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Genetic diversity among human immunodeficiency virus-1 non-B subtypes in viral load and drug resistance assays

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Abstract

The tremendous diversity of human immunodeficiency virus (HIV)-1 strains circulating worldwide has an important impact on almost all aspects of the management of this infection, from the identification of infected persons, through treatment efficacy and monitoring, and prevention strategies such as vaccine design. The areas where HIV-1 genetic diversity is highest are those where the majority of patients in need of treatment and biological monitoring live. With increased access to treatment in these areas, it is expected that the demand for monitoring tools such as viral load assays and resistance tests will also increase, and their reliability will be critical. Regular updates of these assays during the last two decades have aimed at improving their performances in different ways that include their reliability with different HIV-1 strains. We here review to what extent HIV-1 genetic diversity still limits or not the use of currently available viral load and resistance tests.

Keywords: Drug resistance test, genetic diversity, HIV-1, review, viral load
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Introduction

The global human immunodeficiency virus (HIV)/AIDS epidemic is characterized by high diversity of HIV. On the basis of phylogenetic analyses of numerous isolates obtained from diverse geographical sources, HIV is subdivided into types, groups, subtypes, sub-subtypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs) [1]. This viral diversity has implications for possible differences in disease progression, responses to antiretroviral therapy (ART) (including the development of resistance), vaccine development and diagnosis [2]. Here, we review selected aspects of the genetic diversity of HIV, with particular emphasis on tests to monitor the efficiency of ART.

Classification and Molecular Epidemiology of HIV

AIDS is caused by two viruses: HIV-1 and HIV-2. The initial genetic diversity of HIV is tightly associated with its origin; the different groups of HIV-1 (M, N, O and P) and HIV-2 (A–H) are the results of cross-species transmission events from different primate sources, namely chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla*) in West Central Africa for HIV-1, and sooty mangabeys (*Cercocebus atys*) in West Africa for HIV-2 [3–5]. HIV-1 group M can be further subdivided into nine subtypes (A–D, F–H, J and K), denoted by letters, and subtypes A and F can be further subdivided into sub-subtypes, A1–A4, F1 and F2. Numerous intersubtype recombinant viruses are also observed. When such recombinant viruses spread further within the human population they become CRFs, and when they remain restricted to a limited number of individuals they are called URFs. Today, at least 45 CRFs and numerous URFs are recognized (http://www.hiv.lanl.gov). Fig. 1 illustrates the genetic diversity of HIV-1.

The classification of HIV strains has helped in tracking the course of the HIV pandemic. HIV-2 is restricted to West Africa, and only two variants, HIV-2 groups A and B, are represented in the HIV-2 epidemic, the others being documented in one or few individuals only [6]. HIV-1 group O is endemic in Cameroon, where it represents about 1% of HIV infections, and HIV-1 groups N and P have been described in

