Journal of Clinical Virology 97 (2017) 18–21

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Short communication

Hepatitis C Virus (HCV) RNA screening and sequencing using dry plasma spots

Anna Maria Geretti[a,*], Simon King[a], Kwabena Adjei-Asante[a,b], Lambert Tetteh Appiah[b], Dorcas Ohui Owusu[b], Fred Stephen Sarfo[b], David Chadwick[c], Richard Odame Phillips[b,d], Apostolos Beloukas[a]

[a] Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom
[b] Komfo Anokye Teaching Hospital University, Kumasi, Ghana
[c] James Cook University Hospital, Middlesbrough, United Kingdom
[d] Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ARTICLE INFO

Keywords:
HCV RNA
Sequencing
Dried plasma spots
Sub-Saharan Africa
Epidemiology

ABSTRACT

Background: HCV RNA screening of large sample repositories provides data on HCV epidemic patterns that may help guide control policies. In resource-limited settings, shipment of frozen samples to molecular laboratory facilities and testing of individual samples may be prohibitively expensive.
Objective: Our aim was to detect and sequence HCV RNA in a large HIV-positive cohort from Kumasi, Ghana, using pooled and individual dried plasma spots (DPS) produced from samples stored at −80 °C.
Study design: In the validation phase, replicate DPS were prepared with six dilutions (500–10,000 IU/ml) of the 4th International Standard for HCV and tested in three independent experiments. In the testing phase, DPS prepared with plasma samples from 875 HIV-positive subjects were pooled for screening, followed by testing of individual DPS of positive pools. Input from individual DPS was two 6 mm punches; pools comprised two punches from each of five DPS. Genotypes were determined by Sanger sequencing of HCV core and NS5B.
Results: With the dilution series, sensitivity of HCV RNA detection was ≥2500 IU/ml. Replicate DPS gave intra-assay and inter-assay coefficients of variation ≤1.4%. With the stored samples, HCV RNA was detected in 5/175 DPS pools and in one DPS from each positive pool, yielding a HCV RNA prevalence of 5/875 (0.57%; 95% confidence interval 0.07-1.07%). The five samples were sequenced as HCV genotypes 2l and 2r.
Discussion: DPS allowed reproducible HCV RNA detection, and pooling effectively contained the cost and labour of screening a previously untested, low-prevalence cohort. DPS were also suitable for HCV sequencing.

1. Background

Approximately 50% of HCV carriers worldwide remain undiagnosed [1]. There have been calls for expanded HCV testing, based upon the proven efficacy of treatment in reducing morbidity and mortality and preventing transmission [2–4]. Recommendations for HCV screening are based on testing needs in different populations [2], which must be informed by epidemiological data.

The epidemiology of HCV infection in sub-Saharan Africa (SSA) is poorly defined. Studies have relied largely on antibody testing without confirmation, raising doubts about the reliability of prevalence estimates [5–9]. HCV antibody assays show variable performance in the African setting, requiring confirmatory algorithms that are not easy to implement with limited infrastructure [7–9].

The use of dried sample spots for virus detection offers the advantage of nucleic acid stability at room temperature, low biohazard risk, and ease of sample storage and transport [4,10–20]. Previous studies have reported on HCV RNA detection in spots prepared with venous or capillary blood [14,15,21] or with serum in one study [10]. There are circumstances where frozen plasma is available (e.g., from HIV monitoring), which may be accessed for HCV screening. Where prevalence is expected to be low, sample pooling reduces cost and labour, as demonstrated for HIV RNA testing using pooled dry blood spots (DBS) or dry plasma spots (DPS) [16,20].

2. Objectives

Our aim was to investigate the use of DPS to detect and sequence

* Corresponding author at: Department of Clinical Infection, Microbiology and Immunology Institute of Infection and Global Health University of Liverpool, 8 West Derby Street, L69 7BE, United Kingdom.
E-mail addresses: geretti@liverpool.ac.uk, a.beloukas@liverpool.ac.uk (A. Beloukas).

http://dx.doi.org/10.1016/j.jcv.2017.10.012
Received 15 February 2017; Received in revised form 1 October 2017; Accepted 20 October 2017

