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The Evolving Landscape of HIV Drug Resistance Diagnostics for Expanding Testing in Resource-Limited Settings

Seth C. Inzaule[1], Raph L. Hamers[1,2], Roger Paredes[3], Chunfu Yang[4], Rob Schuurman[5] and Tobias F. Rinke de Wit[1]

[1]Department of Global Health, Academic Medical Center of the University of Amsterdam, and Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands; [2]Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center of the University of Amsterdam, The Netherlands; [3]HIV Unit & irsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona & Universitat de Vic-UCC, Badalona, Catalonia, Spain; [4]Division of Global HIV/AIDS and TB, Center for Global Health, Centers for Disease Control and prevention, Port-au-Prince, Haiti; [5]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands

Abstract

Global scale-up of antiretroviral treatment has dramatically changed the prospects of HIV/AIDS disease, rendering life-long chronic care and treatment a reality for millions of HIV-infected patients. Affordable technologies to monitor antiretroviral treatment are needed to ensure long-term durability of limited available drug regimens. HIV drug resistance tests can complement existing strategies in optimizing clinical decision-making for patients with treatment failure, in addition to facilitating population-based surveillance of HIV drug resistance. This review assesses the current landscape of HIV drug resistance technologies and discusses the strengths and limitations of existing assays available for expanding testing in resource-limited settings. These include sequencing-based assays (Sanger sequencing assays and next-generation sequencing), point mutation assays, and genotype-free data-based prediction systems. Sanger assays are currently considered the gold standard genotyping technology, though only available at a limited number of resource-limited setting reference and regional laboratories, but high capital and test costs have limited their wide expansion. Point mutation assays present opportunities for simplified laboratory assays, but HIV genetic variability, extensive codon redundancy at or near the mutation target sites with limited multiplexing capability have restricted their utility. Next-generation sequencing, despite high costs, may have potential to reduce the testing cost significantly through multiplexing in high-throughput facilities, although the level of bioinformatics expertise required for data analysis is currently still complex and expensive and lacks standardization. Web-based genotype-free prediction systems may provide enhanced antiretroviral treatment decision-making without the need for laboratory testing, but require further clinical field evaluation and implementation scientific research in resource-limited settings. **(AIDS Rev. 2017;19:179-89)**

Corresponding author: Seth Inzaule, s.inzaule@aighd.org

Key words

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Correspondence to:
Seth Inzaule
Trinity Building C
Pietersbergweg, 17
1105 BM Amsterdam, The Netherlands
E-mail: s.inzaule@aighd.org

Introduction

By the end of 2015, over 18.2 million people infected with HIV were receiving combination antiretroviral treatment (ART), reflecting an unprecedented scale-up of ART over the past decade[1]. This increased access to ART has been a major public health success, especially in resource-limited settings (RLS), with significant reductions in HIV transmission and HIV-related mortality[2]. However, access to monitoring of diagnostic laboratory tests has not matched the rapid ART scale-up[3-5]. Until recently, most programs in RLS have relied mainly on clinical and immunological criteria to monitor ART efficacy, despite their inaccuracy and late detection of treatment failure when compared to the gold-standard viral load criteria[3,6].

Following the recent World Health Organization (WHO) recommendations, implementation of routine viral load (VL) monitoring is now being prioritized in large-scale ART programs to enable early detection of treatment failure, thus enhancing timely switching to next-line drug regimens[7,8]. In 2015, the Joint United Nations Programme on HIV/AIDS (UNAIDS) further launched an ambitious target for achieving a 90% VL suppression rate among all patients on ART by 2020[9]. This brings in focus the need for expanding access to HIV diagnostic tools.

With expansion of routine VL tests, the number of patients switched to second-line therapy is forecasted to increase to about 4-6 million by 2030, and this will comprise of nearly 20% of all patients on ART[10]. Limited available data have indicated that about 10-40% of the patients on protease inhibitor-based second-line have treatment failure[11] and a majority (70-90%) of them lack resistance mutations to the key drug, protease inhibitors (PI)[12,13]. Due to the limited third-line drug options in most RLS, the clinical management of these patients is increasingly complex in the absence of HIV drug resistance (HIVDR) tests to determine those harboring drug resistant viruses and guide the selection of optimal regimens.

Rising levels of pre-treatment HIVDR in sub-Saharan Africa also poses a threat to the success of national ART programs[14-16], especially in the era of 'treat all' and pre-exposure prophylaxis (PrEP) scale-up recommendations[17,18]. Recognizing these challenges, the recent WHO guidelines recommend active surveillance monitoring of HIVDR in patients initiating ART for pre-treatment HIVDR and in those on ART for acquired HIVDR[8,19].

