Journal of Clinical Virology 112 (2019) 40–44

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Performance evaluation of the Hologic Aptima HCV Quant Dx assay for detection of HCV RNA from dried blood spots

Beth Catlett[a,b,*], Alex Carrera[a], Mitchell Starr[a], Tanya L Applegate[b], Peter Lowe[c], Jason Grebely[b,1], H. Philip Cunningham[a,1]

[a] NSW State Reference Laboratory for HIV, St Vincent's Centre for Applied Medical Research, Darlinghurst, Sydney, Australia
[b] The Kirby Institute, UNSW Sydney, Sydney, New South Wales, Australia
[c] Hologic Australia, Macquarie Park, New South Wales, Australia

ARTICLE INFO

Keywords:
Diagnostic
DBS
People who inject drugs
HCV
HCV RNA

ABSTRACT

Background: The availability of effective direct-acting antiviral therapy for hepatitis C virus (HCV) has led to a need for simplified diagnostic pathways. Barriers to treatment uptake, specifically in people who inject drugs and in remote and resource limited settings, may be overcome by utilizing novel collection methods, such as dried blood spots (DBS). However, there are currently no registered assays for HCV RNA testing from DBS samples.

Objectives: To evaluate the sensitivity and specificity of the Aptima HCV Dx Quant assay for HCV RNA detection in DBS samples

Study design: 107 paired venepuncture and DBS samples from HCV antibody positive individuals were analyzed for HCV RNA on the Aptima HCV Dx Quant and Roche CAP/CTM (gold standard) HCV assays.

Results: 78% (n = 83) had detectable HCV RNA in plasma. Sensitivity of the Aptima assay for HCV RNA detection in DBS was 96.4% (95% CI 89.8–99.3%) and specificity was 95.8% (95% CI 78.8–99.9%). Sensitivity for HCV RNA detection in DBS using a quantitative threshold of ≥15 IU/mL in plasma was 95.1% (95% CI 88%–98.7%) and specificity was 96.0% (95% CI 79.7%–99.9%). The sensitivity of HCV RNA detection in DBS using a quantitative threshold of ≥1000 IU/mL (based on a clinically relevant threshold) was 100% (95% CI 95.3–100%) and specificity was 100% (95% CI 88.4–100%).

Conclusions: Our data indicates that the Aptima HCV Dx Quant can detect active HCV infection from a DBS sample with good sensitivity and specificity, particularly when using a threshold of ≥1000 IU/mL.

1. Background

Chronic hepatitis C virus (HCV) infection is a major public health concern, with 71 million people infected worldwide [1]. Treatment options have improved with the availability of interferon-free direct-acting antiviral (DAA) therapies with cure in > 95% of people [2]. However, broadening the availability of DAA therapies will be vital in achieving the WHO target of eliminating HCV as a major global public health threat by 2030 [3]. One of the major challenges will be to address the low rates of HCV testing and diagnosis [4].

Simplifying models of HCV care by reducing the number of visits and time to result is necessary to increase HCV testing and treatment uptake [5]. Alternative sampling and diagnostic options, such as dried blood spots (DBS), have been shown to increase testing and linkage to care [6,7]. Sampling involves the finger-stick collection of a capillary whole-blood sample (50–100 µl, typically 70 µl), onto a filter paper. DBS samples allow for reflex viral testing, testing of other viral analytes (such as HIV), they are easy to biobank and store, they have good stability, and require no cold chain transport. DBS sampling also avoids the need for venepuncture, which is a barrier to sample collection and testing in settings where venepuncture is unavailable or presents a major barrier to testing, such as among people who inject drugs, who often have poor venous access [5,8].

