

Short Communication

Dried Blood Spots (DBS) for Hepatitis C (HCV) Molecular Diagnostic Testing

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Abstract

In resource limited settings, the use of DBS for HCV diagnosis and monitoring has become a necessity for improved patient management. Several studies have evaluated the performance of the RealTime HIV-1 assay using the Whatman 903 device however no data to date has been presented on the RealTime HCV viral load and HCV genotype detection. This study evaluated the use of DBS for HCV viral load testing and viral genotyping. A linear response with a coefficient of determination greater than 0.99 was observed for 903 between viral loads of 3.16 log IU/mL and 6.16 log IU/mL (Figure 1a/b). A 100% detection rate was achieved at HCV RNA down to 3.16 log IU/mL (Figure 2). HCV RNA was stable in DBS at RT and 2–8°C for up to 10 weeks which would allow for convenient storage and shipment of patient samples in resource limited settings. The use of DBS with Abbott RealTime HCV viral load and RealTime HCV Genotype II assays may prove clinically useful for the diagnosis of active HCV infection and the selection of appropriate therapeutic regimens.

Keywords: HCV RNA stability; Dried blood spots, Abbott real time HCV; Abbott Real Time HCV GTII

Introduction

HCV is one of the most common blood borne pathogens in the world and a major source of morbidity globally [1]. The majority of cases of HCV are found in resource-limited settings in Asia, Africa and Latin America. In the US there are an estimated 2.7–3.9 million individuals living with HCV [2] while 49.3–64.0 million individuals are living with HCV in Asia [3]. Current diagnostic tests require phlebotomy followed by refrigeration or freezing until the time of testing. This limits the implementation of HCV diagnosis and initiation of therapy in resource-limited settings where venipuncture and cold chain transportation may not be available. The use of specimen type so then the frozen blood products for HCV diagnostic assays may eliminate this logistical limitation and improve clinical management of patients. In resource-limited settings, the collection of DBS represents an alternative which is minimally invasive, does not require a trained phlebotomist and has proven effective in the diagnosis and monitoring of patients with HIV-1 [4–6]. HCV proteins and nucleic acids from DBS have been successfully quantified using immune assay and polymerase chain reaction (PCR) assays [7–11]. Limited data exist for the quantification of HCV RNA from DBS using modified protocols for commercially available viral load assays. With the advent and introduction of more manageable antiviral therapies for the treatment of HCV, HCV RNA testing will increase dramatically with hopes of identifying and linking patients to care. Overcoming logistical hurdles will be essential if infected populations in high-prevalence regions are to have access to life-saving therapies. In clinical settings where phlebotomy and cold chain storage is a challenge, the use of DBS could be considered.

This study evaluated the detect ability and stability of HCV RNA DBS stored at room temperature and at 2–8°C utilizing Whatman 903 collection device. Assays were run using the Abbottm 2000 system and

RealTime HCV RNA (RTVL) and RealTime Genotype II (RTGT) assays (Abbott Molecular Diagnostics Des Plaines, IL, USA). Citrated whole blood was collected from ten HCV seropositive patients by a commercial vendor (Pro Med Dx LLC, Norton, MA). Patient plasma was attained by centrifugation of whole blood at 1500 rpm for 20 minutes and tested per manufacturer recommendations with RTVL and RTGTII assays to establish the HCV viral load and genotype. RTVL and RTGT plasma controls were included on each genotype run to demonstrate run validity. Standard amplification and detection protocols were used for all samples processed. HCV RNA detect ability and stability in DBS were evaluated using a five-member HCV panel targeting viral loads from 5.16 – 1.16 log₁₀ IU/mL. These panels were generated by spiking citrated whole blood with HCV seropositive plasma followed by serial dilution in HCV sero-negative citrated whole blood. DBS were stored at (RT) or 2 –8°C in zip lock bags with desiccant until testing. HCV RNA detect ability for 903 was assessed by testing multiple replicates of panels with expected viral loads from 5.16–1.16 log₁₀ IU/mL. Stability of HCV on 903 was evaluated by testing two of these panels with expected viral loads of 5.16 and 3.16 log₁₀ IU/mL at regular intervals up to 10 weeks following storage.