Thus, to improve individual patient management, protect the durability of available drug regimens, and ensure the rational use of scarce third-line drugs, there is a need for low-cost strategies to measure HIVDR. In this review we have assessed the landscape of current HIVDR tests and evaluated the strengths and limitations for their use in both individual ART patient management and population-based surveillance and monitoring in RLS.

Landscape of HIV drug resistance technologies

HIV drug resistance tests are broadly classified into genotypic and phenotypic assays. Genotypic resistance tests (GRT) are sequencing-based assays that identify specific amino acid changes, or mutations, known to be associated with resistance to specific antiretroviral drugs. Genotypic resistance assays can be further categorized into sequencing-based approaches such as Sanger and next generation sequencing and into assays based on the detection of specific point mutations. Although phenotypic assays may sometimes provide improved resistance assessment quality over genotypic tests[20], they are prohibitively expensive, require sophisticated laboratory setups such as biosafety level III facilities, and have long turn-around times. Thereby, their use in resistance testing in RLS has not been realized and will not be discussed further in this review.

An ideal GRT for use in RLS has previously been described[5]. The specifications for such a test includes low-cost, compatibility with dried blood spots (DBS) specimen type, having broad subtype coverage, open access to use readily available consumables and reagents, and low-cost instruments for sequencing/detection systems, as well as requiring minimal complexity in terms of equipment, analysis method, and skills, and a reasonable turnaround time and acceptable testing sensitivity. It should also be able to pass certification and proficiency testing by the appropriate regulatory authorities.

In the sections below we discuss the current landscape of HIVDR tests, first from a technical point of view, followed by an assessment of their applicability in RLS.

Sanger sequencing assays

Sanger sequencing, also known as first-generation sequencing assays, are based on di-deoxynucleotide

