Journal of Clinical Virology 117 (2019) 80–84

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

The new Xpert HCV viral load real-time PCR assay accurately quantifies hepatitis C virus RNA in serum and whole-blood specimens

Mélanie Wlassow[a,e], Lila Poiteau[a,e], Françoise Roudot-Thoraval[b,e], Isabelle Rosa[c], Alexandre Soulier[a,e], Christophe Hézode[d,e], Valérie Ortonne[a,e], Jean-Michel Pawlotsky[a,e], Stéphane Chevaliez[a,e,*]

[a] National Reference Center for Viral Hepatitis B, C, and Delta, Department of Virology, hôpital Henri Mondor, France
[b] Department of Public Health, hôpital Henri Mondor, France
[c] Department of Hepatology and Gastroenterology, hôpital Henri Mondor, France
[d] Department of Hepatology and Gastroenterology, Centre Hospitalier Intercommunal de Créteil, France
[e] INSERM U955, Créteil, France

ARTICLE INFO

Keywords:
Hepatitis C
Dried blood spot
Screening
Diagnosis
HCV RNA

ABSTRACT

Background: Sensitive and accurate hepatitis C virus (HCV) RNA detection and quantification are essential to diagnose and monitor the virological response to antiviral treatment and the emergence of resistance. *Objective and study design:* The aim of this study was to assess the ability of the new Xpert HCV Viral Load assay to accurately detect and quantify HCV RNA in serum and in whole blood collected on dried blood spot (DBS). Serum and whole blood from a large series of patients chronically infected with different HCV genotypes were tested in parallel for HCV RNA detection and quantification. *Results:* A significant relationship between HCV RNA levels measured with the Xpert HCV Viral Load assay and the two commercial real-time PCR comparators (Abbott RealTime HCV test and Cobas AmpliPrep/Cobas Taqman HCV 2.0 test) was found in serum as well as in whole blood specimens. *Conclusions:* The Xpert HCV Viral Load assay accurately quantifies HCV RNA regardless of the HCV genotype and can thus confidently be used to detect active HCV infection in serum and in whole blood specimens.

1. Background

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that affects approximately 71 million individuals worldwide [1]. Chronic HCV infection leads to cirrhosis, decompensation liver failure, and hepatoceullular carcinoma, all of which carry mortality and morbidity with more than 700 000 HCV-related deatchs each year [2]. Despite the development of highly effective, all-oral, well tolerated, short duration drugs with high cure rates (> 95%), chronic hepatitis C remains a global public health concern.

The use of sensitive nucleic acid amplification technologies is recommended by the international liver clinical practice guidelines for HCV RNA detection and quantification [3,4]. The Cobas AmpliPrep/Cobas TaqMan HCV Test, version 2.0 (CAP/CTM HCV v2.0, Roche Molecular Systems, Pleasanton, California) and Abbott RealTime HCV (Abbott Molecular, Des Plaines, Illinois) are the most widely used assays. They show satisfactory performances for HCV RNA detection and quantification in clinical practice [5–7]. However, currently available HCV RNA platforms and assays are generally designed for batch testing of multiple specimens within a run.

Although none of commercial HCV RNA assays is approved for use with whole blood recovered from dried blood spot (DBS), The WHO guidelines on hepatitis B and C testing indicate that DBS can be used for HCV RNA testing [8].

The Xpert HCV Viral Load Assay (Cepheid, Sunnyvale, California) is a polymerase chain amplification (PCR)-based assay making use of the fully automated GeneXpert platforms that eliminates the need for batch processing of samples and automates all aspects of nucleic acid testing in a single step with a result available in 105 min, and with the simplicity and ease of use of a point-of-care test.

