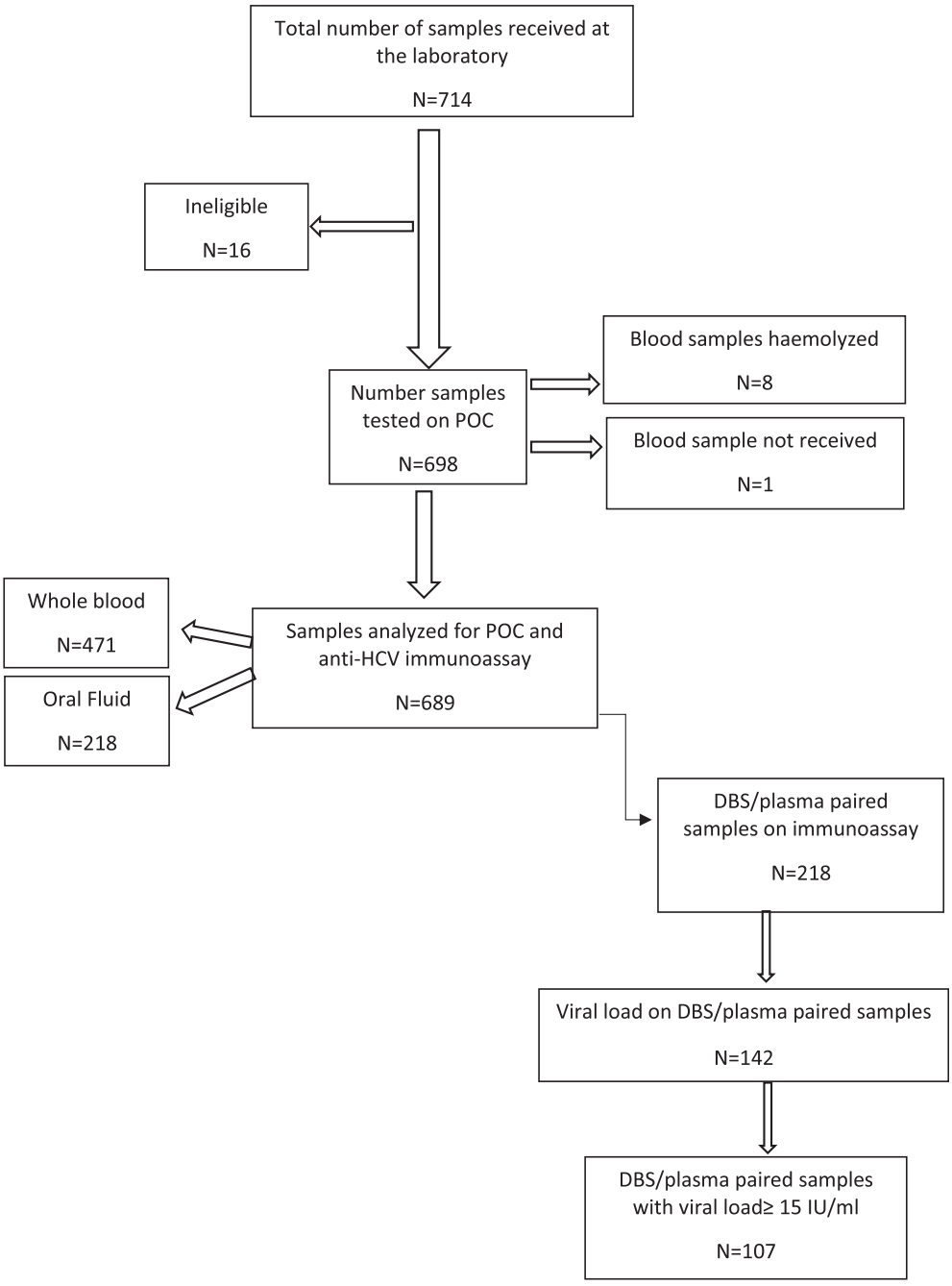


FIGURE 1 Flow diagram indicating the number of sample sets from collection to testing and analyses

percentage concordance was calculated on 142 dB/plasma pairs. Overall, a sensitivity of 107/113 (94.7%, 95%CI 91-98.4) and specificity of 100% was calculated (Table 4). If we consider a cut-off value of ≥100 IU/mL for HCV RNA on plasma, then sensitivity on DBS would be 100%.

