

# Dried Blood Spots: A Tool to Ensure Broad Access to Hepatitis C Screening, Diagnosis, and Treatment Monitoring

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(See the editorial commentary by Thursz and Lacombe on pages 1055–6.)

**Background.** With the advent of highly efficient antiviral therapies for hepatitis C virus (HCV) infection, providing broad access to diagnosis and care is needed. The dried blood spot (DBS) technique can be used to collect, store, and ship whole-blood specimens. Our goal was to assess the performance of standardized HCV diagnostic and monitoring tools in the analysis of DBS.

**Methods.** Serum specimens and whole-blood specimens collected using the DBS technique from >500 patients were tested for virological markers used to diagnose and monitor HCV infection.

**Results.** Enzyme immunoassay detection of anti-HCV antibodies in specimens from DBS was reliable after establishment of a new signal-to-cutoff ratio. HCV RNA was detected DBS from the vast majority of patients with active replication, but HCV RNA levels were substantially lower than in serum specimens, implying that only the presence or absence of HCV RNA or changes in the HCV RNA level should be taken into consideration for therapy. Detection of HCV core antigen in specimens from DBS was not a sensitive marker of chronic HCV infection. HCV genotype determination was possible in the vast majority of DBS.

**Conclusions.** This study shows that whole-blood specimens collected using the DBS technique can be confidently used to diagnose and monitor HCV infection. DBS could help improve access to care for HCV infection because they are suitable for use in large-scale screening programs, diagnosis, and therapeutic monitoring.

**Keywords.** dried blood spot; hepatitis C virus; diagnosis; screening; monitoring of HCV infection.

Approximately 170 million people are chronically infected with hepatitis C virus (HCV) worldwide [1]. HCV infection and its complications, including cirrhosis and hepatocellular carcinoma, collectively represent the 10th most frequent cause of death of infectious origin, with approximately 350 000 deaths per year [2]. Because chronic hepatitis C is often asymptomatic until advanced stages of liver disease develop, the vast majority of infected patients are unaware of their infection and related liver disease. Yet, early diagnosis and treatment of HCV infection is needed to effectively prevent its long-term complications, reduce mortality, and avoid further transmission.

Anti-HCV antibody detection is the first-line tool for screening and diagnosis of infection. HCV RNA detection is necessary to identify viremic patients. HCV genotype determination is important before treatment because it guides treatment

decisions. The outcome of antiviral therapy is classically assessed by measuring HCV RNA levels during and after treatment. Because HCV core antigen levels strongly correlate with HCV RNA levels, they can also be used as an alternative to HCV RNA detection and quantification to diagnose HCV infection and monitor therapy [3, 4].

The HCV virological markers are classically assessed in serum or plasma specimens collected from venous puncture. The advent of new antiviral therapies based on the use of direct-acting antiviral drugs that yield very high HCV infection cure rates [5] emphasizes the need for broad access to diagnosis and care. Drug prices are not the only obstacle; virological tools must also be widely accessible for large-scale screening campaigns targeting individuals unaware of their infection, to diagnose and characterize their infection, to guide treatment decisions, and to monitor the effect of antiviral therapy. Virological testing of serum or plasma specimens requires syringes, tubes, centrifuges, and skilled labor. This is costly and may not be adapted to populations or settings with limited access to care and laboratory facilities.

The dried blood spot (DBS) technique can be used to collect venous blood specimens for biological analyses [6, 7]. Little is known about the performance of standard commercial HCV diagnostic assays when using specimens collected using the DBS

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technique [8, 9]. This could be due to the difficulty to pre-extract biological materials from filter paper without altering the performance of the assays. Nucleic acid pre-extraction reagents have been recently developed. To provide easier access to diagnosis and treatment of HCV infection, especially in settings not equipped for classical virological diagnosis and monitoring, this study evaluated the performance of DBS specimens in the detection and eventual quantification of anti-HCV antibodies, HCV core antigen, and HCV RNA and the determination of HCV genotype.

## METHODS

In this prospective study, >500 HCV-seronegative or HCV-seropositive subjects were included. During a medical consultation, a whole-blood specimen from each subject was collected in a tube containing an anticoagulant and was processed using the DBS technique.

### Clinical Specimens

#### Serum Samples

Serum samples were obtained from patients recruited from September 2012 to November 2013 in our institutions. Group A comprised 170 individuals who were HCV seronegative (defined as having no markers of past or ongoing HCV infection). Group B comprised 26 patients with resolved infection (defined as the presence of anti-HCV antibodies in the absence of detectable HCV RNA), including 13 patients who spontaneously cleared HCV after acute infection and 13 patients who cleared infection after antiviral therapy. Group C comprised 315 treatment-naïve patients with chronic HCV infection (defined as

detectable anti-HCV antibodies and detectable/quantifiable HCV RNA). The study followed the principles of good clinical practice and was approved by the local ethics committee, in accordance with the Helsinki Declaration. All individuals gave written informed consent to their inclusion in the study.