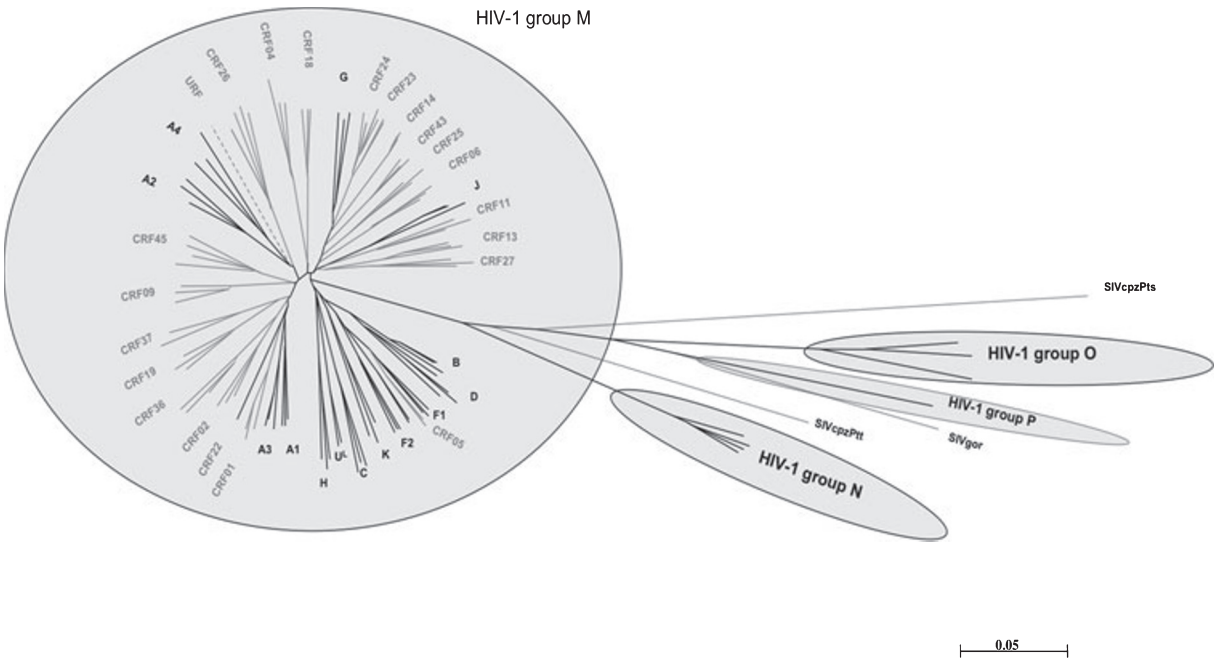

FIG. 1. Phylogenetic tree of near full-length genome sequences representing the genetic diversity of the human immunodeficiency virus (HIV)-1/SIVcpz/SIVgor lineage. Representative HIV-1 isolates from groups M, O, N and P were used to perform the phylogenetic analysis (neighbour-joining method). Within group M, subtypes and sub-subtypes are highlighted in black and circulating recombinant forms (CRFs) in grey. Unique recombinant forms (URFs) are indicated by dotted grey lines. Branch lengths are drawn to scale (the bar indicates 5% divergence).

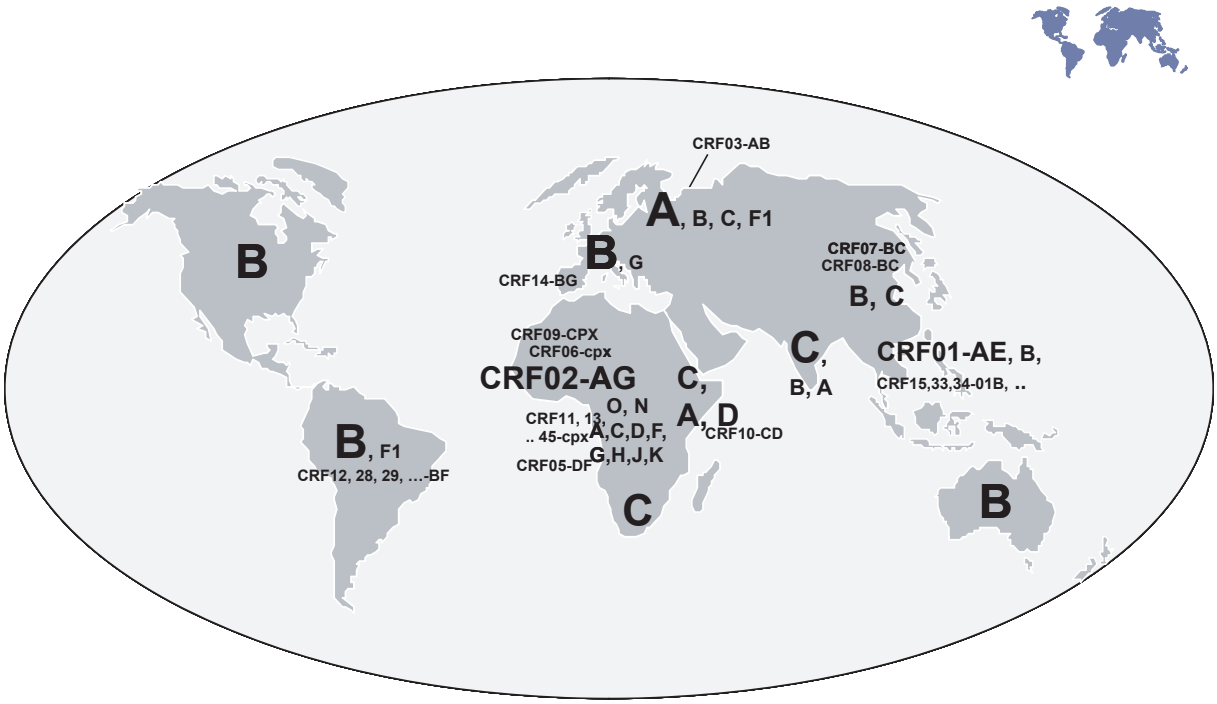

FIG. 2. Schematic representation of the geographical distribution of human immunodeficiency virus-1 variants worldwide.

