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Performance of Celera RUO integrase resistance assay across multiple HIV-1 subtypes

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Background: HIV-1 sequence variation is a major obstacle to developing molecular based assays for multiple subtypes. This study sought to independently assess performance characteristics of the ViroSeq™ HIV-1 Integrase RUO Genotyping Kit (Celera, US) for samples of multiple different HIV-1 subtypes.
Methods: 264 samples were tested in the validation, 106 from integrase inhibitor naïve patients' sent for routine HIV-1 drug resistance testing after failing a 1st- or 2nd-line regimen, and 158 samples from an external virology quality assurance program (VQA). For the latter, 53 unique VQA samples were tested in two to five different laboratories to assess assay reproducibility. For all assays, viral RNA was extracted using the ViroSeq extraction module, reverse transcribed, and amplified in a one-step reaction. Four sequencing primers were used to span codons 1–288 of integrase. The Rega subtyping tool was used for subtype assignment. Integrase polymorphisms and mutations were determined as differences from the HXB2 sequence and by the Stanford database, respectively. Sequences obtained from the different laboratories were aligned and sequence homology determined.
Results: HIV-1 RNA in the 264 samples ranged from 3.15 to 6.74_{log}copies/ml. Successful amplification was obtained for 97% of samples (n = 256). The 8 samples that failed to amplify were subtype D (n = 3), subtype C (n = 1), CRF01_AE (n = 1), subtype A1 (n = 2), and an unassigned subtype (n = 1). Of the 256 that successfully amplified samples, 203 (79%) were successfully sequenced with bidirectional coverage. Of the 53 unsuccessful samples, 13 (5%) failed sequencing and 40 (16%) did not have full bidirectional sequence, as a result of failure of sequencing primers: Primer A (n = 1); Primer B (n = 18); Primer C (n = 1); Primer D (n = 7) or short sequences (n = 16). For the 135 VQA samples (30 unique samples) that were assayed by different laboratories, homology of the sequences obtained ranged from 92.1% to 100%. However, Laboratory 2 detected more mixtures (74%) compared to the other four laboratories, whereas Laboratory 1 detected the least number of mixtures (35%), likely due to differences between the labs in the methods of sequence analysis. Mutations associated with integrase resistance were observed in seven of the 106 (7%) clinical samples [one sample: Q148 K; E138 K; G140A; two samples: T97A and four samples: L74I]. Of the four samples with L74I, 3 were subtype G.

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Conclusion: Of the total 264 samples tested, 243 (92%) of samples were able to be amplified and sequenced to generate an integrase genotype. Sequencing results were similar between the testing laboratories with the exception of mixture detection. Mutations associated with integrase inhibitor resistance were observed in only 7% of integrase inhibitor naive samples, and some of these mutations are likely to be due to subtype-specific polymorphisms rather than selection by an integrase inhibitor.

1. Introduction

Global access to antiretroviral therapy (ART) has increased rapidly over the last ten years in developing countries, with over 15.8 million infected individuals receiving ART by June 2015 (UNAIDS, 2015). ART can fail to suppress HIV-1 as a result of transmitted drug resistance, medication toxicity, incomplete medication adherence, suboptimal pharmacokinetics or inadequate regimen potency, resulting in the emergence of drug-resistant HIV-1 (Boulle et al., 2007; Carr and Cooper, 2000; Haas et al., 2004; Haas et al., 2003; Rhee et al., 2015). When HIV drug resistance develops, ideally, a new regimen containing at least three active drugs should be administered. Optimal third-line options are still being investigated in studies such as the AIDS Clinical Trials Group (ACTG) Protocol A5288: "Management Using the Latest Technologies in Resource-limited Settings to Optimize Combination Therapy After Viral Failure (MULTI-OCTAVE)" (ClinicalTrials.gov Identifier: NCT01641367). This study includes the drug raltegravir from the integrase class of inhibitors.