The Aptima HCV Quant Dx assay (subsequently referred to as Aptima) for the detection of HCV RNA uses real-time TMA technology which has many advantages [9], such as integrated platform automation and random access with continuous sample and reagent loading. It is CE-marked and has previously demonstrated similar performance to

* Corresponding author at: NSW State Reference Laboratory for HIV, St Vincent's Centre for Applied Medical Research, Darlinghurst, Sydney, Australia.
E-mail address: b.catlett@amr.org.au (B. Catlett).
[1] Equally contributed to the work.

https://doi.org/10.1016/j.jcv.2019.01.010
Received 25 October 2018; Received in revised form 14 January 2019; Accepted 24 January 2019

other platforms, such as the COBAS TaqMan HCV2 assay or Abbott RealTime HCV assay [9–12]. However, there is a gap in the literature for evaluating the performance of HCV RNA assays on DBS samples.

HCV serological and nucleic acid testing from DBS samples has been successfully performed within clinical trials, epidemiological surveillance programs, screening programs and in limited clinical settings [13–20]. However, there are currently no registered assays for HCV RNA testing from DBS samples. As such, there is a growing need for validated, standardized manufacturers protocols and registration of HCV quantitative and qualitative molecular diagnostic assays with DBS as a sample type [1].

2. Objectives

The main objective of this study was to evaluate the sensitivity and specificity of the Aptima HCV Quant Dx assay for detection of HCV RNA in DBS samples compared with plasma samples tested on the CAP/CTM HCV assay as the gold standard.

3. Study design

3.1. Study participants

Samples were collected from patients ≥18 years of age with known HCV infection. They were selected for this study if sufficient volume was available from whole blood EDTA primary collection tubes; to obtain paired EDTA plasma aliquots and a venous blood DBS card. This study was reviewed and approved by the St Vincent's Human Research Ethics Committee (IRB/HREC) (LNR/14/SVH/359).

3.2. Methods

DBS specimens were prepared using 70 µl of EDTA whole blood per circle, pipetted five times onto filter paper (Munktell, TNF paper [order number 323046], Ahlstrom-Munksjö, Helsinki, Finland). DBS were then air dried in a biological safety cabinet for 24 h. Once dried, DBS were stored in sealable gas impermeable bags (Fisherbrand™, Bitran™, 6 x 6 inch, [order number 19240119], Thermofisher, Scoresby, VIC, Australia) containing 2 desiccant packs (1gm Silica gel [order number SG23401], Desicco, Sydney, NSW, Australia), with each card separated by wax paper (Glad™, Freezer Film Go Between, 15 m x 33 cm [order number 147,803 P], Coles, Glen Iris, VIC, Australia). DBS were then subsequently batched and stored at −80 °C for up to 6 months prior to testing, either immediately post-drying or left at room temperature for the period described in the stability sub-study.

EDTA primary tubes containing whole blood were stored at 2 °C–30 °C for up to 24 h prior to processing the paired samples. EDTA whole blood was centrifuged for 10 min at 1800 x g at 4 °C, and two 1 mL aliquots of plasma were stored in 2 mL cryovials (Nunc™, [order number 374513], Thermofisher, Scoresby, VIC, Australia) at −80 °C for a minimum of 12 h prior to testing for HCV RNA.

In a subset of patients, where EDTA plasma was available for testing, HCV genotype was determined using the HCV amplification kit 2.0 with the Versant HCV genotype assay [21].

Prior to DBS testing, the following preparation was required. A 1-cm disk was punched-out manually with a 1-cm hole punch (1/2″, [order number 448], hankypankycrafts, Blue Earth, MN, USA) and placed into a aliquot tube (specimen aliquot tube, [order number 503762], Hologic, Inc. San Diego, California, United States) containing 1 mL of sample transport media (Aptima Specimen Transfer Kit, [order number 301154C], Hologic, Inc. San Diego, California, United States). Tweezers and 1-cm hole punches were flame-sterilized between samples to eliminate the risk of cross-contamination between samples. Specimen tubes were then capped (Transport Tube Cap, [order number 504415], Hologic, Inc. San Diego, California, United States) and placed on a rocking platform (Medium Rocking Platform Mixer, [order number RPM5], Australian Scientific Pty Ltd, Kotara, NSW, Australia) and incubated at room temperature for 30 min before centrifugation for 10 min at 2000 rpm.

