

Evaluation of an affordable Assay for Drug-Resistance Genotyping of All Major HIV-1 Subtypes

Susan C. Aitken^{1,2}, Aletta Kliphuis^{1,3}, Carole L. Wallis², Mei Ling Chu¹, Wendy Stevens^{2,4}, Tobias F. Rinke de Wit³ and Rob Schuurman¹, on behalf of the ART-A* consortium.

¹ University Medical Centre, Utrecht, The Netherlands; ² University of the Witwatersrand, Johannesburg, South Africa; ³ Center for Poverty-related Communicable Diseases (CPCD), PharmAccess Foundation, Academic Medical Center, Amsterdam, The Netherlands; ⁴ National Health Laboratory Services, South Africa.

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Corresponding author: S.C.Aitken@umcutrecht.nl

Introduction

Current commercial kits for HIV-1 drug resistance genotyping are expensive and in some cases feature reduced detection sensitivity of HIV-1 non-B subtypes, thus limiting application in resource-constrained settings. The aim of our research was the development and evaluation of an affordable, subtype-independent genotyping assay for use in monitoring HIV drug resistance in Africa.

Methods

A subtype-independent nested PCR encompassing the entire protease and the reverse transcriptase up to amino acid 321 of HIV-1 (figure 1) was designed to detect all group M subtypes of HIV-1. The nested PCR was evaluated using a panel of reference viruses for subtypes A, B, C, D, A/E, F, G, and H (figure 2). In addition the analytical sensitivity of the nested PCR and a set of sequencing primers compatible with all subtypes were determined (table 1). This assay was evaluated on 155 plasma samples from HIV infected females from Africa harbouring various viral loads. Sequence reactions were analyzed on an ABI3100 automated sequencer and SeqScape data analysis software (Applied Biosystems) (figure 3).

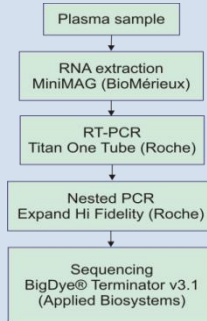


Figure 3: Flow diagram of sample processing steps.

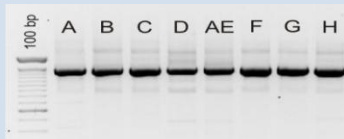


Figure 2: Specificity of amplification primers for representative subtypes in a subtype panel.

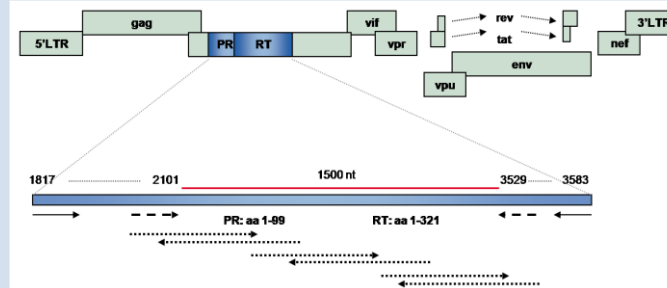


Figure 1: Positions of the amplification primers in the HIV-1 genome. Base locations numbered according to HXB-2

Table 1: Assay sensitivity. A dilution series, given in copies/ml, was performed in quadruplicate for subtypes A-H in a panel. Successful amplification is indicated by a plus, negative by a minus. Assay sensitivity was determined to be 500 copies/ml in plasma.

| Subtype | 4000 | 2000 | 1000 | 500 | 250 |
|---------|------|------|------|------|-----|
| A | ++++ | ++++ | ++++ | ++++ | -+ |
| B | ++++ | +++ | +++ | ++++ | -+ |
| C | ++++ | ++++ | ++++ | ++++ | ++ |
| D | ++++ | ++++ | ++++ | +--- | -+ |
| AE | ++++ | ++++ | ++++ | ++++ | ++ |
| F | ++++ | ++++ | ++++ | ++++ | +- |
| G | ++++ | ++++ | ++++ | ++++ | ++ |
| H | ++++ | ++++ | ++++ | ++++ | +- |

Results

All major HIV-1 subtypes could be detected in the nested PCR with an analytical sensitivity of 500 RNA copies/ml. Application of the genotyping assay on 155 primarily African clinical samples (table 2) comprising of subtypes A (27%), B (8%) C (42%), D (11%), AE (6%) and AG (6%), with a viral load range from 434-30,700,000 (median 365,000) RNA copies/ml was 94% successful: 84% were genotyped using the primary set of 6 sequencing primers, and an additional 10% using 1 or 2 second choice primers. Of the 155 sample, 7 were unable to give a full bi-directional sequence in the PR region, and 2 in the RT region.

Table 2: Subtype distribution and viral load ranges of clinical samples. The percentage success refers to the percentage of samples for which full bi-directional PR-RT sequencing was achievable.

| Subtype | n | % Success | Viral Load (copies/ml) |
|--------------|------------|-----------|-----------------------------------|
| A | 41 | 100 | 72.600 (828 - 416.000) |
| B | 12 | 92 | 931.000 (671 - 8.630.000) |
| C | 65 | 97 | 761.000 (434 - 30.700.000) |
| D | 17 | 100 | 52.300 (665 - 222.000) |
| AE | 10 | 80 | 177.000 (8.900 - 75.000) |
| AG | 10 | 80 | 198.000 (5.550 - 1.100.000) |
| Total | 155 | 94 | 365.300 (434 - 30.700.000) |

Conclusions

We have developed an affordable genotyping assay for HIV drug-resistance. The assay can be applied to all group-M HIV-1 subtypes with adequate analytical sensitivity and is potentially suitable for use in resource-constrained settings. Due to the sensitivity of this assay, it has potential application in combination with dried fluid spot samples.