Methods for HIV Resistance Determination and Interpretation

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Description of Two Commercially Available Assays for Genotyping of HIV-1

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HIV-1 · Genotyping · Drug resistance · Mutations · Assays

Abstract
HIV-1 resistance testing is one important part in the diagnostics of antiretroviral treatment and is commonly done by genotyping. Currently, two systems are commercially available and, despite being far from easy to use, these have achieved a high degree of sophistication. Modifications of standard kit protocols might be necessary based on the clinical situation. Although resistance reports based on decision rules are a part of both systems, considerable knowledge and skills are nevertheless required by the user to establish useful clinical data out of detected resistance patterns. Both systems described here have their advantages and disadvantages; a decision for one or the other system needs to be based on individual requirements. The future might lie in so-called 'next-generation sequencing' systems based on pyrosequencing, which enable a high throughput and the detection of minor variants of less than 1%.

A major reason for keeping HIV replication as low as possible is to avoid the selection and emergence of resistant viral quasispecies carrying drug resistance-associated mutations (DRAMs). According to current treatment guidelines, first-line highly active antiretroviral combination therapy consists of at least 2 nucleoside reverse transcriptase inhibitors in combination with 1 protease (PR) inhibitor, normally boosted with ritonavir, or 1 non-nucleoside reverse transcriptase inhibitor:

Germany/DAIG:
www.daignet.de/site-content/hivtherapie/leitlinien-1/Leitlinien_28-05-2010_V_late.pdf

Europe/EACS:
www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf

USA/DHHS:
http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf.

However, some patients do not respond at all or only insufficiently to highly active antiretroviral combination therapy [1–3]. Poor adherence, maladsorption, other pharmacogenetic differences, or missing potency of the therapy regimen in the given patient context (high viral load, history of drug resistance) might contribute to insufficient drug levels allowing the virus to replicate in the presence of drugs and, consequently, might lead to the development of viral drug resistance [4, 5]. In addition, the transmission of drug-resistant virus isolates in the context of primary HIV infection has been observed [6–11].

A crucial point in genotypic resistance testing is the accuracy of the test system used for the detection of

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DRAMs as well as the subsequent interpretation of the mutation pattern found. There are currently two commercial assays available for HIV-1 genotyping which can be performed in one's own laboratory, the ViroSeq™ HIV-1 genotyping system version 2 (ViroSeq; Abbott GmbH, Wiesbaden, Germany) and the TruGene HIV-1 genotyping kit (TruGene; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) [12, 13], both accompanied by interpretation programs employing rules-based algorithms. Although other assays are available, this review describes only these systems, and as they are most commonly used [14].

ViroSeq and TruGene are FDA-approved and – in the case of ViroSeq – CE-labeled systems for HIV-1 genotyping. They include modules for nucleic acid extraction (ViroSeq only), reverse transcription-polymerase chain reaction (RT-PCR), sequencing reaction and software for sequence alignment and drug resistance interpretation [12, 13]. An automated sequencer for analyzing the sequencing products must be purchased separately; for ViroSeq capillary sequencers from Applied Biosystems Deutschland GmbH, Darmstadt, Germany, are suitable [15], the TruGene sequencing products were analyzed on the slab gel Opengene™ DNA sequencing system (Opengene), which is also available from Siemens [12, 13].