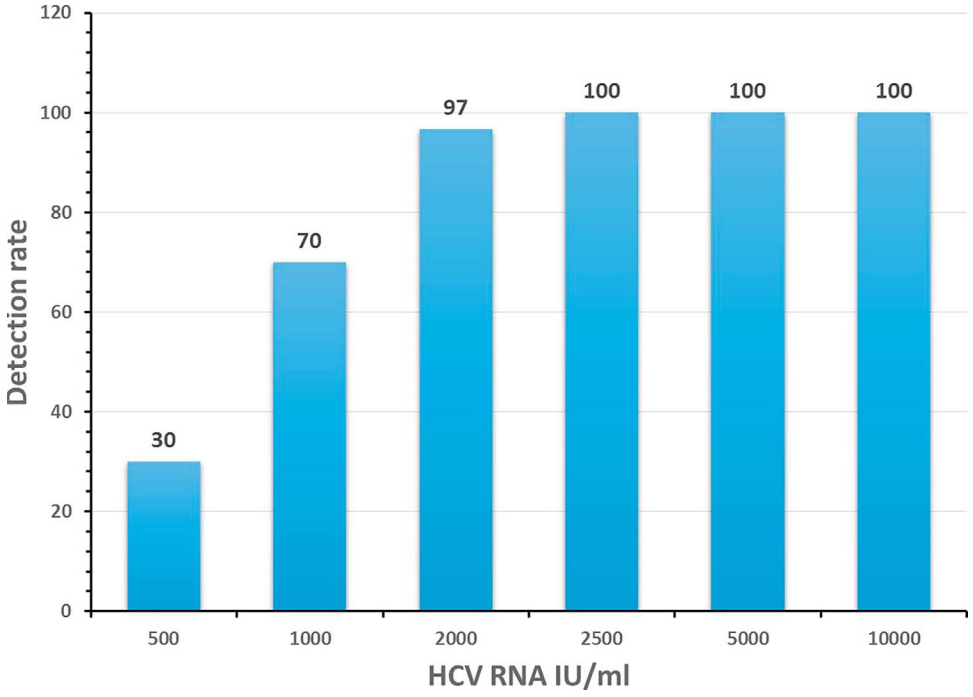

Fig. 1. HCV RNA detection rate expressed as the total proportion of HCV RNA positive dried plasma spots (DPS) across three independent experiments, each including 5 to 10 DPS replicates per dilution. DPS were prepared with dilutions of the 4th International Standard for HCV RNA (HCV genotype 1a). The total number of replicates detected and tested for each dilution was 15/15 at 10,000 IU/ml, 15/15 at 5000 IU/ml, 30/30 at 2500 IU/ml, 29/30 at 2000 IU/ml, 21/30 at 1000 IU/ml, and 9/30 at 500 IU/ml. The intra-assay coefficient of variation ranged from 0.7% to 1.3%; the mean inter-assay coefficient of variation across the three experiments was 1.4% (SD 0.5).

HCV RNA in a large sample repository obtained from consecutive patients receiving HIV care at the Komfo Anokye Teaching Hospital in Kumasi, Ghana.

3. Study design and results

3.1. DPS preparation

Each 12 mm disc of Protein Saver 903 cards (Whatman, Maidstone, UK) was spotted with 50 µl of sample. After drying at room temperature (20–25 °C) for 12–48 h, cards were sealed individually in plastic bags containing desiccant sachets, and stored at room temperature for no longer than two weeks prior to transfer to −80 °C. DPS were brought to room temperature prior to elution.

3.2. HCV RNA detection and quantification

From each DPS, two 6 mm punches were eluted for 2 h at room temperature with gentle agitation in 3 ml NucliSENS lysis buffer (bioMérieux, Boxtel, Netherlands) containing 40 µl Proteinase K (Qiagen, Hilden, Germany) and the internal control Phocine Distemper Virus (PDV). After incubation at 56 °C for 30 min and 100 °C for 30 min, the elute was loaded on the EasyMAG extractor (bioMérieux) (Specific B protocol; 60 µl final elute). The real-time PCR was described previously [9]. It targets a 90 bp region in HCV 5′-UTR and with plasma (1 ml) shows 100% HCV RNA detection at 110 IU/ml. Positive control DPS prepared with six dilutions (500–10,000 IU/ml) of the 4th International Standard for Hepatitis C Virus (Gt1α; 06/102,NIBSC, Potters-Bar, UK) in basematrix (Seracare,Milford, MA, USA) were tested individually in three independent experiments (total of 15–30 replicates per dilution).

3.3. Kumasi cohort

This comprised 875 consecutive subjects that underwent sampling in 2010 and tested negative for hepatitis B surface antigen (HBsAg). Plasma separated from venous EDTA blood within two hours of collection was stored at −80 °C prior to DPS preparation. Dried DPS were shipped to the UK at room temperature, and stored at −80 °C prior to pooled screening as described above, followed by retesting of individual DPS from positive pools.

