Biosensors and Bioelectronics 78 (2016) 358–366

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

HIV-1 Tat biosensor: Current development and trends for early detection strategies

M.F. Fatin [a], A.R. Ruslinda [a,*], M.K. Md Arshad [a], K.K. Tee [b], R.M. Ayub [a], U. Hashim [a], A. Kamarulzaman [b], Subash C.B. Gopinath [a]

[a] Institute of Nano Electronic Engineering (INEE), Universiti Malaysia Perlis, Kangar, 01000 Perlis, Malaysia
[b] Center of Excellence for Research in AIDS (CERiA), Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form
20 November 2015
Accepted 24 November 2015
Available online 25 November 2015

Keywords:
HIV-1 Tat
Biosensor
Aptamer
Antibody

ABSTRACT

Human immunodeficiency virus (HIV) has infected almost 35 million people worldwide. Various tests have been developed to detect the presence of HIV during the early stages of the disease in order to reduce the risk of transmission to other humans. The HIV-1 Tat protein is one of the proteins present in HIV that are released abundantly approximately 2–4 weeks after infection. In this review, we have outlined various strategies for detecting the Tat protein, which helps transcribe the virus and enhances replication. Detection strategies presented include immunoassays, biosensors and gene expression, which utilize antibodies or aptamers as common probes to sense the presence of Tat. Alternatively, measuring the levels of gene transcription is a direct method of analysing the HIV gene to confirm the presence of Tat. By detection of the Tat protein, virus transmission can be detected in high-risk individuals in the early stages of the disease to reduce the risk of an HIV pandemic.

Contents

1. Introduction

Human immunodeficiency virus (HIV) has spread around the

* Corresponding author.
E-mail address: ruslinda@unimap.edu.my (A.R. Ruslinda).

http://dx.doi.org/10.1016/j.bios.2015.11.067
0956-5663/

globe, and infection levels have increased throughout the years. According to the World Health Organization (WHO), by the end of 2013, over 35 million people had been infected. HIV was discovered soon after the first case of Acquired Immune Deficiency Syndrome (AIDS) was reported in the USA in the 1980s and was given the name of HTLV-III/LAV (human T-cell lymphotropic virus-type III/lymphadenopathy-associated virus), which was changed to HIV later (Shaw et al., 1984). There are two types of HIV viruses, known as HIV-1 and HIV-2, which have been classified according to their origin. Scientists believe that HIV-1 was originally transmitted from Simian immunodeficiency virus (SIV) in chimpanzees in Central or West Central Africa, while HIV-2 originated from monkey species from a coastal range in West Africa (Van Rensburg, 2000). The virus was then mutated to HIV when it became capable of entering the human body. HIV infection in humans is a cross-species infection originally caused by the slaughter and consumption of the HIV-carrying primates. However, most of the cases of HIV infection are due to HIV-1, which is more viral and pandemic.

HIV is a type of virus that slowly integrates into the immune system in the human body. Human infections occur through direct contact with bodily fluids, such as blood transfusion, sexual intercourse or syringe sharing among drug addicts. There are three stages of HIV infection: the early infection stage, the clinical latency stage and AIDS. Once HIV enters the human body, HIV integrates and replicates inside $CD4^+$ T cells and activates DNA-dependent protein kinase (DNA-PK) (Cooper et al., 2013). $CD4^+$ T cells, known as T helper cells, are white blood cells covered with the CD4 glycoprotein on their surfaces that are a part of the infection-fighting immune system. DNA-PK acts to destroy the infected $CD4^+$ T cells to eliminate the virus but at the same time it weakens the immune system due to a lack of immune cells to guard the human body from disease. The virus also has the ability to evolve, rapidly, replicating and producing 10^{10} virions/day (Dangeti, 2014) while evolving to suit the current host conditions. Due to the rapid replication of the virus, early detection of HIV is very important in order to eliminate the virus as early as possible with antiretroviral therapy (Basavaraj et al., 2010; Siegfried et al., 2010). Fig. 1 illustrates the described regulation process of HIV and the roles of its proteins.

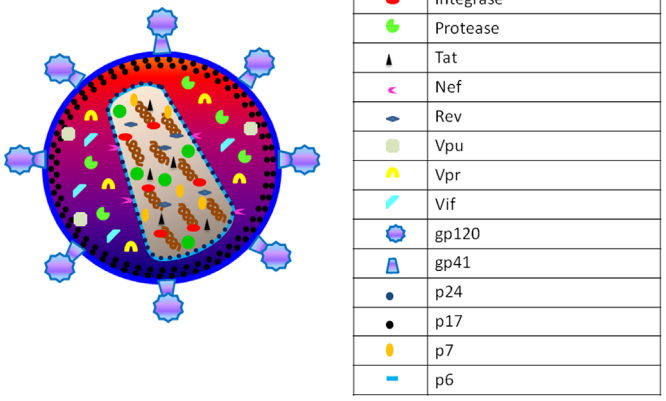

Fig. 2. Structure of human immunodeficiency virus with a diameter of approximately 120 nm.

HIV is an almost round-shaped virus (Fig. 2) with a diameter of almost 120 nm. It is composed of two RNA strands and fifteen different types of proteins with their own unique functions in promoting HIV replication and integration into human cells (Frankel and Young, 1998). There are viral structural proteins/enzymes, structural proteins and accessories proteins. The viral structural proteins or viral enzymes have three subunits, including reverse transcriptase (RT), integrase (IN) and protease (PR). RT (p55/p65) plays a very important role in viral replication by synthesizing a complementary DNA (cDNA) strand from the viral RNA in a process known as reverse transcription. The viral RNA is then integrated into the host genome by the integrase (p11) enzyme and continues the replication process to become cDNA. This cDNA lies dormant in the host for decades and is difficult to fight. Protease (p15) is essential for the maturation of HIV particles, cleaving the long polyproteins of HIV so that the proteins can function properly. The structural proteins include the matrix, surface (SU), transmembrane (TM), capsid and nucleocapsid proteins. The

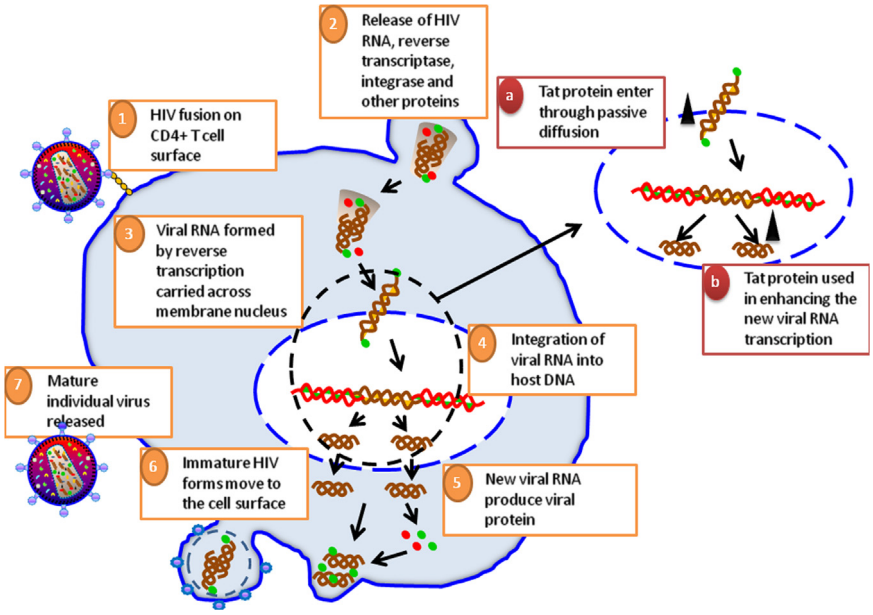

Fig. 1. Steps of HIV regulation in infected cells. (1) Viral fusion with the host cell, (2) release of HIV proteins and RNA into the host cell, (3) viral RNA is carried across the nuclear membrane, (4) viral RNA integration into host DNA, (5) production of new viral proteins, (6) formation of new viruses on the cell surface and (7) release of individual viruses. The function of HIV-1 Tat protein is needed between steps no. 4 and 5 during the transcription of new viral RNA.

