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Research review paper

Molecular diagnosis of HIV and relevant novel technologies in mutation analysis

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ABSTRACT

HIV infection is one of the major threats to human health due to the lack of relevant vaccine and drugs to cure AIDS. Its early diagnosis is thus important in controlling HIV transmission. Molecular diagnosis of HIV can be performed qualitatively and quantitatively. Currently, molecular diagnosis of HIV infection is only used as a complementary diagnosis although viral load test is used to monitor disease progression and responsiveness to antiviral therapy. To optimize HIV assays, a variety of technological advances, such as the introduction of dUTP/UNG system, real-time detection platform, and coupling of more than one enzyme in molecular identification, have been integrated into new methods. With the development of more reliable HIV assays in the future, the molecular diagnosis of HIV is expected to be accepted as one of the standards in determining whether there is a HIV infection in resource-rich laboratories, which will play a crucial role in reducing HIV transmission.

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Human immunodeficiency virus (HIV) is the pathogen causing the acquired immunodeficiency syndrome (AIDS). Since the discovery of

HIV less than three decades ago more than 40 million of people have been currently infected with HIV (Report on the global AIDS epidemic, 2007) and nearly 3 million people have died from AIDS every year. From the prevention point of view, laboratory diagnosis including conventional antibody determination and more sophisticated molecular diagnosis are important approaches for coping with AIDS. As compared to antibody test, nucleic acid-based molecular diagnosis is able to monitor HIV infection at a very early stage, which helps to substantially shorten the 2–6 months window period to 2–7 days. The decrease of the assay window period has great impact in controlling the HIV transmission. Although molecular diagnosis of HIV is not officially recommended as the gold standard in the clinical diagnosis of HIV infection due to the potential false positives from PCR-based assays, recent advances in the development of HIV assays have greatly minimized the laboratory contaminations in molecular diagnosis, rendering the modern molecular diagnosis adequate to replace antibody-based assay which is used only as a complement in selected clinical circumstances in resource-rich laboratories (Swanson et al., 2005). This paper reviews the current applications of molecular diagnosis in HIV qualitative and quantitative determinations, and discusses the relevant novel technologies in mutation assays that can be employed in the development of a new generation of HIV assays with higher accuracy and sensitivity.

1. Genetics of HIV and its implications in molecular diagnosis

Two types of HIV have been identified so far, HIV-1 and HIV-2 (Refseq: NC_001802, and NC_001722). These two types of viruses are homologues with as high as 59% similarity in their conserved regions at the amino acid level. The human AIDS is mainly caused by HIV-1 that is the focus of this review paper. HIV-1 can be further divided into three subtypes: main (M) group, outlier (O) group, and new (N) group (Robertson et al., 2000). The M group is the leading pathogen identified in most of the HIV-infected individuals worldwide. Many subtypes labeled from letter A to K and an increasing number of circulating recombinant forms (CRFs) are reported in the M group of HIV-1. The pure forms of subtypes E and I were never used but have only been reported in the CRFs. Different regions of a gene or genes in the HIV genome vary in their mutation rate, with the *gag* and *pol* being more conserved than other genes and the *env* gene having the highest mutation rate. The genetics of HIV is clinically relevant in the development of molecular assays. For example, the more conserved regions of HIV-1 are used to develop assays for the qualitative determination of HIV load and mutations are used as assay targets for the monitoring of drug-resistant patients.

2. Several clinical and technological issues in diagnosis of HIV infection

Laboratory tests related to HIV infection include the identification of anti-HIV-1 or HIV-2 antibodies, nucleic acid-based viral assay, viral load analysis, and CD4+ T lymphocyte counting (Mbanya et al., 2007). The antibody-based assay is temporarily used as the gold standard for laboratory diagnosis of HIV infection, while viral load analysis and the CD4+ T lymphocyte counts are used to evaluate the progress and the prognosis of AIDS as well as the effectiveness of antiviral therapy (Torti et al., 2007).

2.1. Timing and method option for determination of infant HIV infection

Early diagnosis of HIV allows healthcare providers to offer optimal care and treatment of HIV-infected children, assists in decision-making on infant feeding, and avoids needless stress in mothers and families. Given the high risk of disease progression in infected infants, improving options for early diagnosis is essential, particularly in resource-limited settings. Currently, PCR assays or HIV culture techniques can identify at birth about one-third of infants who finally and ultimately prove to be HIV infected. With these techniques, approximately 90% of HIV-infected infants are identifiable by 2 months of age, and 95% by 3 months of age. Virological testing at 6 weeks of age gives a good sensitivity (>98%) with the various methods (http://www.who.int/hiv/paediatric/EarlydiagnostictestingforHIVVer_Final_May07.pdf). In a WHO-supported study, PCR-based assays were performed at the age of 6 weeks in infants (Optimizing paediatric HIV care in Kenya). HIV-negative infants were subjected to an antibody test at the age of 12 months and 18 months respectively to confirm their HIV-negative status (Cherutich et al., 2008).

One innovative new approach to both RNA and DNA PCR testing uses dried blood spot specimens, which should make it much simpler to gather and store specimens in field settings. Although the PCR-based assay is not accepted clinically as a definitive diagnosis, it is practically infants born from a HIV-1 positive mother (Sahni et al., 2005; Tournoud and Ecochard, 2006). According to PAMAB report, a plasma HIV-RNA assay matched or exceeded the diagnostic performance of quantitative peripheral blood mononuclear cell microculture or DNA-based assays (http://www.nichd.nih.gov/publications/pubs_details.cfm?from=&pubs_id=5687). But none of the DNA- or RNA-based assays received licensing approval for use in routine diagnostic services by the United States, European, or Chinese regulators, although this may change in the future. Therefore, in situations such as infants younger than 18 months old, nucleic acid-based HIV testing is employed but the final diagnosis of HIV-negative still depends on an antibody-based assay in infants up to 18 months old (Lujan-Zilbermann et al., 2006; Mbanya et al., 2007).

2.2. The weakness of antibody-based assay

Whereas the current application of antibody-based assay in the diagnosis of HIV infection is not replaceable in clinical practice, it should be pointed out that there are some circumstances where antibody-based assay is less appropriate (Table 1). The antibody-based assays are less trustworthy when used as the gold standard, particularly for viral infections associated with viruses having weak antigenic and hypermutable features. Examples are viral mutations including point mutation which can substantially minimize or eliminate certain antigenic characteristics resulting in neutralization escape or in low level of antibody titer of the host or in no relevant detectable antibody (Kalia et al., 2005; Selvarajah et al., 2005). In summary, the antibody-based HIV assay is not perfect and the development of better nucleic acid-based HIV assays is highly needed.

2.3. Technical advances minimizing laboratory contamination

Although conventional PCR technique is simple and convenient, occasional false positives prevent this nucleic acid-based assay from being used as the "gold standard" in diagnosing HIV infection. In order to overcome the drawbacks of molecular diagnostic technologies, quite a few revolutionary reagents/methods have been developed. The introduction of filter tips together with positive displacement pipette minimizes or eliminates the contamination caused by aerosol drops

Table 1
Clinical circumstances where nucleic acid-based assay is applied in HIV diagnosis

(1)	Neonates born to HIV-positive mothers
(2)	Individuals potentially infected with HIV but are still in the antibody free window period
(3)	Antibody level cannot reflect the infectious seriousness when the immune system is pathologically or pharmacologically suppressed
(4)	No relevant antibody or only very low levels of the antibody yielded in the case of antigenic feature altered from viral mutations
(5)	Individuals under HIV vaccine clinical trial presently or individuals successfully immunized by vaccination in the future