a handful of individuals from Cameroon only [7,8] (Vallari *et al.*, 17th Conference on Retroviruses and Opportunistic Infections, 2010). Only HIV-1 group M has spread across Africa and to all the other continents, and the geographical distribution of the different HIV-1 group M subtypes and CRFs is heterogeneous [9,10] (Fig. 2). The initial diversification of group M may have occurred within or near the Democratic Republic of Congo, where the highest diversity of

group M strains has been observed and the earliest cases of HIV-1 infection (1959 and 1960) were documented [11–13]. The epidemic in the different countries of West, East and southern Africa is probably the result of different founder effects. In southern Africa, the epidemic is almost exclusively attributable to subtype C; subtypes A and D predominate in East Africa, and CRF02-AG in West Africa [14]. The epidemic in Asia is characterized by CRF01-AE and subtype B in the south-east, by subtypes B and C and B/C recombinants (CRF07 and CRF08) in China, and by subtype C in India [15]. In South America, subtypes B and F were initially introduced, and many B/F circulating and unique recombinants are now present [15]. Finally, in North America and Europe, subtype B predominates, but the number of new infections with non-B strains is increasing [16–19].

Overall, non-B HIV-1 variants represent more than 90% of circulating strains globally, with subtype C accounting for 50% of all infections worldwide [9,15]. Subtypes A, B, D and G have been shown to account for 12%, 10%, 3% and 6%, respectively, and CRF01-AE and CRF02-AG for 5% of cases each [9]. Other recombinants have been shown to account for 8% of infections [9]. With increasing mobility and migration, HIV-1 variants inevitably intermix in different parts of the world, and the distribution of different forms of HIV-1 in the world is thus a dynamic process. The likelihood of generating new recombinant viruses will increase and mosaic genomes will become even more complex, as recombination involving viruses that are already recombinant will occur. Even distantly related viruses have been shown to recombine; for example, intergroup recombinants between group O and M HIV-1 strains have been documented in Cameroon and now also in France [8,20].

Genetic Diversity and Monitoring of HIV Infection

ART and patient monitoring in areas where non-B HIV-1 variants predominate

Owing to national programmes and the support of a wide range of international partners, the number of people receiving ART in resource-limited countries has significantly increased in recent years, most notably in sub-Saharan Africa and south and south-east Asia, the two areas where more than 90% of individuals in need of ART reside [21]. In contrast to the situation in high-income countries, monitoring of ART with viral load (VL) and genotypic resistance testing is not yet widely available, owing to the high costs of these tests and the corresponding equipment. However, there are ongoing initiatives to address the lack of HIV-1 RNA monitoring and drug resistance testing in resource-limited countries. The assays developed and used to monitor HIV infection and treatment efficiency are mainly sequence-based, and are thus subject to sequence variability constraints that can significantly impact on their performance and reliability.

Genetic diversity and HIV diagnosis

Although genetic diversity has a higher impact on molecular tests, HIV tests initially showed limitations in detecting HIV-1 group O antibodies, and also some group M variants, especially during the serological window period [22,23]. Considerable efforts have been made to improve the performance of these assays by the inclusion of HIV-1 group O antigens or the use of broadly cross-reactive antigens. The simultaneous detection of HIV antigens (p24) and anti-HIV antibodies by fourth-generation assays reduced the window period. Despite these efforts, the performance of certain serological assays is actually still suboptimal, although reliable HIV testing is a critical entry point for patients in need of ART [24–27].

Genetic diversity and VL testing

Monitoring VL as a marker of disease progression and treatment efficacy is essential to provide clinicians with valuable information on which to base treatment decisions. Since 1995, many nucleic acid assays have been developed for the quantification of HIV-1 RNA in plasma. The first assays were based on target amplification, such as RT-PCR, nucleic acid sequence-based amplification (NASBA), a signal amplification methodology termed branched-chain DNA (bDNA), and ligase chain reaction (LCX) [28,29]. Currently, almost all assays, both commercial and in-house, are based on real-time PCR technologies, allowing the simultaneous detection of amplified products [30]. These technologies have considerably improved the post-amplification process and have reduced problems with contamination by reducing the handling of amplified samples. Newer real-time technology options are also faster, have larger dynamic ranges, have higher throughputs, and can be coupled to fully automated extraction steps [30,31]; however, they are more sensitive to point mutations within the primer/probe target sequences.

