Evaluation of filter paper transfer of whole blood and plasma samples for the HIV-RNA quantification in HIV-infected subjects on antiretroviral therapy in Uganda.

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ABSTRACT

Whole blood (n=306) and plasma (n=218) samples from 402 Ugandan subjects established on antiretroviral therapy were spotted onto filter paper and transported to Europe for viral load testing. These results were compared to those obtained from the same patient sample using a gold standard assay in Kampala. Plasma filter paper results showed excellent agreement with the gold standard whereas whole blood filter paper results yielded a large number of false positive detectable viral loads.

INTRODUCTION & OBJECTIVES

Over 1 million HIV-infected African individuals are now established on antiretroviral therapy (ART) [1]. The vast majority of these treated patients do not undergo routine HIV-RNA quantification due to the cost and complexity of providing reliable assays in resource limited settings [2]. Filter paper transfer (FPT) of dried whole blood spots to central laboratories is currently being used in Africa for the diagnosis of neonatal HIV (HIV-DNA)[3]. FPT of whole blood and plasma spots demonstrated reliability for HIV-RNA quantification ('viral load') in untreated HIV infected adults both in the developed world and in Africa [4,5]. There are no published data describing the performance of FPT (of whole blood or plasma) for HIV-RNA quantification in ART- treated subjects in Africa. If shown to be reliable for this purpose then FPT could enormously simplify virological monitoring, and potentially reduce the associated costs, in Africa and other resource limited settings.

We aimed to investigate the feasibility and accuracy of using FPT viral load quantification compared with the gold standard plasma based assay.

METHODS

Blood samples obtained from 402 Ugandan patients established on ART underwent viral load testing both at the clinic in Uganda and after FPT of whole blood and/or plasma samples to a European laboratory. Local viral load testing was performed on liquid plasma using the Roche standard reverse transcriptase polymerase chain reaction (PCR) based assay (Amplicor HIV-1 Monitor test, v1.5). Whole blood and/or plasma were collected simultaneously and spotted onto filter paper (Whatman 903®), air dried and stored in sealed envelopes at ambient temperature. Filter paper samples were then sent to Europe every 3 weeks for HIV-RNA extraction (Primagen, Holland) and quantification using the Roche real time reverse transcriptase PCR assay (COBAS TaqMan) with a lower limit of detection of 500 copies/ml. FPT results were compared with local (gold standard) results in terms of correct undetectable (<500 copies/ml) and detectable (> 500 copies/ml) viral loads. Plasma filter paper samples were collected only after an interim analysis of the whole blood results hence the whole blood data was analyzed at a more significance level (99%).

RESULTS

- •524 FPT VL were performed in 402 ART-treated patients (median 11 months); all were on first-line NNRTI-based therapy.
- •**39 (9.7%)** had a detectable viral load by local testing (median 15161 copies/ml; range 511-447000 copies/ml).
- •Of 524 filter paper samples, 306 were whole blood (WB), 218 were plasma (PL) and there were 122 WB/PL pairs.
- •Compared with gold standard results, WBFPT specimens yielded 4 false negative (all <2000 copies/ml by local testing) and 64 false positive results (median 1002 copies/ml; range 510-3510 copies/ml). WBFPT sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are described in table 1.
- •McNemar's test showed a significant difference between the gold standard test and WBFPT.
- •PLFPT yielded no false negative and one false positive result (593 copies/ml).
- •The sensitivity, specificity, positive and negative predictive values of PL FPT are illustrated in table 1.
- •McNemar's test showed no significant difference between local viral loads and PLFPT.
- •Bland Altman plots confirm the high number of low viral load false positives for WBFPT but demonstrate good agreement (within 2 SD) between WB & PLFPT for higher viral loads (>5000 copies/ml).
- •122 WB/PL pairs were analysed for agreement using Cohan's Kappa statistic. The observed agreement was fair at 73% (64-80%); z = 3.89 (p<0.001).

Table 1: Results of WB & PL FPT compared with gold standard

	Whole Blood FPT (99% CI)	Plasma FPT (95% CI)
Sensitivity	86 (67-100)	100 (84-100)
Specificty	77 (69-85)	99 (97-100)
NPV	27 (14-40)	95 (77-99)
PPV	98 (95-100)	100 (98-100)
McNemar's Test	53.13 (p <0.0001)	1.0 (p = 0.31)
No. tested	306	218

DISCUSSION

- •FPT of PL specimens for HIV-RNA quantification may provide a practical and reliable means of monitoring ART in resource poor settings.
- •WB samples were associated with a high number of false positive viral loads which could lead to switching therapy unnecessarily
- •Detection of cell associated HIV-DNA is the likely explanation for the high rate false positive results.
- •PL although more accurate in our study, requires venesection and electricity dependent centrifuge. Solutions include manual/battery operated centrifuges, obtaining plasma from EDTA samples 'left to stand' or the use of nucleic acid sequence-based amplification (NASBA) assays which do require HIV-DNA detection in order to quantify HIV-RNA and may therefore be used on WB [6].

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