Plasma samples were tested with the Aptima assay on the Hologic Panther instrument alongside the gold standard CAP/CTM HCV assay on the Roche Cobas TaqMan. Plasma and DBS samples (DBS tested on Aptima only) were tested in singlicate following manufacturer's instructions within the package inserts [22,23]. Run validity and individual sample validity was automatically determined by the assays' software. The reported assays' lower limit of quantification in plasma samples is 10 IU/mL for Aptima and 15 IU/mL for CAP/CTM®; both assays have a linear range of quantitation from the LOQ to ~10^8 copies/mL.

A small sub-study to examine HCV RNA stability and viability of DBS when stored at room temperature was performed using the same collection, off board processing and testing methodology described above. DBS cards were stored for 1, 5, 15, 30 and 60 days at 22°C prior to testing in duplicate on the Aptima assay.

3.3. Statistical analysis

To correct for haematocrit, all post run DBS HCV viral load results in IU/mL and log10 on the Aptima assay were multiplied by a plasma conversion factor of 25.97. This calculation was undertaken to correct for volumetric differences of plasma within whole blood and was based on the following assumptions 45% haematocrit average in whole blood (DBS), 70 µl DBS volume, 1000 µl ATM volume using the following equation: volume of ATM (µl) / [DBS volume (µl), x (1 – haematocrit average)] [24]. All testing was performed in singlicate and expressed as $\log_{10}$ transformed values in IU/mL. A midpoint between zero and the lower limit of quantification (5 IU/mL or 0.7 Log10) for detectable but unquantifiable HCV RNA below the lower limit of quantitation (LLOQ) was applied and results were expressed as $\log_{10}$ transformed values in IU/mL.

Statistical analysis included the assessment of diagnostic sensitivity and specificity of the detection of HCV RNA from DBS on the Aptima assay, compared to EDTA plasma (gold standard) on the CAP/CTM assay using three thresholds in plasma: detectable HCV RNA, quantifiable HCV RNA at > 10 IU/mL and quantifiable HCV RNA above ≥1000 IU/mL.

To assess bias and agreement between plasma and DBS on the Aptima assay, a Deming regression analysis (including Pearson's correlation coefficient R and statistical significance) was conducted to include all detectable (HCV RNA in plasma) samples. Additionally, a Bland Altman analysis was performed for HCV RNA in both DBS and plasma detectable samples. The midpoint between zero and the lower limit of quantification (5 IU/mL or 0.7 Log10) was used for detectable but unquantifiable HCV RNA, whereas those with undetectable HCV RNA in plasma were excluded.

All analyses were performed using Stata 14.2 (StataCorp software, LLC., College Station, Texas) and GraphPad Prism6 (GraphPad software, Inc., San Diego, California).

4. Results

Samples from 116 HCV antibody positive patients were included in this study, of which 107 had available specimen to perform both assays. Genotype was available for 45 of 83 patients with detectable RNA, as follows: HCV-1 (n = 2), HCV-1a (n = 17), HCV-1b (n = 12), HCV-2a/2c (n = 1), HCV-2b (n = 1) and HCV-3a (n = 12).

The sensitivity of the Aptima assay for HCV RNA detection in DBS was 96.4% (95% CI 89.8–99.3%) and specificity was 95.8% (95% CI 78.9–99.9%) when compared to the CAP/CTM assay (Table 1). Sensitivity for HCV RNA detection in DBS above a quantifiable threshold (≥15 IU/mL in plasma) was 95.1% (95% CI 88% to 98.7%) and specificity was 96.0% (95% CI 79.7% to 99.9%) (Table 2). The sensitivity

Table 1
Detection of HCV RNA in DBS versus Plasma samples with detectable HCV RNA from 107 anti-HCV positive patients*.