### DBS

Whole-blood specimens obtained from the same patients from groups A, B, and C were collected using the DBS technique. A 50  $\mu$ L specimen of venous whole blood collected in a tube containing ethylenediaminetetraacetic acid was spotted onto the filter paper card (Whatman 903; GE Healthcare Europe, Freiburg, Germany). The filter paper was then placed onto a horizontal clean dry surface to air dry for at least 1 hour. Each dried DBS was then stored in an individual sealed plastic bag with a desiccant package at  $-80^{\circ}\text{C}$  until analysis. DBS for 25 patients from group C were stored at room temperature ( $24^{\circ}\text{C}$ ) for a mean duration ( $\pm$ SD) of  $19 \pm 1$  months to assess the stability of HCV RNA on this matrix.

### Laboratory Measurements

#### Total Anti-HCV Antibody Detection

Total anti-HCV antibodies were measured in serum samples and specimens from DBS by means of an automated third-generation enzyme immunoassay (EIA; aHCV Vitros ECI; Ortho-Clinical Diagnostics, Raritan, New Jersey). For specimens collected using the DBS technique, a punched disk of 6 mm in diameter was eluted into 1 mL of an extemporaneously prepared solution containing 0.05% Tween 20 in PBS 1X at  $4^{\circ}\text{C}$  with gentle agitation for 60 minutes and then centrifuged at 36 220g for 1 minute before use. A total of 20  $\mu$ L of supernatant was used in the EIA.

**Table 1. Demographic and Virological Characteristics of Subjects Seronegative for Hepatitis C Virus (HCV; Group A), Subjects Seropositive for HCV With Resolved Infection (Group B), and Subjects With Chronic HCV Infection (Group C)**

Characteristic	Group A (n = 170)	Group B (n = 26)	Group C (n = 315)
Age, y, median (range)	42.5 (19–81)	60.5 (29–74)	57.0 (28–73)
Male sex	68 (40.0)	14 (53.8)	200 (63.5)
Treatment naïve	NA	13 (50.0)	289 (91.7)
HCV RNA level, log IU/mL	Less than the LLOQ	Less than the LLOQ	$m2000^a$ : $5.8 \pm 0.8$ ; CAP/CTM: $6.0 \pm 0.9$
HCV RNA level >800 000 IU/mL	0	0	$m2000^a$ : 158 (50.3); CAP/CTM: 202 (64.1)
Undetectable HCV RNA	170 (100)	26 (100)	$m2000$ and CAP/CTM: 0
HCV genotype			
Genotype 1	NA	NA	189 (60.0)
Genotype 2	NA	NA	16 (5.1)
Genotype 3	NA	NA	40 (12.7)
Genotype 4	NA	NA	58 (18.4)
Genotype 5a	NA	NA	3 (0.9)
Genotype 6	NA	NA	4 (1.3)
Not determined	NA	NA	5 (1.6)
Anti-HCV antibody signal-to-cutoff value	$0.1 \pm 0.1$	$26.4 \pm 7.6$	$27.0 \pm 4.3$
HCV core antigen level, log fmol/L	<0.50	<0.50	$3.2 \pm 0.7$

Data are no. (%) of subjects or mean value  $\pm$  SD, unless otherwise indicated.

Abbreviations: CAP/CTM, Cobas Amplicprep/Cobas TaqMan HCV assay, version 2; LLOQ, lower limit of quantification;  $m2000$ ,  $m2000$  platform; NA, not applicable.

<sup>a</sup> Data for 1 patient are missing owing to an insufficient amount of serum available.

### HCV Core Antigen Detection and Quantification

HCV core antigen detection and quantification assays were performed for serum samples and specimens collected using the DBS technique by means of a fully automated chemiluminescent microparticle immunoassay (Architect HCV antigen assay; Abbott Diagnostics, Chicago, Illinois). The same procedure as that for antibody detection was used for preparation of specimens from DBS. A total of 108 µL of supernatant was used in the EIA.

### HCV RNA Detection and Quantification

HCV RNA was quantified with 2 distinct real-time polymerase chain reaction (PCR) assays: including the Cobas Ampliprep/Cobas TaqMan HCV, version 2 (CAP/CTM; Roche Molecular Systems, Pleasanton, California) and the *m2000* platform (Abbott Molecular, Des Plaines, Illinois).

In the CAP/CTM procedure, HCV RNA was extracted from 650 µL of serum by means of the Cobas AmpliPrep automated extractor. The Cobas TaqMan 96 analyzer was used for automated real-time PCR amplification and detection of PCR products according to the manufacturer's instructions. The data were analyzed with Amplilink software, version 3.3.

In the *m2000* procedure, HCV RNA was extracted from 500 µL of serum in the *m2000*<sub>SP</sub> automated extractor. The *m2000*<sub>RT</sub> device was then used for automated real-time PCR amplification and quantification of PCR products, according to the manufacturer's instructions.

For the specimens from DBS, a punched disk with a 6 mm diameter was eluted into 1–1.5 mL of a lysis buffer (Cobas AmpliPrep/Cobas TaqMan Specimen Pre-Extraction [SPEX] from Roche for CAP/CTM or *mLysis* from Abbott for *m2000*) at 56° with gentle agitation for 30 minutes and centrifuged at 36 220g for 1 minute before use. We used 500 µL and 650 µL of pre-extraction supernatant to perform the CAP/CTM and the *m2000* assay, respectively.

