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REVIEW

Nucleic acid testing and molecular characterization of HIV infections

Junpeng Zhao [1,2,3] · Le Chang [1,2] · Lunan Wang [1,2,3]

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Abstract

Significant advances have been made in the molecular assays used for the detection of human immunodeficiency virus (HIV), which are crucial in preventing HIV transmission and monitoring disease progression. Molecular assays for HIV diagnosis have now reached a high degree of specificity, sensitivity and reproducibility, and have less operator involvement to minimize risk of contamination. Furthermore, analyses have been developed for the characterization of host gene polymorphisms and host responses to better identify and monitor HIV-1 infections in the clinic. Currently, molecular technologies including HIV quantitative and qualitative assays are mainly based on the polymerase chain reaction (PCR), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and branched chain (b) DNA methods and widely used for HIV detection and characterization, such as blood screening, point-of-care testing (POCT), pediatric diagnosis, acute HIV infection (AHI), HIV drug resistance testing, antiretroviral (AR) susceptibility testing, host genome polymorphism testing, and host response analysis. This review summarizes the development and the potential utility of molecular assays used to detect and characterize HIV infections.

Keywords Human immunodeficiency virus · Quantitative nucleic assays · Qualitative nucleic acid assays · Nucleic acid testing · Characterization

Introduction

HIV mainly invades the human immune system and causes immunodeficiency syndrome (AIDS), followed by high risk of opportunistic infections and tumors [1]. In 2016, the total number of global infections reached 36.7 million, and 1 million people died of AIDS-related diseases [2]. Detection and treatment are critical in preventing a large-scale epidemic of AIDS. Laboratory and clinical microbiologists perform various tests to determine HIV infection status of a patient, evaluate the progression of disease, and monitor the effectiveness of antiretroviral therapy (ART). HIV infection can be tested by visualization of virions through electron microscopy in cell culture, measurement of HIV-specific antibody and viral antigens, and detection of viral nucleic acids [3–5]. Molecular assays based on polymerase chain reaction (PCR) or alternative target amplification assays were first demonstrated in the late 1980 as homebrew methods in the clinical virology laboratory by amplifying HIV-1 genomic DNA [6]. Molecular methods have improved significantly and, along with serologic assays, are now routinely used to detect and characterize HIV-1 infections. With the introduction of the laboratory-based antigen/antibody combo assays allowing the detection of p24 antigen and IgG-IgM antibodies, the window period has been narrowed from approximately 3–4 to 2–3 weeks [7–11]. AHI phase represents the interval between the appearance of the first detection of antibodies and detectable HIV RNA [12]. During the acute infection, HIV viruses rapidly replicate and HIV RNA is the first and only detectable virus-specific marker, followed by HIV p24 antigen. According to the comparative analysis of Food and Drug Administration (FDA)-approved HIV assays used for screening, HIV-1 nucleic acid laboratory-based test is the most sensitive test available for HIV diagnostic use during AHI [11]. The number of viral RNA molecules can increase to several million copies per ml of plasma and allowing for rapid

✉ Lunan Wang
lunan99@163.com

[1] National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, No.1 Dahua Road, Beijing 100730, People's Republic of China

[2] Beijing Engineering Research Center of Laboratory Medicine, Beijing Hospital, Beijing, People's Republic of China

[3] Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, People's Republic of China

detection by molecular assays, shortening the initial analysis window from 3 to 4 weeks to about 2 weeks [8, 13–15].

At present, molecular assays are being used for clinical companion diagnosis, blood screening, POCT, pediatric diagnosis, AHI, HIV drug resistance testing, antiretroviral susceptibility testing, host genome polymorphism testing, and host response analysis. This review summarizes the development and the potential utility of molecular assays currently used to detect and characterize HIV infections. The following items will be developed: (i) presentation of the variety of molecular tools used for HIV detection or diagnosis; (ii) applications of HIV molecular technologies (Table 1); and (iii) future prospects.

The variety of molecular tools used for HIV detection or diagnosis

The common properties needed for molecular tools aimed to HIV detection or diagnosis are the availability of results in a relative short period of time and a good standardization of the extraction and amplification steps [41]. The detection of the amplification products is performed by probes, which bind to the target DNA or RNA at one or more different genomic regions. Thus, nucleic acid testing (NAT) used for HIV detection or diagnosis requires a high level of automation. Two molecular tools can be used for HIV detection and/or HIV diagnosis: HIV quantitative nucleic assays and qualitative nucleic acid assays (Tables 1 and 2). HIV quantitative nucleic assays can be used for the clinical progress of HIV infection and evaluation of ART outcomes through the detection of viral load, while qualitative nucleic acid assays can be conducted as diagnostic tools of HIV infection [42, 43].

HIV RNA quantitative assays

HIV-1 infection results in lifelong viral persistence. The HIV RNA viral load in the plasma together with CD4+ T cell numbers are the two laboratory markers routinely used to guide ART initiation, monitor treatment effectiveness, verify clinical progression, and determine treatment regimens [44, 45]. Prospective AIDS cohort studies demonstrated that plasma HIV RNA load was a better predictor of progression to AIDS and death than CD4+ T cell counts [46, 47]. The first quantitative assay for determining HIV-1 viral load in plasma or serum was based on end point measurement of the product of reverse transcription-polymerase chain reaction (RT-PCR) or nucleic acid sequence-based amplification (NASBA) [48, 49]. In addition, signal amplification-based techniques such as branched chain (b) DNA test have been developed [50]. They are characterized by high levels of analytical sensitivity, reproducibility and linearity, and can be performed on a large series of samples in a few hours with limited risks of contamination.