		Plasma (CAP/CTM)		Total
		Not Detected	Detected	
DBS (APTIMA)	Not Detected	23	3	26
	Detected	1**	80	81
Total		24	83	107

* Only 107 of the 116 patients had data in both sample types.
** 13 IU/mL DBS (equivalency to plasma)

*Only 107 of the 116 patients had data in both sample types.
**13 IU/mL DBS (equivalency to plasma).

Table 2
HCV RNA detection in DBS (Aptima), in samples with known quantifiable and unquantifiable HCV RNA plasma (CAP/CTM) (> 15IU/mL and 1000IU/mL thresholds).

	Plasma (CAP/CTM)		
DBS (APTIMA)	Not Detected/ Unquantifiable	Detected >15 IU/mL	Total
Not Detected/ Unquantifiable	27	2	29
Detected	1*	77	78
Total	28	79	107
	Not Detected/ Unquantifiable	Detected >1000 IU/mL	Total
Not Detected/ Unquantifiable	30	0	30
Detected	0	77	77
Total	30	77	107

*Detectable >LLOQ (>10IU/mL) in Aptima

*Detectable > LLOQ (> 10IU/mL) in Aptima.

of HCV RNA detection ≥1000 IU/mL in DBS (based on a clinically relevant threshold obtained from EASL guidelines) was 100% (95% CI 95.3–100%) and specificity was 100% (95% CI 88.4–100%) (Table 2). A similar sensitivity and specificity of the Aptima assay for HCV RNA detection in DBS compared to Aptima HCV RNA detection in plasma was observed across all three of the above thresholds (Supplementary Tables 1–2).

CAP/CTM assay results in plasma were in agreement for 102/107 (95.3%) with Aptima DBS (23 undetectable, 80 detectable) (Table 1). Three samples were 'undetectable' in DBS and were detectable at very low HCV RNA concentrations in plasma (27 IU/mL, 22 IU/mL, 20 IU/mL); one DBS sample was detectable, but below the LLOQ, while the paired plasma sample result was quantified at 33 IU/ mL and one DBS sample was detectable (13 IU/mL) while the paired plasma sample result was 'undetectable'.

Correlation between paired plasma and DBS tested on the Aptima HCV Dx Quant assay (HCV RNA detectable in plasma (n = 86)) was assessed using Deming regression analysis (Fig. 1). This demonstrated a strong, positive correlation (R^2 = 0.929) that was stastically significant ($p < 0.001$). Bland-Altman plot analysis (Fig. 2) showed a bias of +0.51 $\log_{10}$ between plasma and DBS HCV RNA concentrations (n = 86). The limits of agreement indicate that 95% of the difference between plasma and DBS on the Aptima are between -0.48 and 1.50 $\log_{10}$. Both Deming and Bland-Altman anlysis showed that HCV RNA tended to be higher in plasma than DBS for samples with HCV RNA above 4.5 $\log_{10}$ IU/mL. Importantly, HCV RNA in DBS could be quantitated as low as 525 IU/mL (2.72 $\log_{10}$ IU/mL), with agreement between sample types.

Stability of DBS over the 60-day time points was encouraging, with a maximum of 0.1 $\log_{10}$ IU/mL degradation from the day 1 value (Supplementary Fig. 1 and Supplementary Table 3).

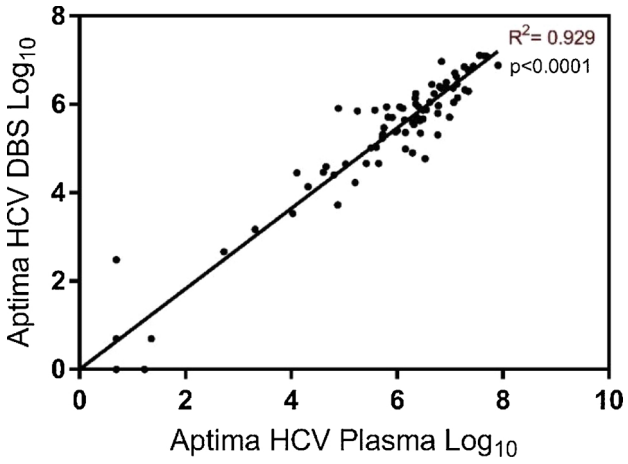

Fig. 1. Deming correlation analysis between paired samples (detectable HCV RNA in plasma) with the Aptima HCV Dx Quant assay (n = 86), Y = 0.9228*X - 0.07554: R^2 = 0.929; the diagonal line represents identical results.