2. Objective

The aim of the present study was to assess the ability of the new

* Corresponding author at: Stéphane Chevaliez, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010, Créteil, France.
E-mail address: stephane.chevaliez@aphp.fr (S. Chevaliez).

https://doi.org/10.1016/j.jcv.2019.06.007
Received 27 February 2019; Received in revised form 11 June 2019; Accepted 21 June 2019

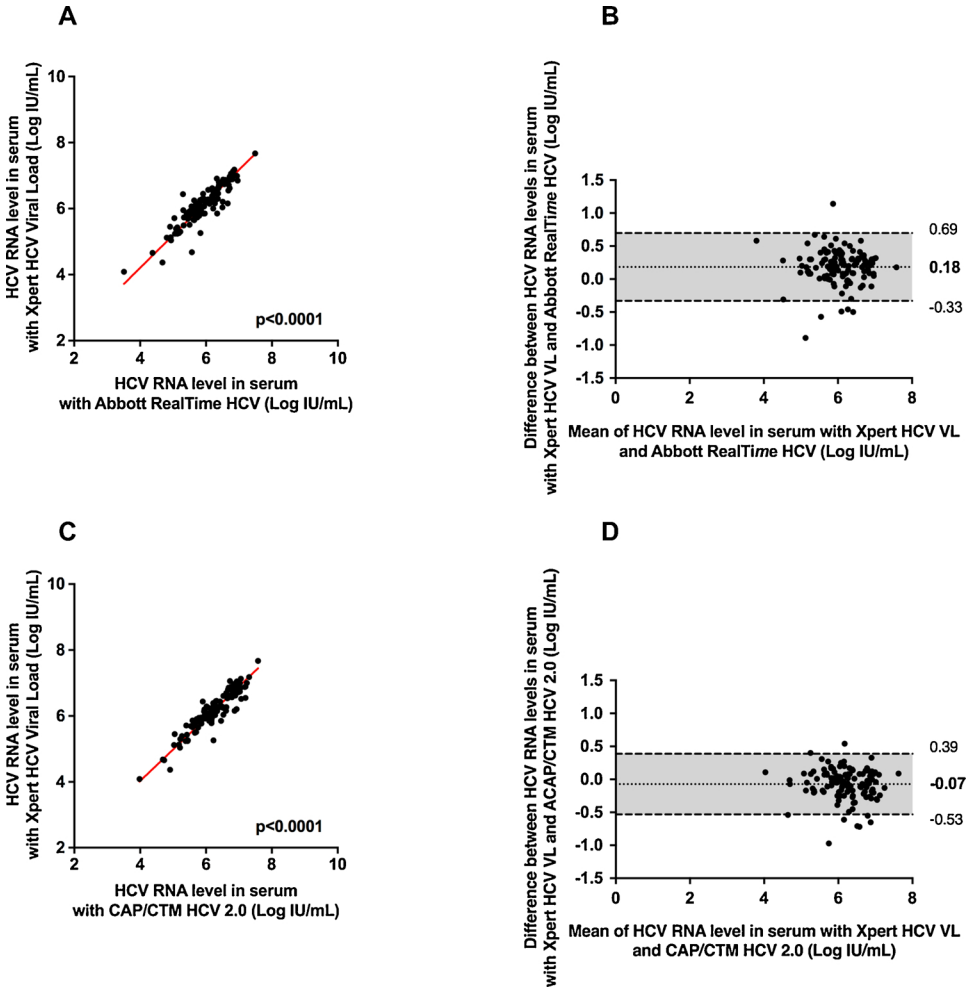

Fig. 1. Deming correlation and Bland-Altman plot analysis of HCV RNA levels measured by Xpert HCV Viral Load assay in 120 serum specimens containing HCV genotype 1 (n = 80), 2 (n = 8), 3 (n = 16), 4 (n = 14), 5a (n = 1) or 6 (n = 1). (A) Deming regression of HCV RNA levels measured by Xpert HCV Viral Load *versus* Abbott RealTi*m*e HCV, respectively. (B) Bland-Altman plot analysis of Xpert HCV Viral Load *versus* Abbott RealTi*m*e HCV. (C) Deming regression of HCV RNA levels measured by Xpert HCV Viral Load *versus* CAP/CTM HCV 2.0. (D) Bland-Altman plot analysis of Xpert HCV Viral Load *versus* CAP/CTM HCV 2.0.

Xpert HCV Viral Load assay to accurately detect and quantify HCV RNA in serum and in whole blood from DBS collected in patients infected with different HCV genotypes frequently encountered in clinical practice.

