Current Topics in
Virology
Vol. 15, 2018

Review

Recognizing the challenge of HIV genetic diversity: Global surveillance program

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ABSTRACT

Human immunodeficiency viruses (HIV) are retroviruses belonging to the primate lentivirus family that consists of at least 11 lineages. Independent zoonotic events of primate lentiviruses crossing into humans resulted in the emergence of HIV-1 and HIV-2. HIV-1 is comprised of four phylogenetic Groups, M (major), N (non-M, non-O), O (outlier) and P, each representing a separate cross-species transmission from primates into humans. HIV-1 Group M is most prevalent and responsible for the global epidemic and is subdivided into 9 subtypes based on sequence heterogeneity. Due to dual or coinfections of subtypes and recombination event between HIV strains, a wide diversity of circulating recombinant forms (CRFs) and unique recombinant forms (URFs) occur worldwide. Genetic divergence, mutations, evolution and the continual global redistribution of HIV continues to pose a significant challenge to the reliability of serology and molecular assays used for diagnosis. Global surveillance program is important for understanding HIV genetic diversity and to ascertain that despite genetic variation, all HIV infections are efficiently diagnosed by serology and molecular assays.

KEYWORDS: HIV, genetic diversity, surveillance, diagnosis.

INTRODUCTION

The isolation of T-Cell lymphotropic, cytopathic retroviruses from patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) in 1983-1984, is one of the most significant discoveries of the modern times. This discovery enabled the development of diagnostic and blood screening tests to identify infected individuals and the development of subsequent therapeutic interventions to treat these patients. The early AIDS retroviruses isolates include lymphadenopathy-associated virus (LAV); human T-cell lymphotropic virus type III (HTLV-III), AIDS-associated retrovirus (ARV-2), and three isolates derived from blood transfusion associated AIDS patients [1-5]. Evidence from several laboratories substantiated the observation that retroviruses isolated from patients with AIDS or AIDS-related complex exhibit restriction-site polymorphism in their genome [6-9]. Of the viruses examined in these early studies, some were isolated from patients in one geographic area: North America, whereas others came from various locations including Europe, North America and Africa [8, 9]. The studies on heteroduplex and restriction map analyses demonstrated that viruses isolated from different AIDS patients had distinct restriction map and exhibit greatest divergence in their envelope genes [10]. The heterogeneity in genomic sequences was predicted to have important implications as it results in changes in antigenic properties and/or pathogenicity by AIDS retroviruses. To gain knowledge regarding the variation in genomic structure and to study the effect on the translational products encoded by the viral genome, at Abbott Diagnostics, in collaboration with Centers for Disease Control (CDC), Atlanta,

nine independent virus isolates from AIDS/ lymphadenopathy syndrome (LAS) patients were compared by restriction-site analysis and the translational products encoded by the viruses were characterized by using specific immunoassays and metabolic labeling experiments [9]. The data indicated that individual retroviruses exhibit significant restriction-site polymorphism in their genome even though they were isolated from patients residing at same geographic location around Atlanta, Georgia. Furthermore, this polymorphism is reflected in the variation of the apparent size of the *gag* (group specific antigen) and *env* (envelope) gene-encoded structural proteins [9]. The results gave an indication that the heterogeneity in AIDS retrovirus-encoded proteins may be due to either substitutions in the primary amino acid sequence of the protein or deletions or additions in the coding regions of proteins. These studies provided early evidence that there is significant diversity among AIDS retroviruses that were designated as human immunodeficiency viruses (HIV) by the International Committee on Taxonomy of Viruses (ICTV) in 1986.

Molecular characterization of HIV

The availability of molecular clones of three isolates, LAV [11], HTLV-III [12-14], and ARV-2 [15], and their primary nucleotide sequence analysis [11-15] were useful for comparison of these isolates and determine how closely related the three strains were. These studies showed that the restriction map and nucleotide sequences of LAV and HTLV-III were closely related, while ARV-2 was more distantly related [15]. The variability was most evident in the envelope glycoprotein (*env*) gene than in the other regions of the viral genome [6, 13-15]. The sequence comparison of three human immunodeficiency virus (HIV) isolates indicated that the genome of LAV and HTLV-III differed from one another by 1.8%, whereas ARV-2 differed from LAV by 9.3% [12-15]. To evaluate genomic relatedness and divergence among HIV isolates, in collaboration with the CDC, we molecularly cloned an independent virus isolate designated as HIV (CDC-451) and generated a complete genome sequence, which at that time was the 4th HIV genome ever sequenced. This foundational work in the HIV field initiated a research program to characterize HIV diversity at Abbott Diagnostics that continues to this day. The fourth HIV genome expanded the known diversity of HIV and comparison to the previous three genomes demonstrated that the envelope (*env*) sequences had most of the changes (around 24% divergence), whereas the group specific antigen (*gag*) was relatively conserved (around 5% divergence) [16]. Since the launch of the first US FDA-approved serology assay in 1985 and subsequent demonstration of sequence diversity among HIV strains, collection of samples from HIV endemic areas around the world was undertaken. Recognizing the importance of accumulating data on HIV genetic diversity, in 1994, a formal Abbott Global Surveillance Program was initiated. By launching this program, Abbott significantly committed resources to acquire and assemble large-volume panels of genetically diverse strains, as well as develop strategies for search of newly emerging strains. Through international collaborations, the program acquires specimens from HIV endemic locations. The specimens collected from diverse locations are subjected to characterization by a variety of serology and molecular techniques. The Abbott Global Surveillance Program efforts have led to the identification and sequence characterization of rare HIV variants of HIV-1 Group O, Group N, Group P and dual infections of Groups M and O that have been submitted to the Los Alamos Database [17]. Thanks to innovation of technology and international commitment to HIV research, as years passed by, the knowledge of HIV strains and genetic diversity has advanced rapidly at an exponential rate. The number of sequences in the HIV database continues to increase; at the end of 2015, there were 675,479 sequences in the HIV Sequence Database, an increase of 8% since the previous year. The number of near complete genomes (>7000 nucleotides) increased to 7667 by the end of 2015 [17].