### HCV Genotype and Subtype Determination

The HCV genotype was determined by directly sequencing a portion of the nonstructural (NS) 5B gene encoding the RNA-dependent RNA polymerase, followed by phylogenetic analysis [10]. The same procedure as for the pre-extraction of nucleic acids for HCV RNA quantification was used for DBS preparation. A total of 400 µL of the pre-extraction supernatant were used for RNA extraction with the QIASymphony DSP Virus/Pathogen kit (Qiagen, Hilden, Germany). DBS samples that could not be amplified in the NS5B region were amplified and sequenced in the HCV E1 region, and sequencing was followed by phylogenetic analysis for genotyping.

### Statistical Analysis

Receiver operating characteristic (ROC) curve analysis was used to estimate the threshold value of the EIA for analysis of whole-blood specimens from DBS. This new signal-to-cutoff value was the value with the best sensitivity and specificity for anti-HCV

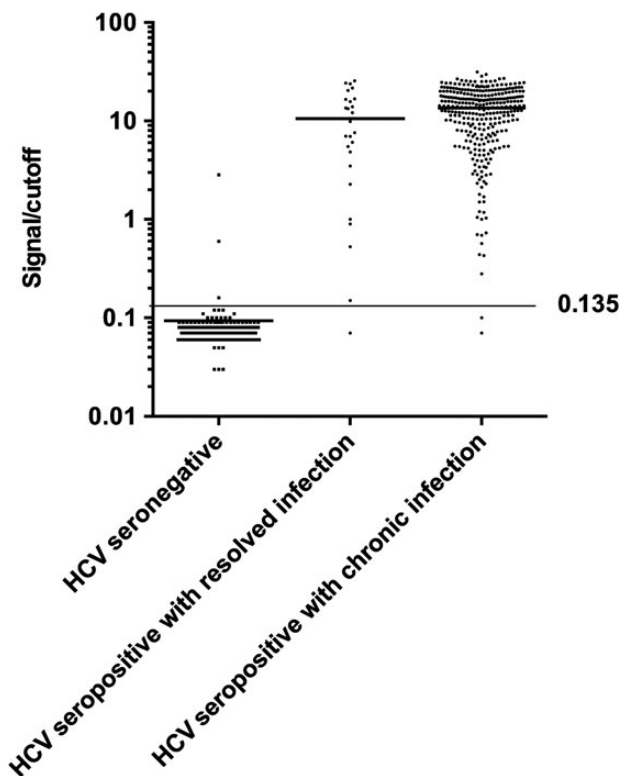
**Table 2. Performance of Anti-Hepatitis C Virus (HCV) Antibody Detection, HCV Core Antigen Detection, HCV RNA Detection, and HCV Genotype Determination on Whole-Blood Specimens Collected With the Dried Blood Spot Technique**

Variable	Specificity, % (95% CI)	Sensitivity, % (95% CI)	PPV	NPV
Anti-HCV antibody detection	98.2 (94.9–99.6)	99.1 (97.4–99.8)	99.1	98.2
HCV core antigen detection	100 (97.8–100)	64.1 (58.5–69.3)	100	64.7
HCV RNA detection				
CAP/CTM	100 (97.8–100)	97.1 (94.7–98.5)	100	95.0
<i>m2000</i>	100 (97.8–100)	98.1 (95.9–99.1)	100	96.6
HCV genotype determination	NA	72.3 (67.0–76.9)	100	NA

Results of serum analysis are the references.

Abbreviations: CAP/CTM: Cobas Ampliprep/Cobas TaqMan HCV assay, version 2; CI, confidence interval; *m2000*, *m2000* platform; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

antibody detection in whole-blood specimens from DBS, compared with serum specimens. Descriptive statistics are shown as mean values ± standard deviations [SDs]. Relationships between quantitative variables were studied by means of regression



**Figure 1.** Dot plot of signal-to-cutoff values assessed with the third-generation enzyme immunoassay (aHCV Vitros ECi; Ortho-Clinical Diagnostics, Raritan, New Jersey), according to hepatitis C virus (HCV) status: HCV-seronegative individuals (group A), HCV-seropositive patients with a resolved infection (group B), and patients with chronic HCV infection (group C).

analysis. For better visualization of differences between the quantification assays, the Bland–Altman plot method was used. Comparisons between groups were made using the Kruskal–Wallis test or the Mann–Whitney *U* test. *P* values of <.05 were considered statistically significant.

