Published in final edited form as:

AIDS. 2010 March 13; 24(5): 785–786. doi:10.1097/QAD.0b013e32833653c5.

Reply to ‘Quantification of HIV-1 RNA on Dried Blood Spots’ *AIDS* 24:475–6

Sarah M. Lofgren^a, Wendy S. Stevens^{e,f}, John A. Bartlett^{a,b}, and John A. Crump^{a,b,c,d}

^aDivision of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, ^bDuke Global Health Institute, Duke University, Durham, North Carolina, USA, ^cKilimanjaro Christian Medical Centre, ^dKilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania, ^eDepartment of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of Witwatersrand, and ^fNational Health Laboratory Service, Parktown, South Africa.

The authors thank Dr Johannessen for the thoughtful comments on our manuscript [1]. In light of growing evidence of the substantial shortcomings of clinical and immunologic monitoring of patients receiving anti-retroviral therapy (ART) for informing decisions on switching regimens [2], we agree that practical and reliable approaches to earlier identification of virologic failure in resource-limited settings are urgently needed. A primary purpose of our study was to extend the promising results seen in laboratory-based studies of a dried blood spot method [3] using an assay that was already in service in our setting for liquid plasma-based HIV-1 RNA testing [4] to a field situation similar to that expected under program conditions in Tanzania. As pointed out by Dr Johannessen and described in our paper, at plasma HIV-1 RNA levels below approximately 3 log copies/ml, dried blood spot samples yielded higher HIV-1 RNA levels presumably related to amplification of pro-viral DNA. Accepting this limitation, we explored the performance of dried blood spot samples against liquid plasma samples at two threshold definitions for virologic failure: a conventional threshold of 400 copies/ml or more and at a threshold of 5000 copies/ml or more, a definition for virologic failure used by some national care and treatment programs [5]. Not surprisingly and as shown in Table 2 of our paper, although sensitivity for detection of virologic failure was similar at both thresholds, specificity was higher at a threshold of 5000 copies/ml or more (0.97) than at a threshold of 400 copies/ml or more (0.87). However, we would dispute the characterization of the dried blood spot HIV-1 RNA resulting in ‘a high number of false-positive results’ at either threshold when viewed in light of the shortcomings of clinical and immunologic monitoring [6] and we disagree that there are any substantial differences in the classification of virologic failure between dried blood spot HIV-1 RNA and plasma HIV-1 RNA at a threshold of 5000 copies/ml or more. Furthermore, it is notable that Dr Johannessen’s own study from Tanzania that tested dried blood spots by nucleic acid sequenced-based amplification using the NucliSENS EasyQ HIV-1 assay (BioMérieux Inc., Durham, North Carolina, USA) showed a similar specificity for virologic failure at a plasma HIV-1 RNA threshold of 5000 copies/ml or more as the Abbott m2000 system (Abbott Laboratories, Abbott Park, Illinois, USA) did in our study [7]. Nonetheless, we applaud efforts to improve the performance of dried blood spot approaches to HIV-1

nucleic acid amplification for ART monitoring at lower HIV-1 RNA levels by refinement of existing assays and by evaluation of alternative assays.

Acknowledgement

This study was funded by Abbott Laboratories (Abbott Park, Illinois, USA). None of the authors was employed by Abbott Laboratories. S.M.L. and J.A.C. have received speaking honoraria from Abbott Laboratories and J.A.B. has received speaking honoraria and has served as a consultant for Abbott Laboratories.

References

1. Lofgren SM, Morrissey AB, Chevallier CC, Malabeja AI, Edmonds S, Amos B, et al. Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities. *AIDS* 2009; 23:2459–2466. [PubMed: 19741481]
2. Hosseinipour MC, Van Oosterhout JJG, Weigel R, Phiri S, Kamwendo D, Parkin N, et al. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. *AIDS* 2009; 23:1127–1134. [PubMed: 19417582]
3. Marconi A, Balestrieri M, Comastri G, Pulverenti FR, Gennari W, Tagliazucchi S, et al. Evaluation of the Abbott Real-Time HIV-1 quantitative assay with dried blood spot specimens. *Clin Micro-biol Infect* 2009; 15:93–97.
4. Crump JA, Scott LE, Msuya E, Morrissey AB, Kimaro EE, Shao JF, Stevens WS. Evaluation of the Abbott m2000rt RealTime HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR 1.5 using specimens from East Africa. *J Virol Methods* 2009; 162:218–222. [PubMed: 19729037]
5. National Department of Health. National Antiretroviral Treatment Guidelines. Johannesburg: Minuteman Press; 2004.
6. van Oosterhout JJG, Brown L, Weigel R, Kumwenda JJ, Mazinganjira D, Saukila N, et al. Diagnosis of antiretroviral therapy failure in Malawi: poor performance of clinical and immunological WHO criteria. *Trop Med Int Health* 2009; 14:856–861. [PubMed: 19552661]
7. Johannessen A, Garrido C, Zahonero N, Sandvik L, Naman E, Kivuyo SL, et al. Dried blood spots perform well in viral load monitoring of patients who receive antiretroviral treatment in rural Tanzania. *Clin Infect Dis* 2009; 49:976–981. [PubMed: 19663598]