Based on accumulating data over the past three decades, we know that HIV is a retrovirus belonging to the primate lentivirus family that consists of at least 11 lineages. The two primate lentiviruses crossed into humans giving rise to HIV-1 and HIV-2; HIV-1 came from transmission

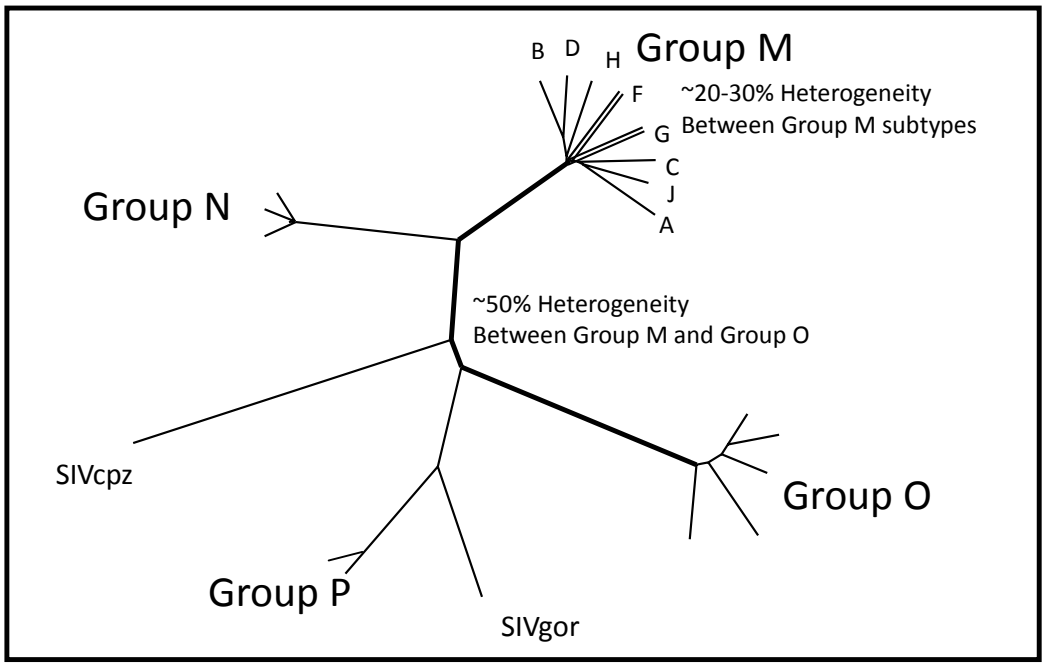

Figure 1. Phylogenetic classification of HIV-1 based on *env* gp41 sequences.

of a simian immunodeficiency virus (SIV) from chimpanzees and HIV-2 came from zoonosis of SIV from sooty mangabeys. HIV-1 is comprised of four phylogenetic groups, M (major), N (non-M, non-O), O (outlier) and P, each representing a separate cross-species transmission from chimpanzees or gorillas into humans [18-28]. HIV-1 Group M, responsible for most worldwide infections, is further subdivided into nine distinct subtypes A-D, F-H, J, K [17]. HIV-1 Group O is endemic to Cameroon and West Central Africa, accounting for 1-6% of the HIV-1 infections in Cameroon. Although rare, Group O infections have been identified in several European countries and the United States [18-20].

The sequence divergence between HIV-1 groups and subtypes is evident from phylogenetic trees that depict the genetic relationship between the strains (Figure 1). For example, within HIV-1 envelope gene, sequences differ by 20-30% between Group M subtypes and up to 50% between Groups M and O. In addition, recombination between subtypes is a relatively frequent event associated with HIV-1 replication, a mechanism that contributes significantly to genetic diversity. In areas where multiple groups/subtypes co-circulate, infection by two independent subtypes or groups in the same individuals leads to the emergence of new transmittable recombinant viruses. Over time, increasing numbers of inter-subtype and intergroup mosaic viruses have been identified, sequenced and deposited in the HIV sequence database. Currently, the Los Alamos Database has recognized 96 circulating recombinant forms (CRFs) of HIV-1 that have been characterized and designated formally [17]. However, the number of CRFs is bound to increase over time as more complete HIV genomes are sequence characterized.

Depending on the founder HIV strains that entered in each continent and spread in various countries, the distribution of HIV strains varies significantly. Moreover, HIV replicates at a rapid pace, 10.3 x 10^9 virions/day [29] and with an extremely high mutation rate of $(4.1 \pm 1.7) \times 10^{-3}$ per base per cell, the highest reported for any biological entity [30]. Selective immune pressure and anti-viral treatment also play a role in the generation of diverse HIV strains by putting selective pressure on HIV variants. HIV-1 Group M, subtype C is the most prevalent (48%) strain

Figure 2. Abbott Global Surveillance Program sites.

in the world, while HIV-1 Group M, subtype B represents 11% of HIV-1 infections [31]. However, the HIV diversity and global viral strain distribution of HIV-1 groups, subtypes and CRFs are dynamic and continue to increase due to international tourism, immigration, expatriate labor, military deployment, migration of population and their sexual and drug abuse practices. In globally connected population, HIV has the ability to jump from one continent to another within hours. The majority of the infections in the US and Europe are by HIV-1 Group M, subtype B. However, an increasing number of non-B infections are being identified in these regions [32, 33]. In Europe, the proportion of newly diagnosed HIV-1 infections due to non-subtype B and recombinant strains exceeds 20% [34-38]. Analogous situation exists in other continents as well [39-49]. The Abbott Global Surveillance Program continues strongly; to date, the program has collected more than 67,000 specimens from over 40 countries in six continents with emphasis on West Africa where all HIV groups and subtypes have been identified. (Figure 2). The panel of over 5,000 well characterized specimens comprises of HIV-1 Group M, subtype A, B, C, D, F, G, H, J and K; as well as circulating recombinant forms (CRFs), and unique recombinant forms (URFs), Group N, Group O and Group P [Ref. 28, 50-54]. With the continual diversification and global redistribution of HIV and the potential for new zoonotic transmissions, it is a challenge to develop serological and molecular assays capable of reliably detecting and monitoring all HIV infections irrespective of groups, subtypes, and recombination leading to significant genetic heterogeneity.