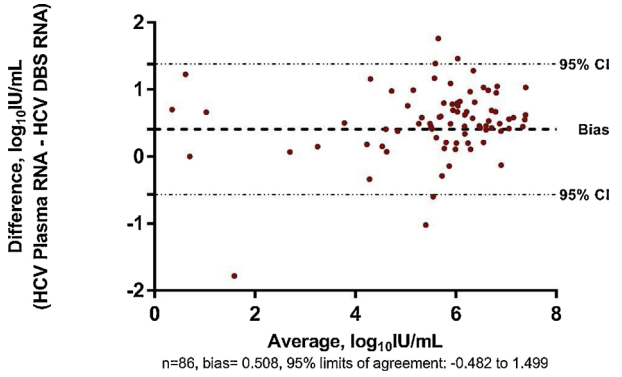

Fig. 2. Bland-Altman analysis to show bias and agreement for HCV RNA detection in plasma samples compared with DBS samples on the Aptima assay, using Bland-Altman Bias plots; the dashed line represents bias (0.508 $\log_{10}$ IU/mL); the two dotted lines represent the 95% limit of agreement (-0.482 to 1.499).

5. Discussion

This study evaluated the sensitivity and specificity of the Aptima HCV Dx assay for HCV RNA detection from spotted whole blood DBS samples compared with the CAP/CTM and Aptima HCV Dx assay in plasma using HCV antibody positive remnant diagnostic samples. A high degree of correlation and diagnostic accuracy was observed in HCV RNA detection in DBS compared to plasma (venepuncture), especially when considering the clinically relevant threshold proposed by EASL [25]. This is one of the first studies to compare the Aptima HCV dx assay in DBS and plasma samples. Finger-stick DBS sample collection and HCV RNA testing using the Aptima assay provides an opportunity for simplified diagnostic strategies to increase testing uptake particularly in settings where venepuncture is a barrier to testing. These findings provide support for further evaluation of the 'real-world' performance of DBS collection to enhance HCV testing, linkage to care, and treatment by simplifying sample collection.

The sensitivity and specificity of the Aptima for HCV RNA detection in DBS when compared to the CAP/CTM ('gold standard') was 96.4% and 95.8% respectively, consistent with similar studies evaluating automated PCR and TMA methods, where sensitivity ranged between 90–98% and specificity was reported as 100% [26–29]. In this study, the false negatives (n = 3) and false positive (n = 1) occurred at the lower assay range (< 33 IU/mL (false negatives) and below the CAP/CTM < LLOQ (false positive). The false negatives could be attributed

to hematocrit correction as 33IU/mL in plasma is equivalent to 1.3 IU/mL in DBS (33/25.97) which falls below the limit of detection. Encouragingly, the sensitivity and specificity increased to 100% when a clinically relevant threshold of 1000 IU/mL was used based on guidance for the minimum detectable threshold for HCV RNA detection proposed in the 2018 EASL recommendations [25]. Furthermore, excellent agreement and a strong correlation ($R^2 = 0.929$, $p < 0.0001$) was observed between both plasma and DBS when analyzed using the Aptima assay with a small bias of 0.51 $\log_{10}$ towards plasma.

To our knowledge, this is the first published study to demonstrate that DBS collection and testing on the Aptima assay may be acceptable for implementing into simplified testing pathways for healthcare settings where venepuncture is difficult or impossible. Further, DBS enables the flexibility for testing algorithms which include HCV RNA testing alone (for determination of active infection) or HCV antibody testing followed by HCV RNA testing. The decision as to which testing algorithm to use will depend on the test price and the prevalence of HCV infection in the population, which will determine which strategy will be more cost-effective.