For the 107 plasma samples, HCV viral load ranged from 1.17 to 7.13 $\log_{10}$ IU/mL, with a median of 5.42 ± 1.06 $\log_{10}$ IU/mL (IQR 4.84, 5.98). The median viral load on DBS, before normalization, was 4.87 ± 0.94 $\log_{10}$ IU/mL (IQR, 4.34, 5.41) and the quantitive range was 2.17 to 6.36 $\log_{10}$ IU/mL. After normalization, the median viral load was higher at 5.21 ± 0.94 $\log_{10}$ IU/mL (IQR, 4.68, 5.75) and viral load ranged from 2.51 to 6.70 $\log_{10}$ IU/mL. The mean titer difference between DBS and plasma before normalization was 0.5 $\log_{10}$ IU/mL and after normalization, improved to 0.16 $\log_{10}$ IU/mL.

4.4 | HCV viral load correlation between DBS and plasma

Correlation between DBS and plasma shows a positive linear relationship with a Pearson's correlation coefficient of R = 0.94. A coefficient of determination, R^2 of 0.90 (P < .0001) for both linear regression curves with and without normalization was calculated (Figure 2A,B).

TABLE 1 Demographics on 689 participants

	PWID/UD	MSM	SWs	Total
No. per risk group	523	70	96	689
Male	454	69	6	529
Female	69	0	89	158
Transgender	0	1	1	2
Median age in years (IQR)	30 (26-35)	32 (26-40)	29 (25-36)	
Anti-HCV POC positive	494	21	1	516
Whole blood	364	5	1	370
Oral fluid	130	16	0	146
Log viral load in IU/mL (IQR)	5 (5-6)	6 (5-6)	NA	
Co-infection HCV/HIV	138	9	0	147

Abbreviations: HCV, hepatitis C virus; IQR, interquartile range; MSM, men who have sex with men; POC, point-of-care; PWID/UD, people who injecting drugs/use drugs; SWs, sex workers.

TABLE 2 Performance characteristics of the OraQuick anti-HCV POC on whole blood or oral fluid

	Anti-HCV immunassay—Laboratory reference test								
	WB or OF			WB			OF		
Anti-HCV POC	Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
Pos	513	3	516	367	3	370	146	0	146
Neg	8	165	173	4	97	101	4	68	72
Total	521	168	689	371	100	471	150	68	218
Sensitivity (%, 95% CI)	98.5 (97.4-99.5)			98.9 (97.9-99.9)			97.3 (95.2-99.5)		
Specificity (%, 95% CI)	98.2 (96.2-100)			97.0 (95.5-100)			100		
PPV (%, 95% CI)	99.4 (98.8-100)			99.2 (98.3-100)			100		
NPV (%, 95% CI)	98.2 (96.2-100)			96.0 (94.3-99.8)			94.4 (91.4-97.5)		
Accuracy (%, 95% CI)	98.4 (96.5-99.3)			98.5 (97.4-99.6)			98.2 (96.4-99.9)		
Kappa value	0.96			0.96			0.96		

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; Neg, negative; NPV, negative predictive value; OF, oral fluid; POC, point-of-care; Pos, positive; PPV, positive predictive value; WB, whole blood.

TABLE 3 Performance characteristics of DBS on the ARCHITECT HCV antibody assay

	Anti-HCV immunassay—PLASMA		
Anti-HCV immunassay—DBS	Pos	Neg	Total
Pos	144	2	146
Neg	6	66	72
Total	150	68	**218**
Sensitivity (%, 95%CI)	96.0 (93.4-98.6)		
Specificity (%, 95%CI)	97.06 (94.8-99.3)		
PPV (%, 95%CI)	98.6 (97.1-100)		
NPV (%, 95%CI)	91.7 (87.9-95.3)		
Accuracy (%, 95%CI)	96.3 (93.8-98.8)		
Kappa value	0.92		

Abbreviations: DBS, dry blood spot; HCV, hepatitis C virus; Neg, negative; NPV, negative predictive value; Pos, positive; PPV, positive predictive value.