The previous algorithm to rapidly identify HIV groups employed a lateral flow serology assay developed at Abbott that readily classified specimens as HIV-1 Group M, Group O or HIV-2 infections [55]. In addition to the rapid test used for preliminary classification, to identify divergent strains of HIV, and a serology algorithm based on HIV peptides was developed [52, 49]. The algorithm first tests specimens using the Abbott ARCHITECT HIV antigen/antibody combination assay that detects antibodies to HIV-1/HIV-2 and HIV-1 antigen. The reactive specimens are further evaluated by peptide enzyme-linked immunoassays (PEIA). PEIAs are developed based on *env* gp120 V3 and *env* gp41 IDR sequences derived from HIV-1 Groups M, O, and N, and from two strains of SIVcpz. The serology algorithm is useful for preliminary classification of specimens as: HIV negative, HIV-1 group M, HIV-1 group O, HIV-2,

and HIV unclassified. The HIV unclassified specimens either do not exhibit reactivity in the PEIAs or show reactivity to the group N, SIV, or multiple peptides. The specimens are characterized further using molecular techniques including viral load determination and sequence analyses [52]. More recently, the complete genome sequencing using next genome sequencing (NGS) technology is being employed that provides insight into genomic structures of HIV [53-54].

HIV-1 Group M

Since the global pandemic is caused by HIV-1 Group M, majority of specimens collected to date by Abbott Global Surveillance Program are HIV-1 Group M. Samples are classified by molecular characterization to determine the group and subtype. Initially, this included nested RT-PCR amplification of regions of the HIV genome, direct sequencing, and phylogenetic analysis. Sets of primers were developed to facilitate amplification of genetically divergent strains of HIV-1 Group M, Group O, and HIV-2. Initial efforts to genetically characterize divergent HIV strains were based on sequencing two regions of the HIV genome: group specific antigen (*gag* p24) and *env* gp41. Based on this analysis many samples obtained from Cameroon were initially characterized as pure HIV-1 Group M, subtype A. However, sequencing of the third region of the genome polymerase (*pol*) integrase (IN) showed them to have subtype G sequence for *pol* IN. Recognizing the phylogenetic similarity of the Cameroonian A/G specimens to CRF02_AG, the complete genome characterization of a subset of the candidate A/G recombinants was undertaken. Phylogenetic analysis revealed that the recombinant breakpoints were identical to CRF02_AG. Thus, 70% of the Cameroonian panel members were CRF02_AG. This was the first formal demonstration that CRF02_AG predominated in Cameroon [56]. Reliance on analysis of only *gag* p24 and *env* gp41 regions would have dramatically shifted subtype assignment (83% subtype A). Thus, successful identification of the CRF02_AG recombinant strains clearly demonstrated the advantages of our algorithm targeting three regions of the genome. It increased the reliability of group/subtype assignments and the probability of identifying recombinant viruses. These three regions are not only important for classification, but for characterizing sequence diversity in regions that are important for diagnostic tests. The *gag* p24 contains HIV antigen detection epitopes critical for detection of antigenemia in an HIV infected individual, while *pol* IN region is the target for Abbott viral load and ViroSeq™ assays that are used for monitoring the efficacy of anti-viral therapy and drug resistance [57]. The *env* gp41 immuno-dominant region (IDR) is the key region of the antigen that is essential for the detection of anti-HIV antibodies [58]. In cases where unique strains are identified or there is a rareness of sequence information available for known subtypes or CRFs, complete viral genomes are amplified and sequenced [28, 32, 33, 59-66]. Recent introduction of the NGS technology has reduced the time required to sequence and characterize complete viral genomes.

HIV-1 Group O

Using the screening algorithm described above, over 200 Group O infections have been identified to date at Abbott. Full-genome sequences have been characterized for over 40 group O isolates, and in addition, sequences were generated for *gag* p24, *pol* IN, and *env* gp160 (roughly half the genome) for over 50 isolates [50, 51]. In 2002-2003, the Abbott Global Surveillance Program observed that although the level of genetic diversity within Group O is analogous to that between Group M subtypes, Group O has not been formally classified into subtypes. Through phylogenetic analyses of the *gag* p24, *pol* p32, and *env* gp160 sequences from 39 group O isolates, our group laid the foundation for the classification of 5 phylogenetic clusters within Group O [50, 51]. We observed that the intra- and intersubtype genetic distances for Group O were similar in magnitude to the corresponding distances for Group M subtypes. Intersubtype recombination was also identified in three of the 23 (13%) Group O genomes [49]. Furthermore, Group M/O dual infections and replication competent Group M/O recombinants have been described by several groups [27, 65-70].

HIV-1 Group N

HIV-1 group N is extremely rare and has only been found in Cameroon [25] or in patients linked to Cameroon. The variant screening algorithm at Abbott led to the identification of 11 HIV-1 Group N infections [28, 33, 59, 62]. All the Group N-infected specimens were HIV seropositive in the Abbott commercial HIV assays. The specimens were identified as Group N infections based on their reactivity in the PEIAs. The group N specimens showed strongest reactivity to the group N peptides in the IDR and V3 PEIAs; some also showed high reactivity to the SIVcpz GAB peptides. Near full-length viral genome sequences were amplified for each Group N virus. Two specimens, drawn from a husband and wife, exhibited close phylogenetic relationship between the Group N sequences amplified from these patients. The direct patient linkage strongly suggested that one spouse infected the other. This study provided the first evidence that Group N can be transmitted horizontally. In addition, the previous documentation of an infection in a seven-year-old orphan revealed vertical transmission of group N [25]. HIV-1 Group N has high replication capacity both *in vivo* and *in vitro*, and its identification in AIDS patients indicates that the virus is pathogenic. However, currently, our knowledge of Group N is too limited to know if it will contribute significantly to the HIV epidemic. Surveillance efforts need to continue to identify additional Group N infections and to monitor changes in the prevalence and sequence diversity of group N. To date only 17 Group N infections have been reported in literature, with 11 of these identified and characterized by Abbott Global Surveillance Program [28-50].