Clinical use of DBS described in published literature is limited and regulatory requirements vary from country to country, adding to the need for validated, standardized manufacturers protocols and registration of HCV quantitative and qualitative molecular diagnostic assays with DBS as a sample type [30]. Further work is urgently needed to have DBS product monograms for in-vitro medical diagnostic devices (outside of research only setting) recognised and approved by regulatory authorities to allow for clinical implementation [28].

There are some limitations to this study, firstly the remnant DBS samples were manufactured in the laboratory by spotting standard of care HCV antibody positive venous whole blood from clinical samples onto filter paper rather than patient collected fingerstick. As such, limited demographic information was available and information on injecting drug use risks were unavailable (e.g. injecting drug use). The sample set was also small and heavily biased (anti − HCV positive), with high HCV RNA detection (80%) and concentration (average viral load 6.04 $\log_{10}$ IU/mL) and genotype was only determined for 45 of the 83 patients with detectable RNA due to sample insufficiency and was restricted to genotypes 1-3. Finally, to replicate 'real-world' testing scenarios, further evaluations with larger sample sizes and fingerstick capillary DBS are required to assess analytical sensitivity and specificity in relevant cohorts (including people who inject drugs), especially at lower HCV RNA concentrations and with genotypes 4-6.

In summary, our data indicates that the Aptima HCV Dx Quant can detect active HCV infection from a DBS sample with good sensitivity and specificity, particularly when using a clinically relevant threshold of 1000 IU/mL. However, further evaluation of 'real-world' performance is required to assist the manufacturer to seek regulatory approval for a DBS package insert claim. This could then offer a simplified diagnostic solution to identify individuals with chronic HCV in decentralized health settings and link them into care.

Author contribution

BC, PL and PC contributed to the study design. PC was the study investigator. BC, MS, PC, AC contributed to the study implementation and study conduct. BC and AC contributed to the laboratory work. BC, JG, PC and TA contributed to the data interpretation and drafted the first version of the manuscript. All authors contributed to the writing and review of the report.

Funding

This work was supported by funding from St Vincent's AMR. Hologic provided Panther equipment and Aptima HCV Quant Dx Viral Load assays.

The sponsors had no rule in the analysis and interpretation of the study results, the manufacturer of reagents used in this study did not influence the study design, analysis of data or reporting of results. The Kirby Institute is funded by the Australian Government Department of Health and Ageing. NSW State Reference Laboratory for HIV, St Vincent's Centre for Applied Medical Research and The Kirby Institute each receive funding from the Australian Government Department of Health and Ageing and the New South Wales Ministry of Health. The views expressed in this publication do not necessarily represent the position of the Australian Government. BC is supported by an Australian Postgraduate Award from UNSW Sydney and an Australian Government Research Training Program Scholarship provided by the Commonwealth Government. JG is supported by a National Health and Medical Research Council Career Development Fellowship (APP1112352). PL is a former employee of Hologic Australia.

TA has received research grants from Abbott Diagnostics and travel grant from Cepheid. JG is a consultant/advisor and has received research grants from AbbVie, Bristol-Myers Squibb, Cepheid, Gilead Sciences and Merck/MSD.

Acknowledgments

The authors would also like to acknowledge Nadika Atapattu (Hologic) for technical assistance and Leon McNally, Joanne Sherring and Charles Crew (St Vincent's Hospital, NSW state reference laboratory for HIV) for laboratory support and Florence Paillard, who contributed toward the original manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.01.010.

References

[1] B. Lange, J. Cohn, T. Roberts, J. Camp, J. Chauffour, N. Gummadi, et al., Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): two systematic reviews and meta-analyses, BMC Infect. Dis. 17 (Suppl 1) (2017) 700.

[2] Polaris Observatory HCVC, Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study, Lancet Gastroenterol. Hepatol. 2 (3) (2017) 161–176.

[3] WHO Guidelines on Hepatitis B and C Testing. WHO Guidelines Approved by the Guidelines Review Committee, (2017) Geneva.