matrix protein (p17) exists on the inner surface of the HIV membrane and associates into trimers to help new virus particles bud on the surface of the infected immune cells. The SU (gp120) and TM (gp41) proteins function to envelope the proteins attached to the surface of the immune cells and form spikes to penetrate the cell wall to deliver the viral RNA into the cell. These proteins are coated with carbohydrates, making it difficult for antibodies to recognise and mediate the elimination of the infected cell. Capsid (p24) is a protein that forms a cone shape in the middle of the viral particle. It coats the RNA and helps deliver the RNA into the cell during infection. Nucleocapsid (p9) functions to protect the viral RNA because RNA is easily degraded. It forms a complex with the RNA to stabilise and avoid the degradation of the RNA by nucleases. The accessory proteins include Tat (trans-activator of transcription), Rev (regulator of virion), Nef (negative regulatory factor), Vpu (viral protein u), Vif (viral infectivity factor), Vpr (viral protein r) and P6. Tat is important in the transcription of viral RNA and enhances the amount of protein produced by attaching itself to the viral RNA (Rubartelli et al., 1998; Bayer et al., 1995). The Rev protein binds to the viral RNA and regulates its splicing and transport. Nef is one of the important proteins that leads to the progression of AIDS by collapsing the human immune system. This negative regulatory factor makes the infected cell stop producing proteins that are important for the body's defence against disease. Vpu is important when the infected cell begins to produce viral buds; it helps to weaken the interaction between the new envelope protein and cell receptors to allow the virus to escape the cell. Vif functions in the newly infected cell to deactivate the cell's defence proteins while Vpr helps to guide the viral genome to the nucleus of the infected cell. P6 is a largely unstructured protein that promotes Vpr incorporation into the new cell. All of the proteins have been classified by their genes and summarised in Table 1 and play an important role in viral replication and survival in the human body.

In this review, we focused on the early diagnosis of HIV using the HIV-1 Tat protein as the target. Currently, the gold standard for HIV testing is the ELISA method, which is highly accurate at detecting anti-HIV antibodies within human blood. The current antibody-based testing requires approximately 3–6 months post-infection to obtain a valid result. In contrast, the Tat protein is an optimal target because it is present in the virus and released in the body at an early stage of infection. Tat helps the virus penetrate the infected host cell nucleus by passive diffusion and increases virus transcription. During early infection, the RNA that integrates into the host cell will produce the Tat protein. Once a certain amount of Tat protein has been produced, HIV transcription in the host cell increases, and at a certain point the patient develops the flu-like acute HIV infection syndrome. This stage usually happens between 2–4 weeks after infection and varies between individuals. This justifies the selection of the Tat protein as an indicator for the early detection of HIV. In this early infection stage, Tat is actively participating in the multiplication process of HIV; thus, the Tat protein is released extracellularly and is detectable in human serum. By detecting the Tat protein, we may be able to eliminate the limitations in detection within the first 3 months of infection and reduce the high risk of transmission during this stage.

2. A current approach to HIV detection

The presence of HIV can be detected by biological tests using various methods. There are several tests for HIV detection that are reliable and accurate to indicate infection in humans. Antibody-based HIV detection is the most common test for HIV screening (Greenwald et al., 2006; Oelrichs, 2004). Another approach to HIV screening would be the antigen test. However, this method is cost-consuming and more complex. These tests detect the HIV itself and eliminate the possibility of a false negative during HIV infection. They detect the presence of either viral proteins (Bukrinskaya, 2007; Tehe et al., 2006; Schpbach et al., 2001; Pedersen et al., 1987) or viral genes. An antigen test works by detecting the viral antigen from the virus using antibodies. HIV consists of 15 different types of proteins and RNA, which is also considered an antigen. Each protein is specifically detected by its own complementary antibodies that can be used for the antigen test. Among these, the P24 antigen is commonly used for the antigen test to detect infection at an early stage in patients with suspected HIV infection (Fearon, 2005). The P24 protein is present in the viral capsid and produced abundantly during early infection before the body develops antibodies against it. Due to its higher copy number in the

Table 1
Types of HIV-1 protein (Frankel and Young, 1998).

Gene	Name of protein (Size)		Function
	Precursor	Products	
gag	Gag (p55)		Gag precursor protein encoding the capsid proteins
		CA (p24)	Viral capsid coating around viral RNA
		MA (p17)	Viral matrix coating the inner surface of the viral membrane
		NC (p7)	RNA-binding protein protecting the viral RNA
		(p6)	Helps with Vpr incorporation into the new cell
pol	Pol (pr $160^{gag\text{-}pol}$)		Pol precursor protein encoding viral enzymes
		RT (p65/55)	Reverse transcriptase that builds a DNA copy of the viral RNA genome
		PR (p15)	Protease helps in the maturation of HIV particles
		IN (p11)	Integrase inserts the viral RNA copy into the infected cell genome
env	Env (gp160)		Envelope precursor protein encoding viral glycoproteins
		SU (gp120)	Envelope surface protein
		TM (gp41)	Transmembrane protein
tat		Tat (p14)	Transactivate viral gene expression
rev		Rev (p19)	Regulates viral mRNA splicing and transport
nef		Nef (p27)	Collapsing the self defence of the infected cell by stopping the production of several proteins
vif		Vif (p23)	Deactivate the host defence proteins
vpr		Vpr (p18)	Guide the viral genome to the host nucleus
vpu		Vpu (p15)	Let the virus escape the host cell receptors during budding

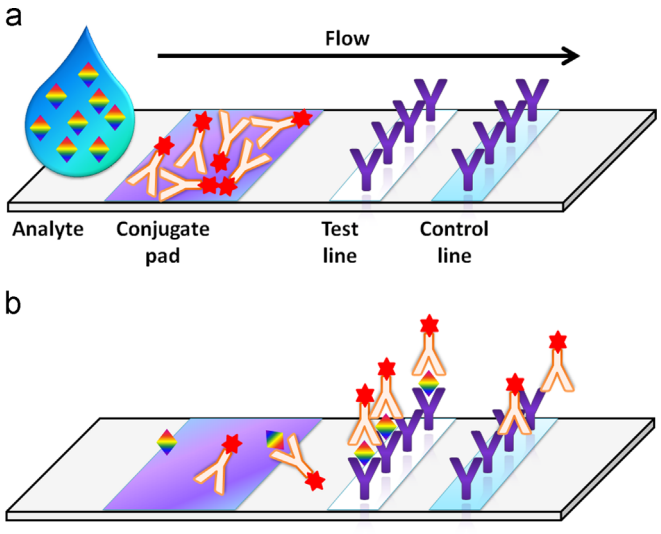

Fig. 3. Schematic reaction of a lateral flow system (a) before analytes pass through the strip and (b) after analytes pass through the strip. When analytes pass through the strip, they bind to the conjugated antibody at the conjugate pad, and the complex will bind an antibody immobilized on the test line. The unbound conjugated antibody will bind to the specific antibody on the control line. This type of reaction will produce two coloured bands on the strip (positive reaction), indicating the present of the analyte in the sample solution.