During the last 10 years, a number of reagents have been available for quantifying HIV viral load, including Cobas AmpliPrep TaqMan HIV-1 (Roche Diagnostics GmbH, Penzberg, Germany), Abbott RealTime HIV-1 M2000rt (Abbott Laboratories, Abbott Park, IL), NucliSens EasyQ HIV-1 (BioMerieux, Inc., Durham, NC, USA), VERSANT HIV-1 RNA (kPCR) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) (Table 2). All these technologies rely on real-time PCR that is more suitable for quantifying HIV RNA in plasma than previous molecular methods based on conventional PCR and ligase chain reaction.

At present, two basic assays from Roche Diagnostics GmbH are commonly used for HIV RNA quantitation: (i) the Amplicor HIV-1 Monitor assay, a manual test performed in 96-microwell plates; and (ii) the Cobas AmpliPrep/Cobas TaqMan HIV-1 assay with full automation of nucleic acid preparation and real-time RT-PCR amplification. The new fully automated version of the COBAS AmpliPrep/COBAS TaqMan HIV-1 system V1.0 (CAP/CTM V1.0) targets the HIV-1 gag gene and can quantify all group M and N viruses and many circulating recombinant forms [51, 52]. However, the major drawbacks for the CAP/CTM V1.0 are limited specificity owing to genetic diversity or genotype inclusivity [16]. Furthermore, low viral load and mismatches in the primer/probe region might result in the detection failure of the NAT [17, 18, 53]. To improve the test's genetic diversity and genotype inclusivity, Roche modified the CAP/CTM V1.0 and upgraded to the CAP/CTM V2.0, which targets the gag and conserved long terminal repeat (LTR) regions of the viral genome and extends subtype coverage to Group O [54]. Apart from HIV quantitative assays based on RT-PCR, the real-time HIV-1 assay in the M2000 system from Abbott also rely on PCR for HIV RNA quantitation. This assay offers a broad detection range and improved sequence variation tolerance and detects a wide coverage of HIV-1 including groups M, circulating recombinant forms, and groups N and O viruses [55]. For M2000 system, nucleic acid extraction and amplification are fully automated. However, the operator must manually add internal standard to "mLysis" and add anhydrous ethanol to "mWash 1" before nucleic acid extraction. The operator involvement should be minimized, and the amplification and detection steps are both carried out in a closed system in order to significantly reduce the risk of carryover contamination. In recent years, two next-generation HIV quantitation assays (APTIMA HIV-1 Quant Assay (Hologic Inc., San Diego, CA) and Cobas HIV-1 (quantitative nucleic acid test for use on the Cobas 6800/8800 systems) (Roche Diagnostics GmbH, Penzberg, Germany)) have been available for HIV RNA quantitation. APTIMA HIV-1 Quant Assay performs TMA and real-time detection on Panther system

Table 1 List of all the US FDA-approved HIV nucleic acid assays (including multiplex assays) [16–19]