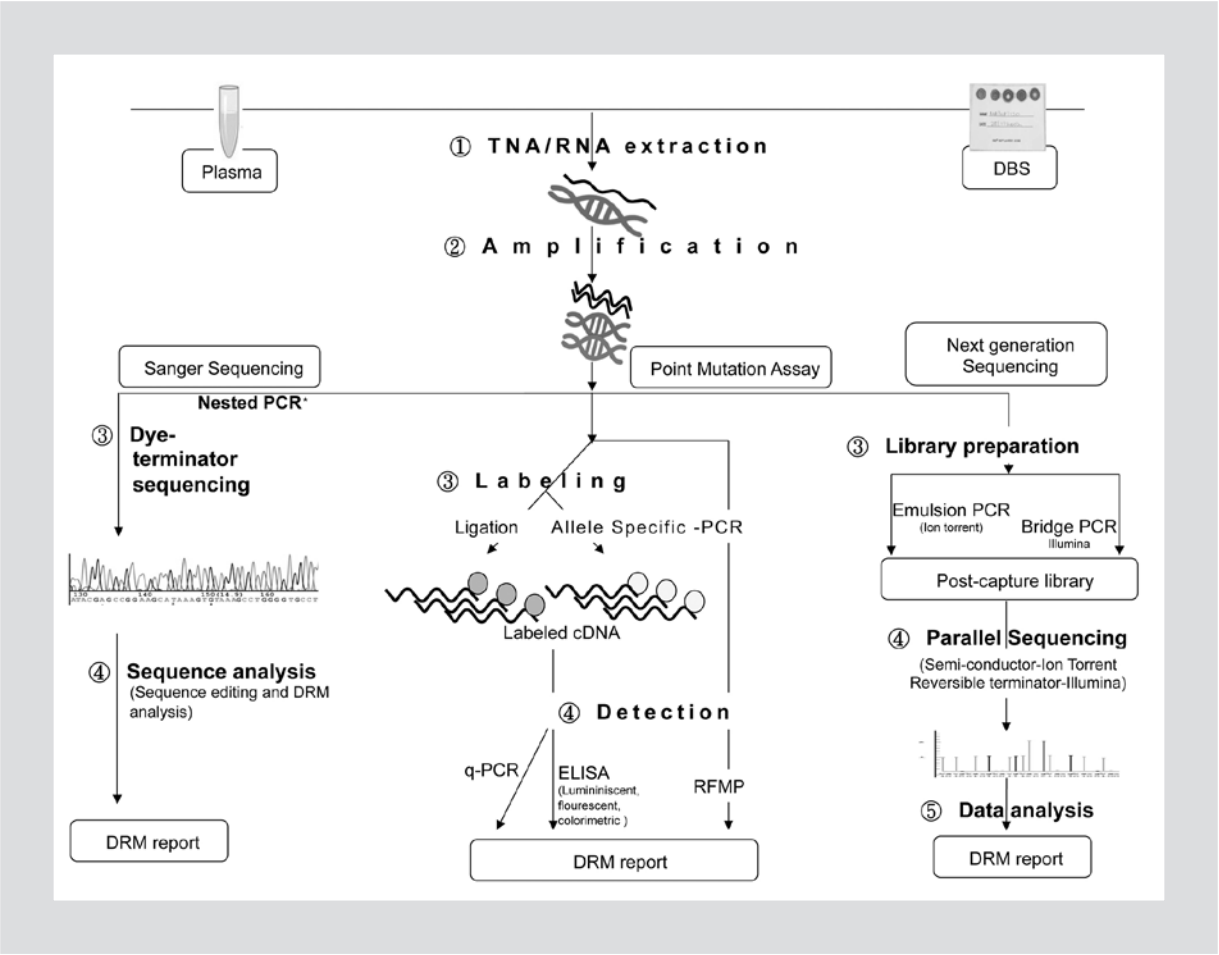

Figure 1. *Overview of Sanger, point mutation, and next generation assays work flow.*
*(1) Nucleic acid is extracted from plasma, RNA, or dried blood spots; TNA, followed by (2) amplification to either cDNA or DNA. *Some assays include a second amplification to increase yield. For Sanger sequencing (3) dye-terminator sequencing involving incorporation of fluorescence-labeled terminator bases to produce dye-labeled nested products, which are resolved in a genetic analyzer to produce a chromatogram. (4) Analysis involves base-calling, sequence editing, and quality checks by use of automated software followed by drug resistance analysis using proprietary or web-based applications. For point-mutation assays, (3) production of labeled target product is done where labeled bases are incorporated in complementary DNA strands using either selective ligation of adjacent oligonucleotide probes by a template-dependent DNA ligase or single base extension by allele-specific primers. (4) Detection of labeled target mutation then occurs by q-PCR or ELISA. Alternatively non-labeled products of different lengths are generated using allele-specific primers of varying lengths. The products are then digested using restriction enzymes and detected through mass-spectrometry. For next-generation sequencing, (3) a library of target nucleic acid is created from the initially amplified DNA by a second round of amplification in a process called target enrichment. First, the DNA or cDNA is randomly fragmented to form short strands that are then joined to adapter sequences template (adaptors contain binding sequences, barcodes and primers). The se then undergo amplification, either by emulsion PCR (Ion Torrent) or bridge PCR (Illumina). (4) Sequencing is by synthesis through semi-conductor sequencing – Ion Torrent or reversible termination sequencing: Illumina (5). Analysis involves a series of steps involving (a) quality assessment of the raw files, (b) removal of adaptor sequences, (c) de-multiplexing to re-identify the samples, (d) mapping of data to reference sequence variant calling and validation, and (e) HIVDR analysis by third-party resistance interpretation databases. cDNA: complementary DNA; DBS: dried blood spots; DRM: drug resistance monitoring; HIVDR: HIV drug resistance; RFMP: restricted fragment mass polymorphism; TNA: total nucleic acid.*

chain-termination technique[21] and are currently the most widely used assays. Sanger sequencing requires four basic steps: nucleic acid extraction, target amplification, sequencing, and data analysis (Fig. 1). Briefly, extracted nucleic acid from either plasma or DBS is first amplified using primers for the target region. The confirmed amplicons are then sequenced using fluorescently labeled chain terminators to generate nested sets of dye-labeled products, which are then resolved by gel or capillary electrophoresis in a genetic analyzer. The sequence data are then edited and the consensus sequences generated analyzed for HIVDR using proprietary software or web-based HIVDR databases that provide automated sequence interpretations[22].

Sanger GRT includes commercialized kit-based and in-house assays (Supplementary Tables 1 and 2)[23-32]. To date, only two genotypic assays for the polymerase gene (protease and reverse-transcriptase) have been FDA-approved, i.e. ViroSeq® assay (Celera Alameda, CA, USA) and TruGene® assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA), with the latter having been discontinued[26]. ViroSeq® covers the entire protease region (1-99 codons) and two-thirds (1-335 codons) of the reverse-transcriptase region[23]. It has a detection limit of 2,000 cps/ml of plasma VL and is approved dedicated for use with certain models of the Applied Biosystems® genetic analyzers and for HIV-1 subtype B viruses only.