3.4. Sequencing

In validation experiments, none of the extracts obtained with EasyMAG yielded a sequence. HCV RNA from positive DPS was instead extracted using the Qiagen Viral RNA Mini Kit (Qiagen), extending the incubation time from 10 min to 30 min, prior to Sanger sequencing, which was performed as described previously [9].

4. Results

4.1. Assay performance using DPS

HCV RNA detection was 100% at ≥2500 IU/ml (Fig. 1). Experiments were run to optimise the size of the pools. The final protocol consisted of pooling two punches from each of five DPS (total of 10 punches) to be eluted together in 3 ml of buffer without risk of drying. To validate the approach, DPS prepared with HCV RNA negative plasma were mixed at a ratio of 4:1 with positive control DPS and tested in three independent experiments. This confirmed the assay performance observed with individual DPS. All validation experiments were run under conditions that reproduced those of clinical DPS.

4.2. HCV RNA detection and sequencing

HCV RNA was detected in 5/175 pools and in one DPS from each positive pool. HCV RNA prevalence was 5/875 (0.57%; 95% confidence interval, CI 0.07%-1.07%). HCV core (403 bp) and NS5 B (380 bp) sequences obtained from HCV RNA positive DPS were characterised phylogenetically as genotype 2 (Fig. 2) [9,22].

5. Discussion

A recent systematic review reported encouraging data on the use of DBS for HCV RNA detection and sequencing in resource-limited settings [16]. Here we show that DPS enable transport to centralised testing facilities without the requirement for refrigeration or dry ice, allow