viral capsid, approximately 2000 molecules per virus particle, it can be easily detected during the early infection stage. However, the sensitivity of this test declines after the development of antibodies, and it does not work very well using blood samples unless they undergo virus culture and isolation. A lateral flow immunoassay is another test that has been widely used since it was introduced in the 1960s, especially in pregnancy tests. Since then, many lateral tests for the detection of various proteins have been developed (He et al., 2014; Novell et al., 2012; Njiru, 2011). The test is basically a strip of a pad containing immobilized biomolecules. Usually, there is a conjugate pad (containing an immobilized conjugated antibody), a test line (containing an immobilized antibody specific to the target analyte) and a control line (containing an immobilized antibody specific to the conjugated antibody) (Sajid et al., 2014), as illustrated in Fig. 3. When the analyte passes through the strip, it will bind to the test and control lines. With the presence of the analyte, both lines will change colour, while without the presence of the analyte, only the control line will change colour. The HIV test kit that has been approved by The Food and Drug Administration (FDA) is a detection strip (OraQuick and Oraquick Advance) that has been developed for HIV screening (Zachary et al., 2012; Holguín et al., 2009; Reynolds and Muwonga, 2004). This strip contains synthetic gp41 for antibody detection. The accuracy of the result is questionable for certain cases and requires confirmation at a hospital to clarify the results due to the inability of the device to detect a low viral load and the possibility of false negative results during the early period of infection (Pavie et al., 2010), when the body is in the process of developing antibodies against the HIV virus. This early stage can vary between 9 days to 3–6 months based on the individual. Any antibody-based detection can result in a false negative due to the inability of the test to sense the presence of antibodies (due to their absence or an ultra-low level in human blood). However, after the immune system produces antibodies, the level of the virus will return to the initial infection level. With time, the virus will advance to the next stage, which is the clinical latency stage. At this stage, the virus will continue to replicate in the body with either no symptoms or only mild symptoms. Antibodies are detectable during this stage. During these two stages, it is important to start taking medication to suppress viral replication. With the proper medication, HIV patients can prolong their lifespan to that of a healthy person. Depending on various factors, viral replication will eventually decrease the $CD4^+$ T cell count to 200 cells per millimetre of blood (cells/mm^2; normal count: 500–1600 cells/mm^2) (Lobritz et al., 2011). This situation will eventually lead to the development of AIDS.

The current approach used for HIV detection for point of care testing (POCT) is similar to the OraQuick lateral flow strip. The main constraint of POCT is the poor sensitivity during the early infection period that may last approximately 3–6 months. An additional concern to the specificity and sensitivity is that POCT needs to be low-cost, easy to use, and have results that are easily interpreted and reliable. To avoid the necessity of further laboratory tests if the POCT test is positive, dual probe-based testing needs to be developed. Detection of both anti-HIV antibodies and antigens can both be performed concurrently on one testing device by immobilizing two different probes for a single sample in order to gain confirmation. This approach will also reduce the sample volume required for testing. POCT with a lower sample requirement (to a microliter or nanoliter level) will add an additional advantage. Moreover, a detection device can also be used for the detection of co-infection in HIV patients, who often suffer from other diseases such as hepatitis, herpes and tuberculosis. POCT should be developed for home testing to increase the frequency of testing among high-risk individuals. The result should be easy to interpret, which will further reduce the manpower required. Additionally, the development of a smartphone dongle for HIV testing is another new technology that enables easy in-situ testing, and the results can be shared online with doctors for result interpretation.

3. Why the HIV-1 Tat protein?

The Tat protein is typically produced by the HIV virus as soon as it enters the human body in order to ensure the sustainability of the virus. It is involved in viral transcription (Romani et al., 2010; Rana and Jeang, 1999), helping the virus to spread by penetrating the host cell membrane (Ziegler and Seelig, 2004; Rudolph et al., 2003). This process begins once the virus enters the human body, as explained in Fig. 1. The Tat protein is present from the early stage of HIV infection. Tat can travel in and out of the cell without being detected through passive diffusion (Cardarelli et al., 2007). It binds to phospholipids and travels freely across the nuclear membrane (Debaisieux et al., 2012). The presence of Tat protein in the extracellular environment is toxic and plays a key role in the progression from HIV to AIDS (King et al., 2006). Tat is released by HIV-infected cells and is detectable in infected human blood serum down to levels of 40 ng/ml (Wang et al., 2011; Campbell and Loret, 2009).

The Tat protein can be divided into five different regions according to its amino acid sequence; amino acids 1–19 (N-terminal activation region), amino acids 20–39 (cysteine-rich domain), amino acids 40–47 (core region), amino acids 48–56 (basic region) and amino acids 57–71 (glutamine-rich region) (Rubartelli et al., 1998; Klostermeier et al., 1997). The protein structure is illustrated in Fig. 4. The cysteine-rich region is believed to be involved in metal-ion binding (Garber et al., 1998; Weeks and Crothers, 1993), while the core, basic and glutamine-rich regions are all involved in RNA binding, which will later be used as the region for Tat protein detection (Karn, 1992).

The P24 and Nef proteins are also produced in early HIV infection. P24 forms the capsid of the virus. It is used as a detection target due to its abundant presence in the bodily fluids of HIV-infected people (Bukrinskaya, 2007). However, antibodies against the P24 protein are mixed with the bodily fluids and bind to the P24 protein if present, making the test less sensitive; thus, P24 detection only works before the body produces antibodies against

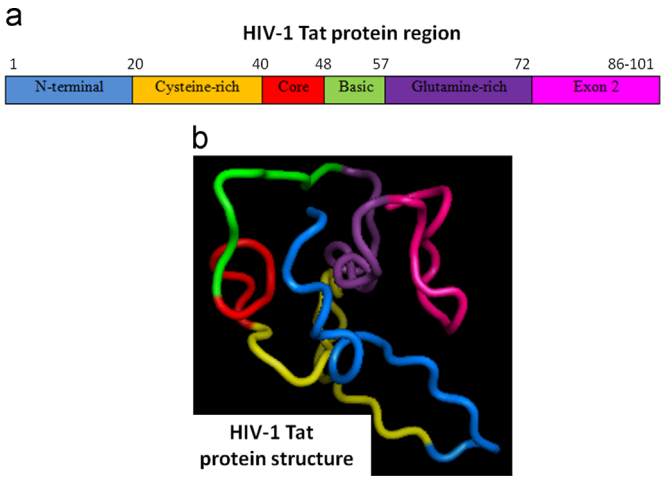

Fig. 4. (a) HIV-1 Tat protein and (b) the protein structure based on its region by colour difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the P24 protein (Pedersen et al., 1987). Nef expression is responsible for approximately three-quarters of the viral mRNA load during the early phase (Das and Jameel, 2005); however, using the Nef protein as an indicator for HIV infection has not been thoroughly explored. Thus, we see the Tat protein as one of the most promising candidates for diagnostic tests for HIV screening.