## RESULTS

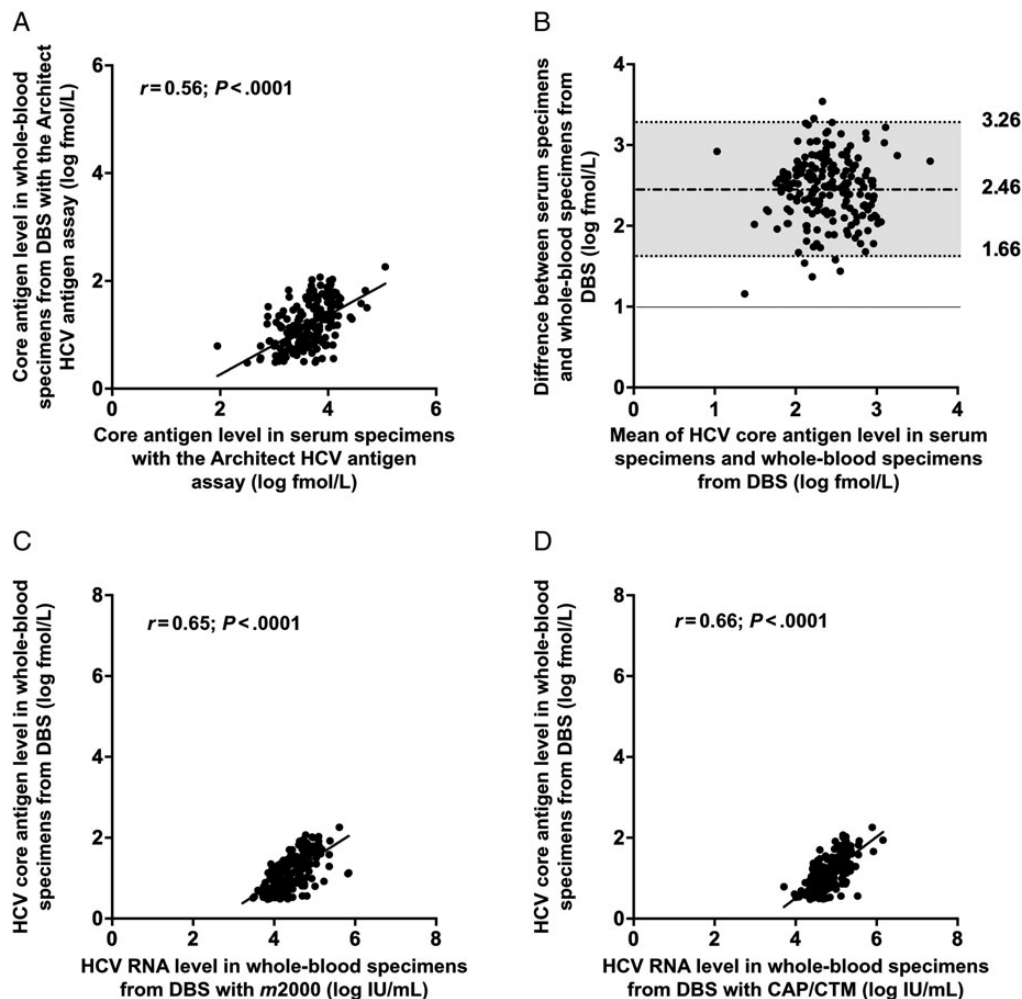
### Characteristics of the Study Population

Table 1 shows the demographic and virological data of the patients from the 3 groups according to their HCV status. A total of 511 individuals were included. The median age was 54 years (range, 19–81 years), and 55.2% were men. There were 170 subjects in group A (mean signal-to-cutoff value, 0.1; HCV RNA

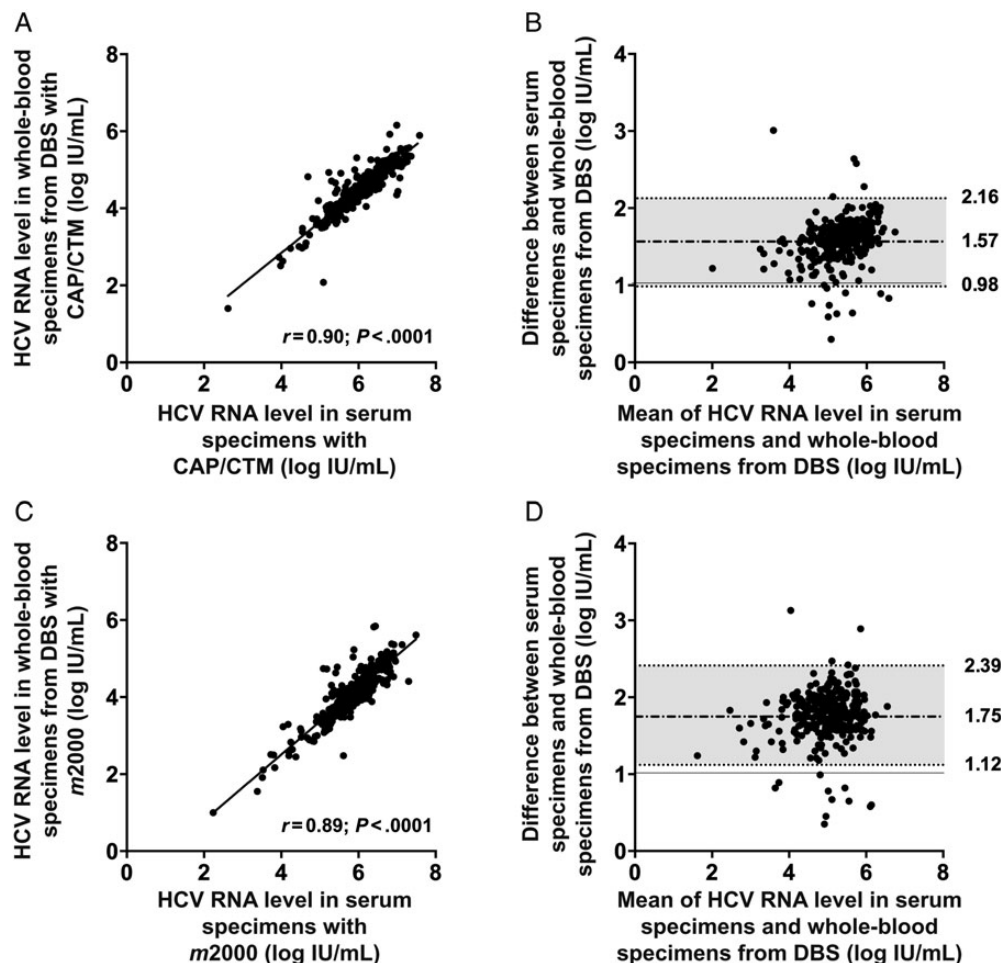
level below the lower limits of detection [LLOD] and quantification [LLOQ]), 26 in group B (mean signal-to-cutoff value, 26.4; HCV RNA level below the LLOD and LLOQ), and 315 in group C (mean signal-to-cutoff value, 27.0; detectable HCV RNA in 2 distinct real-time PCR assays; Table 1).

