Use of Reverse Transcription-PCR-Based Assays for Quantification of HIV-1 in Dried Blood Spots Requires Specific HIV-1 RNA Isolation for Monitoring of Antiretroviral Treatment Efficiency

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The paper published by the ANRS 12235 Study Group (1) showed that monitoring of “real-life” HIV-1 RNA viral load (VL) and drug resistance mutations (DRMs) in dried blood spot (DBS) specimens collected from patients in Africa and Asia was possible, but with some limitations, particularly for HIV-1 RNA quantification. A total of 382 DBS specimens were tested for VLs with two nucleic acid extraction methods: (i) the Abbott extraction protocol followed by the m2000rt RealTime HIV-1 reverse transcription (RT)-PCR test (Abbott) and (ii) the NucliSENS miniMag extraction system followed by either NucliSENS EasyQ HIV-1 v1.2 nucleic acid sequence-based amplification (NASBA) (bioMérieux) or generic HIV viral load (Biocentric/Agence Nationale de Recherches sur le SIDA et les hépatites virales) assays. Overall, a poor specificity was mentioned for the generic HIV viral load RT-PCR-based procedure, with 39.2% of specimens showing levels of ≥ 1,000 copies/ml in DBS but < 1,000 copies/ml in paired plasma specimens. With the Abbott RT-PCR technique, this discrepant pattern was less common (7.3%), whereas it was rare (4.2%) with the NucliSENS NASBA-based assay.

These discrepancies are explainable. When DBS specimens from patients receiving antiretroviral therapy (ART) are tested, careful attention should be paid to the risk of interference due to the presence of HIV DNA copies archived in white blood cells. Indeed, without RNA-specific extraction, HIV DNA may be co-extracted and coamplified by PCR, leading unavoidably to false-positive VL results (2), as well as to discordant DRMs in plasma versus DBS specimens. Due to the unpredictable HIV DNA concentrations in the blood of treated patients (3), the definition of a higher cutoff for HIV-1 RNA DBS quantification is unjustified.

Consequently, the specificity obtained for the NucliSENS assay in this study was high because the NucliSENS technology prevents in itself any amplification of HIV DNA, as recently published (4). For RT-PCR-based assays, specificity was better with a relatively RNA-specific extraction method like the Abbott extraction kit than with total nucleic acid (RNA plus DNA) extraction, such as with the NucliSENS technique (1). This explains why using NucliSENS extraction followed by the Biocentric assay gave very low specificity. In a previous work, we showed that the specificity of the “association” NucliSENS extraction/Biocentric test was even lower (~40%) but reached 100% when a prior DNase treatment step was introduced (5).

In summary, our group would like to emphasize the fact that specific RNA isolation from DBSs, with the Biocentric assay or any other RT-PCR kit, is a prerequisite for accurate routine VL monitoring of ART success in resource-limited settings. When one considers the diagnosis of HIV-1 infection in neonates, in whom HIV DNA and HIV RNA coamplification by PCR may increase the sensitivity of the detection of HIV infection (6), the context and purpose of testing are radically different from those with specimens taken from patients under ART. When RT-PCR-based assays are used for DBS specimens collected from treated patients, enzymatic pretreatments are efficient in overcoming the risk of HIV-DNA coextraction/coamplification, as already demonstrated. However, these methods remain poorly used because of the increased time and manipulation required. That is why research teams and companies have to develop more-specific RNA extraction procedures compatible with any RT-PCR kit if we want to improve the utility of DBS specimens under real-life conditions.

ACKNOWLEDGMENT

All authors are members of the AC12 Working Group, which corresponds to a network of virology laboratories working on viral quantification and resistance testing in resource-limited settings.

REFERENCES


