Quantification of HIV-RNA from dried blood spots using the Siemens VERSANT® HIV-1 RNA (kPCR) assay

Maria Franca Pirillo1, Patricia Recordon-Pinson2, Mauro Andreotti1, Maria Grazia Mancini1, Roberta Amici1 and Marina Giuliano1*

1Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Rome, Italy; 2Laboratory of Virology, Victor Segalen University, Bordeaux, France

*Corresponding author. Tel: +39-06-49903303; Fax: +39-06-49387199; E-mail: marina.giuliano@iss.it

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Objectives: Simplified methods for virological monitoring in resource-limited settings are increasingly needed. We evaluated the performance of the VERSANT® HIV-1 RNA (kPCR) assay for the determination of HIV-1 viral load from dried blood spots (DBS). Assay sensitivity and correlation with plasma quantification values were assessed.

Methods: A total of 98 DBS were prepared from fresh blood samples of HIV-infected patients. DBS were kept at room temperature for 6 weeks or 7 months before processing while the corresponding plasma samples were stored at −80°C. DBS were first pre-treated in a special DBS buffer. The DBS extracts and the plasma samples were then purified and amplified using the VERSANT assay reagents.

Results: In the first series of tests, performed after 6 weeks of storage, there was good correlation between quantification of viral load in plasma and in DBS (r=0.95, P<0.001). The detection rate in DBS was 100% when plasma levels were >1000 copies/mL. The sensitivity and specificity of the DBS assay were 88.2% [95% confidence interval (CI) 79.4–93.6] and 69.2% (95% CI 42.0–87.4), respectively. Using the 5000 copies/mL threshold (defining virological failure in resource-limited settings), both positive and negative predictive values were high (95.2% and 87.5%, respectively). After 7 months of storage there was a modest decrease in the detection rate and less significant correlations for samples with HIV-RNA <5000 copies/mL.

Conclusions: Quantification of HIV-RNA from DBS by the VERSANT automated sample preparation and detection method can be used to diagnose virological failure in HIV-positive patients.

Keywords: resource-limited settings, real-time PCR, automated assays

Introduction

Quantification of HIV-RNA from dried blood spots (DBS) has been evaluated in several studies using different methodologies.1,2 Overall, the results have shown that determination of viral load in DBS is well correlated to plasma levels, especially when HIV-RNA is above a certain threshold (generally ~3.5 log copies/mL), and its clinical utility to detect virological failure in resource-limited settings has been confirmed.3 Particularly good results have been obtained with new methodologies for HIV-RNA quantification based on real-time PCR technology.4–6

In the present study we had two objectives: (i) to evaluate the performance of the new real-time kPCR VERSANT® assay (Siemens Healthcare Diagnostics) in determining viral load from DBS; and (ii) to assess the stability over time of DBS stored at room temperature for viral load determination.

Methods

EDTA blood was collected from 100 HIV-1-infected patients attending the HIV Clinic at the Pellegrin Hospital, Bordeaux, France. EDTA blood was spotted onto each circle of Whatman 903 card and dried at room temperature. After drying, the cards were placed in individual Ziploc bags containing a desiccant and stored at room temperature. Plasma from the same samples was separated and stored at −80°C. Plasma samples (on dry ice) and DBS (at room temperature) were then shipped to Rome (Istituto Superiore di Sanità, Department of Therapeutic Research) where the samples were processed.

Processing of specimens

For DBS, one spot was excised from the paper card, incubated for 30 min at room temperature in 1.25 mL of a DBS-specific lysis buffer (Siemens Healthcare Diagnostics) on a bench-top shaker and 1.1 mL was then processed with the VERSANT Sample Preparation Module for automated
nucleic acid extraction, using a specific DBS protocol (minimum volume required 1 mL) developed and provided by Siemens. The DBS lysis buffer replaced the plasma lysis buffer in the automated nucleic acid extraction. Subsequently, the same amplification software and the same settings of the VERSANT HIV-1 RNA 1.0 (kPCR) assay were used. Dedicated calibrators and controls were prepared for the assay by diluting stock 8E5/LAV non-infectious virus in a whole blood haemolysate matrix and assigning values using the same standardization reference materials and procedures of the VERSANT HIV-1 RNA 1.0 assay. Calibrators and controls were stored frozen with the other amplification reagents and, 1 week before each experiment, freshly spotted on a Whatman 903 card, stored at −20°C and then processed as for the DBS samples. Plasma samples were processed according to the manufacturer’s instructions. The VERSANT HIV-1 RNA kPCR assay for plasma samples has a detection limit of 37 copies/mL (1.57 log10/mL).

Data analysis
All results obtained were converted to log10 values before performing statistical analysis. HIV-RNA values below the detection limit were assigned the value of 1.57 log10 copies/mL. The correlation between plasma and DBS results was determined by Pearson analysis. For concordance between assays, the Bland–Altman approach was used. With this method the differences between individual viral load results from plasma and spots are plotted against the mean of the two results. Stability was assessed by comparing the differences in log10 HIV-RNA levels between the two series of DBS assays using the Wilcoxon signed rank test.