### Detection of Total Anti-HCV Antibodies in DBS

All DBS were tested for the presence of total anti-HCV antibodies by means of the aHCV Vitros Eci. On the basis of ROC curve analysis, the optimal signal-to-cutoff value was established at 0.135. This signal-to-cutoff value was thus used instead of the  $\geq 1.0$  threshold recommended by the manufacturer to identify reactive serum or plasma samples. It was associated with a sensitivity of 99.1% (95% confidence interval [CI],



**Figure 2.** A, Relationship between hepatitis C virus (HCV) core antigen levels determined by the Architect HCV antigen assay in serum specimens or blood specimens collected using the dried blood spot (DBS) technique from 191 patients in group C infected with different HCV genotypes (ie, genotypes 1–6) with detectable HCV core antigen in both matrices. The black line represents linear regression. B, Bland–Altman plot analysis of the differences between HCV core antigen levels determined by the Architect HCV antigen assay in serum specimens or specimens from DBS, respectively. The difference in gray has been plotted as a function of the mean of the 2 values; the numbers correspond to the mean  $\pm 2$  standard deviations. The 1 line represents the reference (ie, there is no difference between values for serum specimens and those for specimens from DBS). C and D, Relationships between the HCV core antigen levels (in log fmol/L) measured by the Architect assay and the HCV RNA levels (in log IU/mL), measured by 2 real-time polymerase chain reaction platforms, the m2000 assay (C) and the Cobas Ampliprep/Cobas TaqMan HCV assay, version 2 (CAP/CTM; D), in whole-blood specimens collected using the DBS technique from 191 patients in group C infected with different HCV genotypes (ie, genotypes 1–6). The black line represents linear regression.



**Figure 3.** A, Relationship between hepatitis C virus (HCV) RNA levels determined by the Cobas Ampliprep/Cobas TaqMan HCV assay, version 2 (CAP/CTM), in serum specimens or whole-blood specimens obtained using the dried blood spot (DBS) technique from 306 patients in group C infected with different HCV genotypes (ie, genotypes 1–6) with detectable HCV RNA in both matrices. B, Bland–Altman plot analysis of the differences between HCV RNA levels determined by the CAP/CTM in serum specimens or whole-blood specimens from DBS. C, Relationship between HCV RNA levels determined by the m2000 assay in serum specimens or whole-blood specimens from DBS for 308 patients from group C infected with different HCV genotypes (ie, genotypes 1–6) with detectable HCV RNA in both matrices. D, Bland–Altman plot analysis of the differences between HCV RNA levels determined by the m2000 assay in serum specimens or whole-blood specimens from DBS. In panels A and C, the black line represents linear regression. In panels B and D, the difference in gray has been plotted as a function of the mean of the 2 values; the numbers correspond to the mean  $\pm$  2 standard deviations. The 1 line represents the reference, (ie, no difference between serum specimens and whole-blood specimens from DBS).

97.4%–99.8%) and a specificity of 98.2% (95% CI, 94.9%–99.6%; Table 2); the area under the ROC curve was 0.996 ( $P < .0001$ ; data not shown). Figure 1 shows the distribution of the signal-to-cutoff values in individuals from the 3 groups. In DBS, the mean anti-HCV antibody signal-to-cutoff values ( $\pm$ SD) were  $0.1 \pm 0.2$ ,  $10.5 \pm 8.2$ , and  $13.5 \pm 7.1$  for groups A, B, and C, respectively. Values were  $0.1 \pm 0.1$ ,  $26.4 \pm 7.6$ , and  $27.0 \pm 4.3$ , respectively, in serum (Table 1).

Three subjects from group A, all with low signal-to-cutoff values ( $\leq 0.12$ ) in serum, had a signal-to-cutoff value of  $>0.135$  when tested using DBS. These results were considered to be falsely positive. One DBS sample from group B was falsely negative (signal-to-cutoff value,  $<0.135$ ); the amount of anti-HCV antibodies in serum was low, with a signal-to-cutoff value of 1.80. This sample

was retested with another EIA, the HCV Ab Plus Access assay (Biorad, Hercules, California), and a low signal-to-cutoff value of 1.30 was also found. HCV RNA was not detected in this subject's serum by means of either real-time PCR assay. Two patients in group C also tested negative when DBS were used. Both were infected with HCV genotype 1b and had HCV RNA levels in serum of  $>5.0$  log IU/mL. Anti-HCV antibodies were detected in serum specimens from these patients, with high signal-to-cutoff values of 28.4 and 32.4.