4. Antibodies and aptamers as biomolecular probes

Detection of the HIV-1 Tat protein has been conducted using a biological probe, which can be either an antibody, an aptamer, a DNA strand or an enzyme. In this review, we are going to evaluate the usage of antibodies and aptamers, which are the two primary probes used. Antibodies are heavy Y-shaped proteins used by the immune system to label targets for cells of the immune system, such as bacteria and viruses. Five different classes of immunoglobulin exist, including IgA, IgD, IgE, IgG and IgM. A single antibody is made up of two heavy chains and two light chains. The light chains contain the region that recognizes an epitope on the targeted antigen. Each antibody consists of three regions, two of which are the Fab regions that act as the antigen identification site. Antibodies are widely used by biologists for assays including enzyme-linked immunosorbence assay (ELISA), lateral flow, Western blot and dot blot. Antibodies are also widely used as probes for protein detection due to their specificity of recognition (Saerens et al., 2008). However, antibodies are easily denatured at room temperature and experience a loss in activity upon freeze and thaw cycles.

Aptamers are oligonucleotides derived from DNA or RNA that can bind to their targets with high selectivity. They have the ability to discriminate between chiral molecules, enantiomers and protein isoforms. Aptamers can be engineered in vitro by the Systematic Evolution of Ligands by EXponential enrichment (SELEX) process, and modifications can be made to produce aptamers with the desired characteristics. There are a few advantages of aptamers compared with antibodies or enzymes as probes. Aptamers remain very stable at high temperatures, extreme pH or in chemically modified environments, and they do not easily denature. Aptamers are usually molecules with low immunogenicity and toxicity because nucleic acids are not typically recognised by the human immune system as foreign agents, which means that the aptamer recognition reaction is free from interference (Song et al., 2012). Since they were first reported in 1990, aptamers have received significant interest from researchers, and the first aptasensor was reported in 1996 (Radi, 2011). Aptamers performed better at detecting Immunoglobulin E than monoclonal antibodies did under similar conditions (Maehashi et al., 2007). A few researchers have demonstrated that the reaction between an RNA aptamer and a protein involves an electrostatic interaction between the arginine region of the protein and G bases from the aptamer (Yamamoto et al., 1998). One study investigated the effectiveness of RNA aptamer binding with the Tat protein using NMR spectroscopy. The strong-affinity reaction was due to the formation of two binding complexes consisting of hydrogen bonds between arginine and the nucleotides from the Tat peptide (Matsugami et al., 2003). Despite the excellent performance of aptamers as recognition probes, antibodies are more widely used than aptamers due to a lack of literature and research development of aptamers for various diseases. The comparison of these two types of probes is shown in Fig. 5.

5. Strategies for HIV-1 Tat detection

Research on the detection of HIV-1 Tat has been conducted in various ways but remains limited (Table 2). Many studies on HIV-1 Tat have focused on the structure and function of Tat (Campbell and Loret, 2009; Pugliese et al., 2005; Jeang, 1996) and reactions with Tat (Comandini et al., 2013; McQueen et al., 2011; Cardarelli et al., 2008; Rudolph et al., 2003; Zhao et al., 2003). Here, we describe the different methods of HIV-1 Tat detection, including immunoassay, biosensor and gene expression.

5.1. Immunoassay

Immunoassay testing is basically a method that implements the antigen–antibody reaction; most immunoassays require a label that acts as the signal producer (Cox et al., 2012). The target antigen present in human serum or urine will react with the epitope from the antibody and produce a measurable signal that may be emitted in the form of light, radiation, fluorescence or colour change. This type of testing is widely used around the world due to the accurate and reliable results produced. However, in most cases an immunoassay requires a moderate sample volume, and it is not a regenerative test. In the case of the HIV-1 Tat protein, there are several studies that have demonstrated the effectiveness of immunoassays in detecting the viral proteins.

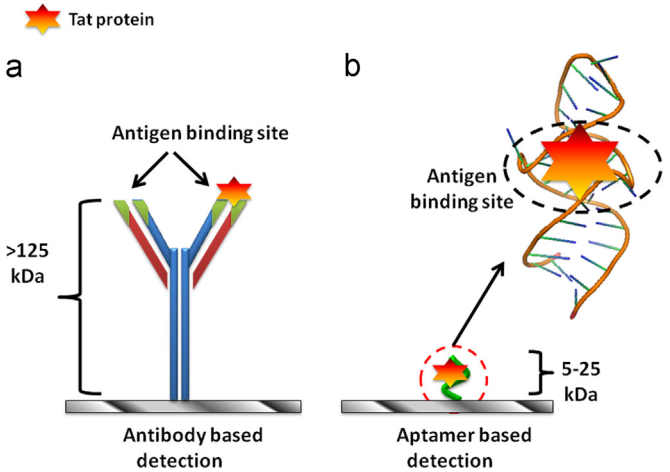

Fig. 5. Probe based detection (a) antibody based detection; (b) aptamer based detection. These probes display different affinities. Other differences between these probes are stability and suitable for chemical modification.

Table 2
HIV-1 Tat immunoassay-based detection.

Method	Probe	Lowest LOD	Advantages	Disadvantages	Reference
Immunoassay					
ELISA	IgG antibody	–	Good signal readout	Usage of an enzyme label	Re et al. (2001)
Western blot	K51 me1 methyl-lysine specific antibody	–	Differentiate between mono-, di- and trimethylated Tat peptides	Need to lyse the cell sample	Pagans et al. (2011)
Immunostaining	Rabbit polyclonal antiserum	–	No extraction	Human evaluation	Del Valle et al. (2000)
Fluorescence immunoassay	RNA aptamer	–	Good affinity and specificity	Label method	Yamamoto and Kumar (2000)
Biosensor					
Frequency-based device (QCM)	Monoclonal antibody anti-Tat / RNA aptamer	0.25 ppm	Low sensitivity	Specific, reliable and reproducible readout	Minunni et al. (2004)
Surface plasmon resonance (SPR)	RNA aptamer	0.25 ppm	Low sensitivity	Specific, reliable and reproducible readout	Tombelli et al. (2005)
SG-FET electrical characterization	Split RNA aptamer	1 nM	Reusable	Electrical detection in lab	Ruslinda et al. (2013)
Gene expression					
Fluorescent bioassay	Fluorescent irradiated cell	–	No extraction procedures, enzymatic assay	Need to culture cells	Daelemans et al. (2001)
Bioinformatics tools	Tat gene	–	Accurate result	Complicated sampling procedure	Mullick et al. (2010)

5.1.1. Enzyme-linked immunosorbence assay

Enzyme-linked immunosorbence assay (ELISA) is one of the most common tests used in the world to detect the presence and concentration of desired analyte solutions. This test typically uses antibodies conjugated with enzymes such as horseradish peroxidase (HRP). The enzyme will eventually give rise to colour changes if there are target analytes present in the sample solution and can be quantified either by the naked eye or using a spectrophotometer. There are four types of ELISA, including direct, indirect, sandwich and competitive ELISA (Fig. 6). In direct and indirect ELISA, the antigen is immobilized on top of microtiter plates. If the sample tested contains an antibody for the specific antigen, it will bind on top of the antigen and the conjugated enzyme will cause a colour change when it reacts with the substrate. The substrate is the solution that is used to elicit the signal readout.