[4] J.V. Lazarus, K. Safreed-Harmon, M. Colombo, T. Reic, E. Schatz, P. van Damme, et al., Many European countries' flying blind' in their efforts to eliminate viral hepatitis, Nat. Rev. Gastroenterol. Hepatol. 14 (8) (2017) 445–446.

[5] J. Grebely, T.L. Applegate, P. Cunningham, J.J. Feld, Hepatitis C point-of-care diagnostics: in search of a single visit diagnosis, Expert Rev. Mol. Diagn. 17 (12) (2017) 1109–1115.

[6] J.T. Coats, J.F. Dillon, The effect of introducing point-of-care or dried blood spot analysis on the uptake of hepatitis C virus testing in high-risk populations: a systematic review of the literature, Int. J. Drug Policy 26 (11) (2015) 1050–1055.

[7] S. Bajis, G.J. Dore, B. Hajarizadeh, E.B. Cunningham, L. Maher, J. Grebely, Interventions to enhance testing, linkage to care and treatment uptake for hepatitis C virus infection among people who inject drugs: a systematic review, Int. J. Drug Policy 47 (2017) 34–46.

[8] C.E. Lee, S. Sri Ponnampalavanar, S.F. Syed Omar, S. Mahadeva, L.Y. Ong, A. Kamarulzaman, Evaluation of the dried blood spot (DBS) collection method as a tool for detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian tertiary referral hospital, Ann. Acad. Med. Singapore 40 (10) (2011) 448–453.

[9] S. Chevaliez, F. Dubernet, C. Dauvillier, C. Hezode, J.M. Pawlotsky, The New Aptima HCV Quant Dx Real-time TMA Assay Accurately Quantifies Hepatitis C Virus Genotype 1-6 RNA, J. Clin. Virol. 91 (2017) 5–11.

[10] K. Schonning, M.S. Pedersen, K. Johansen, B. Landt, L.G. Nielsen, N. Weis, et al., Analytical and clinical performance of the Hologic Aptima HCV Quant Dx Assay for the quantification of HCV RNA in plasma samples, J. Virol. Methods 248 (2017) 159–165.

[11] A.R. Garbuglia, A. Bibbo, R. Sciamanna, M. Pisciotta, M.R. Capobianchi, Performance evaluation of the Aptima((R)) HCV Quant Dx assay for hepatitis C virus (HCV) RNA detection and quantification in comparison to the Abbott RealTime HCV assay, J. Clin. Virol. 92 (2017) 1–6.

[12] A. Worlock, D. Blair, M. Hunsicker, T. Le-Nguyen, C. Motta, C. Nguyen, et al., Analytical characteristics and comparative evaluation of Aptima HCV quant Dx assay with the Abbott RealTime HCV assay and Roche COBAS AmpliPrep/COBAS TaqMan HCV quantitative test v2.0, Virol. J. 14 (1) (2017) 66.