Trade name	Format	Specimen	Use	Manufacturer	Target
Human Immunodeficiency Virus, Type 1 RT PCR Assay	Qualitative PCR	Plasma	Source Plasma Donor Screening: Qualitative detection of HIV-1 RNA. For in-house use only by Baxter Healthcare International	BioLife Plasma Services, L.P. Deerfield, IL US License 1640	–
UltraQual HIV-1 RT-PCR Assay	Qualitative PCR	Plasma	Source Plasma Donor Screening: Qualitative detection of HIV-1 RNA. For in house use only at NGI	National Genetics Institute Los Angeles, CA US License 1582	–
COBAS AmpliScreen HIV-1 Test, ver 1.5	Qualitative PCR	Plasma/Cadaveric serum or plasma	Donor Screening: Qualitative detection of HIV-1 RNA	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	Gag
APTIMA HIV-1 RNA Qualitative Assay	Qualitative TMA	Plasma/Serum	In Vitro Diagnostic: Qualitative detection of HIV-1 RNA	Hologic Inc., San Diego, CA US License 1592	LTR/POL
APTIMA HIV-1 Quant Assay	Quantitative TMA	Plasma/Serum	Patient Monitoring: Quantitation of HIV-1 RNA in plasma of HIV-1 infected individuals	Gen-Probe, Inc., San Diego, CA US License 1592	LTR/POL
cobas HIV-1	Quantitative PCR	Plasma	Patient Monitoring: Quantitation of HIV-1 RNA in plasma of HIV-1 infected individuals	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	LTR/Gag
Amplicor HIV-1 Monitor Test	Quantitative PCR	Plasma	Patient Monitoring: Quantitation of HIV-1 RNA in plasma of HIV-1 infected individuals	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	Gag
COBAS AmpliPrep/COBAS TaqMan HIV-1 Test	Quantitative PCR	Plasma	Patient Monitoring: Quantitation of HIV-1 RNA in plasma of HIV-1 infected individuals	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	Gag
Abbott RealTime HIV-1	Quantitative PCR	Plasma	Patient Monitoring: Quantitation of HIV-1 RNA in plasma of HIV-1 infected individuals	ABBOTT Molecular, Inc., Des Plaines, IL	POL
Versant HIV-1 RNA 3.0 (bDNA)	Quantitative PCR	Plasma	Patient Monitoring: Quantification of HIV-1 RNA in plasma of HIV-1 infected individuals	Siemens Healthcare Diagnostics, Inc.	POL
ViroSeq HIV-1 Genotyping System	Genotyping	Plasma	Patient Monitoring: For detecting HIV genomic mutations (in the protease and part of the reverse transcriptase regions of HIV) that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection	Celera Diagnostics Alameda, CA	PR/RT
Trugene HIV-1 Genotyping Kit and Open Gene DNA Sequencing System	Genotyping	Plasma	Patient Monitoring: For detecting HIV genomic mutations (in the protease and part of the reverse transcriptase regions of HIV) that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection	Siemens Healthcare Diagnostics, Inc.	PR/RT
COBAS TaqScreen MPX Test	Qualitative PCR	Plasma/Cadaveric plasma or serum	Donor Screening: Simultaneous qualitative detection of HBV DNA, HIV-1 Group M and Group O RNA, HIV-2 RNA, and HCV RNA	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	LTR
COBAS TaqScreen MPX Test version 2.0	Qualitative PCR	Plasma	Donor Screening: Simultaneous qualitative detection of HBV DNA, HIV-1 Group M and Group O RNA, HIV-2 RNA, and HCV RNA	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	LTR
cobas MPX Test	Qualitative PCR	Plasma/Cadaveric plasma or serum	Donor Screening: Simultaneous qualitative detection of HBV DNA, HIV-1 Group M and Group O RNA, HIV-2 RNA, and HCV RNA	Roche Molecular Systems, Inc. Pleasanton, CA US License 1636	LTR
Procleix Ultrio Assay	Qualitative TMA	Plasma/Serum/ Cadaveric plasma or serum	Donor Screening: Simultaneous qualitative detection of HBV DNA, HCV RNA, and HIV-1 RNA	Grifols Diagnostics Solution., San Diego, CA US License 2032	LTR/POL
Procleix Ultrio Plus Assay	Qualitative TMA	Plasma/Serum/Cadaveric plasma or serum	Donor Screening: Simultaneous qualitative detection of HBV DNA, HCV RNA, and HIV-1 RNA	Grifols Diagnostics Solution., San Diego, CA US License 2032	LTR/POL
Procleix Ultrio Elite Assay	Qualitative TMA	Plasma/Serum/ Cadaveric plasma or serum	Donor Screening: Simultaneous qualitative detection of HBV DNA, HCV RNA, HIV-1 RNA and HIV-2 RNA	Grifols Diagnostics Solution., San Diego, CA US License 2032	LTR/POL
UltraQual Multiplex PCR Assay	Qualitative PCR	Plasma	Source Plasma Donor Screening: Simultaneous qualitative detection of HBV DNA, HCV RNA, HIV-1 RNA and HIV-2 RNA	National Genetics Institute Los Angeles, CA US License 1582	–

Table 1 (continued)

Trade name	Target	Genotype	Volume (specimen) 95% LOD (IU/mL)[a]	Conversion factor[b]	Reference
Human Immunodeficiency Virus, Type 1 RT PCR Assay	–	HIV-1 M (B)	–	–	[20]
UltraQual HIV-1 RT-PCR Assay	–	HIV-1 M, O	1 mL, 2-Reaction mode: 12.7 2 mL, 4-Reaction mode: 4	1	[21]
COBAS AmpliScreen HIV-1 Test, ver 1.5	Gag	HIV-1 M (A-G), O, N	1 mL, Multi-prep procedure: 61.3 0.2 mL, Standard procedure: 150.3	1.56	[22]
APTIMA HIV-1 RNA Qualitative Assay	LTR/POL	HIV-1 M, O, N	0.5 mL, 30 copies/mL	–	[23]
APTIMA HIV-1 Quant Assay	LTR/POL	HIV-1 M, O, N	1.2 mL, 34.3	2.86	[24]
cobas HIV-1	LTR/Gag	HIV-1 M, O, N	0.65 mL, 33 0.35 mL, 83	1.67	[25]
Amplicor HIV-1 Monitor Test	Gag	HIV-1 M	0.2 mL, Ultra-Sensitive procedure (Dilution Factor: 4): 50 copies/mL 0.2 mL, Standard procedure (Dilution Factor: 40): 400 copies/mL	–	[26]
COBAS AmpliPrep/COBAS TaqMan HIV-1 Test	Gag	HIV-1 M (A-H)	1.0 mL, 80	1.67	[27]
Abbott RealTime HIV-1	POL	HIV-1 M (A-H, AG), O, N	1 mL, 69.6 0.5 mL, 130.5 0.2 mL, 261	1.74	[28]
Versant HIV-1 RNA 3.0 (bDNA)	POL	HIV-1 M (A-G)	1 mL, 245.3	3.27	[29, 30]
ViroSeq HIV-1 Genotyping System	PR/RT	HIV-1	0.5 mL, 2000 copies/mL	–	[31, 32]
Trugene HIV-1 Genotyping Kit and Open Gene DNA Sequencing System	PR/RT	HIV-1	17 μL RNA template, 1000 copies/mL	–	[33]
COBAS TaqScreen MPX Test	LTR	HIV-1 M(A-J, AG), O, HIV-2	1.0 mL, ID-NAT/MP-NAT: 49	1.67	[34]
COBAS TaqScreen MPX Test version 2.0	LTR	HIV-1 M (A-J, AG, BG), O, HIV-2	1.0 mL, ID-NAT/MP-NAT: 46.2	1.67	[35]
cobas MPX Test	LTR	HIV-1 M, O, N, HIV-2	1.0 mL, ID-NAT/MP-NAT: 25.7	1.67	[36]
Procleix Ultrio Assay	LTR/POL	HIV-1 M (A-G), O, N	1.0 mL, Ultrio: 63.0 1.0 mL, Discriminatory: 59.1	1.67	[37]
Procleix Ultrio Plus Assay	LTR/POL	HIV-1 M (A-H), O, N	1.0 mL, Ultrio Plus: 21.2 1.0 mL, Discriminatory: 18.9	1.67	[38]
Procleix Ultrio Elite Assay	LTR/POL	HIV-1 M(A-H), O, N, HIV-2(A, B)	1.0 mL, Ultrio Elite: 18.0 1.0 mL, Discriminatory: 17.3	1.67	[39]
UltraQual Multiplex PCR Assay	–	HIV-1 M, O, HIV-2	1.0 mL, 30.76	–	[40]