A research group from Italy has demonstrated the interaction between anti-Tat antibodies and the HIV-1 Tat full length protein or peptide in human blood serum (Re et al., 2001). They measured the anti-Tat antibodies that reacted with full-length Tat protein or individual Tat peptides in patients with different levels of HIV viremia. Blood samples from HIV-positive patients and healthy controls were taken, and the presence of antibodies and full-length Tat was analysed by ELISA method. The antibody used also reacts to individual Tat peptides with good signal strength, indicating that the antibody is very efficient at detecting the Tat protein. In HIV-1-infected patients, immune response is stronger against the HIV-1 Tat protein and inversely correlated to the viral load in blood which means a spiking level of antibody against Tat peptides is corresponding to an undetectable plasma viral load. These findings make the way to generate vaccines containing anti-HIV-1 Tat.

5.1.2. Western blot

Western blotting is a technique of detecting a protein in a complex solution of proteins by gel electrophoresis (Hnasko and Hnasko, 2015). Usually the sample taken from the cells must be extracted by lysing. This method is divided to three steps, which are separation by size, transfer to solid support and protein marking using a conjugated antibody for visualization (Mahmood and Yang, 2012). The protein is separated by size through gel electrophoresis and the desired protein (band) is transferred to a membrane. The protein is incubated with bovine serum albumin (BSA) first to block non-specific interactions before being detected by a conjugated antibody for signal visualization.

Pagans et.al. have used the Western blot method to detect the presence of HIV-1 Tat protein. They investigate the modification of an antibody to detect the lysine 51 region in Tat protein. Lysine 51 includes mono-, di- and tri-methylated lysine and is a conserved residue located in RNA binding region of Tat. The antibody used in this study was extracted from a rabbit (the rabbit was injected with three different types of modified antibody) and purified, and

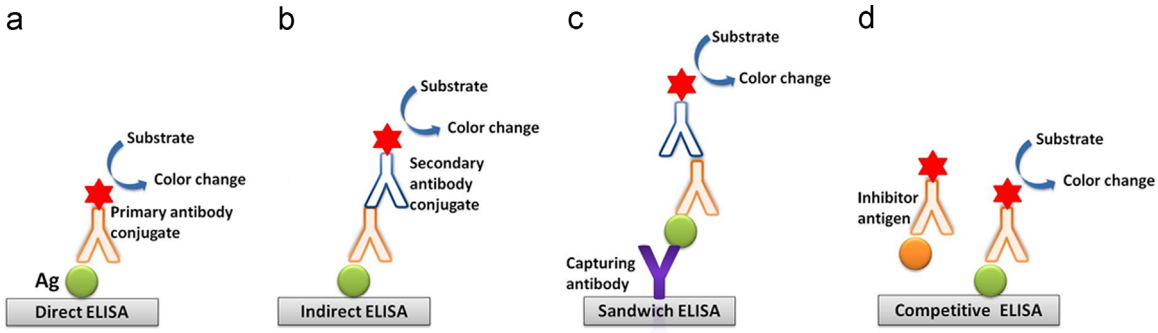

Fig. 6. Different types of ELISA immunoassay. (a) Direct ELISA, (b) Indirect ELISA, (c) Sandwich ELISA and (d) Competitive ELISA. All types of detection will present changes in colour after the addition of the substrate solution if antigen is present. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

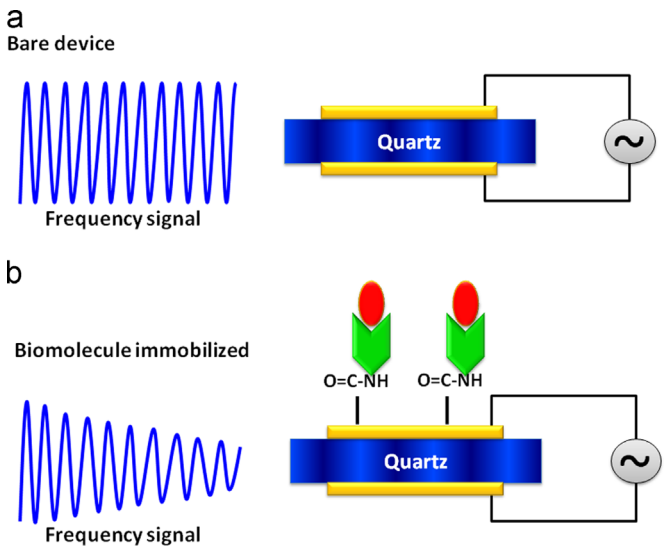

Fig. 7. Schematic structure of QCM measurement. Changes in the frequency signal can be observed on (a) a bare device and (b) after the immobilization of biomolecules (ex.: antibodies and antigens). The presence of the target analyte can be detected by signal changes.

a Western blot was used to determine the specificity of the purified antibodies. This modified antibody (K51 me1 methyl-lysine specific antibody) can differentiate between mono-, di- and trimethylated Tat peptides with similar sensitivity (Pagans et al., 2011). However, methylated Tat was not detected in cells, which may be because Tat is not present in its methylated form within cells, or it is present at levels below the limit of detection.

5.1.3. Immunostaining

Using immunostaining, Del Valle et al. have demonstrated effective Tat protein detection. Immunostaining is a label-based method used to detect the specific protein within tissue or cells using a chemical stain and can either be directly evaluated using a microscope or fluorescence (Maity et al., 2013). Other types of labels used can be non-fluorescent biomolecules such as using enzymes and radioactive elements that can later be visualised using autoradiography.

Del Valle et al. focused on the use of Progressive Multifocal Leukoencephalopathy (PML) to investigate HIV-infected glial cells. Cells containing Tat protein are treated with rabbit polyclonal antiserum generated from recombinant HIV-1 Tat protein. This technique results in changes in colour from blue to red during immunostaining (Del Valle et al., 2000). However, the immunostaining method has a drawback with regards to microscope analysis due to the need for two microscopists to evaluate the results together to achieve consensus.

5.1.4. Fluorescence immunoassay

Fluorescence is the emission of light from a substance that has absorbed light. In most cases, the emitted light has a longer wavelength and therefore lower energy than the absorbed radiation. Fluorescence immunoassays have been used for a long time for the detection of many compounds including viruses, bacteria, antibody–antigen complexes, proteins and drugs (Hicks, 1984). This method involves using fluorescent labelling for detection and visualization under fluorescent light. The fluorescent label will absorb light at certain wavelength and emit light at another wavelength and can therefore be quantified using a fluorometer (Hemmila, 1985).