3. Study Design

3.1. Clinical specimens

Serum and whole blood specimens were obtained from patients recruited between September 2012 and November 2013 in the Departments of Hepatology and Gastroenterology of Henri Mondor University hospital and the Centre Hospitalier Intercommunal de Créteil. One hundred and twenty patients with chronic HCV infection (defined by the presence of both HCV antibodies and detectable HCV RNA) and 50 individuals who were HCV-negative (without detectable HCV antibodies and HCV RNA) were included. Patients with chronic HCV infection were infected with HCV genotype 1 in 80 cases (39 with subtype 1a, 38 with subtype 1b and 3 with another genotype 1 subtype), 8 were infected with genotype 2, 16 with genotype 3, 14 with genotype 4, 1 with genotype 5a and 1 with genotype 6.

Whole blood specimens were collected using the DBS technique. Briefly, a 50 µL specimen of venous whole blood collected in a tube containing ethylendiaminetetraacetic 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 one hour. Each dry DBS was then stored in an individual sealed plastic bag with a desiccant package at −80 °C until analysis.

The study followed the principles of good clinical practice and was approved by the ethic committee (Comité de Protection des Personnes - Ile-de-France IX, approval number: 12-006) in accordance with the Helsinki Declaration. All individuals gave written informed consent to their inclusion.

3.2. Performance of the study

Serum specimens were tested with three different HCV RNA detection and quantification assays, including CAP/CTM HCV v2.0, Abbott RealTi*m*e HCV and Xpert HCV Viral Load, whereas whole blood specimens were tested with the Xpert HCV Viral Load using two pre-extraction buffers including guanidinium thiocyanate-based buffer (lysis reagent) and 20 mM Tris-based buffer (pH8.6).

3.3. Laboratory measurements

In the CAP/CTM procedure, HCV RNA was extracted from 650 µL of serum by mean 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

Table 1
Sensitivity and specificity of the Xpert HCV Viral Load assay for HCV RNA quantification in whole blood collected on DBS compared to serum.

	Xpert HCV Viral Load (serum)		
	No. of quantifiable	No. of unquantifiable	No. of total
Xpert HCV Viral Load (whole blood)			
No. of quantifiable	119	0	119
No. of unquantifiable	0	50	50
No. of total	119		169

instructions. The data were analyzed with Amplilink software, version 3.3. In the *m*2000 procedure, HCV RNA was extracted from 500 µL of serum in the $m2000_{SP}$ automated extractor. The $m2000_{RT}$ device was then used for automated real-time PCR amplification and quantification of PCR products, according to the manufacturer's instructions. In the GeneXpert procedure, 1 000 µL of serum was transferred into the cartridge that contains all reagents needed for sample preparation, nucleic acid extraction, amplification and quantification of PCR products.

For the specimens from DBS, a punched disk with a 12 mm diameter was eluted into 1.5 mL of a pre-extraction buffer (lysis reagent or 20 mM Tris pH8.6) at 56 °C with gentle agitation for 15 min and centrifuged at 36 220*g* for 1 min before use, according to the protocol recently published [9]. We used 1 000 µL of pre-extraction supernatant to perform the Xpert HCV Viral Load assay.

3.4. Statistical analysis

Descriptive statistics are shown as mean values ± standard deviation (SD). Relationships between quantitative variables were studies by means of Deming or regression 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 < 0.05 were considered statistically significant. Results obtained for DBS specimens were not corrected for the hematocrit.

4. Results

4.1. Influence of the HCV genotype on HCV RNA quantification in serum

To assess the influence of the HCV genotype on HCV RNA quantification, serum samples from 120 untreated patients with chronic HCV infection due to genotype 1–6 were tested in parallel with Xpert HCV Viral Load, Abbott RealTi*m*e HCV and CAP/CTM HCV v2.0 assays. All of these samples fell within the dynamic range of quantification of the three assays. Fig. 1 shows the relationships between HCV RNA levels measured with Xpert HCV Viral Load and the two comparators. This figure includes Deming regression and Bland Altman plot analysis for pairwise comparisons of the assays.

