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Short communication

Evaluation of the Abbott *m*2000 system for dried blood spot detection of hepatitis C virus RNA

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ABSTRACT

Background: Hepatitis C virus RNA testing using dried blood spots (DBS) offers a method for detecting ongoing HCV infection in "hard to reach" populations. Abbott Molecular have developed a quantitative HCV RNA DBS protocol (currently for research use only) for extraction and real-time PCR amplification using the*m*2000sp and *m*2000rt system.

Methods: A panel of seventy "mock" DBS were made from patient whole blood; who were known to be either HCV RNA negative or positive. This panel compared the "mock" DBS and the plasma viral load results. A further dilution panel of "mock" DBS made from one HCV positive patient was used to estimate the detection limit of the assay. Abbott was then compared with an in-house real-time Taqman PCR using patient DBS samples.

Results: All "mock" DBS samples with a viral load > 1000IU/ml were detected by Abbott, with only 1/8 detected at < 1000 IU/ml. The dilution panel suggested the limit of detection to be between 178 to 1779 IU/ml. There were two false positive samples detected at low level < 282 IU/ml, both samples were from patients who had been previously positive. The overall sensitivity of the Abbott RUO DBS protocol when compared to plasma was 86% (95 CI 73.76%–74.18%) increasing to 100% (CI 91.59%–100%) when the viral load was > 1000IU/ml. Abbott compared well with the in-house assay with sensitivity of 97.5% (95% CI 86.84%–99.94%) and specificity of 100% (95% CI 91.19%–100%).

Conclusions: The Abbott system is an automated platform which can be used for DBS HCV RNA extraction and amplification. The preliminary data presented here showed a high sensitivity and specificity for DBS with viral loads greater than 1000IU/ml and compared well with a published in-house method.

1. Background

It is estimated that ~0.7% of the Scottish population is chronically infected with hepatitis C virus (HCV) with the majority of infections found among people who inject drugs (PWID) [1]. Dried blood spot (DBS) sampling was introduced in Scotland to improve access to testing and determine HCV prevalence [2]. We introduced DBS testing in 2009 as part of the West of Scotland Specialist Virology Centre (WoSSVC) routine service. Since its introduction there has been a 300% increase in HCV RNA testing using DBS. This increase had been attributed to greater up-take of DBS testing within "hard to reach populations" including the community addiction and prison services. [3,4]. The current DBS protocol used by WoSSVC is a qualitative in-house real-time PCR method comprising of multiple manual steps [5]. Abbott Molecular have provided an HCV RNA extraction and amplification method for DBS (for research use only (RUO)) using the *m*2000sp and *m*2000rt platform. This method is currently an off-label use of the already available CE-marked Abbott RealTi*m*e HCV kits and protocol used to determine HCV viral load in plasma. The CE-marked Abbott RealTi*m*e HCV kit is widely used as a routine assay by many laboratories and its performance has been proven in many studies [6–8].

We evaluated the Abbott RUO DBS protocol using a number of sample panels. First we assessed its performance using a panel of known HCV positive and negative "mock" DBS samples. Then, to determine the detection limit of the new protocol we manufactured a panel of "mock" DBS made from a dilution series of a HCV positive sample. Finally we wanted to directly compare the Abbott RUO DBS protocol to the in-house DBS PCR method already in use within the laboratory. This was carried out using a panel of positive and negative patient DBS samples.

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Table 1
Results of "mock" DBS samples compared to gold standard plasma viral loads.

Tested	HCV plasma data from samples used to make mock DBS		Abbott *m*2000 RealTi*m*e HCV mock DBS results				
	Abbott *m*2000 plasma viral load IU/ml	Median $\log_{10}$ IU/ml	Detected	Negative	DBS viral load range (IU/ml)	Mean $\log_{10}$ IU/ml	Median $\log_{10}$ IU/ml
20	Negative*	Negative	2	18	Negative - < 282 DET	NA	Negative
8	≤1000	< 1.08 DET to 2.31	1	7	Negative - < 282 DET	NA	Negative
7	1001-10,000	3.47	7	0	< 282 DET – 14,125	3.86	2.86
4	10,001-100,000	4.81	4	0	5012 – 6,165,950	6.49	5.58
18	100,001-1000,000	5.55	18	0	13,490 – 1,659,587	5.92	5.11
13	1000,001-10,000,000	6.22	13	0	251,189 -10,000,000	6.71	6.10

NA: not applicable.
* Includes 10 HCV RNA negative samples and 10 HCV specimens that had negative HCV antibody results but were not tested with PCR.