Yamamoto and Kumar investigated the interaction of RNA aptamers with HIV-1 Tat and successfully implemented fluorescence labelling to validate the interaction (Yamamoto and Kumar, 2000; Yamamoto et al., 1998). The aptamer beacon (an aptamer that possesses a stem-loop structure) is modified covalently at each end with fluorescent and non-fluorescent quenchers. The folding structure of the beacon aptamer brings the fluorophore and quencher in close proximity, resulting in no fluorescent signal. When HIV-1 Tat is present together with its complimentary oligomer, the aptamer beacon forms a duplex structure with the oligomer and intercalates with the protein in the middle of the duplex, resulting in the splitting of the beacon aptamer at both ends, permitting the aptamer to exhibit fluorescence.

5.2. Biosensor

In order to develop point of care detection, efficient and low-cost sensor devices known as biosensors have been developed (Mohd Hanafiah et al., 2013; Peeling and Mabey, 2010). A biosensor consists of three major parts: the probe, the transducer and the signal processor. The probe is a biological entity (e.g., aptamer, antibody, or enzyme) that acts as a molecular recognition unit. Usually, intermediate material will be used on the device as the base site for probe immobilization. The material used primarily functions to improve the signal produced during the biological interaction between the probe and the target analyte. The transducer converts the signal from one form of energy to another form of energy that can be easily measured and quantified. The signal produced can be in various forms depending on the type of device, whether it is a frequency-based device that reflects the mass changes on the device surface, an electrical-based device that reflects changes in resistance, capacitance and ion transfer or a colour-based device that reflects fluorescence or UV–vis changes in the wavelength value. The signal processor receives the signal from the transducer and processes it to form the display signal such as the frequency, current or chromatogram. Additionally, a smartphone dongle used with the lateral flow system can be used to install software to detect HIV antibodies (Laksanasopin et al., 2015). Limited research has been performed to evaluate possible electronic biosensors for HIV-1 Tat detection. In this review, we focus on quartz crystal microbalance (QCM), surface plasmon resonance (SPR) and field effect transistor (FET) techniques because they are the most established and explored methods for disease diagnosis and for HIV-1 Tat detection. In addition to that, these methods cover basic fundamental of sensing principles.

5.2.1. Quartz crystal microbalance

Quartz crystal microbalance (QCM) is a device made out of quartz as the substrate and is widely used as a sensor (Vashist and Vashist, 2011). The sensing mechanism is based on the piezoelectric effect of quartz. By applying an electrical field across the quartz, a mechanical oscillation of a characteristic frequency is produced (Marx, 2003). When any material or molecule sits on top of the quartz, there are changes in the mass on the surface, and this can be quantified by the QCM in the form of changes in frequency (Fig. 7).

Minunni et.al. have studied the detection of HIV-1 Tat using QCM (Minunni et al., 2004). They detected the presence of HIV-1 Tat by frequency signal variation with biomolecule immobilization on top of a biosensor crystal surface, demonstrating the limit of protein detection at 0.25 ppm. The fabricated aptasensor has the ability to differentiate between different types of proteins and has a high selectivity towards HIV-1 Tat. The sensor can measure up to fifteen cycles of detection without losing its sensitivity, demonstrating the reliability and repeatability of the frequency-based sensor. A comparison between the probe aptamer and antibodies has also demonstrated that the two probes have the same sensitivity limit, 0.25 ppm, and almost the same performance of the

sensor. These results enable the aptamer to replace antibodies in future research because the aptamer is a more stable molecule with a more efficient handling procedure. However, the sensitivity of the device is still low and cannot reach the detection level necessary with human blood samples.

5.2.2. Surface plasmon resonance chip

The surface plasmon resonance (SPR) chip measures the difference in the reflection of incident light during analyte detection (Sang et al., 2013). A polarised light is directed onto an electrically conductive thin metal coating on the chip and reflected back towards detector. When the light strikes the thin metal coating, an evanescent wave is generated and is adsorbed by the electrons on the gold surface. These adsorbed electrons generate a plasmon that reduces the intensity of the reflected light.

In another study, Tombelli et al. used the same aptasensor approach and compared QCM and SPR, both of which use the concept of frequency-based detection (Tombelli et al., 2005). The dextran-modified SPR chip was immobilized with streptavidin and biotinylated aptamer. Three different chips were modified with the same method, demonstrating the high reproducibility of immobilization. The signal was measured using the Biacore X in a nuclease-free environment. Biacore works by measuring changes in the refracted light after the binding or dissociation interaction of the Tat protein with the biotinylated aptamer. This device shows selectivity towards Tat, with only 25% of the signal towards the HIV-1 Rev protein.

5.2.3. Field effect transistor

Another potential device that has been used for the detection of biomolecular interactions is the field effect transistor (FET). It works by detecting the charge regulation on its active area (channel) (Im et al., 2007). The FET consists of a three-electrode system (source, drain and gate) and a channel. Deposition of material and immobilization of biomolecules taking place on the channel will result in changes in the electron density on the channel surface (Poghossian et al., 2005). The changes are reflected on the current output at the source electrode.

Research conducted by Ruslinda et al. (2013) has implemented the split RNA aptamer as the detection probe for HIV-1 Tat. They have fabricated FET device that is surface modified with diamond to act as the base site of the biomolecular interaction (Fig. 8). This device measures the electron charge regulation during the interaction that comes from the charges that surround the biomolecules and interprets it as an electrical signal. With a limit of detection of up to 1 nM and repeatable results, the device is a start in developing a field effect biosensor for the early detection of HIV infection using detection of the HIV-1 Tat protein.

5.3. Gene expression for HIV-1 Tat detection

In addition to using antibodies and aptamers as the capturing probes for the HIV-1 Tat protein, detection can also proceed by measuring the gene expression in the infected cell. The first method, using a green fluorescence protein (GFP) reporter gen, was developed by Daelemans et al. (2001) for the detection of HIV-1 Tat transactivation inhibitors. Two types of cell, one containing Tat protein and one without, were cultured and transfected with GFP. Fluorescence microscopy was used to monitor the signal of the cells, which are divided into 96-well plates. A low fluorescence signal was measured in the cells without Tat, while cells with Tat exhibited a bright fluorescent signal. However, the main goal of this research was to investigate the usage of GFP as a reported, so there was no quantification of the LOD. Mullick et al. also used the Tat gene for molecular characterization of the HIV-1 Tat protein using bioinformatics tools (Mullick et al., 2010). The samples, taken from the National Center for Biotechnology Information, originated from injection drug users in Manipur, India. The samples were amplified by polymerase chain reaction and the Tat gene present in the samples was visualised by gel electrophoresis.

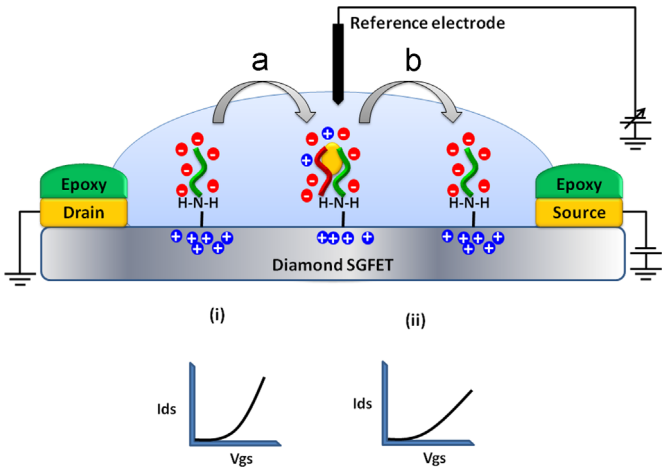

Fig. 8. Solution gate field effect transistor (SGFET) detection mechanism. (a) Addition of Tat protein and (b) regeneration using urea solution. The difference in the Ids Vgs curve after biomolecule immobilization is due to the difference in charge on the channel surface. The changes of the signal are recorded as (i) the curve recorded for the aptamer immobilized on SGFET and (ii) the shift due to the positive charge from the Tat protein and shift back to the initial condition as in (i) due to the removal of the Tat protein.