HIV-1 Group P

In 2009, a new HIV-1 strain was identified in Paris from a Cameroonian woman. This individual was HIV-1 seropositive, however, had undetectable viral load by Group M specific assay but in contrast had high viral load by the Abbott molecular assay that detects and quantitates HIV-1 Group M and Group O isolates. Initially, it was thought to be HIV-1 Group O infection; however, extensive characterization showed that this HIV strain was closely related to gorilla SIV (SIVgor) and exhibited no evidence of recombination with other HIV-1 lineages. This new virus, prototype of a new HIV-1 lineage designated as Group P, was shown to be distinct from HIV-1 Groups M, N and O [26]. Seventeen hundred thirty-six HIV sero-positive samples collected by the Abbott Global Surveillance from Cameroon were screened by the PEIA, followed by molecular analyses. This led to the identification of HIV-1 Group P infection in an HIV-seropositive male hospital patient in Cameroon. This observation confirmed that the HIV-1 group P virus is circulating in humans [66]. Subsequently, Group P was not identified amongst 4575 HIV positive specimens collected in 2011-2015 from Southern Cameroon by the same screening algorithm [53]. To date, there are only two reports of HIV-1 Group P infections in the literature indicating that the prevalence of Group P in HIV-1-infected individuals in Cameroon is low. Alternatively, Group P may be restricted to specific regions of Cameroon that were not represented in our study population or Group P infections may not be efficiently detected by the current HIV screening tests due to cross reactivity to reagents used in the current HIV serology tests in the absence of Group P-specific reagents for antibody detection.

HIV-2

In 1986, a new HIV virus was discovered that was morphologically similar to HIV-1, yet antigenically distinct [67]. This virus, originally designated as LAV-II and later as HIV-2, was shown to be closely related to SIV from *Cercocebus atys atys* or sooty mangabeys (SIVsmm) [68]. HIV-2 is most prevalent in West Africa; however, cases have also been identified globally. The number of HIV-2 infections is declining, even in the West African countries, where it is being replaced by HIV-1. HIV-2 is less pathogenic, with infections being mostly asymptomatic and having lower replication rate and low or undetectable viral loads, and only a small percentage of infections progress to AIDS [69]. Currently, less than 40 complete HIV-2 genome sequences are in the Los Alamos database [17]. Based on sequence comparison, there are 8

known HIV-2 groups (A to H) that have been reported in the literature of which Group A and Group B are the most prevalent. The HIV-2 Group G was first identified and sequence characterized by the Abbott Global Surveillance Program [71]. More recently, using next-generation sequencing, nine HIV-2 genomes were sequenced and classified phylogenetically. These studies at Abbott identified a patient with a series of mutations in an invariant cytotoxic lymphocyte (CTL)-restricted *gag* epitope that is required for retroviral structure/replication and implicated in long-term non-progression to AIDS [72]. The presence of wild-type sequence argues that these mutations are involved in immune escape, whereas its reversion to a sequence seen only in the sooty mangabeys reservoir suggests an alternate means of controlling infection. Surveillance and molecular characterization of circulating strains are essential for continued development of monitoring tools and may provide greater insight into the reduced pathogenicity of HIV-2.

HIV Diversity: Implications on serology and molecular diagnostic tests

Diagnosis of infection in health-care setting and transmission of HIV through blood transfusion continue to be a worldwide concern. During the past three decades, considerable progress has been made to improve HIV assays. The early, first generation HIV assays developed in 1985, used viral antigens derived from HIV-1 Group M, subtype B on the solid phase and polyclonal antibodies to human immunoglobulins conjugated to an enzyme for detection of HIV antibodies. With the discovery of HIV-2 and observation that not all the HIV-2 infections are detected by then available serological tests based on only HIV-1, Group M, subtype B reagents, there was an urgent need to modify the serology test. For efficient detection of all HIV infections, HIV-2 reagents were incorporated in the existing HIV-1 serology tests. The second-generation assays used HIV-1/ HIV-2 recombinant antigens instead of viral lysate resulting in improved specificity and detection of both HIV-1 and HIV-2 infections [73]. The third-generation assays used HIV recombinant reagents on the solid phase and recombinant antigens conjugated to an enzyme or hapten, resulting in the detection of HIV IgM, in addition to IgG resulting in the reduction of the seroconversion window. At the same time, assays were also developed for detection of HIV-1 p24 antigen. Identification of HIV-1 Group O and variable detection of Group O infections led to the incorporation of group O reagents into HIV assays. During the past decade, fourth generation assays have been developed that combine antigen and antibody detection in a single assay, leading to significant improvement of the performance [74]. While these assays detect infections efficiently, one of the major challenges for HIV diagnosis continues to be its genetic variation.

Immunoassays designed to detect antibodies specific for HIV-1 rely primarily on reactivity to the envelope (*env*) gp41 immunodominant region (IDR). The occurrence of natural polymorphisms, associated with the genetic variation of HIV-1 has the potential to modify or eliminate key epitope(s) targeted by these assays, ultimately leading to reduced sensitivity or lack of antibody detection. Cases of reduced seroconversion sensitivity for some non-subtype B strains have been documented [75]. An additional example includes a study that showed natural polymorphisms within the IDR region of a subtype B strain resulted in failure to detect antibodies by a fourth-generation assay [76]. Some tests that simultaneously detect both antigen and antibody showed low sensitivity for detection of p24 antigen for some non-subtype B strains [77-80]. Analysis of specimens from patients infected with Group O viruses revealed that some commercial immunoassays failed to detect Group O infections [20, 81-84]. Incorporation of Group O-specific antigens and/or peptides into assays was critical and led to improved detection of all Group O infections. More recent studies used recombinant virus-like particles with the *gag* gene from diverse HIV-1 strains and demonstrated that there is a significant difference in the detection of non-subtype B HIV-1 variants by commercially available tests [85-88]. The specimens acquired through the Abbott Global Surveillance Program have been effectively used to demonstrate the performance of diagnostic and screening assays [89].

The global surveillance program provides enormous scientific value for assay development

and evaluation. Discordant results between the tests used for diagnosis or monitoring of HIV infections, often inconsistent with clinical status, provide useful specimens for characterization of viruses to determine whether the inconsistency and suboptimal performance of the tests are due to genetic variation. All nucleic acid amplification or signal amplification technologies rely on HIV-1 sequence-specific primers and/or probes. Natural polymorphisms occurring in the target genomic regions have the potential to reduce or abolish hybridization that results in compromising reliable detection or quantification of viral load. In these cases, subtype and target sequence information are unknown at the time of testing and hence, genetically divergent variants may go unrecognized. Sequence analysis of the viral genome of specimens acquired by the Abbott Global Surveillance Program provides insight on the reasons for failure or under-quantification associated with significant mismatches at primer and/or probe binding sites. There are several documented cases in the literature where viral genetic diversity has been shown to influence the performance of nucleic acid-based technologies designed to detect and monitor HIV-1 [59, 90, 91]. Failed detection or unreliable quantitation can have significant consequences [92].