4.4.1 | HCV viral load agreement between DBS and plasma

The Bland-Altman analysis on DBS viral load without application of the normalization coefficient found a mean difference (bias) of 0.50 ± (SD) 0.37 (95%CI 0.43-0.58). The limits of agreement before normalization was found to be −0.23 to 1.24 (Figure 3A). The 95% CI for lower limit of agreement was −0.35 to −0.11, and for upper limit of agreement was 1.11 to 1.36 (Figure 3A).

After application of the normalization coefficient to raw DBS viral load values, a mean difference (bias) of 0.16 ± (SD) 0.37 (95% CI 0.09-0.23). This bias was a 5-fold improvement compared to bias without correction. The limits of agreement after normalization was found to be −0.57 to 0.89 (Figure 3B). The 95% CI for lower limit of agreement was −0.69 to −0.45, and for upper limit of agreement was 0.77 to 1.02.

TABLE 4 Comparison of HCV RNA viral load between plasma and DBS samples on the CAP/CTM

	Viral load—Plasma			
Viral load—DBS	**≥15 IU/mL**	**≤15 IU/mL (LDL)**	**Virus not detected (TND)**	**Total**
≥15 IU/mL	107	1	0	*108*
≤15 IU/mL (LDL)	2	0	0	*2*
Virus not detected	4	1	27	*32*
Total	*113*	*2*	*27*	***142***
Sensitivity (%, 95%CI)	94.7 (91-98.4)			
Specificity (%, 95%CI)	100			
PPV (%, 95%CI)	99.1 (97.5-100)			
NPV (%, 95%CI)	84.4 (90.3-78.4)			
Accuracy (%, 95%CI)	94.4 (98.2-90.5)			
Kappa value	0.77			

Abbreviations: DBS, dry blood spot; HCV, hepatitis C virus; LDL, lower than detectable limit; Neg, negative; NPV, negative predictive value; PPV, positive predictive value; TND, target not detected.

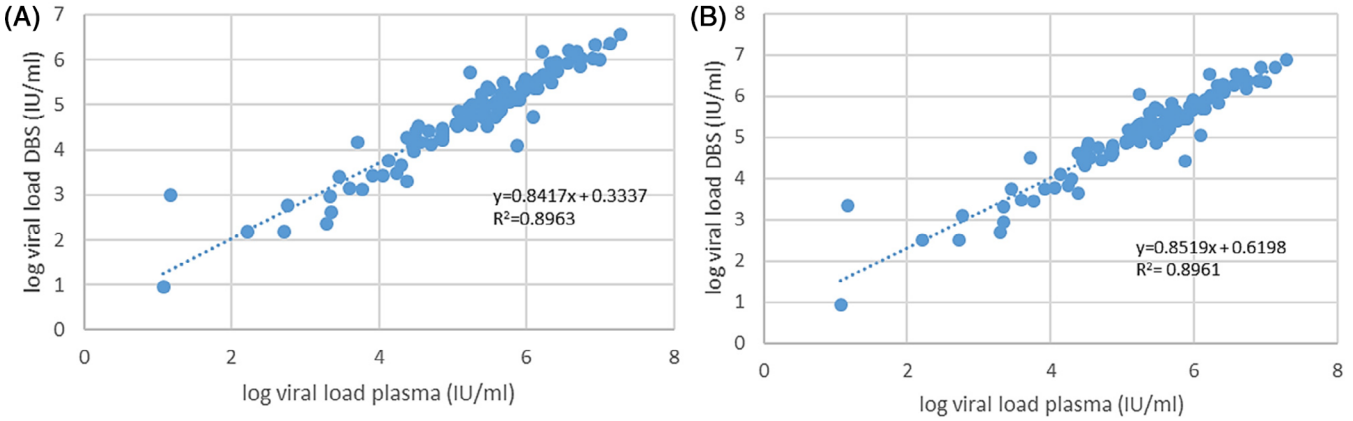


FIGURE 2 Correlation of dried blood spots (DBS) to plasma viral load (log_{10}IU/mL) on CAP/CTM, A, before application of the normalization coefficient to the raw DBS results, B, after application of the normalization coefficient