All the details about kits are summarized through references or package inserts

– information loss, *LOD* limit of detection, *PR* protease, *RT* reverse transcriptase

[a] The concentration of HIV RNA was shown as copies/mL, if the conversion factor was not found in package insert

[b] One copy = conversion factor*IU

Table 2 The current molecular assays used for HIV NAT and molecular characterization in the laboratory

Assay	Manufacturer	Use
Molecular POCT	ALERE Q HIV-1/2 DETECT (Abbott), Liat HIV Quant (IQuum), EOSCAPE-HIV (Wave 80 Biosciences), SAMBA (Real World), geneXpert (Cepheid)	POC diagnosis, for rapid diagnosis, still in scientific research
Phenotypic drug resistance testing	Antivirogram (Janssen Diagnostics BVBA), PhenoSense (Monogram Biosciences)	Antiretroviral drug resistance analysis, direct analyzing HIV ability to grow in presence of drug
Host genome genetics and host response analyses	None	Analysis of host susceptibility to HIV infection, clinical disease progression and monitoring treatment outcome
Digital PCR for HIV viral load quantification	QX200 Droplet Digital PCR (Bio-Rad), QuantStudio 3D (Applied Biosystems), CONSTELLATION Digital PCR System (Formulatrix), BioMark HD (Fluidigm), (RainDance Technologies)	Absolute quantification of HIV viral load, to quantitate HIV viral load for ART initiation and disease monitoring, future research focus
Genome editing	None	Treatment of HIV by inhibiting multiple steps of HIV-1 infection, future research focus

All the molecular assays are still in research in laboratory, not licensed by US FDA

and was proved to be of great performance in linearity and accuracy, consistent with CAP/CTM V2.0 and Abbott RealTime HIV-1 assay [56]. Cobas HIV-1 used on Cobas 6800/8800 has been available since 2014 as an alternative to CAM/CTM V2.0, the analyses demonstrated lower sample volume, improved limit of detection (LOD) and improved throughput capability of the novel cobas HIV-1 test in comparison to CAM/CTM V2.0, and Cobas HIV-1 had great correlation with CAM/CTM V2.0 [57]. A German study made an evaluation in performance between the two novel HIV-1 quantitative nucleic acid assays and established Abbott RealTime HIV-1 assay, the results showed the high agreement of the three assays in individual clinical samples, APTIMA HIV-1 Quant Assay and Abbott RealTime HIV-1 assay showed higher precision than Cobas HIV-1, meeting 5σ-criterion for most of HIV-1 isolates throughout all laboratories and subtypes, and represented a feasible alternative for HIV-1 viral load monitoring [58].

Two other quantitative technologies (b-DNA, NASBA) are also widely applied to quantify HIV viral load. The branched chain DNA-based VERSANT HIV-1 RNA 3.0 Assay is based upon a unique signal amplification technology and provides good reproducibility at the lower end of the detection range [59, 60]. This assay is also less affected by the presence of inhibitors and product carry over contamination problems associated other methods. There are disadvantages of branched chain DNA technique, and these include the requirement for larger plasma volumes and more time-consuming operation [61]. Another molecular tool, the NucliSens HIV-1 RNA QT assay, combines three key technologies: (i) silica-based nucleic acid extraction, (ii) NASBA, and (iii) electrochemiluminescence detection [62]. The NASBA technology is a sensitive and rapid amplification method that does not require a thermocycler and heat-stable enzymes. The next-generation assay (NucliSENS EasyQ HIV-1 V2.0) showed improved sensitivity to non-B HIV M subtypes compared to previous versions [55]. However, BioMerieux recalled the NucliSENS EasyQ HIV-1 V2.0 due to potential inaccurate test results when using NucliSENS easyMAG Magnetic Silica (MagSil) for nucleic acid extraction. The detection problem could lead to a risk of false negative or invalid results for clinical laboratory tests, which may cause serious adverse health consequences, to date. NucliSENS EasyQ HIV-1 V2.0 has not been approved by US FDA [63].

HIV qualitative nucleic acid assays

HIV qualitative nucleic acid assays are mainly designed to screen for the presence of HIV DNA and/or RNA to confirm the safety of blood products, early diagnose infant infection and acute infection [43, 64, 65]. The technologies used rely upon real-time PCR or TMA and are performed under single or multiplex assays in individual or pooled samples. Qualitative, highly sensitive assays with detection limits lower than 100 copies of HIV-1 RNA per ml have been developed for blood screening in order to enable the detection of low-level HIV RNA at early HIV infection [66]. To date, different commercially available qualitative nucleic acid assays can mainly be used for blood screening. Qualitative nucleic acid assays have become the methods of choice for diagnosis in infants born to HIV-1-infected mothers [43]. HIV serological testing in newborns who are below 18 months of age cannot be performed to confirm the source of HIV infection, due to the passage of maternal HIV antibody across the placenta to

the infant or children. Therefore, nucleic acid tests are required to diagnose HIV infection in children below 18 months of age [67]. Furthermore, qualitative nucleic acid assays can be used for the diagnosis of AHI due to nonreactive HIV antibody immunoassay result in its duration, and help initiate ART during early stage of HIV-1 infection can reduce HIV transmission and benefit patients [68].