The large-volume panel developed by the Abbott Global Surveillance Program includes all well-characterized HIV-1 Group M, Group O, Group N, Group P, and HIV-2 as well as HIV-1 group M CRFs and URFs serum or plasma samples. The knowledge of genomic sequences of samples acquired from diverse geographic locations provide a sound basis for the selection of antigens representing key epitopes for detection of antibodies and developing monoclonal antibodies for detection of antigen by serology assays. In the same manner, identification of conserved regions of the viral genome is critical for designing primers and probe for qualitative detection as well as accurate quantitation of viral load by molecular assays. The availability of well-characterized large-volume HIV specimens provides unique opportunities for comprehensive evaluations of assays and to develop highly sensitive and specific future HIV tests using genetically diverse specimens [77-79, 90-94], which is essential to curb the pandemic and eliminate transfusion transmission of HIV. Performance of our serology and molecular assays on theses panels provides additional assurance that all HIV infections, including Group N and Group P are diagnosed accurately and quantified efficiently by the molecular assays [94].

Since the approval of the first HIV test by the US FDA in 1985, Abbott Diagnostics has invested significant efforts to monitor the evolution of HIV over the past decades. Surveillance of global HIV strain diversity provides capability to address the challenge posed by ongoing evolution of HIV and the emergence of new variants. It also allows to generate data and track the strains present in different geographical regions of the world that may challenge the detection limits of the serology and molecular tests. In addition, Abbott Global Surveillance Program data may inform clinical work outside the realms of diagnostics, with HIV-1 diversity having implications for possible differential rate of disease progression, response to anti-viral therapy and vaccine development [95, 96]. The ability to anticipate, identify new HIV strains and respond to this evolving threat will be dependent, to a significant degree, on our level of vigilance for monitoring and characterizing newly emerging strains. The proficiency to characterize full-length genome using next generation sequencing technology by newer methods at Abbott should accelerate surveillance efforts and add to the knowledge of HIV diversity without prior classification for group or subtype [54, 61, 62]. The knowledge gained will be useful for the continual evaluation of assay performance using genetically diverse HIV strains and modifying reagents, primers/probes based on scientific rationale, for efficient detection so that no HIV infection is misdiagnosed by serology and molecular assays. In addition to HIV, the Abbott Global Surveillance Program also tracks and monitors the hepatitis B virus (HBV) and hepatitis C virus (HCV) worldwide. HBV and HCV are the causative agents of global hepatitis epidemics that continue to evolve, generating new strains like HIV, creating challenges for blood screening, diagnostic and monitoring tests. The overall goal of the surveillance program is to remain ahead of the evolving threats of viral diversity and ascertain that all HIV, HBV and HCV infections are accurately diagnosed, despite genetic variations.

Summary

Rapid, ongoing genetic divergence and the continual global redistribution of HIV continue to pose a significant challenge to reliability of serology and molecular assays used for diagnosis, blood screening, and patient monitoring. The Global Surveillance Program plays a critical role in revealing HIV genetic diversity and development of well-characterized specimens' panels that are crucial to ascertain that despite genetic diversity, all strains, and emerging HIV infections are efficiently diagnosed and monitored by serology and molecular assays.

ACKNOWLEDGEMENTS

The author thanks Drs. Mary Rodgers, Michael Berg, Mary Kuhns and Gavin Cloherty, Abbott Diagnostics, for critical reading of the manuscript and helpful suggestions.

CONFLICT OF INTEREST STATEMENT

The author is an employee of Abbott Laboratories, Abbott Park, Illinois, USA.

REFERENCES

1. Barre-Sinoussi, F., Chermann, H. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. and Montagnier, L. 1983, Science, 220, 868-871.
2. Popovic, M., Sarngadharan, M. G., Read, E. and Gallo, R. C. 1984, Science, 224, 497-500.
3. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, F., Foster, P. and Markham, P. D. 1984, Science, 224, 500-503.
4. Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukoro, J. M. and Oshiro, L. S. 1984, Science, 225, 840-842.
5. Ferino, P. M., Kalyanaraman, V. S., Haverkos, H. W., Cabradilla, C. D., Warfield, D. T., Jaffe, H. W., Harrison, A. K., Gattlieb, M. S., Goldfinger, D., Chermann, J. C., Barre-Sinoussi, F., Spira, T. J., McDougal, J. S., Curran, J. W., Montagnier, L., Murphy, F. A. and Francis, D. P. 1984, Science, 225, 69-72.
6. Hahn, B. H., Gonda, M. A., Shaw, G. M., Popovic, M., Hoxie, J. A., Gallo, R. C. and Wong-Staal, F. 1985, Proc. Natl. Acad. Sci. USA, 82, 4813-4817.
7. Wong-Staal, F., Shaw, G. M., Hahn, B. H., Salahuddin, S. Z., Popovic, M., Markham, P., Redfield, R. and Gallo, R. C. 1985, Science, 229, 759-762.
8. Benn, S., Rutledge, R., Folks, T., Gold, I., Baker, L., McCormick, I., Feorino, P., Piot, P., Quinn, T. and Martin, M. 1985, Science, 230, 949-951.
9. Devare, S. G., Srinivasan, A., Bohan, C. A., Spira, T. J., Curran, J. W. and Kalyanaraman, V. S. 1986, Proc. Natl. Acad. Sci. USA, 83, 5718-5722.
10. Hahn, B. H., Shaw, G. M., Arya, S. K., Popovic, M., Gallo, R. C. and Wong-Staal, F. 1984, Nature (London), 312, 166-169.
11. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. and Alizon, M. 1985, Cell, 48, 9-17.
12. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Raflaski, J. A., Whitehorn, E. A., Baumeister, K., Ivanoff, L., Petteway, S. R. Jr., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, I., Chang, N. T., Gallo, R. C. and Wong-Staal, F. 1985, Nature (London), 313, 277-284.
13. Rabson, A. B. and Martin, M. A. 1985, Cell, 48, 477-480.
14. Wong-Stall, F. and Gallo, R. C. 1985, Nature (London), 317, 395-403.
15. Sanchez-Pescador, R., Power, M. D., Barr, P. J., Steimer, K. S., Stempien, M. M., Brown-Shimer, S. L., Gee, W. W., Renard, A., Randolph, A., Levy, J. A., Dina, D. and Luciw, P. A. 1985, Science, 227, 484-492
16. Desai, S. M., Kalyanaraman, V. S., Casey, J. M., Srinivasan, A., Andersen, P. R. and Devare, S. G. 1986, Proc. Natl. Acad. Sci. USA, 83, 8380-8384.
17. Foley, B., Leitner, T., Apetrei, C., Hahn, B., Mizrachi, I., Mullins, J., Rambaut, A., Wolinsky, S. and Korber, B. (Eds.), HIV Sequence Compendium 2017, Published by Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, NM, LA-UR 17-25240.