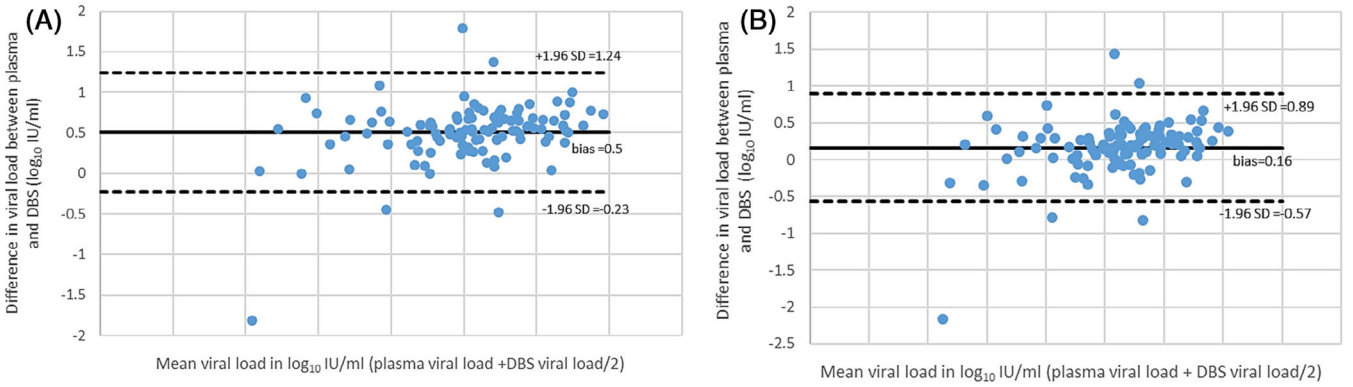


FIGURE 3 Bland-Altman plot analysis of the differences between viral load in plasma and dried blood spots (DBS) (log_{10} IU/mL) on CAP/CTM, A, without application of normalization coefficient, B, corrected DBS (log_{10} IU/mL) viral load

5 | DISCUSSION

With a high HCV seroprevalence and viraemic prevalence in PWID in South Africa,[10] it is of dire need that we simplify hepatitis C screening and diagnostics. This study examined the use of POC tests and DBS for anti-HCV screening and HCV RNA quantification in high risk populations (PWID/UD, MSM and SWs). Although a few studies on HCV Oraquick POC have been conducted in United States and Korea,[25] this is the first study to report on the Oraquick POC on whole blood and oral fluid, as well as antibody and HCV RNA quantification using DBS in high risk populations in SSA. In our country and many others in SSA, there is poor or no access to laboratories in remote regions. DBS can be transported from rural areas to centralized laboratories, as well as, provide a less painful way to get more people tested.

Our results showed that the OraQuick HCV POC testing on either whole blood or oral fluid yielded high sensitivities (97%-99%) and specificities (97%-100%). Similar results of 100% specificity on oral fluid were reported by Reference 41 in Europe, in patients who were at-risk for hepatitis C. In Reference 42 study on whole blood POC in the United States on people at-risk, a lower sensitivity of 92.7% was reported compared to ours of 99% and a higher specificity of 99.8%, compared to ours of 97%. Interestingly, we had higher specificity on oral fluid POC than on whole blood POC. In total, we had three false-positive results which could occur due to poor adherence to decontamination procedures in between testing and handling of devices or incorrect reading of visual results, or autoimmunity.[43] Of the eight false-negative results, five had viral load. Although, co-infection with HIV may diminish HCV antibody responses and could be a reason for false-negative results on rapid assays,[24,42,44] we found only one false-negative test POC result among HIV-coinfected participants. This is an important finding as SSA has the highest HIV prevalence in the world. We did not find any evidence that genotypes, low viral load or serum HCV antibody titer can affect POC test results. Our results do suggest that POC testing using HCV OraQuick, with high levels of accuracy on both whole blood and oral fluid, make it a good option for HCV screening among at-risk groups of people. Oral fluid sampling is easier, particularly for PWID/UD's and is likely to be more acceptable for higher risk populations and for use in community health care settings.[45]

