Journal of Virological Methods 247 (2017) 77–80

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Evaluation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 for HCV viral load monitoring using dried blood spot specimens

Ed. G. Marins*, Keerthi Bodinaidu, Matthew Lin, Alison Deforest

Roche Molecular Systems, 4300 Hacienda Dr, Pleasanton, CA, 94588, United States

ARTICLE INFO

Keywords:
Hepatitis C virus
Dried blood spot
DBS
CAP/CTM
COBAS

ABSTRACT

This study evaluated the use of dried blood spot (DBS) for HCV viral load quantification using the COBAS® AmpliPrep/COBAS® Taqman® HCV Quantitative Test v2.0 (CAP/CTM HCV v2), and compared two different procedures for preparation of DBS samples with a Specimen Pre-Extraction (SPEX) reagent (either heated [SPEX with SH] for 10 min at 56 °C on a thermomixer, or incubated for 1 h at room temperature [SPEX at RT]) against the standard plasma input. Whole blood specimens from 48 patients with chronic HCV infection and Whatman® 903 Protein Saver Cards were used to prepare 35 µL DBS. An aliquot of plasma was spun and frozen from each draw. Mean DBS viral load results were compared to the corresponding results from plasma. Correlation between DBS to plasma was linear for both SPEX with SH (R^2 = 0.96) and SPEX at RT (R^2 = 0.97) procedures, with a constant negative offset of approximately 2.0 $\log_{10}$ IU/mL between whole blood DBS without any adjustments and plasma results. After volume corrections, the mean offset to plasma decreased to −0.39 and −0.36 for the two procedures, respectively. The study demonstrated the use of DBS for HCV viral load correlates well with plasma with a constant offset.

1. Introduction

Hepatitis C virus (HCV) infection is a significant cause of morbidity and mortality worldwide (Mohd Hanafiah et al., 2013). The prevalence of HCV infection is highest in Central and East Asia, the Middle East, and North Africa (estimated to be > 3.5%) (Mohd Hanafiah et al., 2013). Moreover, HCV remains underdiagnosed in certain high-risk populations worldwide (Weinbaum et al., 2005; Hickman et al., 2007; Sturrock et al., 2007).

Following detection of HCV antibody in blood, testing for the presence of HCV RNA is required to identify active infection and initiate appropriate antiviral therapy (CDC, 2013). Quantification of the viral load by a reliable nucleic acid amplification test is important for patients considered for treatment, and, depending on the treatment options available, testing is important to determine treatment duration, monitor clinical outcomes, and guide treatment decisions (CDC, 2013). However, such tests require trained technicians and specialized equipment, neither of which may be widely available in hard to reach or resource-limited areas, thereby requiring samples to be shipped to central laboratories. While plasma or serum specimens are conventionally used for HCV RNA quantification tests, such samples degrade rapidly at room temperature and must be stored under refrigeration until testing, imposing an additional challenge: sample transportation. A dried blood spot (DBS) can be a simple and cost-effective solution in remote regions where transportation to laboratories requires specimen stability over a period of days/weeks (Greenman et al., 2015). The World Health Organization guidelines for screening and treatment of patients with HCV infection, published in 2014, specify that refining rapid screening methods such as DBS-based tests would help increase the count of infected patients identified by expanding access to HCV testing (WHO, 2014).

With the availability of highly efficacious all-oral direct-acting antiviral (DAA) drugs for HCV therapy, treatment accessibility for HCV will also improve (WHO, 2014). Studies have demonstrated that the use of DBS can positively impact the adoption of antiviral therapy for HCV by further simplifying the process for viral load monitoring (McAllister et al., 2014; McLeod et al., 2014; Coats and Dillon, 2015). Also, HCV treatment and monitoring can now be extended into remote areas where an advanced laboratory infrastructure may not be available.