Potential for use in resource-limited settings

Many research or public health laboratories around the globe have developed in-house Sanger assays for HIV GRT to circumvent the high costs associated with the commercial tests and to ensure compatibility with HIV-1 non-B subtypes[24,25,27,29-32]. The following three laboratory developed and validated in-house assays have been commonly used in sub-Saharan Africa: (i) the assay that was developed by the French National Agency for AIDS Research (ANRS)[30], (ii) the assay that was developed by the US Centers for Disease Control and Prevention (CDC) and the technology transferred to the American Type Culture Collection (ATCC) and Thermo Fisher[31,33] scientific for kit productions, and (iii. the Southern African Treatment and Resistance Network (SATuRN) developed assay which is partnering with Thermo Fisher for kit-based production[29,34]. There are other laboratory developed in-house assays, such as the RT only Affordable Resistance Test for Africa (ARTA) assay[32] and low-cost assays from the Asia-Pacific region[24,25,27] (See Supplementary Table 2 for details).

Compared to other potential GRT, these Sanger-based sequencing assays are the ones commonly used for WHO-recommended HIVDR surveys[35]. These assays have demonstrated optimal performance over a wide range of HIV-1 subtypes and circulating recombinant forms (CRF) and some of them performed well on DBS sample type with high accuracy and reasonable (1,000-5,000 cps/ml of VL) genotyping sensitivity[30-32,36] and have a reasonable cost per test (US$ 40-100), (Supplementary Table 2). Due to the relatively low-cost per test and medium-to-high throughput of the genetic analyzers used, they are suitable for both individual patient monitoring and HIVDR surveys.

In addition, a number of open-source sequence editing tools are available for use with Sanger-based assays, including automated tools that minimizes inter-subject variability. This includes Recall (University of British Columbia, Canada) and BioEdit (Tom Hall, CA, USA).

However, there are limitations for Sanger-based assays: they are labor-intensive, require certain laboratory set-ups for preventing contamination, and only detect majority genotypes (> 20%). Moreover, they use sequencers that have a high capital cost (~250,000 US$) and whose infrastructure is mainly suitable for centralized specialized laboratories.

Next-generation sequencing assays

Recent advancements have seen the development of massive parallel high-throughput sequencing technologies, collectively called next-generation sequencing (NGS)[37]. Although NGS technology has rapidly transformed the landscape of most areas in genomic research[38], its introduction into routine clinical application has been much slower. Today only a few commercial virus genotyping assays based on NGS platforms are available: DeepGen™ Assay (University Hospital Case Medical Center) using Ion Torrent™ and Monogram's GeneSure® genotypic assay using Illumina's sequencers[39]. The Illumina and Ion Torrent™ NGS are the most commonly used platforms in HIVDR research and surveillance[40] and are also the potential low-cost bench-top assays discussed in this section (Supplementary Table 3).

The basic workflow for NGS includes nucleic acid extraction, amplification, library preparation, target enrichment, sequencing, imaging and data analysis, as shown in figure 1.

Potential for use in resource-limited settings

Similar to Sanger-based sequencing assays, NGS also has a relatively high capital cost for acquiring instruments, but this is expected to gradually decrease with advancements that allow competition and innovation of even cheaper technologies. Currently, the only commercially available "low-cost" devices are the bench-top Ion Torrent Personal Genome Machine (US$ 50,000), Illumina's MiSeq (US$ 99,000) and MiniSeq™ (US$ 49,500)[41]. The cost per run of NGS is prohibitively high for use in RLS (Supplementary Table 1), but the prices can be significantly lowered through multiplexing[42,43]. A previous study using Illumina MiSeq

(read length ~ 200 bp) demonstrated that 24 samples could be multiplexed with a depth of > 10,000 counts per base[43] at a cost of between US$ 24-31. An even higher level of multiplexing has been demonstrated in a "wide-sequencing" approach (multiplexing of many samples in a run but with lesser coverage depth) on Illumina MiSeq, resulting in sequencing costs of ~ 5 US$ per sample (multiplex of 1,143 samples), with a median read depth of > 9900 counts per base[44]. This study, however, only sequenced a small portion of RT (90-234 bases), but the same depth and costs might not be achieved when longer targets are desired.

Sequencing of longer genome targets is sometimes required, especially when assessing resistance in patients treated with integrase strand-transfer inhibitors (INSTI) plus nucleoside reverse transcriptase inhibitors (NRTI) or PIs without the need for separate assays, as is currently the case for most available Sanger-sequencing assays[39]. This is especially important, given the current recommendation for use of INSTI with NRTI or/and PI combinations in RLS. As with Sanger-based assays, NGS also has the ability to use the widely validated broad subtype primers and DBS[45], as they share similar steps in the upstream procedures (Fig. 1).

Limitations for NGS include the following: the need for high-level multiplexing to achieve significant cost reductions limits their use to high-throughput facilities or for population-based HIVDR surveys. In addition, the assay requires multiple procedures, which increases the complexity of the assay and the laboratory infrastructure. Moreover, the cost of library preparation for longer genome targets is expensive and does not change even with increased order of multiplexing (Fig. 2).