18. Gao, F., Bailes, E., Robertson, D. L., Chen, Y., Rodenburg, C. M., Michael, S. F., Cummins, L. B., Arthur, L. O., Peters, M., Shaw, G. M., Sharp, P. M. and Hahn, B. H. 1999, Nature, 397, 436-441.
19. DeLeys, R., Vanderborght, B., vander Haesevelde, M., Heyndrickx, I., van Geel, A., Wauters, C., Bernaerts, R., Saman, E., Nijis, P., Willems, B., Taelman, H., van der Groen, G., Piot, P., Tersmette, T., Huisman, J. G. and van Heuverswyn, H. 1990, J. Virol., 64, 1207-1216.
20. Gürtler, L. G., Zekeng, L., Simon, F., Eberle, J., Tsague, J. M., Kaptué, L., Brust, S. and Knapp, S. 1995, J. Virol. Meth., 51, 177-184.
21. Hunt, J. C., Golden, A. M., Lund, J. K., Gürtler, L. G., Zekeng, L., Obiang, J., Kaptué, L., Hampl, H., Vallari, A. and Devare, S. G. 1997, AIDS Research and Human Retroviruses, 13, 995-1005.
22. Brennan, C. A., Hackett J. Jr., Zekeng, L., Lund, J. K., Vallari, A. S., Hickman, R. K., Gürtler, L., Kaptué, L., von Overbeck, J., Hampl, H. and Devare, S. G. 1997, AIDS Research and Human Retroviruses, 13, 901-904.
23. Hackett, J. Jr., Zekeng, L., Brennan, C. A., Lund, J. K., Vallari, A. S., Hickman, R. K., Gürtler, L., Kaptué, L. and Devare, S. G. 1997, AIDS Research and Human Retroviruses, 13, 1155-1158.
24. Simon, F., Mauclère, P., Roques, P., Loussert-Ajaka, I., Müler-Trutwin, M. C., Saragosti, S., Georges-Courbot, M. C., Barré-Sinoussi, F. and Brun-Vézinet, F. 1998, Nature Med., 4, 1032-1037.
25. Roques, P., Robertson, D. L., Souquière, S., Apetrei, C., Nerrienet, E., Barré-Sinoussi. F., Mu¨ller-Trutwin, M. and Simon, F. 2004, AIDS, 18, 1371-1381.
26. Plantier, J. C., Leoz, M., Dickerson, J. E., Dr Oliveira, F., Cordonnier, F., Lemee, V., Damond, F., Robertson, D. L. and Simon, F. 2009, Nature Med., 15, 871-872.
27. Bush, S. and Tebit, D. M. 2015, AIDS Rev., 17, 147-15.
28. Bodelle, P., Vallari, A., Coffey, R., McArthur, C. P., Beyeme, M., Devare, S. G., Schochetman, G. and Brennan, C. A. 2004, AIDS Research and Human Retroviruses, 20, 902-908.
29. Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M. and Ho, D. D. 1996, Science, 271, 1582-1586.
30. José, M., Cuevas, J. M., Geller, R., Garijo, R., López-Aldeguer, J. and Sanjuán, R. 2015, PLoS One, 16, 13(9), e1002251. https://doi.org/10.1371/journal.pbio.1002251.
31. Hemelaar, J., Gouws, E., Ghys, P. D. and Osmanov, S. 2011, AIDS, 25, 679-689.
32. Delwart, E. L., Orton, S., Parekh, B., Dobbs, T., Clarke, K. and Busch, M. P. 2003, AIDS Research and Human Retroviruses, 19, 1065-1070.
33. Brennan, C. A. 2007, J. Med. Virol., 79, S27-S31.
34. Barin, F., Courouce, A. M., Pillonel, J. and Buzelay, L. 1997, AIDS, 11, 1503-1508.
35. Barlow, K. L., Tatt, I. D., Cane, P. A., Pillay, D. and Clewley, J. P. 2001, AIDS Research and Human Retroviruses, 17, 467-474.
36. Beloukas, A., Psarris, A., Giannelou, P., Kostaki, E., Hatzakis, A. and Paraskevis, D. 2016, Infect. Genet. Evol., 46, 180-189.
37. UK Collaborative Group on HIV Drug Resistance, 2014, AIDS, 28, 773-780.
38. Pérez-Parra, S., Chueca, N., Álvarez, M., Pasquau, J., Omar, M., Collado, A., Vinuesa, D., Lozano, A. B., Yebra, G. and García, F. 2017, PLoS One, 12(10), e0186928.
39. Yan, M., Zhao, K., Du, J., Li, L., Wu, D., Xu, S., Zeng, X., Wang, G. and Yu, X. F. 2014, PLoS One, 19, 9(6), e100540.
40. Khan, S., Zahid, M., Qureshi, M. A., Mughal, M. N. and Ujjan, I. D. 2018, Arch. Virol., 163, 33-40.
41. Prakash, S. S., Kalra, R., Lodha, R., Kabra, S. K. and Luthra, K. 2012, AIDS Research and Human Retroviruses, 28, 505-509.
42. Boullosa, J., Bachu, M., Bila, D., Ranga, U., Süffert, T., Sasazawa, T. and Tanuri, A. 2014, Viruses, 23, 2495-2504.
43. Li, X., Ning, C., Chen, Y., Feng, Y., Wei, M., Xing, H. and Shao, Y. 2014, AIDS Research and Human Retroviruses, 30, 695-700.

