Author's response to reviews

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

Authors:

Beatrice N Vetter (vetter.beatrice@virology.uzh.ch)
Vanessa Orlowski (orlowski.vanessa@virology.uzh.ch)
Christoph Niederhauser (christoph.niederhauser@bsd-be.ch)
Louise Walter (louise.walter@swissmedic.ch)
Jörg Schüpbach (schupbach.jorg@virology.uzh.ch)

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Author's response to reviews: see over
Dear Editor,

Please find below our answers to reviewers’ comments to the above mentioned manuscript.

Reviewer David Kelso:

Comment: The reviewer wonders why the Alere Determine HIV-1/2 Combo was not included in this study

Answer: The aim of this study was to investigate if the failure of antigen detection of otherwise highly sensitive tests was due to individual amino acid polymorphisms in p24 (and thus linked to antibody epitope detection). The Alere Determine HIV-1/2 Combo had overall very poor antigen sensitivity in our previous study (Vetter et al. DOI: 10.1371/journal.pone.0111552), which was most likely not due to single amino acid polymorphism but rather due to technical weaknesses of the test. When aligning the p24 amino acid sequences of the previously investigated 43 VLPs, no amino acid variation could be observed which might explain the poor performance of the Alere Determine HIV-1/2 Combo. Thus, in all likelihood, testing the mutated VLPs in the present study with the Alere Determine HIV-1/2 Combo would not have resulted in positive detection, as it is the technical poor performance of the test, rather than the employed antibodies, which result in non-detection in the case of this test. Once the overall sensitivity of the test has been improved by the manufacturer, it is indeed worthwhile to investigate epitope-specific antigen sensitivity.

Reviewer Valeria Ghisetti:

Comment: “I strongly suggest and recommend the Authors to assess a panel of p24 WHO antigen preparation”

Answer: There is only a single WHO p24 antigen preparation available (NIBSC 90/636), which was included in our previous study Vetter et al. (DOI: 10.1371/journal.pone.0111552). All immunoassays included in the present study detected the WHO p24 antigen standard at the measured concentrations of 2, 10 and 50 IU/ml, the 2 IU/ml concentration reflecting regulatory requirements. The renewed inclusion of this WHO p24 antigen standard in the present study would have only helped to show that the assays perform according to regulatory sensitivity requirements, which we already tested in our previous study.
Comment: “Alternatively, they could test a panel of HIV serum samples from HIV recent infection to study if variation in assay performance is due to polymorphisms at those two positions”

Answer: As the described point mutations in the present study are relatively infrequent, it seems unlikely that any available panel of HIV serum samples from HIV recent infections contain patients carrying this mutation. Moreover, generally there is no sequence information available for these samples. The very striking variation in S/Co ratios before and after amino acid mutation (see Figure 1), strongly suggests that the amino acid polymorphism, rather than technical variation, is the source variation in assay performance. Different p24 input concentrations can also not be the source of the variation as this has been controlled for by standardizing VLP input based on RT-activity.

Reviewer Jesse Kwiek:
Comment: Lack of information on sequence analysis for table 1.
Answer: A methods section was included, describing search and analysis parameters for p24 sequences downloaded from LANL. In our initial analysis we had based sequence inclusion criteria on unique accession numbers, assuming that each accession number represents an individual patient. During review, we noted that several sequences can be deposited in the LANL database with unique accession numbers but from the same patient. To exclude these duplicate sequences, the LANL database query was run again and only one sequence per patient was subsequently downloaded (as defined by the LANL interface). Consequently, the number of total sequences decreased and table 1 and the main text were adjusted accordingly (lines 153-162 and lines 244-245). The frequency of the amino acid T at position 16 decreased to 1.68% (from previously 2.51%) and the frequency of amino acid R at position 170 decreased to 2.08% (from 2.28%). Thus, particularly the occurrence of the S to T polymorphism was previously overestimated (due to the high number of subtype B and C duplicate sequences). However, this does not alter the overall message of the analysis, namely that these amino acid polymorphisms do occur in HIV variants and this should be taken into consideration when designing HIV diagnostic test.

We hope that this sufficiently answers the reviewers comments and will fulfil the requirements for publication of our manuscript.

Sincerely,

Dr. Beatrice Vetter