#### HCV Core Antigen Detection and Quantification in DBS

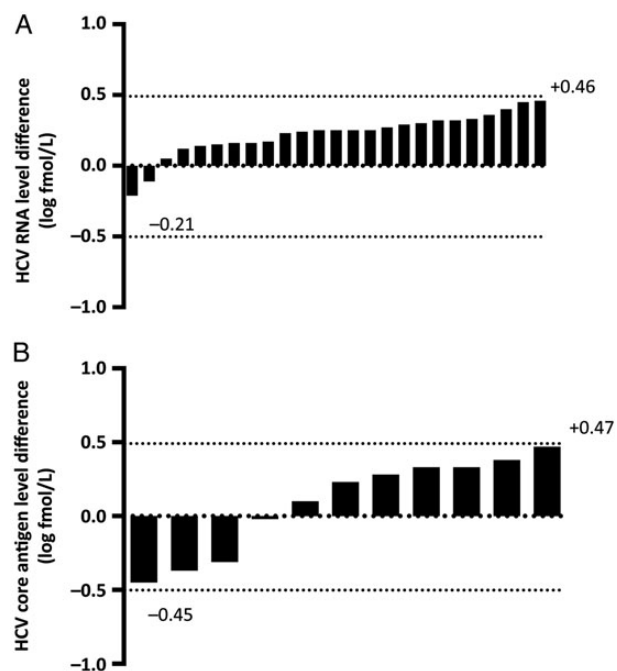
Blood specimens from DBS were tested for the presence and amount of HCV core antigen by means of the Architect HCV antigen assay. None of the individuals from group A had

detectable HCV core antigen. Specificity was thus 100% (95% CI, 97.8%–100%; Table 2). HCV core antigen was also undetectable in the 26 patients from group B. Among the 298 patients (94.6%) in group C with detectable/quantifiable HCV core antigen in serum, only 191 (64.1%) also had quantifiable HCV core antigen in whole-blood specimens from DBS, yielding poor sensitivity (Table 2). As shown in Figure 2A, there was a significant positive relationship between HCV core antigen levels measured in whole-blood specimens from DBS and those measured in serum specimens from the same patient ( $r = 0.56$ ;  $P < .0001$ ), regardless of the HCV genotype. Nevertheless, Bland–Altman plot analysis showed that HCV core antigen levels in whole-blood specimens from DBS were lower than in serum specimens, with a mean difference ( $\pm$ SD) of  $2.5 \pm 0.4$  log fmol/L (Figure 2B). As in serum or plasma specimens [3, 4], the HCV core antigen levels correlated with the HCV RNA levels measured with both real-time PCR assays in whole-blood specimens from DBS ( $r = 0.65$  [ $P < .0001$ ] for *m2000* and  $r = 0.66$  [ $P < .0001$ ] for CAP/CTM; Figure 2C and 2D). No substantial difference in HCV core antigen levels was observed between whole-blood specimens from DBS stored for a mean duration ( $\pm$ SD) of  $19 \pm 1$  months at  $-80^\circ\text{C}$  or at room temperature ( $24^\circ\text{C}$ ), respectively ( $P = .4116$ ; Figure 4B). The mean difference ( $\pm$ SD) in the HCV RNA level was  $0.09 \pm 0.30$  log IU/mL, using the Architect HCV antigen assay.

#### HCV RNA Detection and Quantification From DBS

All DBS were tested for HCV RNA using 2 quantitative real-time PCR assays, the CAP/CTM and the *m2000* platform. None of the individuals from group A had detectable HCV RNA in whole-blood specimens from DBS. Specificity was thus 100% (95% CI, 97.8%–100%; Table 2). HCV RNA was also undetectable in patients from group B.

Among the 315 patients in group C, 315 and 314 had HCV RNA quantifiable by the CAP/CTM and the *m2000* assay, respectively (1 patient lacked serum for HCV RNA quantification by *m2000*). Of these patients, 306 and 308 patients had HCV RNA quantified in whole-blood specimens from DBS by the CAP/CTM and *m2000* assay, respectively. One patient had detectable but not quantifiable ( $<1.1$  log IU/mL) HCV RNA by the *m2000* assay and an HCV RNA level of 1.4 log IU/mL, just above the LLOD/LLOQ, detected by the CAP/CTM. Two patients had no HCV RNA detected by the CAP/CTM and an HCV RNA level of 1.55 and 4.50 log IU/mL detected by the *m2000* assay. A significant positive correlation was found between HCV RNA levels detected by the CAP/CTM and *m2000* assay in DBS and serum specimens from the same patient ( $r = 0.90$  [ $P < .0001$ ] and  $r = 0.89$  [ $P < .0001$ ]), regardless of the HCV genotype (Figure 3A and 3C). As shown by Bland–Altman plot analysis, the mean HCV RNA levels ( $\pm$ SD) detected in whole-blood specimens from DBS were  $1.60 \pm 0.3$  log IU/mL less than those in serum specimens when measured by the