The level of bioinformatics support, expertise, and infrastructure required for on-site data analysis is also still complex and expensive[46]. This includes the need for dedicated data centers with servers and storage center, computing clusters (high-performance computers, high-capacity storage and fast networks) and skilled bioinformaticians, system administrators, and developers[46]. Alternative cloud-based analysis applications are also expensive, for example DeepChek® (Advanced Biologic Laboratories, Luxembourg). This is currently the only commercially available HIVDR customized bioinformatics application, developed for research use only, and has a cost of US$ 65 per sample (~ 3-times the cost of sequencing)[47]. However, there are ongoing efforts to create open-source robust yet user-friendly automated NGS bioinformatics HIVDR analysis platforms that could be used by laboratory technicians with no bioinformatics expertise.

Point mutation assays

Point mutation assays identify only specific mutations as opposed to the sequencing methods, which provide sequence information of almost the entire target genome. A number of point mutation assays have been developed and adapted for use in HIVDR[48-60]. These include allele-specific PCR (ASPCR)[48,51-56,58-60] and oligonucleotide ligation assays[49,50,57]. The ASPCR assays use mutation-specific primers with mismatched 3' residues to selectively amplify viruses containing mutant and wild-type alleles of a given codon. Ligation assays, on the other hand, use the principle of selective ligation of adjacent oligonucleotide probes by a template-dependent DNA ligase[49,50,57]. The oligonucleotides are designed to selectively match the mutation site while mispriming if hybridized to a wild-type template. The target allele is then detected by either ELISA (colorimetric, fluorescent or luminescent)[48-50,53,58,59] real-time q-PCR[54,55,57,60], mass spectrometry[52], or gel electrophoresis[56] (Fig. 1).

Potential for use in resource-limited settings

Compared to the other promising low-cost technologies, point mutation assays are an attractive option as they have a lower capital cost and can also be deployed at or near point-of-care settings. A key challenge is that they are only able to identify a limited number of mutations. Furthermore, a high polymorphism at primer sites affects both the accuracy and sensitivity of these tests. Equally high codon redundancy at mutation site coupled with the high number of possible resistance mutations for the different drugs in the combined regimen increases the cost per sample in the absence of high order multiplexing. Moreover, differential codon usage by HIV strains makes point mutation assays subtype-dependent.

Based on a recent analysis of genotypes available in the Stanford HIVDR database, it was proposed that a point mutation assay capable of detecting a set of six drug resistance mutations in reverse-transcriptase (i.e. K103N, V106M, Y181C and G190A [NNRTIs] and K65R and M184V [NRTIs]) could be sufficient to detect drug resistance in patients failing first-line and in ART-naive patients, with 98.8 and 61.2% sensitivity, respectively[61]. A similar analysis using the same data

