Molecular assays for monitoring HIV infection and antiretroviral therapy

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Infection with HIV results in lifelong persistence of the virus in the body of infected persons, independent of antiretroviral treatment. Therefore, efficient and meaningful therapy monitoring has been developed since its introduction in the 1980s. Whereas, primarily, the measurement of the CD4 cell count was the most important clinical marker of disease progression, nowadays the estimation of plasma viral load with molecular methods plays a major role as a marker of therapy success. To optimize therapy changes in patients failing on antiretroviral therapy regimen, HIV-1 genotyping has been introduced and is now widely accepted as an additional diagnostic tool. Due to this increase in diagnostic parameters, clinicians and virologists have to cope with many different methods. This review should give a brief overview of the current commercially available assays for detection and quantification of HIV, as well as for HIV-1 genotypic resistance testing. Quantitative reverse transcriptase PCR, real-time PCR, nucleic acid sequence-based amplification and the branched DNA system are described in detail, and the advantages and disadvantages are discussed. In addition, two commercially available HIV-1 genotyping assays are compared. However, a general recommendation to favor one system over the other cannot be given, because the final decision of which system to use should be decided on the individual requirements.

At the end of 2006, an estimated 39.5 million (34.1–47.1 million) people worldwide had been infected with the HIV [101], and approximately 25 million have died already. The HIV pandemic is caused by two major types of HIV; while HIV-1 is responsible for the majority of HIV infections worldwide, HIV-2 predominates in parts of West and Central Africa. HIV-2 is divided into seven subtypes (A–G) [1–3], and HIV-1 viruses are further subdivided into three groups (major [M], N and O) based on their genetic make-up. Furthermore, group M is now divided into nine genetic subtypes A–J and over 20 circulating recombinant forms [4].

Routine screening for HIV infection is currently based on the detection of specific antibodies, antigens or both by serological tests. However, there are well-known limitations of these tests. In the phase of primary infection, viral antigens and antibodies are not detectable until 18–22 days after infection. In infants born by HIV-infected mothers, maternal antibodies will be present up to 18 months. To exclude vertical HIV transmission from mother to child, a direct diagnosis of viral antigen (p24 antigen) or the detection of viral RNA in plasma is essential for diagnosis. In chronically infected people, the molecular diagnosis of HIV RNA viral load in plasma in conjunction with the CD4 T-lymphocyte cell number are laboratory markers used to guide both initiation of highly active antiretroviral therapy (HAART), and monitor the effectiveness of treatment and the feasibility of clinical progression [5]. The goal of HAART should be the complete suppression of viral replication below the detection limit of the most sensitive assay, in order to avoid the emergence of
drug-resistant virus mutants and to delay clinical progression [6]. Therefore, continuous testing of HIV-1 viral load (VL) is necessary [7]. The value of the HIV genotypic-resistance test has been investigated and confirmed by several prospective studies [8–13]. Therefore, genotypic drug-resistance testing has been implemented in clinical guidelines as an important tool to guide therapy changes, therapy and more recently, initiation of therapy [14–16]. This recommendation is based on an increase in drug-resistant viral variants found in recently infected patients.

For both measurement of viral and genotypic drug resistance, several commercially available tests are on the market. This review will focus on these assays and discuss the different approaches to assess VL without preference for one or the other system. Furthermore, the two commercially available genotypic drug-resistance assays will be compared, focusing on the interpretation of the detected drug resistance-associated mutations.

Viral load determination
At present, there are four different methods with some commercially available assays for detection and quantification of HIV RNA on the market:

- Reverse transcriptase (RT) PCR; (Cobas Amplicor HIV1 Monitor® UltraSensitive/Cobas Amplicor HIV1 Monitor v1.5; Roche Diagnostics)
- Real-time PCR (Cobas Taqman® HIV-1 Test; Roche Diagnostics/Abbott RealTime® HIV-1; Abbott Laboratories)
- Nucleic acid sequence-based amplification (NASBA; NucliSensEasyQ® HIV-1; bioMérieux Clinical Diagnostics)
- Branched-chain DNA amplification (bDNA; Bayer Versant® HIV-RNA3.0; Bayer Corp.)

All of these commercially available assays have been approved for monitoring HIV-1-infected persons; they are not proposed to be used as screening tests for HIV-1 nor as confirmatory tests for HIV-1 infection, but are often used in this context.

