A Recombinant HIV-1 Gag Virus Panel for the Evaluation of p24-Antibodies and HIV Diagnostic Tests

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Background: Diagnostically, the HIV-1 p24 protein is currently only used in HIV-1/2 Ab + Ag combination screening tests (4th generation). However, there is growing interest in new test development to extend its use to diagnosing paediatric HIV infection and disease monitoring as inexpensive alternatives to PCR-based tests. Regarding the global HIV-1 diversity, such tests should detect all subtypes with similar sensitivity. Readily available, inexhaustible tools for subtype evaluation and test comparisons are a pre-requisite for efficient test development. To this end, we developed a panel of 44 recombinantly expressed virus-like-particles (VLPs), which contain the Gag-Protease region of HIV-1 subtypes A, B, C, D, F, G, H, circulating recombinant forms 01_AE, 02_AG, BF/BG, and group O. We characterised 10 mono- or polyclonal antibodies (mAb, pAb) for their subtype-binding profiles and initiated an evaluation of commercially available 4th generation screening tests.

Methodology: Patient-derived PCR-amplified Gag-PR regions were cloned into pCMVdR8.91, allowing expression of non-infectious VLPs in tissue culture. Concentrations were standardized based on the VLPs’ reverse transcriptase activity. To assess p24 antibody binding, VLPs were lysed and coated on ELISA plates. For testing diagnostic kits, dilutions of VLPs corresponding to 2, 10 and 50 IU/mL (WHO p24 standard) were prepared in HIV-negative plasma.

Results: p24 amino acid divergence of our panel within a given subtype ranged from 4.5-8.9% and was similar to that of the Los Alamos subtype reference sequences (2.9-9.1%). Overall, weighted panel diversity was 6.8%, compared to 4.9% for the subtype-matched reference sequences. Binding profiles of p24 antibodies showed that only one mAb detected 43/44 panel members (97.7%), sensitivity for the other six mAbs ranged from 19/44 - 40/44 (43.2 - 90.9%). Subtype B and CRF01_AE were detected by the 7 mAbs most effectively (mean sensitivity 85.7%), but mean sensitivity to CRF02_AG was low (37.1%). The three pAb tested (one a pooled HIV-serum) showed a sensitivity of 100% for all subtypes except D, G and CRF02_AG (83.3%, 83.3% and 86.7%, respectively).

To date, we have tested two commercial combination screening tests and noted a large difference in performance. The ARCHITECT HIV Ag/Ab Combo assay detected all but one VLP, even at low concentration, whereas the Access HIV combo assay completely missed 16/44 subtypes.

Conclusions: Our HIV-1 Gag subtype panel has a broad diversity and served as a useful tool to assess the breadth of subtype detection. Moreover, initial results indicate that there is a need to carefully assess the capability of existing diagnostic kits for detecting the p24 of diverging subtypes.