To decrease the occurrence of false results, education and training of field operators and healthcare workers at facilities that offer POC testing becomes pivotal.[46,47] It is imperative that correct times for rapid diagnostic tests are adhered to, so to prevent false results. If the time range is too wide (as with the 20-40 minutes for OraQuick HCV test) for field workers to work with, this may account for variations in time keeping. Further studies are on the way to determine the most effective time for a reliable POC result, bearing in mind that quality assurance, calibration of timers and clocks at healthcare facilities is required.[48,49]

DBS can increase the number of people screened, diagnosed and ultimately treated especially among high risk groups.[50,51] In areas, such as SSA, where there is a high drop-off rate in the cascade of care, a once-off sampling on DBS can provide further samples for HCV confirmatory testing.[52] Again in SSA, where there is high endemicity of HIV, HBV and TB, DBS is of particular advantage to surveillance studies as the spots can be used for HCV, HBV, HIV, and TB testing.

Many of the hepatitis C DBS studies have been conducted in Europe, the United States and Brazil.[32,33,53] Our DBS analyses on the HCV ARCHITECT immunoassay showed sensitivity and specificity of greater than 95% and a kappa value of 0.92, even when using the signal to reference cut-off ratios as recommended in the assay. With DBS testing, however, the volume of blood in DBS collection is limited, increasing the possibility of false-negative and false-positive reporting.[54]

On DBS for HCV quantitative testing, a sensitivity and accuracy of 94% was found in our study. Comparatively, in similar studies using the CAP/CTM, a larger cohort of 511 patients, in France, with known HCV serostatus, showed a sensitivity of 97% and specificity of 100%[55] and in a smaller study, in the United States on 48 HCV chronic individuals, a sensitivity of 98% was reported.[40] Much work still remains to be done in terms of standardizing the methods when using DBS and establishing better signal to cut-off ratios for serological and threshold cut-off for molecular tests.

In our study, a strong correlation (R^2 = 0.90) and good agreement between viral load measurements for DBS and plasma samples was observed. This demonstrates that the two testing sample matrices are not markedly different from each other. Similar results with a strong correlation of R^2 = 0.97 in Reference 40 study and R^2 = 0.90 in Reference 55 were shown. By applying the correction factor to our DBS results, the correlation did not improve. Similar findings have also been reported,[40] due to the loss of HCV RNA in the DBS during amplification and quantification.[40]

In the Bland-Altman plot agreement, after normalization, the bias between plasma and DBS improved from 0.5 to 0.16 log10 IU/mL. Outliers were observed in the Bland-Altman analysis; these outliers could be a result of the overcorrected DBS results since some observations on the DBS had HCV viral loads higher than in plasma. Overall, the results indicate that on average, CAP/CTM measures 0.16 $\log_{10}$ IU/mL more on plasma samples compared to DBS. Our results show that for low viral loads on plasma, there was an under-quantification on DBS. Bennet et al[50] reported a low threshold of 250 IU/mL for HCV RNA testing on DBS. We found discordant results on DBS for plasma viral load of ≤100 IU/mL. Usually in clinical disease, HCV viral load is high as seen in our study (~10^5 IU/mL) and for confirming HCV infection, low viral load is not clinically relevant.[56] We noted that by using threshold values of CAP/CTM, we could not use the normalization coefficient to the LDL results and hence, there was no correction for LDL results on DBS. We suggest results with LDL on DBS be interpreted with caution when using the CAP/CTM assay. Further studies are necessary to monitor cut-offs on DBS using the CAP/CTM for monitoring treatment outcomes.