However, all of these assays are based on nucleic acid amplification and hybridization, and the genetic diversity of HIV-1 is thus a major challenge for the quantification of plasma HIV-1 RNA. Therefore, the early tests, designed for subtype B, often failed to detect non-B subtypes [32–38]. The new quantitative HIV-1 assays are designed to cope with increasing molecular diversity of the virus. Most of the currently used VL assays have been frequently updated to improve their ability to correctly detect and quantify the various HIV-1 group M variants, mainly through the design of

primers and probes that are conserved across subtypes and CRFs [39]. However, there is currently no single assay capable of quantifying the whole spectrum of HIV-1 strains circulating worldwide. Significant differences continue to be observed among different tests, because they use different primer/probe sequences, target different genomic regions or use slightly different technologies [28]. Thus, the genetic diversity of HIV continues to pose problems of underquantification (>1 $\log_{10}$ copies/mL in certain cases) or detection failure, which have practical implications for clinical management and detection of treatment failure. Ideally, each laboratory should initially compare different HIV-1 RNA tests and choose the assay that performs best with the HIV variants circulating in the country. It can also be the case that discrepancies are not related to particular HIV variants; therefore, if sufficient resources are available, physicians should also not hesitate to request VL determination to be performed with two different assays to highlight underestimation, particularly in cases of discrepancy between the VL and the CD4 count or clinical observations.

Table 1 shows the characteristics of the VL assays currently used in routine practice. Despite the high degree of diversity between HIV-1 group M, N and O viruses, some tests are able to quantify both HIV-1 group M and O strains, or HIV-1 group M and N strains [40–43]. Because of the relatively low sequence homology between HIV-1 and HIV-2, the development of a VL assay that is able to reliably quantify all subtypes of both viruses is virtually impossible; however, cross-reactivity may occur [40]. Moreover, no commercial assay is available to quantify HIV-2 plasma VL.

Genetic diversity and drug resistance assays

Drug resistance testing can be performed with either phenotypic or genotypic assays. Phenotypic analysis determines the degree to which a drug inhibits replication of the patient's virus *in vitro*, which is expressed as a fold change in 50% inhibitory concentration (IC_{50}) as compared with a wild-type reference HIV strain, and most current assays are based on recombinant virus assay technologies [44–46]. The advantages of phenotypic testing are the relatively easy interpretation, quantitative information on the degree of resistance, the ability to assess interactions among drugs, and the fact that it does not require an understanding of genotypic correlates with resistance [44]. Also, phenotypic assays are less subject to sequence variability.

Most genotypic assays detect resistance mutations by comparing the protease and the reverse transcriptase sequences of the investigated virus with those of a wild-type HIV-1 subtype B reference strain. Although phenotypic and genotypic assays are complementary, genotypic assays are preferred for routine patient management, because they are easier to perform, less expensive and less time-consuming (days vs. weeks) [47,48]. The most frequently used commercial genotyping assays worldwide are the ViroSeq HIV-1 Genotyping System v2.0 (Celera Diagnostics, Alameda, CA, USA) [49] and the TRUGENE HIV-1 Genotyping Kit for Drug Resistance (Siemens Healthcare Diagnostics, Deerfield, IL, USA) [50]. Both tests are based on direct population sequencing, and generally do not detect mutants that constitute less than 20% of the virus population [51,52]. To overcome this limitation, new approaches have been developed, and the most common include the subcloning and sequencing of HIV-1 clones, ultra-deep sequencing, oligonucleotide ligation assay, mutation-specific PCR assays, and the LigAmp assay [53]. However, like standard genotyping assays, all of these techniques are also sequence-based and therefore subject to HIV-1 genome variability.

HIV-1 genetic diversity impacts on sequence-based genotypic drug resistance assays at several levels, including performance and the interpretation of results. Performance is closely associated with the efficacy of the primers used for