Applications of HIV molecular technologies

HIV NAT used for blood screening

Due to the widespread application of NAT among blood donations, a significant decrease was observed in residual risk for transfusion transmitted disease (TTI) (hepatitis B virus (HBV), hepatitis C virus (HCV), HIV) [69]. This strategy was characterized by mini-pools (MP) or by individual donation (ID), for the HBV, HCV, and HIV screening of blood donors [70]. Currently, four systems are mainly used around the world for the viral screening of blood products: the Procleix Tigris system, Procleix Panther system (GenProbe Inc) based on TMA technology and the Cobas s 201 system (Roche Diagnostics) based on RT-PCR technology. Tigris system can perform Procleix Ultrio Assay (GenProbe Inc) and Procleix Ultrio Plus Assay (GenProbe Inc) with mode of individual-donation nucleic acid testing (ID-NAT). Panther system can conduct Procleix Ultrio Elite Assay with lower LOD than the former two assays (Table 2). Cobas s 201 system can perform Cobas TaqScreen MPX test (CTS/MPX) (Roche Diagnostics) characterized as mini-pool nucleic acid testing (MP-NAT). All these assays can detect the majority of HIV-1 subtypes, and the testing targets are aimed at LTR, Gag and Pol integrase regions (Table 1), which are most conserved region in HIV genome [71], and two different targets are suggested to detect HIV RNA for mismatches in the primer/probe region [72]. On May 30, 2018, a new multiplex PCR product, National Genetics Institute's (NGI) UltraQual ® Multiplex PCR Assay was approved by US-FDA to screen source plasma for HCV, HIV-1, HIV-2, and HBV, could detect a variety of HIV-1 genotypes, HIV-1 isolates, and HIV-2 [40].

When the performance of the two assays (Procleix Ultrio Assay and CTS/MPX version 1.0 (V1.0)) were compared for HIV, the two kits had equivalent clinical sensitivity [73]. Another study showed that both two assays mentioned above demonstrated acceptable operational performance, but Ultrio Assay ID-NAT format was significantly more sensitive than CTS/MPX V1.0 MP-NAT in detecting HIV RNA dilution panels [74]. A French research group also found Ultrio Assay ID-NAT detected HIV RNA 2 days earlier than CTS/MPX V1.0 MP-NAT in seroconversion panels [75]. However, the major problem for Ultrio Assay based on Tigris system is the higher operator involvement than CTS/MPX V1.0 based on Cobas s 201, which could be improved to enhance automation. The next generation of Procleix Ultrio Assay, Procleix Ultrio Plus Assay, reported to be of equal sensitivity to CTS/MPX [75, 76] can only detect HIV-1, while the new-generation assay (Ultrio Elite Assay) can detect both HIV-1 and HIV-2, and not affected by other viruses in sensitivity [77]. Ultrio Elite Assay reported to be rapid, automated and specific to detect HIV-1, HIV-2, HBV, and HCV could be feasible for blood, organ, and hematopoietic stem cell donors [78]. Among most of blood banks in China, HIV-1 RNA amplification is currently performed from pooled donors as a more cost-effective alternative to single-sample testing. A decisive factor for the sensitivity of nucleic acid detection is the pool size and the sensitivity of NAT, MP-NAT is less sensitive than ID-NAT for smaller sample volume, a previous study showed that MP-NAT detected HIV RNA 2 days later than ID-NAT in window period [79]. As for CTS/MPX, the biggest improvement of CTS/MPX V2.0 is that results of samples are simultaneously detected and discriminated for HIV, HCV, and HBV, while the positive results detected by CTS/MPX V1.0 cannot be simultaneously discriminated for HIV, HCV, and HBV [34, 35]. Recently, a new multiplex assay (cobas MPX Test ((Roche Diagnostics GmbH, Penzberg, Germany)) used on Cobas 6800/8800 system has been applied for the detection of HIV-1, HIV-2, HBV, and HCV in blood donations. Cobas MPX test showed high specificity and sensitivity in blood donations tested in pools and individually [15]. The excellent specificity and sensitivity were achieved by cobas MPX Test for the detection of HIV-1, HIV-2, HBV, and HCV, and accompanied by the reduction of the turnaround time and costs of NAT for blood screening [80]. In some certain status among HIV-infected populations such as elite controls (EC) and long-term nonprogressors (LTNP) [81, 82], serological tests are more reliable for the detection of HIV. EC and LTNP maintain stable levels of CD4+ T cells and HIV-1 RNA load below the LOD of NAT without ART but are detected HIV positive for antibody [81]. Therefore, serological tests are also important for HIV blood screening, which cannot completely replaced by NAT.

Used for HIV diagnosis of pediatrics and acute infection

HIV is the major pediatric infection acquired through mother-to-child transmission [83]. The maternal HIV viral load is high risk of transmission. Rates of mother-to-child transmission are very low when viral load is at extremely low levels [84]. Early pediatric diagnostics by NAT is crucial to prevent neonatal infection through ART as HIV prophylaxis in pregnant woman [85]. Besides, virological test for newborns who are exposed to HIV is strongly recommended by the World Health Organization (WHO) at the first postnatal visit [67].