Each of the two levels was tested with a minimum of 3 replicates. Standard deviation of the three replicates did not exceed 0.25 log IU/mL. For RTVL testing two whole 50ul spots were excised and placed into 1.2 mL of Abbott mLysis buffer where as for RTGT a single 75ul spot was excised and placed in 1.2 mL of mLysis buffer. Following a 45 minute incubation at RT, the tube was gently mixed and the supernatant was transferred to a sample tube before being processed using the standard CE marked RTVL or RTGT protocol.

A linear response with a coefficient of determination greater than 0.99 was observed for 903 between viral loads of 3.16 log IU/

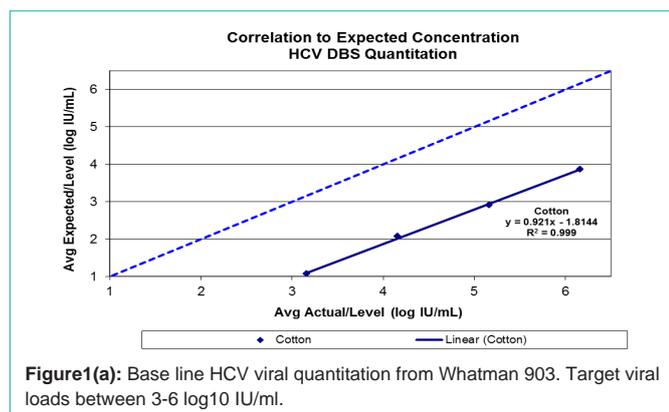


Figure 1(a): Base line HCV viral quantitation from Whatman 903. Target viral loads between 3-6 log₁₀ IU/mL.

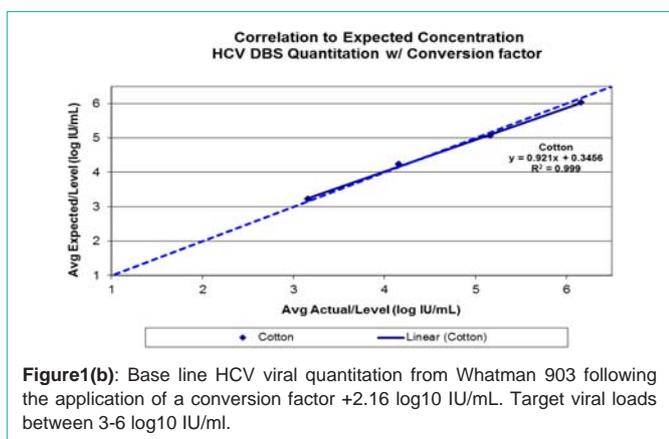


Figure 1(b): Base line HCV viral quantitation from Whatman 903 following the application of a conversion factor +2.16 log₁₀ IU/mL. Target viral loads between 3-6 log₁₀ IU/mL.

mL and 6.16 log IU/mL (Figure 1 a/b). A 100% detection rate was achieved at HCV RNA down to 3.16 log IU/mL (Figure 2). Viral quantification correlated well with standard plasma results (based on expected values of dilution panels) with an estimated conversion factor of +2.16 log₁₀ IU/mL. The conversion factor is specific to the sample collection / extraction protocol used (2x50ul spots and 1.0 mL extraction protocol) and was calculated by subtracting the measured viral load of the DBS from the viral load in the original blood sample (ie expected VL – observed DBS VL). Although, the samples within this study are limited to genotype 1 and 3, there was no difference in genotype result between 903 and standard plasma protocol (Table 1). HCV RNA GT was not evaluated beyond the initial time point. HCV RNA in 903 DBS was stable with 100% detection at both levels tested from samples stored at RT or at 2–8°C for up to 10 weeks (Figure 3).