Reverse transcription PCR
RT-PCR is used for converting RNA to cDNA and the subsequent amplification of the reverse-transcribed DNA. Using RT, dNTPs and a specific primer, a cDNA copy of the extracted RNA is made in a first step, first-strand synthesis. This product (cDNA) is used in a classical PCR and multiple copies are amplified. Taq polymerase is a heat-stable bifunctional enzyme, composed of a polymerization domain and a 5′-3′ nuclease domain. First, the double-stranded DNA is denatured by heating the template up to 90°C. The next step is the annealing of sequence specific primers at a temperature of 50–60°C, dependent on the melting temperature of the primer. To enable the thermostable polymerase to elongate new DNA strands, starting from the partial primer/DNA double strand, the temperature is maintained at 70°C (the optimum temperature of the taq polymerase). Afterwards, the next cycle is started with a new denaturation phase. Detection and quantification is carried out in the commercial assay by hybridization with specific labeled probes and subsequent detection by enzyme immunoassay. An example of an available assay is the Cobas Amplicor HIV1 Monitor v1.5/UltraSensitive (Roche Diagnostics).

Real-time PCR
In general, the real-time PCR principle is based on the standard RT-PCR method. In addition, it combines the quantification by RT-PCR with the optical detection of specific fluorescent reporter molecules to monitor the amount of PCR products in each amplification cycle. The TaqMan principle is only one of the provided quantification methods, and is used
to describe the principle of real-time PCR in this review. TaqMan probes are modified with a reporter fluorophore at the 5’-end, a quencher fluorophore at the 3’-end, and a 3’ blocking phosphate so that the probe is nonextendable during amplification. While the TaqMan probe is intact, the vicinity of the two dyes enables a fluorescence resonance energy transfer (FRET) from the reporter dye to the quencher dye, so that most of the excited state energy of the reporter is transferred to the quencher dye, which is emitted at a wavelength that is not analyzed by the system. During the annealing process, the TaqMan probe binds to the target sequence between the two primer binding sites (the traditional forward and reverse primers). As aforementioned, the Taq polymerase has a 5’–3’ nuclease domain, which is the crucial factor for the TaqMan assay. During strand elongation, the Taq polymerase cleaves the probe by using its 5’–3’ nuclease activity. The reporter dye is now separated from the quencher dye and, consequently, reporter fluorescence emission increases and is detected by the system (FIGURE 1). With each amplification cycle, additional reporter dye molecules are cleaved from their probes, thus the amount of fluorescence increases proportionally with the amount of PCR product generated. Available assays include Cobas TaqMan an HIV-1 Test, Abbott-Real Time HIV-1.

**Nucleic acid sequence-based amplification**

The NASBA assay is a continuous, isothermal, transcription-based amplification system that was originally designed for specific RNA amplification from plasma samples. Deiman and colleagues have given an excellent overview of the NASBA principle as well as primer and probe design rules [17]. Using the extracted RNA as a template, single-stranded RNA is amplified by the simultaneous enzymatic activity of T7 RNA polymerase, RT and RNase H to produce cDNA with a promoter sequence for the RNA polymerase in a first noncyclical phase. Therefore, in a specifically designed primer (Primer 1), the 3’ end is a target-specific sequence and the 5’-end contains a T7 RNA polymerase promoter sequence, which anneals to the target RNA. RT then synthesizes an RNA-DNA hybrid. Next, the RNase H digests the RNA component, the reverse primer (Primer 2) can anneal, and the RT synthesizes a DNA-DNA hybrid that includes the complete T7 RNA polymerase promoter sequence. At this point, the cyclical phase of the NASBA starts by the production of numerous RNA copies through the work of the T7 RNA polymerase (thousands of copies can be produced from one double strand of DNA). The amplified single-strand RNA is now used as the new template in this cyclic phase (FIGURE 2). Detection of the

![Figure 2. Principle of nucleic acid sequence-based amplification (NASBA) with molecular beacons.](image)
single-stranded RNA can easily be achieved by hybridization with sequence-specific probes in different techniques: enzyme-linked gel assay [18], electrochemoluminescence [19] or real-time detection by molecular beacons [20]. Available assays include, NucliSens HIV QT assay (bioMérieux Clinical Diagnostics); first generation assay, NucliSens EasyQ HIV-1 (bioMérieux Clinical Diagnostics).