44. Neogi, U., Sood, V., Banerjee, S., Ghosh, N., Verma, S., Samrat, S., Sharma, Y., Saxena, A., Husain, S., Ramachandran, V. G., Das, S., Sreedhar, K. V., Goel, N., Wanchu, A. and Banerjea, A. C. 2009, Indian J. Exp. Biol., 47, 424-431.
45. Khan, I. F., Vajpayee, M., Prasad, V. V. and Seth, P. 2007, AIDS Research and Human Retroviruses, 23, 934-940.
46. Ljungberg, K., Hassan, M. S., Islam, M. N., Siddiqui, M. A., Aziz, M. M., Wahren, B., Islam, K. B. and Leitner, T. 2002, AIDS Research and Human Retroviruses, 10, 667-670.
47. Brennan, C. A., Bodelle, P., Coffey, R., Devare, S. G., Golden, A., Hackett, J. Jr., Harris, B., Holzmayer, V., Luk, K-C., Schochetman, G., Swanson, P., Yamaguchi, J., Vallari, A., Ndembi, N., Ngansop, C., Makamche, F., Mbanya, D., Gürtler, L. G., Zekeng, L. and Kaptué, L. 2008, J. Acquir. Immune Defic. Syndr., 49, 432-439. http://dx.doi.org/10.1097/QAI.0b013e31818a6561.
48. Castley, A., Sawleshwarkar, S., Varma, R., Herring, B., Thapa, K., Dwyer, D., Chibo, D., Nguyen, N., Hawke, K., Ratcliff, R., Garsia, R., Kelleher, A. and Nolan, D. 2017, PLoS ONE, 12(5), e0170601.
49. Yamaguchi, J., Bodelle, P., Vallari, A., Coffey, R., McArthur, C. P., Schochetman, G., Devare, S. G. and Brennan, C. A. 2004, AIDS Research and Human Retroviruses, 20, 944-957.
50. Yamaguchi, J., Vallari, A., Swanson, P., Bodelle, P., Kaptué, Nagansop, C., Zekeng, L., Gürtler, L. G., Devare, S. G. and Brennan, C. A. 2002, AIDS Research and Human Retroviruses, 18, 269-282.
51. Yamaguchi, J., Bodelle, P., Kaptué, Zekeng, L., Gürtler, L. G., Devare, S. G. and Brennan, C. A. 2003, AIDS Research and Human Retroviruses, 19, 979-988.
52. Brennan, C. A., Bodelle, P., Coffey, R., Harris, B., Holzmayer, V., Luk, K-C., Swanson, P., Yamaguchi, J., Vallari, A, Devare, S. G., Schochetman, G. and Hackett, J. Jr. 2006, J. Medical Virology, 78, S24-S29.
53. Rodgers, M. A., Vallari, A., Harris, B., Yamaguchi, J., Holzmayer, V., Forberg, K., Berg, M. G., Kenmenge, J., Ngansop, C., Awazi, B., Mbanya, D., Kaptue, L., Brennan, C., Cloherty, G. and Ndembi, N. 2017, Virology, 504, 141-151.
54. Rodgers, M. A., Wilkinson, E., Vallari, A., McArtur, C., Sthreshley, L., Brennan, C., Cloherty, G. and de Oliveira, T. 2017, J. Virology, 91, e01841-16. https://doi.org/10.1128/JVI.01841 -16.
55. Vallari, A. S., Hickman, R. K., Hackett, J. Jr., Brennan, C. A., Varitek, V. A. and Devare, S. G. 1998, J. Clinical Micro., 36, 3657-3661.
56. Holzmeyer, V., Zekeng, L., Kaptué., Gürtler, L. Devare, S. G. and Hackett, J. Jr. 2005, AIDS Research and Human Retroviruses, 21, 414-419.
57. Swanson, P., Devare, S. G. and Hackett, J. Jr. 2003, AIDS Research and Human Retroviruses, 19, 625-629.
58. Harris, B. J., von Truchsess, I. V., Schatzl, H. M., Devare, S. G. and Hackett, J. Jr. 2005, AIDS Research and Human Retroviruses, 21, 654-660.
59. Tang, N., Huang, S., Salituro, J., Mak, W. B., Cloherty, G., Johanson, J., Li, Y. H., Schneider, G., Robinson, J., Hackett, J. Jr., Swanson, P. and Abravaya, K. 2007, J. Virol. Methods, 146, 236-245.
60. Luk, K.-C., Berg, M. G., Naccache, S. N., Kabre, B., Federman, S., Mbanya, D., Kaptué, L., Chiu, C. Y., Brennan, C. A. and Hackett, J. Jr. 2015, PLoS ONE, 10(11), e0141723.
61. Berg, M. G., Yamaguchi, J., Alessandri-Gradt, E., Tell, R. W., Plantier, J. C. and Brennan, C. A. 2016, J. Clin. Microbiol., 54, 868-882.
62. Metzner, K. J. 2016, J. Clin. Microbiol., 54, 834-835.
63. Yamaguchi, J., McArthur, C. P., Vallari, A, Coffey, R., Bodelle, P., Beyeme, M., Schochetman, G., Devare, S. G. and Brennan, C. A. 2006, AIDS Research and Human Retroviruses, 22, 453-457.
64. Luk, K-C., Holzmayer, V., Yamaguchi, J., Swanson, P., Brennan, C. A., Ngansop, C., Mbanya, D., Gayum, H., Djuidje, M. N., Ndembi, N., Kamdem, D., Kaptué, L., Gürtler, L., Devare, S. G. and Hackett, J. Jr. 2007, AIDS Research and Human Retroviruses, 23, 297-302.