6. Immunoassay vs biosensor: perspective

We have reviewed several different methods of HIV-1 Tat detection, i.e., immunoassay, biosensors and gene expression, of which the latter involves tedious laboratory procedures. Immunoassay detection relies on an antibody–antigen reaction and has been widely used around the world. This test provides accurate results but requires time, cost and a large sample volume. However, because of the widely used antibody–antigen reaction, this type of detection is easily available from the supplier and is accessible by HIV patients. Biosensors are typically highly sensitive and specific, but still require proper validation and confirmation of the test results. The use of biosensors can be classified as a new technique in medical detection. The signal produced by a biosensor is optimal because it can display variations during the biological interactions among molecules. In a biosensor device, each change in the active area provides quantifiable readouts that can be calculated to obtain the amount of the biomolecules present. However, precautionary steps such as blocking and washing treatments are a must to avoid interference in the signal recorded. Notably, the biosensor device can often be reused, which can provide cost savings and make the technique more environmentally friendly. Researchers are looking forward to biosensor usage in medical detection systems to reduce the manpower, cost and sample quantity required, but there are still many factors that need to be studied to produce a reliable and highly sensitive sensor ready for the market.

7. Conclusion and future perspective

HIV is a rapidly evolving virus, and currently there is no cure for those who have been infected. Nevertheless, HIV can be controlled by medication to slow the progress of viral replication in the human body. There are currently many tests available for HIV screening that aim for POCT, including immunoassays and antigen tests. However, the main constraint of POCT is the sensitivity. Currently, the gold standard for HIV screening uses the ELISA test kit, which can be used at a hospital or clinic and should be

conducted by a medical specialist. The goal is that in the future, HIV tests can be performed with low cost, will be easy to use and will provide simple and reliable results. Although some may consider HIV a non-critical disease due to its slow progression with proper medicinal control, the development of a reliable and accurate POCT for home testing would help to avoid the risk of HIV transmission in high-risk areas.

Antigen detection for HIV screening has been extensively studied. We have summarised the development of HIV detection methods that target the Tat protein. Since Tat is present in the virus and is produced at high copy numbers in the early infection stage, between 2 and 4 weeks, it is a very suitable candidate for early HIV detection to help eliminate the lengthy waiting period for HIV testing. Detection of Tat can occur via immunoassay, biosensors and measuring gene expression. Each method has its own advantages as well as disadvantages that should be improved in order to develop more reliable detection methods. The biosensor method of detection is the most promising for development as a future screening method for HIV due to its ability to produce fast and accurate data, but there are many improvements that should be made in the development of a Tat protein POCT biosensor. Developing a fast and easy detection method for early detection is critical in order to minimise the risk of infection amongst the human population. With early detection systems, a practical treatment method can be developed to halt viral replication and provide a normal life to HIV patients.

Acknowledgements

This work was funded by Fundamental Research Grant Scheme (9003-00375), Research Acculturation Collaborative Effort (9017-00022) and supported by the Institute of Nano Electronic Engineering (INEE), Universiti Malaysia Perlis. We would like to acknowledge the Faculty of Medicine, Universiti Malaya for financial support using collaboration research grant (CR012-2014).