**Branched DNA**

In contrast to nucleic acid amplification assays, the branched DNA method amplifies fluorescence signals originating from the RNA extracted (Figure 3). After lysis, capture of the target RNA to the solid phase is mediated by a mixture of capture extenders. These are immobilized oligonucleotides that are complementary to the target RNA, which are fixed to the surface of each well in the reaction plate. A second series of target-specific probes, so-called extender probes, now hybridize to various, well-defined regions of the captured molecules. To increase specificity, two extender probes serve as the hybridization site for one preamplifier molecule. Finally, the bDNA amplifiers (the key element of the bDNA technology) hybridize to the preamplifiers. These enzymes catalyze the dephosphorylation of a chemiluminescent substrate. The precise definition of hybridization sites enables quantification of the original input RNA copy number; the intensity of the emitted light signal is directly proportional to the amount of target sequence present in the sample. An example of an available assay is Bayer Versant HIV-RNA 3.0.

**Comparison**

Over the years, several methods for the quantitative detection of HIV-RNA in plasma were developed and are now commercially available (Table 1). One of these is RT-PCR as a powerful and low-cost technology. Newer versions, mostly fully automated systems, need less hands-on time, have lower detection limits of approximately 50 copies/ml and, to some extent, have a better performance in detecting different HIV clades.

While traditionally the PCR product is analyzed at the end point (usually in the plateau phase), the principle of detection by real-time PCR allows acquisition of data during the early phases of the reaction (exponential phase), resulting in a more reliable assessment of the input RNA amount. The increase of reporter fluorescence is directly proportional to the number of amplicons generated. Different target nucleic acids can be amplified in one reaction tube by using probes labeled with different reporter dyes that could be distinguished by the assay system. While PCR primers and hybridization probes are usually strain specific, but might tolerate some sequence mismatches, Taqman probes are highly specific. This is a prerequisite for the generation of the fluorescence signal, which is highly dependent on the hybridization between probe and target. There is no requirement for agarose gels or the addition of labeled probes for detecting PCR products; therefore, no post-PCR processing is needed, thereby minimizing the risk of contamination with amplification products. For HIV VL measurements, the real-time assay increases the dynamic range of detection and is useful for qualitative and quantitative detection; however, the high specificity of the primer/target binding could be disadvantageous for the detection of heterogeneous viruses such as HIV.

NASBA is a sensitive, isothermal amplification method that does not require a thermocycler, and there is no need for heat-stable enzymes. As the temperature is permanently at a constant level during the amplification process, each step of the reaction can proceed as soon as an intermediate amplification product is available. Multiple transcription of RNA copies by one-input RNA product results in the exponential kinetic of the amplification process; this is in contrast to the binary increase per cycle of the DNA amplification methods (PCR). Compared with a DNA-based amplification technique, there is no need for a separate RT step, thus reducing the risk of contamination and the loss of RNA, and saving time. As the enzymes used are not thermostable, the reaction temperature may not exceed 42°C without compromising the reaction; in many cases, this temperature is lower than the annealing temperature of the primers used, resulting in a lower specificity of the amplification process. The length of the amplified RNA target sequence should be in the range of 120–250 nucleotides, as shorter or longer sequences would be amplified with lower efficiency.

Branched DNA is a signal amplification method that does not require tedious extraction steps to isolate virus RNA.
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As bDNA amplification is a nonenzymatic process that does not change the original amount of target nucleic acid sequences, sample-to-sample variation and the influence of inhibitory substances contained in a clinical specimen is much lower in comparison with enzyme-mediated amplification systems; therefore, the risk of contamination is reduced. In general, target amplification still has greater analytical sensitivity than signal amplification methods. When novel targets are analyzed, bDNA has a lower viability in designing new assays, because a whole set of hybridization probes, rather than only one appropriate pair of primers, are needed. Additionally, some studies discuss the probability of false-positive results due to specificity problems of the bDNA assay [20,21].

Finally, the level of automation is of great concern, since a couple of years' worth of amplification and detection could be performed automatically with most of the commercially available assays; however, until recently, the automated extraction of nucleic acid remained the critical step. At present, all the commercially available systems presented in this paper have included an automated sample preparation system; however, most of these assays are still based on two separate systems for sample preparation and amplification/detection. Therefore, further manual steps are necessary to transfer the extracted RNA into the amplification and detection system. Nowadays, a fully automated system including all steps is available only for the Roche Cobas Taqman system.

