Author's response to reviews

Title: Impact of naturally occurring amino acid variations on the detection of HIV-1 p24 in diagnostic antigen tests

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Version: 5
Date: 22 September 2015

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Dear Editor,

In response to your query from 21st of September, we would like to outline two changes made to the manuscript to address the concerns and explain the generation of study material.

The starting tool for our study was a set of previously constructed eukaryotic expression vectors encoding virus-like particles (Gag-Pol) from different HIV-1 subtypes. We produced these VLPs for a previous study, were we aimed to investigate p24 subtype sensitivity of HIV diagnostic tests (Vetter et al. PLoS ONE 2014: 9(10): e111552). To highlight this, the first sentence in the Method section was changed as follows: Lines 75-76: “VLPs used in this study were expressed from Gag-Pol encoding eukaryotic expression vectors, which were cloned for a previous study conducted in our laboratory.”

For mutagenesis, the DNA sequence of the VLP expression vectors was changed at the relevant sites and VLPs carrying these mutations were expressed and spiked into human plasma. To highlight this, the following part (highlighted in bold here) in the method section was changed as follows: Lines 93-95: “Amino acid changes at position 16 and 170 in p24 were introduced by side-directed-mutagenesis of the VLP eukaryotic expression vectors, using the QuickChange II XL Site-Directed Mutagenesis Kit (200521, Agilent Technologies).”

For Western Blot, only VLPs in PBS were used for protein separation on SDS-PAGE. No further amendments were made to the manuscript as the initial statement in the method section (lines 75-76) referring to the production of VLPs explains that these are not patient samples but recombinantly expressed virus-like-particles.

We hope that these changes clarify that our study did not use any patient samples but only VLPs expressed from eukaryotic DNA expression vectors.
encoding for Gag-Pol and constructed during a previous study in our laboratory.

With best regards,

Dr. Beatrice Vetter