2. Study design

2.1. Mock DBS sample panel

The Abbott RUO DBS protocol was evaluated using a panel of "mock" DBS samples made from whole blood that had been sent for routine HCV RNA testing. A plasma aliquot was extracted on the Abbott *m*2000sp and amplified using the Abbott RealTi*m*e HCV assay on the *m*2000rt [6]. The DBS panel consisted of 50 HCV RNA positive patients (viral load range " < 12 detected" to 9,812,515 IU/ml) and 10 HCV antibody positive/ HCV RNA negative patients. A further 10 whole blood samples which were HCV antibody negative by Abbott Architect (RNA not tested) were also evaluated. The 50 known positive HCV RNA patients used to make the "mock" DBS had the following genotypes: 1 A (n = 23), 2 (n = 5), 3 (n = 19), 4 (n = 1) and unknown (n = 2).

To make the mock samples, 1 ml of patient whole blood (stored at 4 °C) and 0.5 ml of plasma (which has been taken from the original whole blood tube and stored at −80 °C), were mixed together gently and 50µl spotted onto Protein Saver 903 cards (Whatman). The cards were left to dry overnight at room temperature. The blood spots were punched from the card using a 1 cm hole puncher into an empty 5 ml sarstedt tube and stored at −80 °C until use. The results generated by the Abbott RUO DBS protocol were compared to the results generated on plasma by the current Abbott HCV RealTi*m*e assay.

2.2. Mock DBS dilution panel

A whole blood sample with a high HCV viral load (1,779,139 IU/ml) was used to generate a dilution panel of "mock" DBS specimens using HCV negative whole blood. The diluted sample was HCV genotype 3. The panel consisted of 6 samples ranging from 1.78 IU/ml (0.25 $\log_{10}$ IU/ml) to 1,779,139 IU/ml (6.25 $\log_{10}$ IU/ml). The method for manufacturing the mock DBS samples was outlined above. The panel was tested four times on two separate runs.

2.3. Mock DBS using a 1000 IU/ml HCV control

The 5th WHO International Standard for HCV nucleic acid amplification techniques supplied by the National Institute for Biological Standards and Control (NIBSC) was diluted using viral transport medium (VTM) to 1000 IU/ml. Fifty microlitres was spotted onto DBS cards and one spot was tested per assay run. Six spots were tested in six different runs.

2.4. Comparison of the Abbott RUO DBS protocol and the in house DBS method

Eighty DBS samples, previously tested for HCV antibody and HCV RNA and stored at −80 °C, were also tested in this study. Forty of these samples were RNA positive and forty were RNA negative by the in-house method. The HCV genotype was only known for 23 of the 40 RNA positive patients, with 13 genotype 1 A and 10 genotype 3.

2.5. Abbott RUO DBS protocol

To prepare the DBS spots for extraction, 1.3 ml of Abbott *m*Sample preparation system DBS buffer was added to an Abbott Master mix tube. One DBS spot was added to this buffer and left at room temperature for 30 min with the tube being inverted every 10 min. The samples were then placed on the Abbott *m*2000sp for extraction along with the necessary RUO controls and RUO calibrators. Abbott *m*Sample Preparation System kit reagents and internal control were prepared according to manufacturer's instructions. The extracted samples were then amplified using Abbott RealTi*m*e HCV RUO amplification reagent kits. The protocol, amplification reagents, controls and calibrator kits are identical to the CE-marked products of the Abbott *m*Sample Preparation System and the Abbott RealTi*m*e HCV used for plasma HCV viral load determination. However, DBS testing is currently an off-label use of this protocol and is therefore referred to as RUO DBS protocol throughout.

2.6. In house PCR protocol

The in house PCR method has been described elsewhere [5].

3. Results

3.1. Plasma viral load compared to mock DBS

In total 70 "mock" DBS were tested (Table 1). Of the 20 negative samples, 2 were false positive at low level (< 282 IU/ml detected) by the Abbott RUO DBS protocol. Both of these samples were found to be negative on repeat testing. Forty-three of the 50 positive samples were detected by the Abbott RUO DBS protocol. The 7 samples not detected had a HCV viral load < 1000IU/ml. A scatter plot, comparing the plasma viral loads (> 1000IU/ml) and the mock DBS viral loads, indicated the $R^2 = 0.751$ (Fig. 1). The overall sensitivity and specificity of the Abbott RUO DBS protocol was 86% (95% CI 73.26–94.18) and 90% (95% CI 68.30–98.77). The sensitivity increased to 100% (95% CI: 91.59%–100%) when comparing only "mock" DBS with viral loads > 1000 IU/ml.

3.2. Dilution panel

All dilutions ≥1779 IU/ml were detected all four times using the Abbott RUO DBS protocol. The results suggested a detection limit between 178 IU/ml to 1779 IU/ml based on the estimated plasma viral load in the dilution series. The viral load of 1779 IU/ml was detected four times and the viral load of 178 IU/ml was detected two of the four times.