As shown in Fig. 1, a significant relationship was found between HCV RNA levels measured in the same serum specimens with Xpert HCV Viral Load and Abbott RealTi*m*e HCV (Deming regression equation: Xpert HCV Viral Load = 0.99x Abbott HCV RealTi*m*e + 0.25; Fig. 1A) or CAP/CTM HCV v2.0 (Deming regression equation: Xpert HCV Viral Load = 0.96 x CAP/CTM HCV v2.0 + 0.21; Fig. 1C). A modest bias (0.18 ± 0.26 IU/mL) was observed between Xpert HCV Viral Load and Abbott RealTi*m*e HCV (Fig. 1B). A lower HCV RNA level was observed with Xpert HCV Viral Load than with Abbott RealTi*m*e HCV in 19 of the 120 samples (15.8%), with no relationship with the HCV RNA level or the HCV genotype. In 6 samples, the difference between HCV RNA levels measured with Xpert HCV Viral Load and Abbott RealTi*m*e HCV was above 1.96 times the bias, including 5 samples (one with genotype 1a, 2 with genotype 2, and 2 with genotype 4) that were lower and one genotype 1a sample that was higher with Xpert HCV Viral Load assay.

When the CAP/CTM HV v2.0 was used as a comparator, 69 out of 120 serum samples (57.5%) were slightly lower with Xpert HCV Viral Load (bias: −0.07 ± 0.23). In 9 samples, the difference between assays was greater than 1.96 times the bias, including 7 (3 with genotype 1a, one with genotype 2, one with genotype 3a and 2 with genotype 4) that were lower and 2 genotypes 1 that were higher with Xpert HCV Viral Load as compared to CAP/CTM HCV v2.0. The difference was less than 1.0 Log UI/mL in all cases (Fig. 1D).

4.2. HCV RNA detection and Influence of the HCV genotype on HCV RNA quantification in whole blood

Among 170 participants enrolled between September 2012 and November 2013, 169 had Xpert HCV Viral Load testing in whole blood from DBS and one (0.6%) had no valid result due to the internal control being out of range. The sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in whole blood specimens collected on DBS was 100% [95% confidence interval (CI), 96.9%–100%] and the specificity was 100% (95%CI, 92.9%–100%) (Table 1). The sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in whole blood specimens collected on DBS was 100% (95% CI, 96.9%–100%) and the specificity was 90.0% (95%CI, 78.6%–95.6%) (Table 2). Five samples demonstrated discrepant results: HCV RNA in whole blood from DBS was detected but below the limit of quantification (< 10 IU/mL) while it was not detected in serum by the with Xpert HCV Viral Load assay. The five samples except two were all found to be HCV RNA negative on retesting with the Xpert HCV Viral load assay. The specificity was similar when whole blood specimens were pre-extracted with Tris-based buffer (90.0%). A significant positive correlation was found between HCV RNA levels detected by the Xpert HCV RNA Viral Load assay in whole blood and serum specimens from the same patients ($r = 0.80$, $p < 0.001$), whatever the HCV genotype (Fig. 2A). As shown by Bland-Altman plot analysis, the mean difference in HCV RNA levels detected in whole blood specimens were 1.93 ± 0.38 Log IU (Fig. 2B). A substantial difference in HCV RNA levels was observed in whole blood specimens that were pre-extracted with Tris-based buffer. The mean difference in the HCV RNA level was 2.78 ± 0.55 Log IU (data not shown).

5. Discussion

Nucleic acid testing (NAT) are the reference method for detection and quantification of HCV RNA in clinical practice [3,4]. Among these techniques, the Abbott RealTi*m*e HCV and the Cobas TaqMan HCV assays are the most widely used tests worldwide, with a satisfactory clinical performance [5,6,10]. The main concern with these assays is the time required to complete full analysis. New generations of real-time assay platforms have been recently developed. These new platforms generate results in less than 2–3 hours [7,11–13]. Dried blood

Table 2
Sensitivity and specificity of the Xpert HCV Viral Load assay for HCV RNA detection in whole blood collected on DBS compared to serum.

