Short communication

Suitability of an open automated nucleic acid extractor for high-throughput plasma HIV-1 RNA quantitation in Gabon (Central Africa)

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Nucleic acid extraction using the open automated EZ1 (Qiagen) instrument, in combination with the Generic HIV Viral Load assay, gave highly concordant HIV-1 RNA viral load results among 181 Gabonese subjects infected with HIV-1, compared to those obtained when performing a manual extraction. Since people living with HIV-1 are being treated with antiretrovirals in Gabon, this automated extraction technique represents an excellent technical method for high-throughput monitoring of HIV-1 RNA viral load.

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Extraction of viral nucleic acids from clinical samples is a critical step in plasma human immunodeficiency virus type 1 (HIV-1) RNA viral load quantitation. In many laboratories from resource-limited countries, this step is unfortunately still performed manually, reducing the large-scale implementation of HIV-1 RNA viral load testing that is required urgently for monitoring the efficacy of highly active antiretroviral therapy (HAART). Indeed, in sub-Saharan Africa, there is an urgent need for high-throughput monitoring of HIV-1 RNA viral load where HIV prevalence rates are the highest and where subjects infected with HIV-1 and treated with HAART are more and more numerous. For instance, in Gabon, it is estimated that 63,000 more people are infected with HIV-1 (HIV-1 prevalence rate, ~6.0%) (Mintsa Ndong et al., 2009). Around 10,000 were receiving HAART by the end of 2009.

Recently, beside closed extractors commercialized by some manufacturers (Gueudin et al., 2007; Scott et al., 2009; Bourlet et al., 2010; Sizmann et al., 2010), other companies have developed open automated extractors utilizing innovative technology for the extraction/purification of nucleic acids from a variety of specimen types. Most extractors are able to read barcode, allow variable sample input volumes, and use self-contained pipetting systems coupled with UV-decontamination. They are easy-to-use and reduce considerably time and effort for extraction at a relatively affordable price. However, using these instruments requires a validation since their performance is not guaranteed for each virus species (Dundas et al., 2008), particularly in the case of viral quantitation techniques which must be accurate and precise.

This study, conducted in a Gabonese reference laboratory, evaluated the performance of the EZ1 Virus Mini Kit v2.0 using the EZ1 Advanced XL instrument for HIV-1 RNA extraction in plasma specimens, in comparison with a manual Qiagen technique. In both cases, HIV-1 RNA viral load measurements were then performed using the Generic HIV Viral Load assay (Biocentric, Bandol, France) which accurately amplifies non-B HIV-1 subtypes/CRFs (Rouet et al., 2007, 2010).

Between March 2010 and April 2011, whole blood specimens were collected by venipuncture (5 ml of EDTA-anticoagulated peripheral blood) from 181 patients infected with HIV-1 and followed-up in two ambulatory treatment centers located in Franceville and Koulamoutou (two cities in the South-East of Gabon). Specimens were shipped to the Retrovirology Laboratory of the Centre International de Recherches Médicales de Franceville (CIRMF), and processed within 6 h. Plasma was separated by centrifugation and frozen at −80 °C. RNA was first isolated from 200 μl of plasma using a Qiagen procedure (QiAamp Viral RNA Mini Kit, Qiagen, Courtaboeuf, France) (~3 € per an extraction). It was performed by at least four laboratory operators. A 0.2 ml
plasma extraction protocol was thereafter realized on the same specimens using the silica-based EZ1 Virus Mini Kit v2.0 (Qiagen, Courtaboeuf, France) in combination with the EZ1 advanced XL instrument (~6 hr per an extraction). This instrument has a capacity of 14 samples per run in 45 min. Paired extracted samples from both methods were then amplified and quantified using the Generic HIV Viral Load assay targeting a conserved region in the LTR gene. An external standard and a low positive control (LPC quantified at 6300 copies/ml, i.e., $3.8 \log_{10}$ copies/ml) (both included in the kit) were extracted together with clinical specimens, by using the manual or automated procedure, respectively. The extracted standard was diluted serially (10-fold) to concentrations from 6,300,000 to 630 copies/ml. With 0.2 ml of plasma, and by using the manual extraction protocol, this assay has a sensitivity threshold of 300 copies/ml (Rouet et al., 2007). All runs were done on the open IQ5 real-time PCR instrument (BioRad, Marnes-la-Coquette, France). Agreement in viral load values obtained between the different extraction methods was measured using the Spearman correlation coefficient and the Bland–Altman methods (Bland and Altman, 1995).

Whatever the extraction method used, the Ct values obtained for the 10-fold dilutions of the external standard and the LPC were very similar (data not shown). When testing the LPC with the automated extraction, the median value was $3.7 \log_{10}$ copies/ml (range, 3.4–3.9) for between run-assays. For clinical specimens, when using the manual Qiagen extraction kit, a total of 155 showed a positive ($\geq300$ copies/ml) HIV-1 RNA result. Among these, 144 (144/155, 92.9%) were also HIV-1 RNA positive ($\geq300$ copies/ml) with the automated extraction. For the remaining 11 samples showing quantifiable results with the manual extraction method only, HIV-1 RNA values were low (between 2.48 and 3.36 $\log_{10}$/ml). When considering samples with concordantly positive HIV-1 RNA values with both extraction methods, HIV-1 RNA levels were highly correlated (Fig. 1A) ($R = 0.8280; P < 0.001$). The overall mean difference ($d$) in the HIV-1 RNA values obtained with the manual and automated protocols was $0.11 \log_{10}$/ml. As shown in Fig. 1B, 138 (out of 144, 95.8%) fell into the 95% confidence interval (CI) of the mean difference. Fig. 1B also suggested that the majority of viral load values ($<4.0 \log_{10}$/ml) generated slightly higher values after manual extraction whereas viral load levels ($\geq4.0 \log_{10}$/ml) were distributed more homogeneously whatever the extraction method used. Finally, among 26 specimens found negative for HIV-1 RNA with the manual extraction protocol, three (3/26, 11.5%) were found positive using the EZ1 instrument (viral load values, 3.24, 3.69 and 4.07 $\log_{10}$/ml) whereas the remaining 23 samples were undetectable.

To our knowledge, this is the first study demonstrating that an open automated extraction technique/instrument can provide a higher throughput contribution to the monitoring of HAART effectiveness in a resource-limited country, compared to a manual extraction method. In our experience, some samples with low viremia by using the manual extraction were found undetectable with the EZ1 machine, suggesting that low viral load values may be more readily quantified after manual extraction. Similar results have been reported by other researchers using the Abbott extraction (manual versus automated)/quantitation systems (Scott et al., 2011). Nevertheless, we also obtained few specimens being positive with the automated procedure only. This suggests that automated protocols may prevent false negative results which may be generated with manual extraction techniques due to human errors. Since May 2011, we have been using this automated extraction routinely in our busy molecular diagnostic laboratory for the analysis of all plasma specimens from many subjects infected with HIV-1 and treated with antiretrovirals and followed-up in different ambulatory treatment centers from Gabon. This allows individuals with virological failure ($\geq5000$ copies/ml) to be more quickly identified, strengthening virological patient management required in sub-Saharan Africa.

Conflict of interest

We declare that we have no conflict of interest.

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