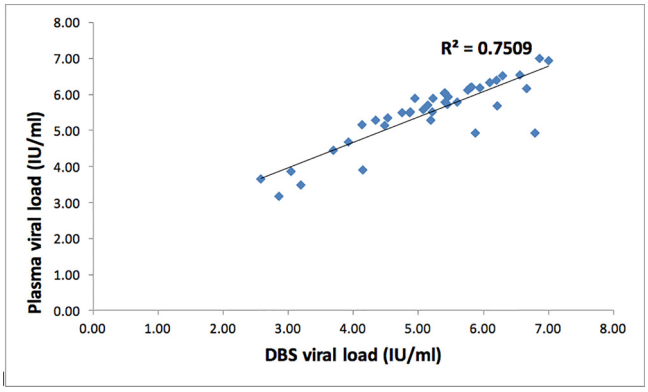

Fig. 1. Comparison of the plasma viral load and the "mock" DBS viral load.

3.3. Mock DBS using a 1000 IU/ml HCV control in VTM

The NIBSC control diluted to 1000 IU/ml in VTM and spotted on to DBS card was tested on six separate assay runs. The mock DBS sample was detected on all six runs, always at a level below quantification (< 282 IU/ml).

3.4. Abbott RUO DBS protocol for detecting patient samples

A total of forty HCV RNA positive and forty HCV RNA negative patient DBS samples, as determined by the qualitative in-house assay, were tested using the Abbott RUO DBS protocol. There were no false positive results among the forty negative DBS samples. The Abbott protocol detected 39/40 RNA positive patient samples. The false negative with the Abbott RUO DBS protocol had a cycle threshold (C_T) of 36.98 (real-time PCR cut-off is C_T 40). The overall sensitivity and specificity of the Abbott RUO DBS protocol compared with the current in-house assay was 97.5 (95% CI: 86.84%–99.94%) and 100% (95% CI: 91.19%–100%).

4. Discussion

Abbott performed well against the panel of 70 "mock" DBS samples. There were two false positive samples among 20 negative "mocks". These two were from patients who had been previously positive and were receiving HCV treatment. Abbott detected 43 of the 50 positive samples, with the false negative samples all < 1000 IU/ml. Preliminary data using the dilution panel suggested the limit of detection for the Abbott RUO DBS protocol may be between 178 IU/ml to 1779 IU/ml. The sample used in the dilution series was genotype 3, one of the genotypes commonly found among our PWID population. Further dilution series and dilution panels consisting of different HCV genotypes are required to narrow down the limit of detection. Interestingly the Abbott RUO DBS protocol generated higher quantitative viral loads in some "mock" DBS samples compared to plasma. This was also seen with the Abbott HIV-1 RealTi*m*e assay and DBS samples [9].

Abbott performed well when compared to the in-house real-time PCR method. Forty patient DBS samples were tested. One was not detected by the Abbott protocol. This sample may be a false negative by Abbott or may be a false positive with the in-house assay, the patient had been previously HCV antigen negative in plasma and there was no follow up samples at the time of writing. The Abbott architect HCV antigen assay has an accurate detection limit of 1000 IU/ml [10]. The current in-house assay was originally reported to have a limit of detection of 250 IU/ml. However, experience over the years with this assay, has suggested a detection limit closer to 1000 IU/ml. Therefore, the Abbott RUO DBS protocol compares well with the in-house assay already in use. The Abbott RUO DBS protocol is a quantitative assay compared to the qualitative in-house assay. Though the Abbott RUO DBS assay is not as sensitive as the Abbott RealTi*m*e HCV plasma viral load assay, it still fulfils the most recent "EASL Recommendations on Treatment of Hepatitis C 2018" that allow the a lower limit of detection ≤1000 IU/ml (3.0 $\log_{10}$ IU/ml) for HCV RNA monitoring in low- or middle-income countries as well as in specific settings in high-income countries to facilitate access to HCV diagnosis and care [11].

The time difference between the two protocols for 24 extractions and amplifications was 35 min, with the Abbott system taking longer. However, Abbott is more automated than the in-house assay with less room for error. The HCV DBS protocol also has the benefit of 96 extractions in one assay run, whereas the in-house protocol would require four separate extraction runs to process the same amount.

Limitations to this study are the lack of inter- and intra- assay reproducibility data. The NIBSC control diluted to 1000 IU/ml in VTM was tested on six different runs and was detected on all six runs highlighting the reproducibility of the assay, but the viral load fell below the level of quantification. The use of VTM, a more fluid medium compared to whole blood, may have generated a greater area of diffusion on the filter paper resulting in a reduced concentration in the area cut for testing. Further work should be conducted to determine the coefficient of variation of the assay.

The HCV DBS protocol, an off-label use of the CE-marked Abbott RealTi*m*e HCV protocol, was easy to use, required less manual input and analysed the results automatically reducing user error and allowed experienced technical staff to be redeployed to other areas of the laboratory. This system would be of value to any busy laboratory where work load is likely to increase with the move towards HCV elimination [12].

Author contribution

The project was devised by Dr Rory N Gunson and Dr Samantha J Shepherd, the work was carried out by Rachel E Baxter and the manuscript was written by Dr Samantha J Shepherd. All authors contributed to the final draft.

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