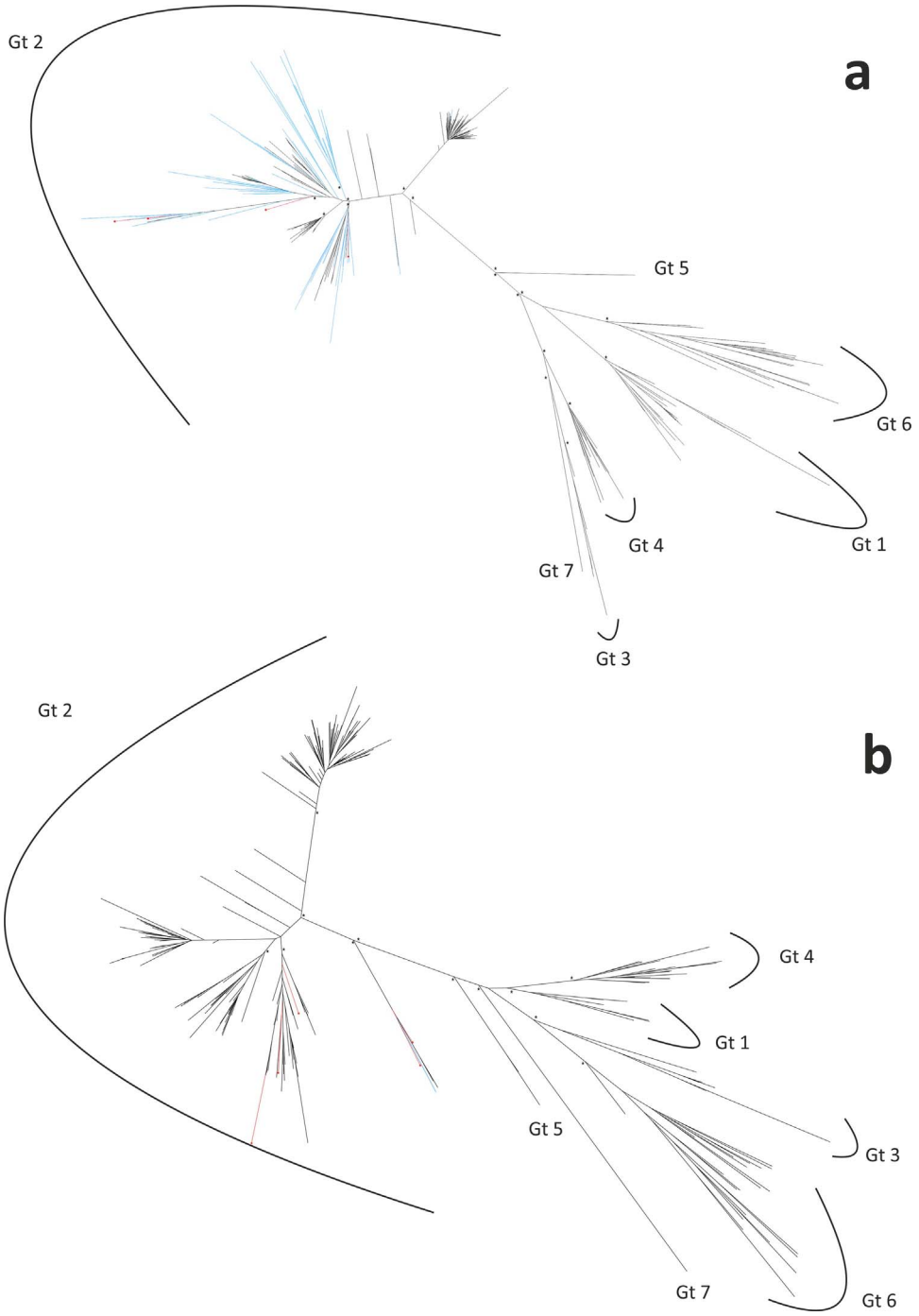

Fig. 2. Distribution of HCV genotypes determined from HCV NS5 B (a) and HCV core (b) sequences recovered from HCV RNA positive DPS and analysed phylogenetically as previously described [22]. The Ghanaian sequences recovered from DPS are in red (GeneBank accession numbers: KJ642631-KJ642634 for NS5 B sequences and KJ642622-KJ642626 for core sequences). Other NS5 B genotype 2 sequences previously reported from Ghana are in light blue [7,9,30]; global reference sequences from the HCV Los Alamos database are in black (Genebank accession numbers: Supplementary Table 1). Bootstrap values ≥85% are indicated with an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reproducible HCV RNA detection, and under appropriate conditions can be used successfully for HCV sequencing.

We used two 6 mm punches from each DPS and eluted them in 3 ml of lysis buffer. Use of larger elution volumes would have caused loss of sensitivity, as shown previously for HIV RNA [20]. Pooling samples is preferred for large-scale screening where prevalence is low and most samples are expected to test negative [9,16,20]. Ten punches were eluted together in the same buffer volume without drying, and eluting together two punches from each of five DPS substantially reduced the number of tests required to screen the large repository. The sensitivity of HCV RNA detection was ≥2500 IU/ml, with excellent intra- and inter-assay reproducibility. The strategy was therefore suitable for screening an undiagnosed, low-prevalence population in a two-step approach.

The sample repository excluded HBsAg-positive patients, as these were recruited for a different study [23]. The population was otherwise unselected and comprised consecutive adults attending for out-patient HIV care. HCV RNA prevalence was 0.57%(95% CI 0.07-1.07%), which is in agreement with the prevalence previously reported(0.6%; 95% CI 0.0-1.8%) in HIV-positive/HBsAg-negative subjects from the same centre [9]. Our prevalence estimate is also in line with HCV seroprevalence data (with or without HCV RNA testing) obtained with conventional sampling of urban populations (predominantly blood donors) in the Ashanti region of Ghana [7,8,24].

Using DPS for HIV RNA detection generally shows good correlation with plasma when allowing for the reduced sensitivity that results from a smaller input in DPS testing [11,25–27]. Storage conditions may impact on detection rates of low-level HIV RNA [28,29] and by parallel

HCV RNA. Some studies observed a deterioration of HCV RNA in DBS stored at room temperature, while others failed to detect such deterioration [14]. With dried serum, HCV RNA was previously shown to be stable for four weeks at room temperature; although a decay in viral titres occurred, this did not result in loss of virus detection [10]. Our DPS were kept at room temperature for up to two weeks in a room with air conditioning and low humidity, which should have prevented significant HCV RNA decay [28,29]. Nonetheless, it is possible that DPS with low viral load tested falsely negative, for instance in very recent or clearing infections.

Previous studies have employed DBS for HCV RNA sequencing and DPS for HIV RNA sequencing [14,17,26,28,29]. We were able to recover HCV sequences from all HCV RNA positive samples, although this required a different extraction method. The presence of synthetic poly-A RNA-carrier during the lysis step may have improved performance with the Qiagen spin column, particularly with long amplicons. The sequences were assigned phylogenetically to genotype 2, subtypes 2l and 2r. Clustering was observed with previously reported sequences from the same setting (Fig. 2), and the diversity was consistent with Ghana being the origin of HCV genotype 2 [8,9,30,31].