### Available assays in detail

The Cobas Amplicor HIV-1 Monitor v1.5/UltraSensitive (Roche Diagnostics) is an RT-PCR-based system available both with manual extraction in combination with an automatic amplification and detection system, and as a fully automated version (UltraSensitive assay) including an automated RNA extraction step; however, this assay will gradually be replaced with assays such as the Cobas TaqMan HIV-1 Test or the Abbott RealTime HIV-1, which are fully automated real-time PCR systems, with a reported dynamic range of between 40 and 10,000,000 HIV-1 RNA copies/ml. Both assays use three external controls (negative, low positive and high positive) and one internal control, which is introduced into the sample lysis buffer and processed simultaneously with each sample. In the Abbott assay, the armored pumpkin RNA (similar to the HIV-1 sequence) is only used as an internal control, with no influence on the quantification result. By contrast, the Roche assay is based on a competitive co-amplification with a non-infectious armored RNA construct that contains HIV sequences with identical primer-binding sites as the HIV-1 target RNA [23]. This internal control has a known concentration of RNA and is used as quantification standard in parallel. The Abbott system must be recalibrated with every new batch, using the provided external calibration standards to create calibration curves. All systems are able to quantify HIV-1 group M subtypes A–H [24–29]. Additionally, the Abbott Real Time HIV-1 assay demonstrated the ability to detect group M subtype J and

### Table 1. Commercially available HIV-1 monitoring assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen</th>
<th>Input (µl)</th>
<th>Sensitivity (copies/ ml)</th>
<th>Dynamic range of quantification</th>
<th>HIV-1 subtype detection</th>
<th>Level of automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative reverse transcriptase PCR</td>
<td></td>
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<tr>
<td>Cobas Amplicor HIV-1 Monitor®v1.5</td>
<td>EDTA plasma</td>
<td>200</td>
<td>400</td>
<td>750,000</td>
<td>Group M (all subtypes)</td>
<td>Manual extraction</td>
</tr>
<tr>
<td>Cobas Amplicor HIV-1 Monitor UltraSensitive</td>
<td>EDTA plasma</td>
<td>500</td>
<td>50</td>
<td>50-75,000</td>
<td>Group M (all subtypes)</td>
<td>Manual extraction or automated extraction (AmpliPrep)</td>
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<tr>
<td>Real-time PCR</td>
<td></td>
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<tr>
<td>Cobas TaqMan® HIV-1 Test</td>
<td>EDTA plasma</td>
<td>1000</td>
<td>40</td>
<td>40-10,000,000</td>
<td>Group M (subtypes A-H)</td>
<td>Automated extraction (AmpliPrep), complete automation with the AmpliPrep/Cobas Taqman instrument</td>
</tr>
<tr>
<td>Abbott-RealTime® HIV-1</td>
<td>ACD and EDTA plasma</td>
<td>1000, 500 and 200</td>
<td>40, 75 and 150</td>
<td>40-10,000,000</td>
<td>Group M (subtypes A-H and Group O plus N)</td>
<td>Automated extraction with the m2000sp</td>
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<tr>
<td>Nucleic acid sequence-based amplification</td>
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<tr>
<td>NucliSens EasyQ® HIV-1</td>
<td>EDTA plasma</td>
<td>1000 and 2000</td>
<td>50</td>
<td>50-3,000,000</td>
<td>Group M (subtypes A-J)</td>
<td>Automated extraction with the EasyQ system</td>
</tr>
<tr>
<td>Branched-chain DNA amplification</td>
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<tr>
<td>Bayer Versant® HIV-RNA 3.0</td>
<td>EDTA plasma</td>
<td>1000</td>
<td>50</td>
<td>50-500,000</td>
<td>Group M (subtypes A-G)</td>
<td>Manual centrifugation and lysis; alternatively, complete automation</td>
</tr>
</tbody>
</table>
the groups N and O, because it targets the highly conserved integrase region of HIV-1 compared with the TaqMan assay, which uses a region in the HIV-1 gag gene. However, the diversity of HIV complicates VL measurement for patient management and treatment monitoring. Numerous studies have demonstrated that non-B group M variants can be underestimated, and that group O strains are not detected by some commercial tests. A comparative evaluation of both the real-time systems from Abbott and Roche demonstrates that the Roche system underestimates the VL in a few samples by more than 1 log (CRF02 strain and subtype A). Furthermore, the system failed to detect a subtype G and H strain. VL values were similar in both assays within subtype B and D plasma samples. Another difference is the possibility to run four assays (for HIV-1, as well as for hepatitis C or B virus) simultaneously on the totally automated Roche system, owing to four independent onboard thermal cyclers. Both assays enable high-throughput analyses (48–96 samples), short turnaround times and low hands-on time, because they have a high level of automation. After automatic extraction, the amplification plate must be transferred in the thermocycler unit in both systems, but in contrast to Abbott, Roche additionally offers a fully automated system.