2. Methods and materials

The COBAS® AmpliPrep/COBAS® Taqman® HCV Quantitative Test v2.0 (CAP/CTM HCV v2, Roche Molecular Systems, Pleasanton, CA) is an FDA-approved nucleic acid amplification test for quantification of HCV viral load using plasma and serum samples as detailed in the

* Corresponding author.
E-mail address: ed.marins@roche.com (E.G. Marins).

http://dx.doi.org/10.1016/j.jviromet.2017.05.016
Received 19 April 2017; Received in revised form 24 May 2017; Accepted 25 May 2017
Available online 30 May 2017

respective product information. This study evaluated the use of DBS as a sample material for HCV viral load monitoring using the CAP/CTM HCV v2 and compared the results to those obtained using corresponding matched plasma samples. The study also compared the use of two different procedures for preparing DBS samples with a chaotropic Specimen Pre-Extraction Reagent (SPEX) i.e., SPEX with heating and shaking (SPEX with SH) and SPEX without heating or shaking at room temperature (SPEX at RT). This latter method was included to simulate conditions in laboratories without access to specialized equipment.

A total of 48 patients with chronic HCV infection were enrolled in this study. Blood samples were collected and used to generate DBS. All patients had given their informed consent for collecting blood for this particular protocol, which was approved by the Independent Review Board.

Each patient provided two tubes of whole venous blood that was treated with anticoagulant EDTA. One tube was centrifuged and the separated plasma obtained was aliquoted into two nuclease-free tubes and stored immediately at −80 °C until testing. From the other tube, ten 35 μL spots of whole blood were pipetted onto Whatman® 903 Protein Saver Cards (GE Healthcare, Piscataway, NJ). Namely, two cards of five 35 μL spots each were prepared for each subject and left to dry overnight at room temperature, then each card was stored in individual zip bags with one desiccant pack for each bag at room temperature for 4 weeks. For each procedure, (i.e., SPEX with SH and SPEX at RT), three DBS of the same subject were cut along the dotted lines using a sterilized and cleaned pair of scissors (with 10% bleach and 70% ethanol, wiped using clean lint-free kimwipes and dried completely between uses), folded in half with the blood spot facing inwards and inserted into three COBAS® Ampliprep sample input tubes (S-tubes) (Roche Molecular Systems, Pleasanton, CA).

The HCV DBS were incubated with SPEX buffer and either subjected to heating and shaking at 56 °C for 10 min on a thermomixer (shaking at 1000 rpm) (SPEX with SH), or a simple 1-hour room temperature incubation (SPEX at RT). For both procedures, 675 μL of Roche SPEX were added to each S-tube. DBS specimen were tested in triplicate for each procedure (SPEX with SH and SPEX at RT). After incubation, all S-tubes were loaded onto the COBAS® AmpliPrep/COBAS® Taqman® (CAP/CTM) instrument for nucleic acid extraction, amplification, and detection in accordance with the instructions for use as provided in the product information of the CAP/CTM HCV v2 test COBAS (2013).

Correlation of whole blood DBS to plasma for HCV viral load quantification was determined using Bland-Altman plots.

3. Results

All 48 samples provided valid results with plasma input, while HCV RNA could not be quantified for 1 sample with a plasma viral load of 4.75 $\log_{10}$ IU/mL.

This specimen generated result of target not detected (TND) for all three DBS using the two procedures.

The mean DBS viral load results, with and without normalization for volume differences between the sample input volumes, were compared to the corresponding plasma viral load results. Because the instrument software algorithm calculates titers assuming 650 μL of plasma as the input volume, the DBS results were multiplied by a normalization coefficient (c) where c = 40.57 to adjust for the difference in sample input volume and type (35 μL of whole blood as DBS vs 650 μL of fresh plasma).