	Xpert HCV Viral Load (serum)		
	No. of detectable	No. of undetectable	No. of total
Xpert HCV Viral Load (whole blood)			
No. of detectable	119	5	124
No. of undetectable	0	45	45
No. of total	119	50	169

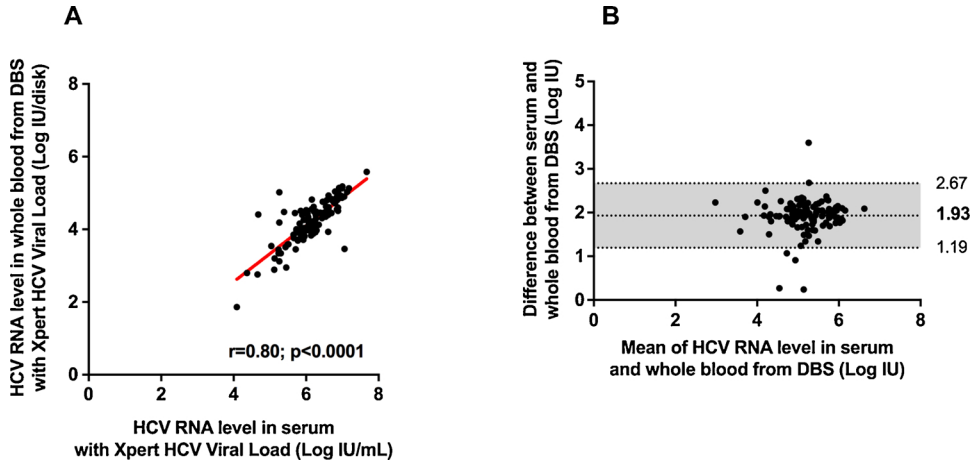

Fig. 2. (A) Linear regression and (B) Bland-Altman plot analysis of HCV RNA levels measured by Xpert HCV Viral Load assay in 120 paired serum and whole blood samples containing HCV genotype 1 (n = 80), 2 (n = 8), 3 (n = 16), 4 (n = 14), 5a (n = 1) or 6 (n = 1) using guanidinium thiocyanate-based buffer (lysis reagent).

spot collection is a promising tool for whole blood sampling in low- and middle-income settings as well as in marginalized populations with low access to health-care system. DBS offer the main advantage to store desiccated blood that can be easily transported to reference centers where state-of-the-art molecular are used. Therefore, DBS sampling has the potential to substantially simplify HCV diagnosis algorithm and to increase HCV testing and linkage to care in different settings.

In the present study, based on a relative large number of clinical specimens covering the different HCV genotypes encountered in HCV-infected patients, we showed the Xpert HCV Viral Load assay accurately quantifies HCV RNA level in serum and whole blood specimens. Its performance was comparable to that of two real-time PCR platforms frequently used in clinical practice, including the Abbott RealTime HCV and CAP/CTM HCV v2.0 assays [14]. Whole blood specimens collected on DBS can be confidently used for active HCV infection detection by means of standardized, commercially available methods, even though these methods were not validated for DBS use. Diagnostic companies have developed specific reagents for pre-extraction of nucleic acids from DBS. Both reagents (guanidinium thiocyanate- or Tris-based buffer) allowed to detect and quantify HCV RNA in all DBS. However, the HCV RNA levels measured in whole blood specimens from DBS were lower by about 2 Log than those in serum specimens. This difference could be attributed to the volume were used for HCV RNA extraction (500–1 000 µL serum compared to 50 µL whole blood eluted from DBS). Only a weak negative bias was observed by Bland Altmann analysis (−0.37 Log) when results from DBS were corrected for the hematocrit. However, this difference was smaller with the guanidinium thiocyanate-based buffer (lysis reagent) than that observed with the Tris-based buffer, suggesting that the use of appropriate pre-extraction buffer improved the performance of quantification. In addition, HCV RNA was detected in five DBS and two DBS after retesting, whereas serum was negative. These results were unexpected and may represent false-positive DBS. Similar findings were observed with the Hologic Aptima HCV Quant Dx assay, a TMA based assay [15]. Further studies assessing the specificity of Xpert HCV Viral Load on DBS are warranted.

This study has several limitations. The small proportion of samples containing genotypes 5 and 6 tested. This reflects the HCV genotype distribution in our area, where genotypes 1–4 and 2 are the most prevalent. However, a recent study suggested that the Xpert HCV Viral Load assay accurately quantified HCV RNA in genotype 6-infected patients in Cambodia [12]. The number of clinical specimens was relatively small (170 specimens). The number of samples with low viral load was small, that limits the ability to robustly assess the lower limit of detection. Finally, DBS samples was manufactured by spotting venous whole blood from clinical samples onto filter paper rather than samples obtained by fingerstick.