For many years, Roche AmpliScreen HIV-1 Test version 1.5 (Roche Diagnostics) has been a confirmatory test for early infant diagnosis (EID) and recommended by WHO [86]; however, this assay will be discontinued by Roche in the next few years [87]. Currently, there are many alternatives. Almost all HIV NAT assays approved by FDA can be applied for HIV companion diagnosis of pediatrics, but the APTIMA HIV RNA Qualitative Assay (GenProbe Inc) based on TMA is the only US FDA-approved nucleic acid test for HIV in vitro diagnostic, which performed with the Procleix Tigris system can be used for HIV diagnosis of pediatrics and acute infection [65]. The result analyses of HIV-1 dilution panels demonstrated the improvement in sensitivity of APTIMA HIV RNA Qualitative Assay compared with Roche Amplicor assay [42]. APTIMA HIV-1 RNA qualitative assay was evaluated as an alternative to confirmatory assay (WB) using HIV serological reactive samples, and the results showed that APTIMA HIV-1 RNA qualitative assay can reduce the number of indeterminate results in Western blot (WB) by the detection of HIV-1 RNA [88]. A recent study compared four commercial virological assays (Roche CAP/CTM V2.0; CTS/MPX test V1.0 and V2.0; Siemens VERSANT HIV-1 RNA 1.0 kPCR assay) for infant HIV-1 diagnosis using dried blood specimens. Twenty-five percent (17/68) discordant results were observed among four assays, which were required for clear guidelines of HIV NAT for infant diagnosis [89]. However, another research showed the high agreement between Abbott RealTime HIV-1 Qualitative assay and CAP/CTM HIV-1 Qual test. An American research demonstrated the great performance of APTIMA HIV RNA Qualitative Assay for HIV infant diagnosis using plasma [65]. Furthermore, a lot of POC molecular assays have also been developed in resource-limited areas for EID. Susanna Ceffaa et al found the Xpert HIV-1 Qualitative assay had identical results as the Abbott M2000 HIV-1 real-time molecular assays for EID, and both Xpert HIV-1 qualitative and quantitative assays were promising molecular tools to diagnose HIV-exposed infants [90]. Another POC assay (ALERE Q HIV-1/2 DETECT, ABBOTT DIAGNOSTICS) also performed well for EID [91]. Therefore, HIV NAT is critical way to prevent HIV spread from mother-to-child transmission.

AHI characterized as plasmas with nonreactive serological results and reactive NAT results should be diagnosed to reduce the risk of HIV transmission, despite its short duration, can result in 10–50% of all new spread of HIV [12, 92, 93]. Evidence showed that 1% of outpatients with nonreactive or discordant HIV serological tests in Durban, South Africa, were diagnosed as AHI using COBAS AmpliScreen HIV-1 Test version 1.5 MP-NAT [94]. A similar pooled NAT research of 13, 226 individuals with HIV-1 seronegative results showed a AHI diagnosis rate of 0.12% [95]. A MP-NAT (APTIMA HIV-1 RNA qualitative assay) was performed to screen HIV RNA among 148, 888 samples, of which 161 were diagnosed as AHI [96]. Hence, AHI dignosis is extremely crucial in preventing the transmission of the virus and initiating ART.

Molecular POCT for HIV

POCT, which implies an analysis of clinical specimens as close as possible to the patient, is being used more and more in clinical microbiology [97]. Recently, NAT tests were developed for this purpose [98] (Table 2). In contrast to many other POCT than can be used by patients or doctors, NAT tests rely on very sophisticated technologies and require a minimum of laboratory training. However, the role of the technician consists mainly in the deposition of the clinical sample in the apparatus, so the machines can be installed in areas distant from central laboratories, notably when the transportation of samples is difficult or the biological resources scarce as in developing countries. In the field of HIV, there are currently no FDA-approved POCT on the market, but different platforms have been developed during the last few years or are still in development for the detection of HIV nucleic acids.

The Alere NAT system is a generic platform that can employ different NAT assays for the quantitative measurement of HIV-1 and HIV-2 viral load from approximately 25 μL, and the system can detect HIV-1 groups M, N, and O and HIV-2 [99, 100]. The Liat HIV Quant, developed by IQuum, Inc., is an automated sample-to-result NAT platform that performs sample nucleic acid extraction, purification, reverse transcription, polymerase chain reaction (PCR) amplification, and real-time detection to detect and/or quantify pathogens. HIV Quant plasma assay showed good performance, with a 2.7% similarity coefficient of variation (CV) compared to the Abbott assay (Abbott RealTime HIV-1) and a 1.8% similarity CV compared to the Roche test (CAP/CTM V2.0) on the verification panel, and 100% specificity [101]. However, LIAT cartridges currently require cold storage, increasing the cost of storage and transportation. Another new POC viral load assay, EOSCAPE-HIV HIV Rapid RNA Assay System, is being designed by Wave 80 Biosciences for use in resource-limited settings. The EOSCAPE-HIV system will be capable of providing either a qualitative or a quantitative HIV-1 RNA test result (detection threshold of 1000 copies/mL) in less than an hour [102]. Other molecular HIV POCT, such as SAMBA, geneXpert from Real World, Ltd. and Cepheid Innovative Bioscience Ltd. respectively, are also being developed for the determination of HIV viral load. An important advantage of all these technologies relies on their ability to be used as a single test, which is particularly adapted for emergency use.

