COMPARISON OF SENTOSA® SQ DEEP SEQUENCING-BASED HIV-1 GENOTYPING COUPLED TO INTEGRATED WORKFLOW WITH SANGER SEQUENCING METHOD FOR DETECTION OF DRUG RESISTANCE MUTATIONS

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Abstract. Sanger sequencing of viral quasispecies has limited sensitivity in detecting drug resistance mutations (DRMs) at frequencies less than 20%. On the other hand, deep sequencing is effective in detecting such mutations, but the protocol still requires manual and time-consuming working steps. Sentosa® SQ HIV-1 Genotyping Assay based on deep sequencing provides an integrated workflow, a robotic liquid handling system for automatic RNA extraction and library preparation, an Ion-torrent-based deep sequencing system and software for data analysis. Thus, we evaluated the performance of deep sequencing assay and compared the results with those from Sanger sequencing for determining DRMs of 120 previously genotyped clinical samples. Deep sequencing assay took 27.7 hours to complete, including 2.3 hours of manual working steps. DRM analysis revealed a total number of 913 and 789 mutations by deep sequencing assay and Sanger sequencing, respectively. Deep sequencing assay detected 99.4% of all DRMs found by Sanger sequencing and additional 129 DRMs at frequencies below and above 20%. Thus, with an integrated workflow, the deep sequencing assay provides a user-friendly platform and has a relatively short turnover time, requirements suitable for adoption in a routine clinical laboratory.

Keywords: deep sequencing, drug resistance mutation, HIV-1, Sanger dideoxy sequencing

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