In conclusion, the present study showed that the newly developed real-time PCR-based Xpert HCV Viral Load assay accurately quantifies HCV RNA in serum samples as well as in whole blood samples collected on DBS from patients with chronic HCV infection. The Xpert HCV Viral Load assay can thus confidently be used to detect active HCV infection as recommended by the 2017 World Health Organization testing guidelines [8]. Whole blood specimen collection using the DBS technique appears to be a promising alternative to classical blood sampling. Its use will improve worldwide screening, diagnosis and access to HCV care.

Funding

This work was supported by the.National Agency for Research on AIDS and Viral Hepatitis (ANRS)

Ethical Approval

The study followed the principles of good clinical practice and was approved by the ethic committee (Comité de Protection des Personnes - Ile-de-France IX, approval number: 12-006) in accordance with the Helsinki Declaration.

References

[1] Polaris Observatory HCV. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study, Lancet Gastroenterol. Hepatol. 2 (2017) 161–176.
[2] Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet. 385 (2015) 117–171.
[3] 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. (69) (2018) 461–511.
[4] Panel A-IHG. Hepatitis C Guidance, Update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection, Clin. Infect. Dis. 2018 (67) (2018) 1477–1492.
[5] S. Chevaliez, M. Bouvier-Alias, J.M. Pawlotsky, Performance of the Abbott real-time PCR assay using m2000sp and m2000rt for hepatitis C virus RNA quantification, J. Clin. Microbiol. 47 (2009) 1726–1732.
[6] S. Chevaliez, M. Bouvier-Alias, C. Rodriguez, A. Soulier, J.D. Poveda, J.M. Pawlotsky, The Cobas AmpliPrep/Cobas TaqMan HCV test, version 2.0, real-time PCR assay accurately quantifies hepatitis C virus genotype 4 RNA, J. Clin. Microbiol. 51 (2013) 1078–1082.
[7] 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.
[8] M.E. Hellard, R. Chou, P. Easterbrook, WHO guidelines on testing for hepatitis B and C - meeting targets for testing, BMC Infect. Dis. 17 (2017) 703.
[9] N. Gous, L. Scott, L. Berrie, W. Stevens, Options to expand HIV viral load testing in South Africa: evaluation of the GeneXpert(R) HIV-1 viral load assay, PLoS One 11

(2016) e0168244.
[10] H. Zitzer, G. Heilek, K. Truchon, S. Susser, J. Vermehren, D. Sizmann, et al., Second-generation Cobas AmpliPrep/Cobas TaqMan HCV quantitative test for viral load monitoring: a novel dual-probe assay design, J. Clin. Microbiol. 51 (2013) 571–577.
[11] E. Gupta, P. Agarwala, G. Kumar, R. Maiwall, S.K. Sarin, Point -of -care testing (POCT) in molecular diagnostics: performance evaluation of GeneXpert HCV RNA test in diagnosing and monitoring of HCV infection, J. Clin. Virol. 88 (2017) 46–51.
[12] M. Iwamoto, A. Calzia, A. Dublineau, F. Rouet, J. Nouhin, S. Yann, et al., Field evaluation of GeneXpert((R)) (Cepheid) HCV performance for RNA quantification in a genotype 1 and 6 predominant patient population in Cambodia, J. Viral Hepat. 26 (2019) 38–47.
[13] L. Izquierdo, C. Pregermain, C. Hottelet, G. Decombe, A.M. Roque-Afonso, Clinical performance of the VERIS HCV assay for hepatitis C virus RNA quantification, J. Clin. Virol. 93 (2017) 1–7.
[14] 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 (2016) 1087–10895.
[15] B. Catlett, A. Carrera, M. Starr, T.L. Applegate, P. Lowe, J. Grebely, et al., Performance evaluation of the Hologic Aptima HCV Quant Dx assay for detection of HCV RNA from dried blood spots, J. Clin. Virol. 112 (2019) 40–44.