HIV-1 drug resistance and susceptibility testing

The global scale-up of ART has led to dramatic reductions in HIV-1 mortality and incidence. However, HIV drug resistance

poses a potential threat to the long-term success of ART and is emerging as a threat to the elimination of AIDS as a public health problem. In high-income countries, standard genotypic resistance testing (SGRT) is recommended in patients with virological failure when the viral load is > 1000 copies/mL [103]. HIV-1 drug resistance testing can be performed phenotypically or genotypically. HIV phenotypic antiretroviral susceptibility testing is used to investigate the growing ability of HIV in the presence of antiretroviral drugs through clinical cutoffs for HIV-1 phenotypic resistance estimates [103–105]. Firstly, protease and reverse transcriptase genes are amplified by PCR after nucleic acid extraction and then inserted are into HIV-1 vectors to produce a recombinant with ability of replication, finally the replication ability of new HIV-1 recombinant will be estimated by a reporter system through 50% or 90% inhibitory concentrations (IC_{50} or IC_{90}) [103]. The two most widely used phenotypic assays (Antivirogram (Janssen Diagnostics BVBA) and PhenoSense (Monogram Biosciences, Inc.)) [106] identify resistance mutations associated protease (PR) and reverse transcriptase (RT) inhibitor by amplification and sequencing of HIV-1 PR and RT region (Table 2). Currently, drug resistance mutations associated the HIV inhibitors of the two HIV-1 genotyping systems have been provided a coverage of integrase and entry inhibitors [107, 108].

In the clinical setting, genotypic resistance testing is more usual than a phenotypic assay, which involves the use of dideoxy terminator Sanger sequencing of protease, reverse transcriptase and integrase to identify established clinically significant drug resistance mutations (DRMs) [109]. Currently, there are two FDA-approved commercial drug resistance assays; these are Trugene HIV-1 Genotyping Kit and Open Gene DNA Sequencing assay from Siemens Healthcare Diagnostics and the ViroSeq HIV-1 Genotyping System from Abbott Molecular (Table 1). Both systems contain modules for sample extraction, RT-PCR, cycle sequencing, sequence assembly, and DRM interpretation. Furthermore, two systems generate only reverse transcriptase and protease gene sequences following RT-PCR amplification; mutations associated with fusion inhibitors were not identified [110]. The systems use different sequencing chemistry. Resistance genotyping was performed by using large dye terminator chemistry provided by the ViroSeq Genotyping System, so the ViroSeq system requires an additional purification step to remove dye terminators, while the Trugene uses a dye primer system, so it is not necessary to remove the dye primers [111]. In addition, six sequencing reactions are needed to analyze one patient for ViroSeq, whereas 12 are needed for Trugene. Trugene system work well for the HIV-1 B subtype [112], other studies showed the performance of the HIV-1 ViroSeq HIV-1 Genotyping System method may be influenced by polymorphism between B and non-B subtypes, so the use of these assays on non-B subtype drug resistance testing requires continuous evaluation.

Furthermore, technologic advances have led to the development of POCT that detect gene mutations associated with HIV drug resistance [113]. Currently, oligonucleotide ligation-based assay (OLA)_Simple V1.0 is being developed for HIV drug resistance in laboratory by detecting mutant codons to predict ART failure [113]. Allele-specific PCR (ASPCR) can also detect HIV drug resistance mutations using laboratory-based qPCR, which was performed to identify K65R, K103N, Y181C, etc. in HIV-1 [114, 115]. In addition, Multiplex allele–specific (MAS) assays and Pan-degenerate amplification and adaptation (PANDAA) developed in recent years have been used for the detection of HIV drug resistance mutations [116, 117].

Nowadays, several next-generation sequencing (NGS) technologies are currently being explored for HIV drug resistance testing [118, 119]. Depending on the experimental design, the roll-out of NGS drug resistance testing at a larger scale is feasible, providing better characterization and understanding of the evolving population of viral variants within a patient and potentially improving the prognostic value of drug resistance testing [120].

Host genome genetics and host response analyses

Some evidences showned that variability in HIV-1 susceptibility was related to host genetics [121, 122]. The risk of HIV infection and progression of AIDS may be greatly influenced by polymorphisms in some AIDS restriction genes including C-X-C chemokine receptor type4 (CXCR4), C-C chemokine receptor type 5 (CCR5), and CCR2 [123–126]. CCR5 Delta32, CCR2-64I, stromal cell-derived factor-1 3'A (SDF-1 3'A), and human leukocyte antigen-B*35 (HLA-B*35) alleles were described to preventing the progression to AIDS [127–129]. Furthermore, an authoritative study demonstrated over 300 single-nucleotide polymorphisms among the major histocompatibility complex and suggested that the main genetic determinants of HIV control is HLA–viral peptide interaction. Therefore, the testing of host polymorphisms among HIV infection can help analyze the potential risk of rapid disease progression and initiate early treatment. Currently, with the rapid development of molecular technologies, millions of polymorphisms at high sample throughput can be detected using Illumina or Affymetrix platforms [130]. Besides, real-time PCR assays are also applied to detect sequence polymorphisms [131]. However, the high costs of these assays limit their applications among large-scale studies. Nucleic acid sequencing remains the most accurate method to detect the host genome polymorphisms [132]. Apart from identifying the potential clinical progression through the detection of host genome polymorphisms, host response analysis can also be conducted to monitor the progression of AIDS

using molecular assays [133]. The episomal double-stranded DNA, T cell-receptor-chain rearrangement excision circles (TREC), is produced in the thymus during T cell maturation, which constantly present in newly matured T cells but the TREC gradually degraded during mitosis after TREC-bearing T cells entering the blood circulation [134]. A rapid decrease in thymus function resulted by HIV infection may contribute to the decrease of TREC; hence, apart from the levels of CD4+ T cells, TREC tested through PCR has been applied to estimate the progression of AIDS [135, 136].