TABLE 1. Molecular viral load assays currently found in routine practice

Assay name	Manufacturer	Principle	Target in HIV-1 genome	Dynamic range	Detected HIV strains	Extraction
Cobas Amplicor HIV-1 Monitor v1.5	Roche	RT-PCR, endpoint detection	*gag*	400–750 000 copies/mL	HIV-1 group M (A–G)	Manual and automated
Cobas AmpliPrep-Cobas TaqMan HIV-1 v2.0	Roche	RT-PCR, real-time detection	*LTR* + *gag*	20–1 × 10^7 copies/mL	HIV-1 groups M, N and O	Manual and automated
Abbott RealTime HIV-1	Abbott	RT-PCR, real-time detection	*pol*	40–1 × 10^7 copies/mL	HIV-1 groups M (A–H), N and O	Manual and automated
NucliSENS EasyQ HIV-1	bioMérieux	NASBA, real-time detection	*gag*	100–3 × 10^6 UI/mL	HIV-1 group M (A–K, CRF01-AE, CRF02-AG, CRF14-BG, AG-GH, CRF11-cpx). May detect HIV-2	Manual and automated
Versant HIV-1 RNA 3.0	Siemens	bDNA	*pol*	50–500 000 copies/mL	HIV-1 group M (A–G)	No extraction
G2 real-time PCR 'in-house'	ANRS-Biocentric	RT-PCR, real-time detection	*LTR*	Not provided	HIV-1 group M (A–H)	Manual

HIV, human immunodeficiency virus.

amplification and sequencing, which are generally optimized for HIV-1 subtype B, especially for the commercial assays [48,54]. Many studies have reported limitations of these conventional assays in correctly amplifying and sequencing non-B strains; for example, studies evaluating the ViroSeq System have reported amplification failure and failure rates for sequencing primers on non-B subtypes [55,56]. The initial version of the TRUGENE HIV-1 genotyping system also showed clear limitations in generating usable sequences from non-B strains (A–J) [57,58]. Several 'in-house' methods have been developed for the genotyping of non-B strains, either for a large spectrum of non-B strains in areas where these variants predominate, such as Central and West Africa [59], or for specific variants that cannot be correctly tested with commercial assays, as is the case for subtype C in South Africa and neighbouring countries [56]. However, care should be taken with 'in-house' techniques, as they are not always well evaluated and validated. Moreover, a recent publication on contaminated commercial enzymes clearly showed the risks and limitations of 'in-house' approaches, stressing the need to put in place a good-quality management system when using these assays [60].

Also, HIV-1 genetic diversity has an impact on the interpretation of the observed mutations as compared with the subtype B reference strain. Interpretation of genotypic drug resistance mutations is based on three algorithms (Stanford, ANRS and Rega) (http://hivdb.stanford.edu), mainly developed on the basis of clinical and/or virological data obtained from patients infected with HIV-1 subtype B. As a consequence, these algorithms can produce important discordances when applied to non-B variants [61,62]. They can misinterpret mutations that are present in non-B variants as natural polymorphisms [59,63]. In addition, although most major resistance mutations in subtype B have also been found in non-B subtypes, few novel mutations in non-B subtypes have been recognized and they are not always identified by the genotypic drug resistance algorithms [64]. Despite regular updates and a recent tendency to incorporate non-B data in the development of these algorithms, important discordances remain.

Furthermore, the highly sensitive approaches developed to detect minor viral populations are even more subject to HIV-1 sequence variability, which is clearly the key limitation of point mutation assays, as they are subject to both intrasubtype and intersubtype variability [65]. Indeed, only subtype-specific oligonucleotide probes are able to hybridize to the targeted codon with high specificity, and even within the same HIV-1 subtype the presence of mismatches between the probes and tested viruses can impact greatly on the assay performance [66–68].

Conclusions

Two decades of experience in the management of HIV infection have demonstrated the usefulness of VL and drug resistance testing for the monitoring of ART in patients. Despite many efforts, HIV-1 genetic variability continues to have a significant impact on the performance and the reliability of these assays. Although VL assays have been regularly updated to encompass the genetic diversity for the pandemic HIV-1 group M strains, very few assays can quantify the other HIV-1 groups, and there is currently no commercial assay for HIV-2 quantification. Commercial genotypic drug resistance assays are less frequently updated than VL assays, although they clearly show limitations in correctly amplifying and providing usable sequences for HIV-1 non-B strains. Moreover, none of them is applicable to the other HIV-1 groups or HIV-2. Actually, the only affordable sensitive techniques for the detection of minor populations are mainly point mutations assays. Their use will probably remain restricted, because they are difficult to implement in areas with high HIV-1 genetic diversity. With the increasing number of patients receiving ART in areas where only non-B variants predominate and with the increasing number of non-B infections in the USA and Europe, the demand for VL and drug resistance tests for non-B strains will increase significantly, and it is thus important that these tests have, as much as possible, equal performance with all HIV-1 variants.

Transparency Declaration

The authors have no conflicts of interest to declare.

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