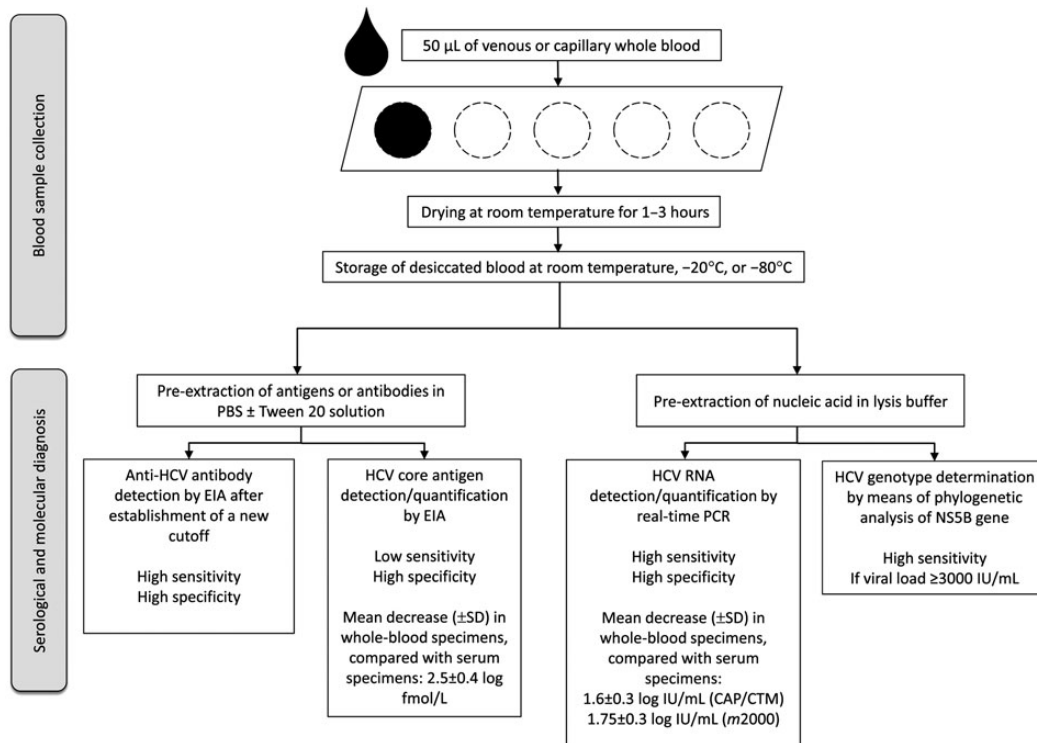


**Figure 4.** A, Individual differences between hepatitis C virus (HCV) RNA levels measured in whole-blood specimens from dried blood spots (DBS) stored at  $-80^\circ\text{C}$  and those from DBS stored at room temperature ( $24^\circ\text{C}$ ) for a mean duration ( $\pm$ standard deviation [SD]) of  $19 \pm 1$  months, using the *m2000* platform, ranked in increasing order for the 25 samples. B, Difference in HCV core antigen levels between whole-blood specimens from DBS and stored for a mean duration ( $\pm$ SD) of  $19 \pm 1$  months at  $-80^\circ\text{C}$  or at room temperature ( $24^\circ\text{C}$ ).

CAP/CTM and  $1.75 \pm 0.3$  log IU/mL less when measured by the *m2000* assay (Figure 3B and 3D). No substantial difference in HCV RNA levels was observed in whole-blood specimens from DBS stored at  $-80^\circ\text{C}$  or at room temperature ( $24^\circ\text{C}$ ) for a mean duration ( $\pm$ SD) of  $19 \pm 1$  months ( $P = .2218$ ; Figure 4A). The mean difference ( $\pm$ SD) in the HCV RNA level was  $0.2 \pm 0.15$  log IU/mL, according to the *m2000* assay.

#### HCV Genotype Determination in DBS

Among the 315 patients in group C, the HCV genotype was identified in serum specimens from 310. The HCV RNA level was too low for amplification of the NS5B region in the remaining 5 cases. Among the corresponding 310 samples collected using the DBS technique, 224 (72.2%) could be amplified in the NS5B region. In the remaining 86 samples, PCR amplification of a portion of the E1 region was performed; 38 (12.2%) yielded positive amplification, whereas 48 (15.5%) did not. Among these 48 samples, HCV RNA was undetectable in whole-blood specimens from DBS in 3 cases, whereas HCV RNA levels in whole-blood specimens varied from 1.5 log IU/mL to 5.2 log IU/mL (mean HCV RNA level [ $\pm$ SD],  $3.7 \pm 0.8$  log IU/mL) in the remaining 45 cases. The inability to determine the HCV genotype in whole-blood specimens from DBS was not related to the HCV genotype.



**Figure 5.** Flow diagram illustrating optimal use of dried blood spots in the diagnosis and management of hepatitis C virus (HCV) infection. Abbreviations: CAP/CTM, Cobas Ampliprep/Cobas TaqMan HCV assay, version 2; EIA, enzyme immunoassay; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

In total, 262 samples (84.5%) could be genotyped from blood collected using the DBS technique. Among the 224 samples genotyped in the NS5B region, the genotype and subtype were identical in serum and DBS in all cases but 3, which displayed mixed viral populations on DBS sequence analysis. Among the 38 samples positive in E1 amplification, the genotype and subtype were identical in serum specimens and whole-blood specimens from DBS in all cases.

## DISCUSSION

The DBS technique is promising for the collection of venous or capillary blood specimens and their shipment by regular mail at room temperature to a central laboratory well equipped for classical virological diagnosis and monitoring. Thus, the use of DBS has the potential to substantially simplify and decentralize hepatitis services in low- or middle-resource areas and thereby expand screening and access to care. On the other hand, in high-risk populations, such as people who inject drugs, venipuncture can be complicated by the difficulty in finding an accessible vein, making capillary blood specimens collected by finger stick easier to obtain. Other advantages of the DBS technique over venipuncture include lower cost, minimal storage facility and transport requirements, and decreased donor discomfort.