For the 48 tested plasma samples, HCV viral loads varied from 3.47 to 7.71 $\log_{10}$ IU/mL, with an overall average of 5.79 $\log_{10}$ IU/mL. With whole blood DBS, the detected HCV viral load without normalization ranged from 1.35 to 5.35 $\log_{10}$ IU/mL for the SPEX with SH method and 1.02 to 5.44 $\log_{10}$ IU/mL for the SPEX at RT method, with overall average viral loads of 4.81 $\log_{10}$ IU/mL and 4.83 $\log_{10}$ IU/mL for SPEX with SH and SPEX at RT, respectively.

The mean titer difference between whole blood DBS measurements to those from plasma was −2.00 $\log_{10}$ IU/mL for the SPEX with SH method and −1.97 $\log_{10}$ IU/mL for SPEX at RT method (Table 1). After normalizing for volume and sample type differences between the whole blood DBS and plasma sample using the formula described above, the detected HCV viral load for whole blood DBS ranged from 2.96 to 6.96 $\log_{10}$ IU/mL for SPEX with SH and 2.63 to 7.06 $\log_{10}$ IU/mL for SPEX at RT and the mean titer difference to plasma improved to −0.39 and −0.36 for SH and RT, respectively.

Table 1
Mean difference (in log IU/mL) between DBS and plasma samples before and after correction for volume and sample type influences.

	Before correction	After correction
Difference between SPEX with heating and plasma	−2.00	−0.39
Difference between SPEX without heating and plasma	−1.97	−0.36

SPEX: Specimen pre-extraction reagent.

Correlation between whole blood DBS to plasma was linear for both DBS methods (for SPEX with SH method, $y = -0.011 \times - 1.9515$; $R^2 = 0.96$ and for SPEX at RT method, $y = -0.0081 \times - 1.9301$; $R^2 = 0.97$) (Figs. 1 and 2a and b). The correlation of whole blood DBS to plasma after volume correction was improved for both SPEX with SH ($y = -0.011 \times - 0.3344$; $R^2 = 0.96$) and SPEX at RT ($y = -0.0081 \times - 0.3154$; $R^2 = 0.97$) (Fig. 3a and b).

4. Discussion

This study examined the use of DBS as a specimen for the quantification of HCV viral load from the blood of patients with chronic HCV infection in comparison to plasma and also evaluated two different procedures for DBS preparation in chaotropic SPEX buffer, one employing a 10-min incubation at 56 °C with shaking (at 1000 rpm) and the other a simple 1 h room temperature incubation. The results of this study demonstrated a strong correlation between viral loads measured using DBS prepared with either SPEX with SH or SPEX at RT procedure, and no significant differences in the results obtained with SPEX with SH and SPEX at RT. The study also demonstrated linear correlation between the results generated with the two experimental procedures and those obtained using plasma, with a constant negative offset of approximately 2.0 $\log_{10}$ IU/mL without adjusting for differences in sample input volumes and type between whole blood DBS and plasma.

Normalizing for differences in input volume between DBS and plasma reduced the offset to 0.36–0.39 $\log_{10}$ IU/mL, indicating the normalization alone did not completely fix the correlation suggesting there is a potential loss of efficiency in the amplification and quantification of HCV RNA using DBS. The reasons for the remaining offset may vary from HCV RNA degradation to entrapment of the target material in the paper among others, but since this was a constant offset it may be overcome with the introduction of a correction factor.

Based on the results obtained without normalizing for volume or sample type differences, the addition of a correction factor of 2 $\log_{10}$ IU/mL to the results generated by the instrument after DBS testing using the described protocol could further improve the correlation to plasma and can be easily applied. The comparison between the two DBS methodologies (SPEX SH and SPEX RT) showed comparable results indicating the use of a thermomixer may be replaced by room temperature incubation, simplifying the procedure and the requirements for the laboratory to work with DBS which may be relevant in resource limiting settings.

The utility of DBS as a specimen type for diagnosis and screening of various infectious agents such as human immunodeficiency virus, hepatitis B virus and HCV has been evaluated in multiple studies (Parker and Cubitt, 1999; Judd et al., 2003; Sherman et al., 2005; Castro et al.,