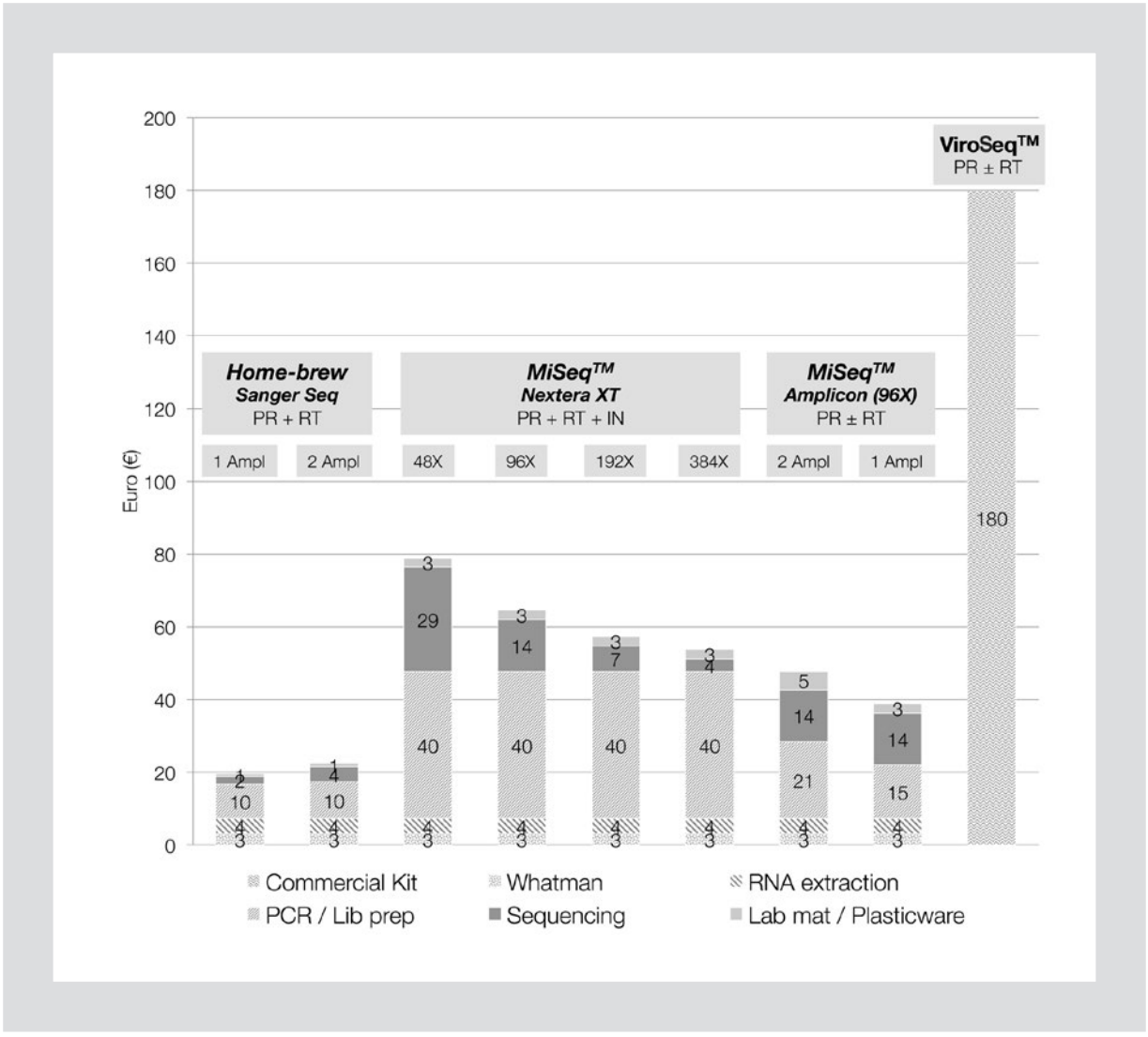

Figure 2. *Reagent costs of HIV-1 genotyping using different technical approaches in a European laboratory.*
These are real reagent costs in a specific European laboratory in 2015 and may vary from lab to lab. They do not include workforce costs, upfront spending on equipment acquisition, or maintenance. This comparison shows that both homebrew Sanger sequencing and next-generation sequencing can reduce sequencing costs by at least twofold compared with current standards. A MiSeqTM Nextera XT approach would provide resistance data for protease, reverse transcriptase, and integrase, being optimal for current genotyping needs. However, such an approach remains significantly more expensive than homebrew Sanger sequencing. Of note, the current main driver of sequencing costs for the MiSeqTM Nextera XT approach is library preparation. Whereas sample multiplexing might reduce sequencing costs to some extent, truly significant cost reductions must come from reducing library preparation costs. Lab mat: laboratory materials; Lib prep: library preparation; IN: integrase; PR: protease; RT: reverse transcriptase; Seq: sequencing; 96X: 96 reactions.

indicated that there are up to 42 distinct codons for the proposed set of six drug resistance mutations in sequences from seven of the most common subtypes (A, B, C, D, G, CRF01_AE, and CRF02_AG)[62]. This ranged from four codons at the 184 position, to eleven at position 190.

A proposed solution to use degenerate primers (a mix of primer sequences with some positions having a number of different possible bases) is feasible[63,64] to cater for polymorphisms near the target binding sites, but this may affect specificity and will still require a medium-to-high level multiplexing capability due to high codon redundancy at target sites.

Most point mutation assays described in the literature have a low multiplex ability due to limitations in available technology and instrumentation[49-51,54,55,57,58]. Some assays are able to compensate for this by using high-throughput instruments, but at an increased labor, complexity, and cost which makes it difficult to deploy them at point of care. Only the array based assays

show promise with medium-to-high multiplex ability, but they still rely on molecular systems that are difficult to deploy at point of care[48,52,59]. Examples include the micro array-based multiplex allele specific drug resistance assay (MAS-DR)[59] and the Matrix Assisted Laser Desorption/Ionization–Time Of Flight (MALDI-TOF) mass array assay[52]. Of these assays, only the MAS-DR assay has both a high multiplex ability and a low capital cost. A study by Zhang, et al. used MAS-DR multiplexed 45 allele targets (20 wild type and 25 mutants)[59] at a cost of US$ 40.90 using a low-cost Luminex MAGPIX® (US$ ~ 27,500) device. The mass array assay has also been shown to have a low-cost at US$ 30 for 18 target alleles, but the mass spectrometers are costly ranging from US$ 150,000 to 850,000 (Supplementary Table 4).