[13] V. Saludes, C. Folch, A. Morales-Carmona, L. Ferrer, L. Fernandez-Lopez, R. Munoz, et al., Community-based screening of hepatitis C with a one-step RNA detection algorithm from dried-blood spots: analysis of key populations in Barcelona, Spain, J. Viral. Hepat. 25 (3) (2018) 236–244.
[14] J. Iversen, J. Grebely, B. Catlett, P. Cunningham, G.J. Dore, L. Maher, Estimating the cascade of hepatitis C testing, care and treatment among people who inject drugs in Australia, Int. J. Drug Policy 47 (2017) 77–85.
[15] J.B. Parr, E.K. Lodge, V. Holzmayer, J. Pepin, E.H. Frost, M.W. Fried, et al., An efficient, large-scale survey of hepatitis C viremia in the Democratic Republic of the Congo using dried blood spots, Clin. Infect. Dis. 66 (2) (2018) 254–260.
[16] V.D. Hope, M. Hickman, S.L. Ngui, S. Jones, M. Telfer, M. Bizzarri, et al., Measuring the incidence, prevalence and genetic relatedness of hepatitis C infections among a community recruited sample of injecting drug users, using dried blood spots, J. Viral Hepat. 18 (4) (2011) 262–270.
[17] S. Vazquez-Moron, P. Ryan, B. Ardizone-Jimenez, D. Martin, J. Troya, G. Cuevas, et al., Evaluation of dried blood spot samples for screening of hepatitis C and human immunodeficiency virus in a real-world setting, Sci. Rep. 8 (1) (2018) 1858.
[18] A. McLeod, A. Weir, C. Aitken, R. Gunson, K. Templeton, P. Molyneaux, et al., Rise in testing and diagnosis associated with Scotland's Action Plan on Hepatitis C and introduction of dried blood spot testing, J. Epidemiol. Community Health 68 (12) (2014) 1182–1188.
[19] M. Hickman, T. McDonald, A. Judd, T. Nichols, V. Hope, S. Skidmore, et al., Increasing the uptake of hepatitis C virus testing among injecting drug users in specialist drug treatment and prison settings by using dried blood spots for diagnostic testing: a cluster randomized controlled trial, J. Viral Hepat. 15 (4) (2008) 250–254.
[20] P.H. Wales, Cardiff HPaSVU (Ed.), Diagnostic Testing for Hepatitis C, Hepatitis B and HIV: Information for Substance Misuse Services 2014, NHS, UK, 2014.
[21] Siemens, VERSANT HCV Genotype 2.0 Assay (LiPA), [Available from: DOA, 2018 18/12/2018 https://www.healthcare.siemens.com.au/molecular-diagnostics/molecular-diagnostics-in-vitro-diagnostics/versant-hcv-genotype-2-assay-lipa/technical-details.
[22] [Website]. [updated 2017. Aptima® HCV Quant Dx Assay package insert]. Available from: https://www.hologic.com/sites/default/files/2018-02/AW-14498_001_001.pdf, DOA:01/09/2018.
[23] Roche Molecular Systems, Inc, COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (package insert), DOA, 2013 01/09/2018.
[24] M.K. Sahoo, V. Varghese, E. White, M. Winslow, D.A. Katzenstein, R.W. Shafer, et al., Evaluation of the Aptima HIV-1 Quant Dx Assay Using Plasma and Dried Blood Spots, J. Clin. Microbiol. 54 (10) (2016) 2597–2601.
[25] European Association for the Study of the Liver, Electronic address eee, European Association for the Study of the L. EASL Recommendations on Treatment of Hepatitis C 2018, J. Hepatol. (2018).
[26] E.K. Dokubo, J. Evans, V. Winkelman, S. Cyrus, L.H. Tobler, A. Asher, et al., Comparison of Hepatitis C Virus RNA and antibody detection in dried blood spots and plasma specimens, J. Clin. Virol. 59 (4) (2014) 223–227.
[27] E.G. Marins, K. Bodinaidu, M. Lin, A. Deforest, Evaluation of the COBAS((R)) AmpliPrep/COBAS((R)) TaqMan((R)) HCV Test v2.0 for HCV viral load monitoring using dried blood spot specimens, J. Virol. Methods 247 (2017) 77–80.
[28] B.K. Mossner, B. Staugaard, J. Jensen, S.T. Lillevang, P.B. Christensen, D.K. Holm, Dried blood spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life, World J. Gastroenterol. 22 (33) (2016) 7604–7612.
[29] A. Soulier, L. Poiteau, I. Rosa, C. Hezode, F. Roudot-Thoraval, J.M. Pawlotsky, et al., Dried blood spots: a tool to ensure broad access to hepatitis C screening, diagnosis, and treatment monitoring, J. Infect. Dis. 213 (7) (2016) 1087–1095.
[30] T.L. Applegate, E. Fajardo, J.A. Sacks, Hepatitis C virus diagnosis and the holy grail, Infect. Dis. Clin. North Am. 32 (2) (2018) 425–445.