Future prospects

Digital PCR for measurement of HIV infection

Digital PCR is based on isolated replicate PCRs from a sample in multiple partitions. In the sufficiently diluted samples, the few DNA templates in different partitions follow Poisson distribution. Therefore, the direct absolute quantification can be performed through the measurement of ratio of negative and positive partitions [137]. Currently, digital PCR has been conducted to measure the effects of latency reversing agents [138, 139], HIV vaccination [140, 141], immunization by neutralizing antibodies [142, 143], hematopoietic chimerism after allogeneic stem cell transplantation [144], structured treatment interruptions [145], and early ART initiation [146, 147]. Besides, digital PCR can also be used as a quantitative tool to measure HIV reservoir [148]. Nowadays, six common digital platforms are available for HIV quantification with different production of partitions amounts of partitions. QX200 Droplet Digital PCR (Bio-Rad, USA) produce partitions through wate-in-oil emulsion chemistry (Table 2). RainDrop plus Digital PCR system (RainDance Technologies) is similar to QX200 system. However, contrary to water-in-oil emulsion chemistry based assays, other digital PCR systems are based on arrays, such as the QuantStudio 3D (Applied Biosystems, US) utilizes a silicon ship made up of a single array of isolated reaction wells, CONSTELLATION Digital PCR System (Formulatrix, USA) divides connecting channels into individual microfluidic areas through seal-compressing roller using a microplate. Furthermore, BioMark HD can dispense the sample in a well and distribute a lot if isolated reaction through the digital integrated fluidic circuits arrays [149]. Strain et al. found droplet digital PCR (QX100) was more precise and sensitive than in-house qPCR [150].

A Dutch study showed that QX100 (former generation of QX200), QuantStudio 3D, and qPCR were able to detect the lowest levels of 2.5 HIV DNA copies [148].The QX100 had the highest precision, efficiency and quantitative linearity. However, the observation of false positive signals among QX100 and QuantStudio 3D platforms. qPCR still cannot be replaced by digital PCR in low viral load of HIV. Furthermore, another research found the similar false-positives [151]. Contaminations or disturbed droplets may result in false-positive droplets [149]. Although, the concept of digital PCR has been well-developed but automated platforms for HIV quantification are not relatively sophisticated. Digital PCR cannot give scientists information about the replication competent viruses; therefore, digital PCR for detection of HIV infections need more advancements in the future [152].

Apart from HIV nucleic acid quantification, digital PCR system (QX200) can be used to conduct single-cell-droplets, after HIV RNA was quantitated by digital PCR. Furthermore, the operators can manually pick up positive droplets for sequencing HIV ENV and CCR5. In the future, digital PCR may focus on the host genome genetics and host response analyses through investigation of HIV integration site and viral protein creation [152]. Digital PCR has developed to be a new molecular tool and with additional advancements in prospect, which may be an indispensable tool for future HIV study.

Genome editing for HIV treatment

Homozygosity for a deletion of 32 base pairs in CCR5 (CCR5 Delta32) has been shown to prevent HIV [153, 154]. A study reported by Hutter G transplanted mesenchymal stem cells (MSCs) with CCR5 Delta32 mutation to HIV-infected populations, and no viral rebound was identified 20 months after discontinuation of ART and transplantation [154]. Three gene editing strategies including zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPRs/Cas9) target to CCR5 for HIV treatment. Currently, CRISPRs/Cas9 has replaced the first two genome editing technologies, which recruit the Cas9 endonuclease to edit gene by hybridizing with a guide RNA and binding a target DNA site [155]. Many evidences showed that CRISPRs/Cas9 can inhibit multiple steps of HIV-1 infection. Insertions and deletions of HIV DNA due to Cas9 cleavage and blockage of pre-integrated proviral double-stranded DNA (dsDNA) will led to marked degradation of viral DNA, and disruption of co-receptor CCR5 brought by CRISPRs/Cas9 can prevent virus to enter the host cells, besides, CRISPRs/Cas9 can help remove HIV infection during the ART through reactivation of latent provirus [156, 157]. Recently, scientists found that CRISPRs/Cas9 can induce viral escape, apart from inhabitation of HIV replication [157, 158]. Viral escape via CRISPR system is caused by mutations in viral DNA sequence targeted by sgRNA. Then, the target sequence cannot be identified by T cells so that viral escape occurs [157, 159].

Conclusions

The development and application of molecular technologies have initiated a revolution in the detection and

characterization of many infectious diseases. Molecular technologies have quickly become mainstream for the laboratory diagnosis and assessment of HIV-1 infections, which are classified as quantitative and qualitative assays.

Molecular technologies have played important roles in early detection, early diagnosis, and early treatment of HIV-infected populations. Furthermore, molecular assays can be used for the observation of therapeutic effects and drug resistance monitoring in the clinical setting. In recent years, commercially molecular assays for HIV detection have made great progress in specificity, sensitivity, reproducibility, and automation. POC nucleic acid testing is also being developed with the potential to make rapid, evidence-based therapeutic intervention at or near the site of patient care. Furthermore, the characterization of HIV including host genome polymorphism and host response analyses tested by molecular tools can identify the potential progression of HIV and initiate ART. Along with the development of digital PCR, the viral load quantification of HIV will be more precise and sensitive. In the future, we may cure HIV infection through genome editing strategies, though no one with HIV infection was currently cured via molecular tools.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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