The NucliSens EasyQ (BioMérieux) is an updated version of the NucliSens HIV VT assay, which uses the molecular beacon detection technology and real-time NASBA amplification with a range of 50–3,000,000 copies/ml; the assay quantifies all HIV-1 group M subtypes A–J reliably. The risk of amplicon contamination is reduced by the single closed-tube format. Each tube has its own internal controls and is calibrated separately, and thus the quantitative result is corrected for any loss of RNA during isolation or less efficient amplification. With its real-time NASBA amplification technology, hands-on time is reduced to less than 1 min per sample, and only 90 min is required for amplification and real-time detection of 48 samples. Automated data analysis and reporting is achieved by using Windows- based software. Comparative evaluation studies demonstrated a relatively good concordance of the result values with the Roche Cobas Monitor v1.5 assay. However, the disparity between the results of the two assays was highly significant in subtype C samples; in the vast majority of these samples, higher VL values were obtained by the Roche assay.

The Bayer Versant HIV-1 RNA 3.0 assay in combination with the Bayer system 440 is a fully automated branched DNA system with a reported dynamic range of 50–500,000 HIV-1 RNA copies/ml, and is able to quantify HIV-1 group M subtypes A–G reliably. The risk of cross-contamination is reduced because no viral extraction is required. Three external controls are processed simultaneously (high positive, low positive and negative). The Versant 440 molecular system processes 12–168 patient samples per run and its load-and-go BDNA automation improves workflow and testing flexibility for mid- to high-volume laboratories. The amplification is followed by an automated data analysis and report output using the included software. The broad, linear range of quantification is one main advantage of the BDNA assay in comparison with classic target amplification methods. This has been lost with the introduction of the real-time amplification-based assays. Nevertheless, the advantage of a comparably low inter- and intra-assay variation still remains. Comparative evaluation studies of the BDNA system with the Roche Monitor Test, v1.5 reveals a relatively good correlation of both assays. Furthermore, Swanson and colleagues demonstrated that VL measured by the Abbott real-time assay correlated very well with both BDNA and the Roche Monitor Test, v1.5 for group M subtypes. By contrast, the group O viruses were not detected by the Roche Monitor Test, v1.5 and were quantified substantially lower, with approximately 2 log_{10} copies/ml in the BDNA assay in comparison with the Abbott real-time assay. The comparison of the Roche TaqMan assay with the BDNA assay revealed no significant differences between both assays, even in the genetically diverse populations of HIV-1 group M.

**HIV-1 genotyping**

There are currently two commercial assays available for HIV-1 genotyping that 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; Bayer Diagnostics, Fernwald, Germany). Both ViroSeq and TruGene are US FDA-approved systems and are CE-labeled. Modules for nucleic acid extraction, RT-PCR, sequencing reaction and software for sequence alignment and drug-resistance interpretation are included. An automated sequencer for analyzing the sequencing products must be purchased separately. For ViroSeq, the capillary sequencers from Applied Biosystems, Foster City, USA, are usable; the TRUGENE sequencing products were analyzed on the slab gel O-pengene™ DNA sequencing system (O-pengene), which is also available from Bayer.

Both systems have been compared recently, and thus a detailed description will not be given here. Briefly, in a head-to-head comparison, they are similar with respect to the complexity, but different in regards to the protocols. The extraction module is either based on isopropanol precipitation (ViroSeq) or silica-based gel columns (TRUGENE), which appear to be more effective. The RT-PCR modules are comparable, but the PCR product purification step and the agarose gel quantification necessary for ViroSeq are omitted for TRUGENE. One major difference between both systems is the sequencing chemistry, although both systems are based on the Sanger sequencing method. ViroSeq uses a four-dye terminator system, which is more robust than the dye terminator system used in TRUGENE, and results in better sequence quality. In addition, six samples are needed to analyze one patient for ViroSeq, compared with 12 samples for TRUGENE. On the other hand, it is not necessary to remove the dye primers in the TRUGENE system compared with the purification step for removal of the dye terminators in the ViroSeq system. Another crucial point is the sample throughput. Depending on the sequencer used for the analysis of the ViroSeq sequencing products, a high sample throughput can be achieved. With a 16-capillary sequencer running for
Detection of the viral variants present at a frequency below 20% has been published investigating the sensitivity of ViroSeq for drug-resistant variants, in-house tests, which have been described in the literature (e.g., the LigAmp assay), must be used. Although the clinical consequences of these findings are not clear, an improvement of the 20% cut-off for the detection of minor viral variants is necessary. Recently, one study has been published investigating the sensitivity of ViroSeq for detection of the viral variants present at a frequency below 20%. They found that ViroSeq frequently detects the analyzed mutation at lower levels; for example, 1-5% of the minor variant was detected in 16.9% of the samples. However, this is not sufficient for routine screening, and to date, no modifications for ViroSeq or TruGene have been published. For those who want to perform HIV-1 genotyping with the sensitive detection of minor viral variants, in-house tests, which have been described in the literature (e.g., the LigAmp assay), must be used.