Raltegravir was the first FDA-approved integrase inhibitor that blocks viral replication by inhibiting integration of HIV-1 DNA into the host genome (Cahn and Sued, 2007; Colquitt and Pham, 2007; Grinsztejn et al., 2007). Decreased susceptibility to raltegravir develops as a result of several different mutations in the integrase gene. To date, the majority of work in detecting integrase resistance has centred on HIV-1 subtype B. However, the subtypes circulating in developing countries, where the need for treatment is greatest, are generally non-subtype B. The subtypes that predominate in these developing countries include sub-subtype A, subtypes C, D, F and several recombinant forms. Sequence variations in the binding sites for PCR amplification primers and sequencing primers are a major obstacle to developing molecular based assays for this range of subtypes. One commercially available test for integrase resistance is available (ViroSeq™ HIV-1 Integrase RUO Genotyping Kit [Celera, US]). In the current study, we sought to assess the performance characteristics of this HIV-1 Integrase RUO Genotyping Kit with patient samples across a broad range of subtypes and across different testing laboratories.

2. Materials and methods

2.1. Samples

Two hundred and sixty four plasma samples were tested in the assessment. One hundred and six of the 264 (40%) plasma samples were from integrase inhibitor naïve patients that were sent for routine HIV-1 drug resistance testing between 2013 and 2014, after failing either a first-line NNRTI based regimen or a second-line PI based regimen. All samples selected had plasma HIV-1 RNA > 1000 copies/ml.

One hundred and fifty eight well characterised plasma samples with a variety of HIV-1 subtypes were obtained from the National Institutes of Health (NIH) Division of AIDS (DAIDS) Virology Quality Assessment Program (VQA) Laboratory, Rush Institute, USA. Of the 158 samples from the VQA, 53 were unique with the following subtypes: B (n = 26); C (n = 15); CRF-1_AE (n = 8); D (n = 2); F (n = 2). Moreover, the six samples had minor and major integrase mutations. Thirty of these 53 were identical samples (panels 23–28), which were tested in at least two and up to five different laboratories to assess the reproducibility of the ViroSeq™ HIV-1 Integrase RUO Genotyping Kit. Twenty three samples (panels 20–22 and NED [NIH-ENVA-DOD]) of which seven were subtype AE were tested in one laboratory.

2.2. Population genotype analysis

Population based genotyping was performed using the ViroSeq™ HIV-1 Integrase RUO Genotyping Kit (Celera, US) according to the manufacturer's instructions. Viral RNA was extracted from plasma using the ViroSeq extraction module. Briefly, 500 μl of plasma was centrifuged at 23000 × *g* for 1 h at 4 °C and the viral pellet resuspended in 600 μl of lysis buffer supplied in the extraction module. Viral RNA was then precipitated with 100% isopropanol and washed with cold 70% ethanol. The extracted viral RNA was resuspended in 50 μl kit provided buffer. The extracted viral RNA was used to generate a 1.1 kb amplicon in a one-step reverse transcribed (RT)-initiated polymerase chain reaction encompassing the entire integrase region. After column or enzymatic purification, the amplicon was used as a template to sequence 0.9 kb of the integrase region using four sequencing primers. Sequencing was performed with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, USA). If sequencing primers failed they were repeated. Sequences were analysed using the ViroSeq™ Integrase Software V1.0.0 to give the drug resistance profile across codons 1–288 of integrase (Fig. 1).

2.3. Data analysis

The Rega HIV-1 subtyping tool (http://dbpartners.stanford.edu/RegaSubtyping/) was used to identify the HIV-1 subtype of each sample. Integrase polymorphisms and mutations were determined using both the ViroSeq™ Integrase V1.0.0 algorithm and the Stanford database to determine the level of predicted susceptibility to raltegravir, elvitegravir and dolutegravir. A phylogenetic tree analysis using MEGA 6.0[11] was performed on the sequences generated from the two different testing laboratories to ensure all duplicate samples clustered together.

To access reproducibility, the paired samples from the two to five different laboratories had the nucleotide sequences aligned and the level of disagreements compared. The level of disagreement was compared after a consensus sequence from the different laboratories was generated as follows: a) number of sequencing differences (complete difference of nucleotide at the same position); b) the number of partial-mismatches (for example R vs. G). Sequence similarity was calculated for each of the parameters evaluated and a greater than 98% for complete mismatches was considered acceptable. Lastly, the mutation lists obtained through the Stanford database were compared for sequences derived from all the laboratories.