Genotype-independent predictions systems of treatment response

The exponential growth in the collection of biomedical data has the potential for the development of personalized treatment decision-making. An example of where "Big Data" or artificial intelligence could inform individualized medicine is that of HIV treatment. These so-called genotype-free systems for predictions of ART responses provide a practical and affordable alternative to laboratory-based strategies to enhance ART decision-making[22,65]. Typically, the data used to train the underlying computational models to make predictions include a complete ART history, VL and CD4 count on the failing ART regimen. The most advanced initiative to date is the HIV Treatment Response Prediction System (HIV-TRePS) developed by the HIV Resistance Response Database Initiative (RDI) and it is available as a free web service[65]. Using biological, clinical, and treatment outcome data from more than 180,000 HIV-1 patients derived globally, the RDI has developed computational models that can accurately predict HIV treatment outcomes, which may be used to identify optimal and individualized therapies for patients experiencing ART failure[65]. The latest models have achieved an accuracy of predicting ART response at the level of 82%, which is statistically significantly more accurate than GRT's rules-based interpretation, typically at 55-65%[65].

Potential use in resource-limited settings

It seems that the non-genotype prediction systems are the most cost-effective method in drug resistance, apart from the requirement for Internet infrastructure. Studies also showed that the methods resulted in more accurate prediction of treatment outcomes than the traditional genotype-dependent rule-based algorithms incorporated in most HIVDR interpretation databases[22,65]. The accuracy is, however, lower for regions in which there are limited datasets in the database, such as sub-Saharan Africa[65].

In addition, the prerequisite of the model systems for a recent VL and CD4 test result[65] may have limited its utility in RLS as most countries have adopted the 2015 WHO recommendation for a "test and start" treatment strategy and VL-based monitoring, leading to reductions in CD4 testing[8]. In addition, not all patients with virological failure have clinically relevant resistance mutations; thus these methods might result in unnecessarily switching to costlier regimens, potentially making them more expensive than GRT. Lastly, these systems also have limitations in providing data at the population level to guide programmatic decisions and hence may only be useful for individual patient management.

Expanding testing within the current health infrastructure

At present, HIVDR testing in Africa is limited to only a few laboratories, mainly research facilities and national reference laboratories, some of which are WHO-designated national or regional HIVDR laboratories[5]. Expansion of drug resistance testing to new sites may be hampered by the high capital costs, limited infrastructure, quality assurance requirements, and a shortage of highly skilled and experienced personnel. In the current landscape, incorporation of HIVDR testing in the public health system could best follow a centralized approach within the WHO-recommended tiered framework for healthcare delivery[66]. This tiered approach incorporates an integral laboratory system aligned within the country's public health delivery structure of the four-level hierarchal health system.

The WHO-designated national/regional HIVDR laboratories belong to the top tier of the four-level pyramid system[66]. These laboratories serve as HIVDR referral facilities for in-country peripheral sites and nearby countries where genotyping capacity is lacking. Appropriate technologies for use in these laboratories would include the Sanger sequencing-based assays, multiplex point mutation assays, and NGS. These could mainly be the high-throughput facilities, which may in addition have bioinformatics capacity to support NGS analysis.

For level III facilities (provincial/ regional laboratories within a country), these can also incorporate upcoming point mutation assays such as the Luminex MAGPIX®. Level III facilities can also perform PCR tests and then send the products to level IV laboratories for sequencing as these laboratories have the capacity for performing molecular tests, such as VL tests and DNA-PCR for early infant diagnosis. Moreover, facilities at level III could also serve as sample collection sites to the referral centers.

As with the WHO recommendations, the level II (district) and level I (primary healthcare center) laboratories could serve as sample collection sites for referrals to the level III and IV facilities. In addition to the tiered approach, the genotype-free prediction systems could be incorporated directly at the clinician's office to support decision-making in selecting the most effective treatment regimen if the prerequisite recent VL and CD4 test results are available at the sites for patients.

Quality assurance

The expansion of HIVDR testing will also require the strengthening of quality management systems to ensure accurate, timely, and reproducible results reporting. Consideration should be given to the entire quality management cycle: quality-assured sample collection and timely sample transportation (pre-analytic); standardized and valid testing procedures (analytic); and systematically reviewing and timely reporting results process (post-analytic). This requires the use of standard operation procedures, sample and results tracking devices, coupled with continuous training and supervision[5,67]. In addition, laboratories also need to implement sequence quality assurance systems to ensure and monitor consistency in assay performance quality, which includes sequence editing, assessing for contamination and other sequence quality aspects such as sequence length, stop codons, unexpected insertions, and unusual residues.