The low HCV viral load observed in DBS (accounting to the low kappa value of 0.77) can be due to the different input volumes in whole blood (50 μL) and plasma (650 μL) when testing on the CAP/CTM instrument. However, techniques to elute virus from DBS

for the CAP/CTM method may require further optimization. With DBS, there may be a reduced sensitivity of viral RNA amplification, as shown for HIV.[57,58] A possibility of nucleic acid entrapment in the DBS cards and suboptimal elution of the nucleic acid from the filter paper could be another explanation for the difference in HCV RNA levels observed in DBS.[40] Increasing the number of DBS spots (±50 μL) to test could be a feasible solution.[40,59] For optimization studies, the number of DBS cards collected per individual may be increased from one to two so as to perform repeat testing. Increasing DBS spots from two to three may alleviate the under-quantitation seen on DBS at lower plasma viral loads. However, as with all capillary blood spotting, spots should be completely covered to give correct and reproducible results.[60] We also recommend the use of the perforated spots, provided the spot is properly filled with blood. The perforation makes for easy removal and eliminates any carry-over from cutting or punching of spots.

Our study was performed in a well-controlled setting with DBS delivered in cold chain. It is recommended that DBS should be delivered without refrigeration within 2 days after collection to the centralized laboratory as storage conditions of DBS have been reported to have an impact on the stability of nucleic acid.[32,59] For diagnostic DBS testing, as with the HIV early infant diagnosis[61] in our country, it is then imperative to have an integrated, efficient and reliable laboratory transport system.

Our study has provided a working methodology on how to perform HCV antibody and viral load testing on DBS using the ARCHITECT HCV assay and CAP/CTM, respectively. For the latter, we have also provided a working calculation on normalization coefficients using the hematocrit value of 50% as an assumption. We have, nevertheless, shown how vital it is to correct for sample type and volume difference by applying normalization coefficients to the DBS results. We recommend that DBS be considered for use as a sample matrix for molecular testing in high risk populations, bearing in mind, that the procedure of using DBS on testing platforms may be considered off-label, as it has not been validated by the manufacturer. More evaluation studies are needed to provide supporting evidence for DBS use on commercial assays, with proper standardized methods for correction factors, elution and testing.

There were limitations to our study. It was performed in a reference laboratory with controlled temperatures on instruments and laboratories. We did not investigate the impact of environmental conditions, such as temperature and humidity on DBS storage, transport and testing. Our study cannot generalize to field settings where transport and storage of DBS samples from clinics to centralized laboratories may vary. More studies are needed to assess these factors in areas with more constrained health systems or transporting of samples over greater distances. We assessed the performance of DBS tests using venous whole blood. Future studies should look at spotting capillary (finger-stick) blood in real-life clinical settings to note any difference in test performance.

In conclusion, we have demonstrated that POC testing on whole blood or oral fluid is feasible and accurate. POC testing for HCV antibody should replace the current screening algorithms in South Africa for at-risk populations. If oral fluid is more acceptable and comfortable for POC testing, then this sample should be used, particularly where vein access is difficult. In parts of SSA, where decentralization and POC viral load tests are not options, then a positive anti-HCV POC result should be followed by venous whole blood or DBS collection and sent to a central laboratory to assess HCV viremia. This simplifies the testing algorithm at the centralized laboratory to only one test. We propose this testing algorithm in a country, such as South Africa, with a mix of a well-established central laboratories in many parts of the country and a reasonably efficient laboratory transport system to transport DBS/blood samples from outlying rural areas to central testing laboratories.

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CONFLICT OF INTEREST

Andrew Scheibe received an honourarium from Gilead for presenting work in this field.

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All authors have read and approved the final version of the manuscript.

Nishi Prabdial-Sing had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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