Expert commentary

Since the introduction of assays for the detection and quantification of HIV-RNA, the technology has been optimized in regard to accuracy, high-throughput capability, wide dynamic range, performance, sensitivity, potential for multi-target detection in a single tube and eliminating the need for post-PCR processing. However, this improvement resulted in high costs for the available assays, leading to problems for countries with a lower budget for healthcare; in these settings, only a few of these industrial developed assays could be used. Therefore, many laboratories have developed their own in-house PCRs, resulting in varying quality, insufficient standardization and loss of comparability between different laboratories. Although the new automated real-time PCR systems offer an attractive solution to increase laboratory throughput, enhance overall efficiency and reduce operator-associated errors (while providing excellent sensitivity, a broad dynamic range and reliable quantitation of most of the genetically diverse HIV strains), a general recommendation to favor one system over the other cannot be given, because the final decision of which system to use should be reached on the individual requirements.

Five-year view

The introduction of the PCR has revolutionized the laboratory diagnosis of viral infection. In a very short time period, this method developed from very laborious and time-consuming home-brewed assays to fully automated real-time PCR systems with a turnaround time of only a few hours. In the future, we expect that the improvements and changes will be achieved mainly in the field of standardization and in the expansion of the amount of different pathogens that could be detected. To date, one of the major problems is that the assays must be adapted to new pathogens, especially to detect all members of the often very heterogeneous virus families equally. In the case of HIV, there is an increased need to improve commercially available assays in their ability to equally detect the different HIV-1 subtypes. The degree of sensitivity has reached a very high level; therefore, the possibilities for further decreasing the detection limit might be difficult. However, efforts are underway to improve the detection limit and especially to reduce inter- and intra-assay variation of test results.

The same approach to lower the detection limit as far as possible must be performed for the commercially available genotypic assays to detect drug-resistance-associated mutations as soon as possible in the case of virological failure. Although some modifications have already been developed, slight changes in the manufacturers’ instructions have not been published. The commercially available systems include a drug-resistance interpretation report, but the frequency of updates must be improved. In general, the interpretation of mutations is still an issue that needs standardization. In addition to the reports mentioned earlier, many interpretation systems are freely available, but the level of concordance between them is suboptimal. However, the most important task to be performed for the improvement of the commercially available assays will be optimization for the detection of minor viral variants.
Key issues

- There are many different commercially available assays for viral load determination.
- These assays should:
  - Be optimized to avoid post PCR processing
  - Have high-throughput capability
  - Have a wide dynamic range
  - The assays currently have relatively high costs
- In-house methods have insufficient standardization.
- HIV-1 genotyping has been implemented in national and international treatment guidelines.
- Two US FDA-approved commercial assays are available to be performed in one's own laboratory.
- Both assays are labor-intensive and highly complex, with many steps.
- Both assays have comparable accuracy for detecting drug resistance-associated mutations (DRAMs) in subtypes A–J and several circulating recombinant forms.
- Interpretation of DRAMs into clinically useful data is provided by both systems and has not been associated with major discrepancies compared with other algorithms.
- The detection of minor variants is the major improvement yet to be made for the commercially available genotypic assays.

References

Papers of special note have been highlighted as:

• of considerable interest
  • of considerable interest

11. Demonstrates the benefit of HIV genotyping in treated patients, independent of therapeutic regimes.
Monitoring HIV infection and antiretroviral therapy


36. Reviews the great variation between genotypic interpretation systems.


38. Modification of the ViroSeq reverse transcriptase (RT) PCR module resulting in increased sensitivity.


40. Modification of the ViroSeq extraction module, resulting in increased sensitivity.