Testing facilities will need to be accredited with appropriate standards such as ISO-15189 for medical laboratories, in addition to obtaining WHO designation for facilities within the WHO-HIVDR laboratory network. Facilities also need to participate in routine external quality assurance (EQA) schemes for proficiency testing. Laboratories can depend on existing regional agencies to facilitate both accreditation and external quality assurance schemes, as is the case of the TREAT Asia quality assessment scheme (TAQAS)[68] and the WHO-mediated step-wise laboratory improvement towards accreditation (SLIPTA) scheme[69].

Discussion

ART management in RLS continues to be challenging due to the emergence of HIVDR and limited available drug options. As with high-income countries, the use of GRT can play a vital role in managing patients with suspected treatment failure[5,8,70]. As VL monitoring for ART patients becomes a routine care and treatment package in RLS[7], drug resistance testing for patients with confirmed virologic failure will become a reality. In fact, several PEPFAR-supported countries in sub-Saharan Africa are recommending drug resistance testing for patients with second-line treatment failure (South Africa and Kenya national treatment guidelines)[71,72]. This is in addition to the WHO recommended population-based surveys; pre-treatment HIV drug resistance, acquired HIV drug resistance (12 months and ≥ 48 months) as well as surveillance of HIV drug resistance in children < 18 months of age[19]. These routine surveys are vital in guiding the choice of first-line treatment, pre- and post-exposure prophylaxis prevention regimens and subsequent second- and third-line regimens[19].

Within the current technology landscape, expansion of HIVDR testing in RLS may depend on low-cost Sanger sequencing-based in-house assays, low-cost bench-top NGS, and possibly point mutation assays with medium-to-high level multiplex capability. Each of these technologies has their strengths and limitations. While Sanger-based in-house assays are the most commonly available, the capital cost for the sequencers and per test cost is comparatively high and this may limit their use, especially in sites considering starting GRT. On the other hand, they have been widely validated and there is considerable expertise for their use in RLS. Moreover, some of the assays, such as the CDC/ATCC/Thermo Fisher Scientific assay, are already commercially available as a testing kit or are being developed (SATuRN collaboration with Thermo Fisher Scientific) into kit-based assays, which is not the case with the other low-cost technologies.

Next-generation sequencing has an equally high capital cost, but it is projected that this may gradually decline as more novel technologies become available. Though the cost per run for NGS is high, significant cost reduction can be achieved through multiplexing

of many individually barcoded samples. On the other hand, the need for multiplexing implies that its utility is limited to high-throughput facilities. Moreover, they also have a high analysis cost, with cloud-based computing tools being up to threefold that of the actual lab test. A number of open source analysis pipelines are available, but the level of expertise and computing infrastructure may still be limiting for most RLS. Different academic laboratories are, however, making efforts to simplify the analysis process and minimize the analysis costs.

While point mutation assays might seem the likely choice for point of care testing, they are highly limited by the multiplexing ability of the technique used. Although there are various variants of point mutation assays, optimization of these assays to accommodate HIV variability and polymorphisms as well as adapting these assays for use with low-cost high-multiplex instruments, could be difficult to implement in the short-term. Further basic and technological research, coupled with engineering advances, is needed to make point of care assays truly feasible.

Lastly the genotype free prediction systems could easily be adapted with limited capital cost in RLS to improve the management of patients with suspected treatment failure. A setback of this method is the potential for switching patients without resistance to the expensive next line of treatment and the need for CD4 results, which may not be available under the current strategy for test and start and VL-based monitoring. In general, all these technologies may require additional field evaluation to assess their suitability in given settings as well as their cost-effectiveness.

To date, implementing HIVDR testing within the current landscape could best follow a centralized approach embedded in the recommended WHO tiered approach with testing at national or regional centers. The lower facilities could then serve as sample collection sites, supported by sample referral and data management systems to ensure quality of sample collection, timely shipment, and results reporting. In addition, the level III laboratory tiers with molecular-based systems for VL and DNA-PCR for early infant diagnosis can also incorporate low-cost point mutation assays like the Luminex MAGPIX® assay or serve as PCR amplification laboratories for drug resistance testing. As an alternative to laboratory-based tests, genotype-free prediction systems can also be used directly at the clinician's office, although these predictions are based on indirect parameters, i.e. VL and CD4, rather than the direct detection of resistance mutations.

In conclusion, the current landscape of HIVDR technologies shows promise with low-cost assays that can be used to expand testing for both clinical management and surveillance in sub-Saharan Africa. However, more implementation research is urgently needed to operationalize the use of these technologies within the public health system in RLS. This type of research should be planned in light of the ongoing global expansion of HIV viral load testing in RLS.

Supplementary Data

Supplementary data is available at AIDS Reviews journal online (http://www.aidsreviews.com).

This data is provided by the author and published online to benefit the reader. The contents of all supplementary data are the sole responsibility of the authors.

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Declaration of interest

The authors declare that they have no conflicts of interest.

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