65. Luk, K-C., Holzmayer, V., Ndembi, N., Swanson, P., Brennan, C. A., Ngansop, C., Mbanya, D., Kaptué, L., Gürtler, L. G., Devare, S. G. and Hackett, J. Jr. 2008, AIDS Research and Human Retroviruses, 24, 1309 -1314.
66. Vallari, A., Holzmayer, V., Harris, B., Yamaguchi, J., Ngansop, C., Makamche, F., Mbanya, D., Kaptué, L., Ndembi, N., Gürtler, L., Devare, S. and Brennan, C. A. 2011, J. Virol., 85, 1403-1407.
67. Clavel, F., Guetard, D., Brun-Vezinet, F., Chamaret, S., Rey, M. A., Santos-Ferreira, M. O., Laurent, A. G., Dauguet, C., Katlama, C., Rouzioux, C., Klatzman, D., Champalimaud, J. L. and Montagnier, L. 1986, Science, 233, 343-346.
68. Sharp P. M. and Hahn, B. H. 2011, Cold Spring Harb. Perspect. Med., 1, a006841.
69. Adam MacNeil, A., Dieng Sarr, A., Sankale´, J-L., Meloni, S. T., Mboup, S. and Kanki, P. 2007, J. Virol., 81, 5325-5330.
70. Brennan, C. A., Yamaguchi, J., Vallari, A. S., Hickman, R. K. and Devare, S. G. 1997, AIDS Research and Human Retroviruses, 13, 401-404.
71. De Oliveira, F., Mourez, T., Vessiere, A., Ngoupo, P. A., Alessandri-Gradt, E., Simon, F., Rousset, D. and Plantier, J. C. 2017, Retrovirology, 14(1), 1. doi:10.1186/s12977-016-0324-3.
72. Yamaguchi, J., Brennan, C. A., Alessandri-Gradt, E., Plantier, J. C., Cloherty, G. A. and Berg, M. G. 2017, AIDS Research and Human Retroviruses, 33, 347-352.
73. Devare, S. G., Desai, S. M., Dawson, G. J., Hampl, H. and Hunt, J. C. 1990, in N. C. Khan and J. L. Melnick (Eds), Monographs in Virology, Human Immunodeficiency Virus, Basel, Karger, Switzerland, 18, 105-121.
74. Devare, S. G. 2007, J. Medical Virology, 79, S11-S15.
75. Apetrei, C., Loussert-Ajaka, I., Descamps, D., Damond, F., Saragosti, S., Brun-Vezinet, F. and Simon, F. 1996, AIDS, 10, F57-F60.
76. Gaudy, C., Moreau, A., Brunet, S., Descamps, J-M., Deleplanque, P., Brand, D. and Barin, F. 2004, J. Clin. Microbiol., 42, 2847-2849.
77. Ly, T. D., Edlinger, C. and Vabret, A. 2000, J. Clin. Microbiol., 8, 2459-2461.
78. Ly, T. D., Martin, L., Daghfal, D., Sandridge, A., West, D., Bristow, R., Chalouas, L., Qui, X., Lou, S. C., Hunt, J. C., Schochetman, G. and Devare, S. G. 2001, J. Clin. Microbiol., 39, 3122-3128.
79. Ly, T. D., Laperche, S., Brennan, C., Vallari, A., Ebel, A., Hunt, J. C., Martin, L., Daghfal, D., Schochetman, G. and Devare, S. G. 2004, J. Virol. Meth., 122, 185-194.
80. Mühlbacher, A., Schennach, H., van Helden, J., Hebell, T., Pantaleo, G., Bürgisser, P., Cellerai, C., Permpikul, P., Rodriguez, M., I., Eiras, A., Alborino, F., Cunningham, P., Axelsson, M., Andersson, S., Wetlitzky, O., Kaiser, C., Möller, P. and de Sousa, G. 2013, Med. Microbiol. Immunol., 202, 77-86,
81. Loussert-Ajaka, I., Ly, T. D., Chaix, M. L., Ingrand, D., Saragosti, S., Couroucé, A. M., Brun-Vezinet, F. and Simon, F. 1994, Lancet, 343, 1393-1394.
82. Schable, C., Zekeng, L., Pau, C-P., Hu, D., Kaptué, L., Gürtler, L., Dondero, T., Tsague, J-M, Schochetman, G., Jaffe, H. and George, J. R. 1994, Lancet, 344, 1333-1334.
83. Simon, F., Ly, T. D., Baillou-Beaufils, A., Schneider-Fauveau, V., de Saint-Martin, J., Loussert-Ajaka, I., Chaix, M. L., Saragosti, S., Couroucé, A. M., Ingrand, D., Janot, C. and Brun-Vezinet, F. 1994, AIDS, 8, 1628-1629.
84. Henquell, C., Jacomet, C., Antoniotti, O., Chaib, A., Regagnon, C., Brunet, S., Peigue-Lafeuille, H. and Barin, F. 2008, J. Clin. Microbiol., 46, 2453-2456.
85. Vetter, B. N., Orlowski, V., Fransen, K., Niederhauser, C., Aubert, V., Brandenberger, M., Ciardo, D., Dollenmaier, G., Klimkait, T., Regenass, S., Schmid, P., Schottstedt, V., Suter-Riniker, F., Yerly, S., Shah, C., Böni, J. and Schüpbach, J. 2014, PLoS One, 24, 9(10), e111552.
86. Barin, F., Courouce, A. M., Pillonel, J. and Buzelay, L. 1997, AIDS, 11, 1503-1508.

87. Barlow, K. L., Tatt, I. D., Cane, P. A.., Pillay, D. and Clewley, J. P. 2001, AIDS Research and Human Retroviruses, 17, 467-474.
88. Delwart, E. L., Orton, S., Parekh, B., Dobbs, T., Clark, K. and Busch, M. P. 2003, AIDS Research and Human Retroviruses, 19, 1065-1070.
89. Qiu, X., Sokoll, L., Yip, P., Elliott, D. J., Dua, R., Mohr, P., Wang, X. Y., Spencer, M., Swanson, P., Dawson, G. J. and Hackett, J. Jr. 2017, J. Clin. Virol., 92, 62-68.
90. Swanson, P., Harris, B. J., Holzmayer, V., Devare, S. G., Schochetman, G. and Hackett, J. Jr. 2000, J. Virol. Meth., 89, 97-108.
91. Swanson, P., de Mendoza, C., Joshi, J., Golden, A., Hodinka, R. L., Soriano, V., Devare, S. G. and Hackett, 2005, J. Cin. Microbiol., 43, 3860-3868.
92. Geelen, S., Lange, J., Borleffs, J., Wolfs, T., Weersink, A. and Schuurman, R. 2003, AIDS, 17, 781-782.
93. Eshelman, S., Hackett, J. Jr., Swanson, P., Cunnigham, S. P., Drews, B., Brennan, C., Devare, S. G. Zekeng, L., Kaptué, L. and Marlowe, N. 2004, J. Clin. Microbiol., 42, 2711-2717.
94. Rodgers, M. A., Vallari, A., Yamaguchi, J., Holzmayer, V., Harris, B., Toure-Kane, C., Mboup, S., Badreddine, S., McArthur, C., Ndembi, N., Mbanya, D., Kaptué, L. and Cloherty, G. 2018, AIDS Research and Human Retroviruses, 34, 314-318.
95. Taylor, B. S., Sobieszczyk, M. E., McCutchan, F. E. and Hammer, S. M. 2008, N. Engl. J. Med., 358, 1590-1602.
96. Korber, B., Gaschen, B., Yusim, K., Thakallapally, R. and Kesmir, V. C. 2001, Br. Med. Bull., 58, 19-42.