Author’s response to reviews

Title: Stability of unfrozen whole blood DNA for remote genotypic analysis of HIV-1 coreceptor tropism

Authors:

Genny Meini (meini4@unisi.it)
Angelo Materazzi (amaterazzi81@gmail.com)
Francesco Saladini (saladini6@unisi.it)
Andrea Rosi (andrearosi4@gmail.com)
Ilaria Vicenti (vicenti@unisi.it)
Michele Mancini (michele.a.mancini@viivhealthcare.com)
Antonella Pirazzoli (antonella.a.pirazzoli@viivhealthcare.com)
Cinzia Caudai (caudai@unisi.it)
Maurizio Zazzi (maurizio.zazzi@unisi.it)

Version: 2 Date: 11 September 2013

Author’s response to reviews: see over
We would like to thank the reviewers for their helpful comments allowing us to improve the manuscript.

Our point-to-point responses follow below.

REVIEWER: JACQUES IZOPET

REVIEWER’S COMMENT 1.

The data do not show tropism results i.e. presence of R5 or X4 viruses. It seems important to demonstrate similar performance for detecting X4 viruses for each storing conditions.

AUTHORS’ REPLY.

Although a differential selection of specific virus variants under different processing conditions is not expected, we agree with the reviewer that there is a need to demonstrate that sequencing amplification products derived from whole blood stored at +4°C (WB4) and at -20°C (WB20) yields comparable results. To comply with this requirement, we randomly selected 20 paired WB4 and WB20 sample extracts and subjected them to sequencing and prediction of coreceptor tropism based on the reference geno2pheno[coreceptor] interpretation algorithm. Due to the well-known variability of the V3 region with replicate testing via Sanger sequencing, we performed duplicate analysis of these samples (i.e. two different amplification products were sequenced both for the WB4 and for the WB20 sample). The results show that the agreement is in the range of what expected with the highly variable V3 domain. Indeed, 18/20 samples yielded the same coreceptor tropism in WB4 and WB20 while two cases were discordant (one R5 in WB4 and non-R5 in WB20, one the other way round). This discrepancy was comparable to that detected within WB4 and within WB20 duplicates, confirming that the different storing conditions do not affect the distribution of virus variants with different coreceptor tropism. We have inserted these results in the revised manuscript.

REVIEWER’S COMMENT 2.

The clinical relevance of tropism testing from HIV-DNA in HIV patients with suppressed viremia is probably supported by published data. References must be provided.

AUTHORS’ REPLY.

While the use of maraviroc as a treatment simplification strategy in patients with suppressed viremia is certainly gaining attention, we are not yet aware of any solid proof of the clinical relevance of HIV DNA tropism testing in this context. There are a few clinical trials focusing on this use of maraviroc and currently recruiting patients. To our knowledge, the only published works in this setting are a small observational study by Vitiello et al. (Intervirology 2012; 55:172-8) and a pilot trial reported by Bonjoch et al. (J Antimicrob Chemother 2013, 68:1382-7). We have added both references in the revised manuscript.