These findings demonstrate that HCV RNA is stable in DBS at

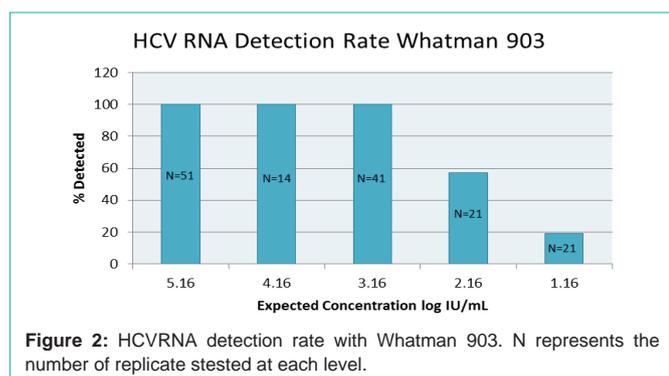


Figure 2: HCV RNA detection rate with Whatman 903. N represents the number of replicate tested at each level.

Table 1: Comparison of HCV Genotype obtained from 10 clinical samples. Results From DBS are compared to those from plasma samples using the standard RTGII protocols reference.

SampleType	Plasma sample	WholeBlood 1x75 µl(903)
Extraction	Standard HCV GT	Standard HCV GT
11622668	1a	1a
11622669	1a	1a
11622670	1a	1a
11622671	1a	1a
11622672	1a	1a
11622673	1a	1a
11622674	1a	1a
11622675	1a	1a
11622676	3	3
11622677	1a	1a

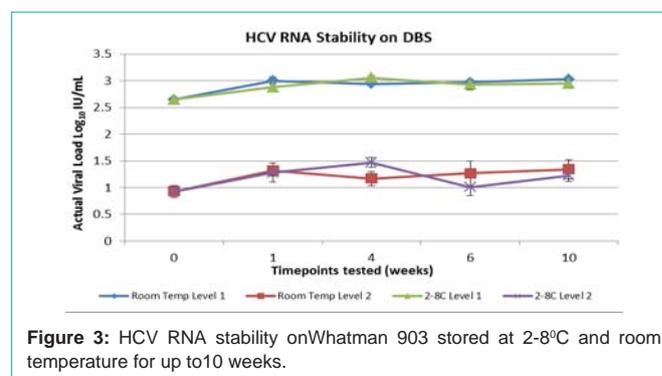


Figure 3: HCV RNA stability on Whatman 903 stored at 2–8°C and room temperature for up to 10 weeks.

RT and 2–8°C for up to 10 weeks, which should allow for convenient storage and shipment. Preliminary results indicate that the Abbott RTVL and RTGT assays are compatible with samples collected as DBS. Furthermore, DBS is an effective method for HCV screening and initiation of patients into care in resource-limited settings and in patients with limited venous access. Also, on therapy monitoring of the first generation of DAA’s (Telaprevir) could be implemented by applying the 2.16 log IU/mL conversion factor to the final result. This would allow for monitoring of Telaprevir stopping rules i.e. 1000 IU/mL. Additional optimization of DBS pre-analytical processing steps may increase the sensitivity of the RTVL with this sample type and should be further investigated. This optimization would further allow the DBS device to be used in the SVR assessment of even the second generation DAA’s as most patients that fail therapy or are not adherent to therapy would have a viral load > 3.0 log IU/mL. The use of DBS with Abbott RealTime HCV viral load and RealTime HCV Genotype II assays may prove clinically useful for the diagnosis of active HCV infection, the selection of appropriate therapeutic regimens and confirmation of post treatment sustained virologic response in developing areas with minimal logistic and technical investment.

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interest declarations: GAC and DL are employees and shareholders of Abbott Molecular. TPY is a former employee and share holder of Abbott Molecular but has no current conflicts to report. SL has received laboratory materials and support for research protocols. CS has no conflict of interest to report.

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