Competing interests

AMG is employed by the University of Liverpool and by Roche Pharma Research and Early Discovery (pRED). Roche pRED was not involved in the study.

Ethical approval

The study conformed to the standards of the Helsinki Declaration and was approved by the Committee on Human Research Publications and Ethics at the Kwame Nkrumah University of Science and Technology of Kumasi, Ghana. Written consent was obtained from all participants.

Acknowledgement

This study was funded by the Leverhulme-Royal Society Africa Award.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2017.10.012.

References

[1] A.P. Thrift, H.B. El-Serag, F. Kanwal, Global epidemiology and burden of HCV infection and HCV-related disease, Nat. Rev. Gastroenterol. Hepatol. 14 (2017) 122–132.
[2] American Association for the Study of Liver Disease, HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C, (2017) Available at: http://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance_April_12_2017_b.pdf (Accessed Oct 2017).
[3] European Association for the Study of the Liver, Recommendations on Treatment of Hepatitis C, (2016) Available at: http://www.easl.eu/medias/cpg/HCV2016/English-report.pdf (Accessed Jan 2017).
[4] 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) 1087–1095.
[5] R.E. Barth, Q. Huijgen, J. Taljaard, A.I. Hoepelman, Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis, Int. J. Infect. Dis. 14 (2010) e1024–31.
[6] J. Riou, M. Ait Ahmed, A. Blake, S. Vozlinsky, S. Brichler, S. Eholie, et al., Hepatitis C virus seroprevalence in adults in Africa: a systematic review and meta-analysis, J. Viral Hepat. 23 (2016) 244–255.
[7] W. Ampofo, N. Nii-Trebi, J. Ansah, K. Abe, H. Naito, S. Aidoo, et al., Prevalence of blood-borne infectious diseases in blood donors in Ghana, J. Clin. Microbiol. 40 (2002) 3523–3525.
[8] D. Candotti, J. Temple, F. Sarkodie, J.P. Allain, Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa, J. Virol. 77 (2003) 7914–7923.
[9] S. King, K. Adjei-Asante, L. Appiah, D. Adinku, A. Beloukas, M. Atkins, et al., Antibody screening tests variably overestimate the prevalence of hepatitis C virus infection among HIV-infected adults in Ghana, J. Viral Hepat. 22 (2015) 461–468.
[10] K. Abe, Konomi N. Hepatitis, C virus RNA in dried serum spotted onto filter paper is stable at room temperature, J. Clin. Microbiol. 36 (1998) 3070–3072.
[11] W. Ayele, R. Schuurman, T. Messele, W. Dorigo-Zetsma, Y. Mengistu, J. Goudsmit, et al., Use of dried spots of whole blood, plasma, and mother's milk collected on filter paper for measurement of human immunodeficiency virus type 1 burden, J. Clin. Microbiol. 45 (2007) 891–896.
[12] S.N. Balinda, P. Ondoa, E.A. Obuku, A. Kliphuis, I. Egau, M. Bronze, et al., Clinical evaluation of an affordable qualitative viral failure assay for HIV using dried blood spots in Uganda, PLoS One 11 (2016) e0145110.
[13] D. Brambilla, C. Jennings, G. Aldrovandi, J. Bremer, A.M. Comeau, S.A. Cassol, et al., Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability, J. Clin. Microbiol. 41 (2003) 1888–1893.
[14] J. Greenman, T. Roberts, J. Cohn, L. Messac, Dried blood spot in the genotyping, quantification and storage of HCV RNA: a systematic literature review, J. Viral Hepat. 22 (2015) 353–361.
[15] B.L. Marques, M.P. do Espirito-Santo, V.A. Marques, J.C. Miguel, E.F. da Silva, C.A. Villela-Nogueira, et al., Evaluation of dried blood spot samples for hepatitis C virus detection and quantification, J. Clin. Virol. 82 (2016) 139–144.
[16] P. Pannus, E. Fajardo, C. Metcalf, R.M. Coulborn, L.T. Duran, H. Bygrave, et al., Pooled HIV-1 viral load testing using dried blood spots to reduce the cost of monitoring antiretroviral treatment in a resource-limited setting, J. Acquir. Immune Defic. Syndr. 64 (2013) 134–137.