Little information was available on the performance of standardized HCV virological assays on specimens collected using

the DBS technique [8, 9, 11]. This is particularly important in the context of the new highly effective interferon-free antiviral therapies [5] that will make it necessary to expand HCV screening and diagnosis to improve access to care, especially in settings where modern virology facilities are not easily accessible.

Our study shows that samples from DBS can be confidently used for anti-HCV antibody detection and for HCV RNA detection and quantification by means of standardized, commercially available methods, even though these methods were not originally validated for DBS use. Blood collected using the DBS technique, including venous or capillary whole-blood specimens that were shown to display equivalent sensitivities for the detection of antibodies and nucleic acids [12, 13], can also be used to determine the HCV genotype. A flow diagram illustrating optimal sample use is shown in Figure 5.

The detection of anti-HCV antibodies with a commercial EIA in blood specimens collected using the DBS technique required optimization because of the reduced sensitivity, compared with testing of serum or plasma specimens. Indeed, the use of the signal-to-cutoff value recommended by the manufacturer tended to increase the number of false-negative results. As previously recommended [14], we defined a new signal-to-cutoff value by means of ROC curve analysis. With this signal-to-cutoff value, only 6 patients were not correctly classified, with only 3 yielding a false-negative result.

Real-time PCR assays are recommended by the international liver society guidelines to diagnose HCV infection and monitor replication during and after antiviral treatment, particularly for assessing the sustained virological response (undetectable HCV RNA level 12 or 24 weeks after therapy), which corresponds to a definitive cure of infection [15, 16]. Molecular diagnostic companies developed specific reagents for the pre-extraction of viral nucleic acids from DBS. Using these reagents, we could quantify HCV RNA in almost all DBS. However, the reported HCV RNA levels in whole-blood specimens from DBS were lower by  $>1.5$  log IU/mL on average than those in serum specimens. The difference could be explained by the fact that serum specimens of 500  $\mu$ L and 650  $\mu$ L were used for RNA extraction by the *m2000* system and the CAP/CTM, respectively, whereas only a 50- $\mu$ L blood specimen eluted from DBS into 1 mL of buffer was used. However, this difference was smaller than that observed in a previous study [9], suggesting that the use of appropriate pre-extraction reagents improved the performance of quantification.

Our results imply that the absolute amount of HCV RNA should not be considered when quantification is performed on whole-blood specimens from DBS. This is particularly the case when capillary whole-blood specimens have been collected using the DBS technique, implying that  $<50$   $\mu$ L of blood is available for testing. Conversely, HCV RNA level changes can be reliably monitored in specimens from DBS. This will not be an issue with the new interferon-free anti-HCV therapies, which essentially require the ability to monitor viral level declines during treatment and assess the sustained virological response. Twelve or 24 weeks after therapy, the HCV RNA levels are high in patients who do not eradicate infection; thus, the DBS result, if negative, can be trusted as indicative of a sustained virological response.

Because HCV core antigen detection and quantification is one third less expensive than nucleic acid testing, HCV core antigen detection could theoretically replace HCV RNA testing in the decision to treat and in the monitoring of antiviral therapy, particularly, but not only, in resource-constrained areas. Our results show that HCV core antigen can be easily detected and quantified in specimens from DBS. However, the sensitivity of detection was substantially reduced in specimens from DBS, compared with serum specimens (64.1%), and could be further reduced when  $<50$   $\mu$ L of capillary whole blood is collected. Thus, when whole-blood specimens from DBS are used, HCV RNA testing should be preferred until more-sensitive procedures are developed. Importantly, we showed that HCV RNA and core antigen levels remain stable in DBS stored at room temperature (24°C) for  $>1$  year.

We have also assessed the performance of the DBS technique for determination of HCV genotype and subtype. Compared with serum samples, all patients were correctly genotyped. DBS can thus be confidently used to determine the HCV genotype, to guide treatment decisions. However, a low HCV RNA

level can prevent the determination of the HCV genotype in blood specimens from DBS, and the subtype may remain undetermined, a potential problem when the treatment indication differs between subtypes 1a and 1b. Further studies are needed to assess whether HCV resistance can be confidently assessed in specimens from DBS in patients who did not respond to an interferon-free anti-HCV treatment regimen.

In conclusion, whole-blood specimens collected using the DBS technique can be confidently used to diagnose and monitor HCV infection. A new cutoff must be established for anti-HCV antibody detection, to increase specificity. Sensitivity for the detection of HCV RNA is altered in specimens from DBS, compared with serum or plasma specimens, and the HCV RNA levels are substantially underestimated. Thus, dynamic changes rather than absolute values should be taken into account, and the risk exists that HCV RNA is undetectable despite active replication in patients with low-level viremia. HCV core antigen detection and quantification in specimens from DBS should not be used as an alternative to HCV RNA level monitoring. HCV genotype determination can be performed in specimens obtained using the DBS technique but may be problematic in cases of low-level viremia. Overall, whole-blood specimen collection using the DBS technique appears to be a promising alternative to classical blood sampling. Its use will substantially improve worldwide screening, diagnosis, and access to care.

## Notes

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