The RNA extraction module supplied by ViroSeq is based on isopropanol precipitation, and both systems use two enzymes to reversely transcribe RNA into cDNA and to perform the subsequent PCR for target amplification. For ViroSeq, each RT-PCR reaction needs to be analyzed by agarose gel electrophoresis, a step not necessary when using TruGene. The sequencing reactions in both systems are based on the chain termination method by Sanger et al. [16], but use different chemistries. ViroSeq uses fluorescence-labeled chain terminators, each with a different dye. In conjunction with an automated sequencer capable of detecting the four different dyes, each sample can be sequenced in one tube. Seven primers (A–D forward direction; F–H reverse direction) are provided by ViroSeq to analyze the complete PR and most of the reverse transcriptase (RT) region (amino acids 1–335). The D primer is a backup primer in case of failure of the A primer, and has an overall poor performance rate [17–20]; therefore, we do not recommend using this primer in the initial testing. The TruGene system uses fluorescence-labeled primers; the complete PR and part of the RT (amino acids 38–247) are covered by 4 primer pairs, with differently labeled forward and reverse primers. The PR is covered by 2 primer pairs, but due to the pure performance of the PR primers, we recommend using only the P2 primer set [21]. Forward and reverse sequencing can be carried out in one tube, but each nucleotide must be analyzed in a separate reaction which means four reactions for each primer pair. The PCR product can be sequenced directly using the respective sequencing mix containing the sequencing primer, the CLIP™ reaction buffer, and the sequencing enzyme AmpliTaq FS. In contrast to ViroSeq, which needs a purification step to remove the not-incorporated chain terminators, no further purification steps are needed for TruGene, and the gel-loading buffer can be applied directly after the sequencing reaction is finished. Both systems are provided with sequence analysis software, which assembles all partial sequences for 1 patient into 1 project. They facilitate comparison with a drug-sensitive wild-type virus, editing of the consensus sequence and finally generate a drug resistance report with regard to the efficacy of the anti-HIV drugs.

To rule out a system-dependent bias in the detection of DRAMs, both systems have been compared extensively regarding general concordance, performance of the drug resistance reports and the detection of unusual insertions [22, 23]. Both systems are comparable and no major discordances regarding the general performance have been described [14, 24, 25]. However, interpretation of DRAMs is a crucial point in the clinical application of genotypic resistance tests. It has been shown that using different interpretation systems on the same sequence results in varying resistance scores [26]. However, neither the ViroSeq nor the TruGene report was associated with serious errors in interpretation [27–29].

Additionally, ViroSeq and TruGene have been tested for performance in analyzing all group M subtypes [14, 17–20, 30, 31]. Both assays are approved for the use in HIV subtype B only, and although subtype B is highly prevalent in Europe and North America, it represents only 11% of HIV-1 infections worldwide [32]. The performance in analyzing non-B subtypes is comparable for both systems, but a higher failure rate compared to subtype B has been reported. Adaptation to non-B subtypes might be a challenge for future updates.

Some modifications were necessary to take current clinical needs into account. Both systems are labeled for a viral load >1,000 copies/ml. Nowadays, clinical routine is quite different, and hence the commercial systems needed adaptations. Therefore, both assays have been tested intensely in terms of performance characteristics on different blood collection systems [33], including dried blood spot specimens [34–36], different extraction procedures [33, 37–40] and modified PCR reactions [41]. Using dried blot samples as a source for genotyping is of major

interest in the resource-limited setting. Both systems are able to reproducibly analyze samples with a viral load >10,000 copies/ml, which might be acceptable for resistance testing in this context. Modifications of the extraction procedure for ViroSeq and TruGene by either applying an ultracentrifugation step or by using sample preparation systems before the start of the procedure allow drug resistance testing in samples with viral loads down to 100 copies/ml.

As mentioned above, both the original ViroSeq and TruGene modules only cover the PR and RT regions. However, new antiretroviral drugs targeting different viral enzymes have been recently approved. Therefore, additional modules for analyzing the integrase region are available for TruGene and ViroSeq [42, 43]; these modules are, however, for research only.

Taken together, we do not favor 1 system over the other. Among other things the individual choice for using ViroSeq or TruGene should depend on sample throughput and equipment which may already be available in the laboratory. Regardless of the system used, it has to be kept in mind that regular updates of the drug resistance reports are mandatory to include new drugs and/or newly described mutational pathways. Finally, all genotypic data should be accompanied by expert advice and need to be used only in the context of individual patient-related parameters including treatment history.

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