[17] C.M. Parry, N. Parkin, K. Diallo, S. Mwebaza, R. Batamwita, J. DeVos, et al., Field study of dried blood spot specimens for HIV-1 drug resistance genotyping, J. Clin. Microbiol. 52 (2014) 2868–2875.
[18] I.J. Snijdewind, J.J. van Kampen, P.L. Fraaij, M.E. van der Ende, A.D. Osterhaus, R.A. Gruters, Current and future applications of dried blood spots in viral disease management, Antiviral Res. 93 (2012) 309–321.
[19] K. Stene-Johansen, N. Yaqoob, J. Overbo, H. Aberra, H. Desalegn, N. Berhe, et al., Dry blood spots: a reliable method for measurement of hepatitis B viral load in resource-limited settings, PLoS One 11 (2016) e0166201.
[20] G.U. van Zyl, W. Preiser, S. Potschka, A.T. Lundershausen, R. Haubrich, D. Smith, Pooling strategies to reduce the cost of HIV-1 RNA load monitoring in a resource-limited setting, Clin. Infect. Dis. 52 (2011) 264–270.
[21] A. Tejada-Strop, J. Drobeniuc, T. Mixson-Hayden, J.C. Forbi, N.T. Le, L. Li, et al., Disparate detection outcomes for anti-HCV IgG and HCV RNA in dried blood spots, J. Virol. Methods 212 (2015) 66–70.
[22] A. Beloukas, S. King, K. Childs, A. Papadimitropoulos, M. Hopkins, M. Atkins, et al., Detection of the NS3 Q80 K polymorphism by Sanger and deep sequencing in hepatitis C virus genotype 1a strains in the UK, Clin. Microbiol. Infect. 21 (2015) 1033–1039.
[23] A.J. Stockdale, R.O. Phillips, A. Beloukas, L.T. Appiah, D. Chadwick, S. Bhagani, et al., Liver fibrosis by transient elastography and virologic outcomes after introduction of tenofovir in lamivudine-experienced adults with HIV and hepatitis B virus coinfection in Ghana, Clin. Infect. Dis. 61 (2015) 883–891.
[24] A.A. Agyeman, R. Ofori-Asenso, A. Mprah, G. Ashiagbor, Epidemiology of hepatitis C virus in Ghana: a systematic review and meta-analysis, BMC Infect. Dis. 16 (2016) 391.
[25] M. Andreotti, M. Pirillo, G. Guidotti, S. Ceffa, G. Paturzo, P. Germano, et al., Correlation between HIV-1 viral load quantification in plasma, dried blood spots, and dried plasma spots using the Roche COBAS Taqman assay, J. Clin. Virol. 47 (2010) 4–7.
[26] R.L. Hamers, P.W. Smit, W. Stevens, R. Schuurman, T.F. Rinke de Wit, Dried fluid spots for HIV type-1 viral load and resistance genotyping: a systematic review, Antivir. Ther. 14 (2009) 619–629.
[27] S. Sawadogo, A. Shiningavamwe, J. Chang, A.D. Maher, G. Zhang, C. Yang, et al., Limited utility of dried-blood- and plasma spot-based screening for antiretroviral treatment failure with Cobas Ampliprep/TaqMan HIV-1 version 2.0, J. Clin. Microbiol. 52 (2014) 3878–3883.
[28] J.G. Garcia-Lerma, A. McNulty, C. Jennings, D. Huang, W. Heneine, J.W. Bremer, Rapid decline in the efficiency of HIV drug resistance genotyping from dried blood spots (DBS) and dried plasma spots (DPS) stored at 37 degrees C and high humidity, J. Antimicrob. Chemother. 64 (2009) 33–36.
[29] M. Monleau, C. Butel, E. Delaporte, F. Boillot, M. Peeters, Effect of storage conditions of dried plasma and blood spots on HIV-1 RNA quantification and PCR amplification for drug resistance genotyping, J. Antimicrob. Chemother. 65 (2010) 1562–1566.
[30] J.E. Layden, R.O. Phillips, S. Owusu-Ofori, F.S. Sarfo, S. Kliethermes, N. Mora, et al., High frequency of active HCV infection among seropositive cases in west Africa and evidence for multiple transmission pathways, Clin. Infect. Dis. 60 (2015) 1033–1041.
[31] M.A. Purdy, J.C. Forbi, A. Sue, J.E. Layden, W.M. Switzer, O.K. Opare-Sem, et al., A re-evaluation of the origin of hepatitis C virus genotype 2 in West Africa, J. Gen. Virol. 96 (2015) 2157–2164.