Results
First series of tests
The first series of tests was performed 6 weeks after spotting and storage at room temperature. In two cases results could not be obtained from the plasma samples because of the lack of amplification of the internal control; thus paired results were available for 98 samples. HIV-RNA was detected in 85/98 (86.7%) of the plasma specimens and in 79/98 (80.6%) of the DBS samples (P = 0.33). When considering samples with detectable HIV-RNA with both methods we observed a strong correlation between measures of viral load in plasma and in the DBS (r = 0.95, P < 0.001) (Figure 1). Categorizing the samples according to the 5000 copies/mL threshold we found a highly significant correlation for samples >5000 copies/mL (r = 0.96, P < 0.001), while for samples below this threshold the correlation was less significant (r = 0.38, P = 0.048).

In 84/98 cases (85.7%) there was agreement between detectable and undetectable levels of HIV-RNA. In 10 cases HIV-RNA was not detectable in DBS, while measurable levels were present in plasma (56–597 copies/mL); 4 samples with undetectable viral load in plasma had values between 162 and 514 copies/mL from DBS. The sensitivity and specificity of the assay were 88.2% [95% confidence interval (CI) 79.4–93.6] and 69.2 (95% CI 42.0–87.4), respectively (Table 1). Using the 5000 copies threshold, sensitivity and specificity were 85.1% and 96.1%, respectively, and both positive and negative predictive values were high (Table 2).

Overall, viral load values in DBS tended to be lower than in plasma, with a mean (SD) difference of 0.23 log (0.36) and differed from those in plasma by <0.5 log unit in 82.7% of the cases and by <1 log unit in 98.7% of the samples. A total of 20 samples (20.4%) had higher HIV-RNA from DBS than from plasma: 12/51 (23.5%) samples with plasma HIV-RNA <5000 copies/mL and 2/47 (4.3%) samples with plasma HIV-RNA >5000 copies/mL (P = 0.008).

The Bland–Altman analysis (Figure 1) performed on samples with detectable viral loads with both methods (n = 75) showed that all but five samples were within the 1.96 SD limits, showing good agreement between the two methods.

Second series of tests
The second series of tests was performed after 7 months of storage at room temperature (between 20 and 28°C). HIV-RNA was detected in DBS in 68/85 (80%) samples with plasma viral load above the threshold, compared with 79/85 (93%) of the first set of assays (P = 0.02).

The HIV-RNA results obtained with DBS in the second series (considering only samples with detectable HIV-RNA with both
The VERSANT HIV-RNA 1.0 (kPCR) is a sensitive, real-time automated assay that has been recently introduced in clinical use. Its use to determine viral load from filter paper has not been previously demonstrated.

In the present study we have shown that the system is highly sensitive (all samples with >800 copies/mL were detected) and yielded results highly correlated with those obtained from plasma.

For samples with HIV-RNA plasma values ≥5000 copies/mL, which defines virological failure according to the recent WHO guidelines for resource-limited settings, both positive and negative predictive values were very high. However, although virological failure is defined by the 5000 copies/mL threshold, the availability of a sensitive assay is crucial to identify virological failure in a timely manner. In our study the highest plasma RNA value at which DBS RNA was not detected was 597 copies/mL.

Previous studies have suggested that the presence of pro-viral HIV-DNA could affect the results obtained from DBS. Vlijoen et al. in particular, testing DBS with and without DNase pre-treatment, concluded that a prior DNA treatment step was a prerequisite for accurate monitoring. In our study we found that ~80% of the HIV-RNA values obtained from DBS were lower than those obtained from plasma, although this proportion was lower in samples with <5000 copies/mL, suggesting that the possible DNA contamination may play a role, especially in samples with low levels of HIV-RNA. Overall however, our results were highly concordant with those obtained from plasma, suggesting that a further step in DBS processing could be avoided in order to make the procedure easier and feasible in resource-limited settings.

Other previous studies have evaluated the stability over time of DBS for viral load quantification. Some of them have shown significant stability over 3–12 months of storage at room temperature. Others have suggested slight to significant decreases. In our study, 7 months of storage at room temperature did actually cause a change in quantification, with a significantly higher mean difference between plasma and DBS HIV-RNA values. However, the most important differences were observed for the lowest viral loads (and indeed a higher variability for low copy samples was also observed in the first series), suggesting that the use of DBS stored for several months, although not recommended, could still be considered in specific circumstances to detect virological failure.

In conclusion, HIV-RNA quantified from DBS was strongly correlated with that measured in plasma. The detection rate in DBS was 100% when the plasma level was ≥3.0 log. Quantification of HIV-RNA from DBS by the VERSANT assay can be used to diagnose virological failure in patients with HIV.

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### Transparency declarations

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References


