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Journal of Clinical Virology 39 (2007) 125–131

JOURNAL OF CLINICAL VIROLOGY

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Evaluation of an in-house genotyping resistance test for HIV-1 drug resistance interpretation and genotyping

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Received 7 October 2006; received in revised form 22 February 2007; accepted 12 March 2007

Abstract

Introduction: The human immunodeficiency virus type 1 (HIV-1) genotyping resistance test (GRT) has been considered essential for HIV-1 drug resistance monitoring. However, it is not commonly used in some developing countries in Asia and Africa due to its high running cost.
Objective: This study aims to evaluate a new low-cost in-house GRT for both subtype B and non-B HIV-1.
Study design: The in-house GRT sequenced the entire protease and 410 codons of reverse transcriptase (RT) in the *pol* gene. Its performance on drug resistance interpretation was evaluated against the FDA-approved ViroSeq™ HIV-1 Genotyping System. Particularly, a panel of 235 plasma samples from 205 HIV-1-infected patients in Hong Kong was investigated. The HIV-1 drug resistance-related mutations detected by the two systems were compared. The HIV-1 subtypes were analyzed through the REGA HIV-1 Genotyping Tool and *env* phylogenetic analysis.
Results: Among the 235 samples, 229 (97.4%) were successfully amplified by both in-house and ViroSeq™ systems. All PCR-negative samples harbored viral RNA at <400 copies/mL. The in-house and ViroSeq™ system showed identical drug resistance-related mutation patterns in 216 out of 229 samples (94.3%).

The REGA *pol* genotyping results showed 93.9% (215/229) concordance with the *env* phylogenetic results including HIV-1 subtype A1, B, C, D, G, CRF01_AE, CRF02_AG, CRF06_cpx, CRF07_BC, CRF08_BC, CRF15_01B and other recombinant strains.

The cost of running the in-house GRT is only 25% of that for the commercial system, thus making it suitable for the developing countries in Asia and Africa.
Conclusions: Overall, our in-house GRT provided comparable results to those of the commercial ViroSeq™ genotyping system on diversified HIV-1 subtypes at a more affordable price which make it suitable for HIV-1 monitoring in developing countries.

Keywords: Genotyping resistance test; HIV-1; In-house; Drug resistance; CRF01_AE

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) genotyping resistance test (GRT) has been widely used to monitor the antiretroviral treatment on HIV-1 patients (Hirsch et al., 2000; EuroGuidelines for HIV Resistance, 2001). This assay could provide early diagnosis of drug resistance in patients adhered to antiretroviral therapy and prevent the cause of treatment failure (Carpenter et al., 2000). In addition, GRT results would be an important factor for Highly Active Antiretroviral Therapy (HAART) regimen selection (Hirsch et al., 2000). Notably, although GRT is essential for HIV treatment, its high running cost hinders its diagnostic application in developing countries.

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doi:10.1016/j.jcv.2007.03.008

Most of the current FDA-approved antiretroviral drugs target the protease (PR) and reverse transcriptase (RT) of HIV-1. The currently available commercial genotyping resistance systems, such as the ViroSeq™ Genotyping System and Trugene™ HIV-1 Genotyping System are based on sequencing the PR and RT region in the *pol* gene from plasma virus (Eshleman et al., 2004; Grant et al., 2003). The sequencing covering regions of the commercial systems included the whole PR and partial RT region (up to codon 335) in the *pol* gene, where the well-defined protease inhibitor (PI) and reverse transcriptase inhibitor resistance-related mutations are positioned (Eshleman et al., 2004; Johnson et al., 2006). In 2000, a novel T386I mutation in the RT region positioned beyond the commercial GRT sequencing covering region was identified in a few HIV-1 strains in Brazil. This mutation was found to abrogate the M184V suppression of L210W and L210W/G333D/E (Caride et al., 2000). Among all commercially available HIV-1 drug resistance interpretation systems, T386I was recognized as a drug resistance-associated mutation only in VircoTYPE HIV-1 analysis version 4.0.00 (Virco, Belgium).