References

Basavaraj, K.H., Navya, M.A., Rashmi, R., 2010. Indian J. Sex. Transm. Dis. 31, 75–80.
Bayer, R., Kraft, M., Ejchart, A., Westendorp, M., Frank, R., 1995. J. Mol. Biol. 247, 529–535.
Bukrinskaya, A., 2007. Virus Res. 124, 1–11.
Campbell, G.R., Loret, E.P., 2009. Retrovirology 6, 50.
Cardarelli, F., Serresi, M., Bizzarri, R., Beltram, F., 2008. Traffic 9, 528–539.
Cardarelli, F., Serresi, M., Bizzarri, R., Giacca, M., Beltram, F., 2007. Mol. Ther. 15, 1313–1322.
Comandini, A., Naro, C., Adamo, R., Akbar, A.N., Lanna, A., Bonmassar, E., Franzese, O., 2013. Mol. Immunol. 54, 181–192.
Cooper, A., García, M., Petrovas, C., Yamamoto, T., Koup, R. a, Nabel, G.J., 2013. Nature 498, 376–379.
Cox, K.L., Devanarayan, V., Kriauciunas, A., Manetta, J., Montrose, C., Sittampalam, S., 2012. Immunoassay Methods, in: Sittampalam, G.S., Coussens, N.P., Nelson, H, et al., (Eds.), Assay guidance manual. Bethesda (MD): Eli Lilly & company and the National Center for Advancing Translational Sciences, pp. 26-28.
Daelemans, D., De Clercq, E., Vandamme, A.-M., 2001. J. Virol. Methods 96, 183–188.
Dangeti, S.R., 2014. HIV AIDS Rev. 13, 1–5.
Das, S.R., Jameel, S., 2005. Indian J. Med. Res. 121, 315–332.
Debaisieux, S., Rayne, F., Yezid, H., Beaumelle, B., 2012. Traffic 13, 355–363.
Del Valle, L., Croul, S., Morgello, S., Amini, S., Rappaport, J., Khalili, K., 2000. J. Neurovirol. 6, 221–228.
Fearon, M., 2005. Can. J. Infect. Dis. Med. Microbiol. 16, 26–30.
Frankel, A.D., Young, J.A., 1998. Annu. Rev. Biochem. 67, 1–25.
Garber, M.E., Wei, P., KewalRamani, V.N., Mayall, T.P., Herrmann, C.H., Rice, A.P., Littman, D.R., Jones, K.A., 1998. Genes. Dev. 12, 3512–3527.
Greenwald, J.L., Burstein, G.R., Pincus, J., Branson, B., 2006. Curr. Infect. Dis. Rep. 8, 125–131.
He, L., Nan, T., Cui, Y., Guo, S., Zhang, W., Zhang, R., Tan, G., Wang, B., Cui, L., 2014. Malar. J. 13, 127.
Hemmila, I., 1985. Assays. Clin. Chem. 31, 359–370.
Hicks, J.M., 1984. Hum. Pathol. 15, 112–116.
Hnasko, T., Hnasko, R., 2015. The Western Blot, ELISA SE-9, Methods in Molecular Biology. Springer, New York.
Holguín, Á., Gutiérrez, M., Portocarrero, N., Rivas, P., Baquero, M., 2009. J. Clin. Virol. 45, 150–152.
Im, H., Huang, X.-J., Gu, B., Choi, Y.-K., 2007. Nat. Nanotechnol. 2, 430–434.
Jeang, K. 1996. HIV-1 Tat : structure and function, in: Proceedings of the Human Retroviruses and AIDS 1996 Compendium. pp. 3–18.
Karn, J. 1992. Tat, a novel regulator of HIV transcription and latency. pp. 2–18.
King, J.E., Eugenin, E. a, Buckner, C.M., Berman, J.W., 2006. Microbes Infect. 8, 1347–1357.
Klostermeier, D., Bayer, P., Kraft, M., Frank, R.W., Rijsch, P., 1997. Biophys. Chem. 63, 87–96.
Laksanasopin, T., Guo, T.W., Nayak, S., Sridhara, A. a, Xie, S., Olowookere, O.O., Cadinu, P., Meng, F., Chee, N.H., Kim, J., Chin, C.D., Munyazesa, E., Mugwaneza, P., Rai, A.J., Mugisha, V., Castro, A.R., Steinmiller, D., Linder, V., Justman, J.E., Nsanzimana, S., Sia, S.K., 2015. Sci. Transl. Med. 7, 273re1.
Lobritz, M.A., Lassen, K.G., Arts, E.J., 2011. Curr. Opin. HIV AIDS 6, 214–220.
Maehashi, K., Katsura, T., Kerman, K., Takamura, Y., Matsumoto, K., Tamiya, E., 2007. Anal. Chem. 79, 782–787.
Mahmood, T., Yang, P.C., 2012. N. Am. J. Med. Sci. 4, 429–434. http://dx.doi.org/10.4103/1947-2714.100998.
Maity, B., Sheff, D., Fisher, R.A., 2013. Methods Cell. Biol. 113, 85–105.
Marx, K.A., 2003. Am. Chem. Soc. 4, 1099–1120.
Matsugami, A., Kobayashi, S., Ouhashi, K., Uesugi, S., Yamamoto, R., Taira, K., Nishikawa, S., Kumar, P.K.R., Katahira, M., 2003. Structure 11, 533–545.
McQueen, P., Donald, L.J., Vo, T.N., Nguyen, D.H., Griffiths, H., Shojania, S., Standing, K.G., O'Neil, J.D., 2011. Proteins 79, 2233–2246.
Minunni, M., Tombelli, S., Gullotto, A., Luzi, E., Mascini, M., 2004. Biosens. Bioelectron. 20, 1149–1156.
Mohd Hanafiah, K., Garcia, M., Anderson, D., 2013. Biomark. Med. 7, 333–347.
Mullick, R., Sengupta, S., Sarkar, K., Chakrabarti, S., 2010. Virus Res. 147, 195–201.
Njiru, Z.K., 2011. Diagn. Microbiol. Infect. Dis. 69, 205–209.
Novell, M., Parilla, M., Crespo, G.A., Rius, F.X., Andrade, F.J., 2012. Anal. Chem.
Oelrichs, R., 2004. Sex. Health 1, 1–11.
Pagans, S., Sakane, N., Schnölzer, M., Ott, M., 2011. Methods 53, 91–96.
Pavie, J., Rachline, A., Loze, B., Niedbalski, L., Delaugerre, C., Laforgerie, E., Plantier, J. C., Rozenbaum, W., Chevret, S., Molina, J.M., Simon, F., 2010. PLoS One 5.
Pedersen, C., Nielsen, C.M., Vestergaard, B.F., Gerstoft, J., Krogsgaard, K., Nielsen, J.O., 1987. Br. Med. J. 295, 567–569.
Peeling, R.W., Mabey, D., 2010. Clin. Microbiol. Infect. 16, 1062–1069.
Poghossian, a, Cherstvy, a, Ingebrandt, S., Offenhäusser, a, Schöning, M.J., 2005. Sens. Actuators B Chem. 111–112, 470–480.
Pugliese, A., Vidotto, V., Beltramo, T., Petrini, S., Torre, D., 2005. Cell. Biochem. Funct. 23, 223–227.
Radi, A., 2011. Int. J. Electrochem. 2011, 1–17.
Rana, T.M., Jeang, K.T., 1999. Biochem. Biophys. 365, 175–185.
Re, M.C., Vignoli, M., Furlini, G., Gibellini, D., Colangeli, V., 2001. J. Clin. Virol. 21, 81–89.
Reynolds, S.J., Muwonga, J., 2004. Expert Rev. Mol. Diagn. 4, 587–591.
Romani, B., Engelbrecht, S., Glashoff, R.H., 2010. J. Gen. Virol. 91, 1–12.
Rubartelli, A., Poggi, A., Sitia, R., Zocchi, M.R., 1998. Immunol. Today 19, 543–545.
Rudolph, C., Plank, C., Lausier, J., Schillinger, U., Müller, R.H., Rosenecker, J., 2003. J. Biol. Chem. 278, 11411–11418.
Ruslinda, A.R., Tanabe, K., Ibori, S., Wang, X., Kawarada, H., 2013. Biosens. Bioelectron. 40, 277–282.
Saerens, D., Huang, L., Bonroy, K., Muyldermans, S., 2008. Sensors 8, 4669–4686.
Sajid, M., Kawde, A.-N., Daud, M., 2014. J. Saudi Chem. Soc., Article in press
Sang, S., Zhang, W., Zhao, Y., 2013. Rev. Des. Art. Biosens., 89–110.
Schpbach, J., Bni, J., Flepp, M., Tomasik, Z., Joller, H., Opravil, M., 2001. J. Med. Virol. 65, 225–232.
Shaw, G.M., Hahn, B.H., Arya, S.K., Groopman, J.E., Gallo, R.C., Wong-Staal, F., 1984. Science 226, 1165–1171.
Siegfried, N., Uthman, O.A., Rutherford, G.W., 2010. Cochrane Database Syst. Rev. 3, CD008272.
Song, K., Lee, S., Ban, C., 2012. Sensors 12, 612–631.
Tehe, A., Maurice, C., Hanson, D.L., Borget, M.Y., Abiola, N., Maran, M., Yavo, D., Tomasik, Z., Böni, J., Schüpbach, J., Nkengasong, J.N., 2006. J. Clin. Virol. 37, 199–205.
Tombelli, S., Minunni, M., Luzi, E., Mascini, M.T., 2005. Bioelectrochemistry 67, 135–141.
Van Rensburg, E.J., 2000. S. Afr. J. Sci. 96, 267–269.
Vashist, S.K., Vashist, P., 2011. J. Sensors 2011, 1–13.
Wang, J., Zhang, W., Nardi, M. a, Li, Z., 2011. J. Thromb. Haemost. 9, 562–573.
Weeks, K.M., Crothers, D.M., 1993. Drug. Discov. Des. 1, 225–234.
Yamamoto, R., Kumar, P.K.R., 2000. Genes Cells 5, 389–396.
Yamamoto, R., Murakami, K., Taira, K., Penmetcha, K.R., 1998. Gene Ther. Mol. Biol. 1, 451–466.
Zachary, D., Mwenge, L., Muyoyeta, M., Shanaube, K., Schaap, A., Bond, V., Kosloff, B., de Haas, P., Ayles, H., 2012. BMC Infect. Dis. 12, 183.
Zhao, H., Dai, D., Li, J., Chen, Y., Jiang, L., 2003. Biochem. Biophys. Res. Commun. 312, 351–354.
Ziegler, A., Seelig, J., 2004. Biophys. J. 86, 254–263.