It is well known that commercial GRTs are optimized for subtype B strains while non-B strains are causing the major global pandemic. Other than HIV-1 subtype B, the circulating recombinant form AE (CRF01_AE), and subtype C is the prevalent strain circulating in Hong Kong and other Asian countries (Ariyoshi et al., 2003; McCutchan, 2006; Yam et al., 2006). Recent studies showed that the ViroSeq™ and TruGene™ HIV-1 Genotyping Systems (Eshleman et al., 2004; Jagodzinski et al., 2003) are applicable to various HIV-1 subtypes; however, problems with non-B strains have occasionally been reported (Beddows et al., 2003; Fontaine et al., 2001; Mracna et al., 2001).

In this regard, different in-house GRTs with low running cost have been designed (Lindstrom and Albert, 2003; Steegen et al., 2006) and they were found to have a high successful rate (>85.3%) of amplifying and sequencing subtype B and non-B HIV-1 samples (Steegen et al., 2006). However, their performance was not validated against internationally approved reference systems.

Therefore, the aim of this study is to evaluate a cost-effective in-house GRT for routine drug resistance-related mutation detection on genetic diversified HIV-1. The genotyping results of the in-house system were compared with those of the FDA-approved ViroSeq™ Genotyping System. The frequencies of T386I development among patients in Hong Kong were also investigated. Moreover, HIV-1 genotyping using the *pol* sequences was further evaluated.

2. Materials and methods

2.1. Samples

A panel of 235 EDTA whole blood or archived plasma samples was collected from 205 HIV-1-infected patients in Hong Kong between July 1996 and May 2005. Among the 235 samples, 143 were pre-treatment and 92 were post-treatment samples. Patient plasma separated from EDTA blood samples was stored at −80 °C. The HIV-1 plasma viral loads (400 to 2×10^6 copies/mL) of all samples were monitored by the COBAS Amplicor HIV-1 Monitor Test version 1.5 with lower quantitation limit at 400 copies/mL (Roche Diagnostic Systems, 1996).

2.2. RNA extraction, RT-PCR amplification

Total RNA was extracted from 420 μL patient plasma using three times the volume of lysis buffer in the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). Viral RNA was eluted in a 60 μL elution buffer provided in the kit. The entire PR and 410 codons of the RT in the *pol* gene was reverse-transcribed and amplified by using *C. therm* Polymerase One-Step RT-PCR System (Roche Diagnostics, Germany) with primer HIVF04 and HIVR03 (Table 1). A 2200 bp fragment encompassing the PR and RT regions was further amplified with FastStart High Fidelity PCR System (Roche Diagnostics, Germany) by a nested PCR with the inner primers HIVF03 and HIVR04 (Table 1). Parallel amplification was performed on each RNA extract using the ViroSeq HIV-1 Genotyping System version 2.0 (Celera Diagnostics, CA, USA) following the manufacturer's instructions. The 369 base pair *env* gp41 immunodominant region was amplified for HIV-1 genotyping as described previously (Swanson et al., 2003). Positive and negative controls were included in each run and all precautions to prevent cross-contamination were observed (Kwok and Higushi, 1989).

2.3. Sequencing and purification

The nested PCR products were purified with QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were direct sequenced in both directions with 1/4 dilution of the BigDye Terminator Cycle Sequencing Ready Reaction kit version 1.1 (AppliedBiosystems, CA, USA) with five specific primers (Table 1). These primers provided overlapping, bidirectional sequences covering the region where all defined PI and RT inhibitor resistance-related mutations positioned.

For the ViroSeq™ system, PCR products were sequenced with the sequencing module according to the manufacturer's instructions (Celera Diagnostics, CA, USA). Excess dye terminator after the cycle sequencing was removed by AutoSeq96 (Amersham Biosciences, USA) before loading into the Prism 3700 Genetic Analyzer (Applied Biosystems, CA, USA).

2.4. Sequencing and phylogenetic analysis

The individual sequence fragments of each sample were aligned and edited with Staden Package (version 2003.0.1) (Staden et al., 2000). Following the sequencing analy-