RAPID DETECTION KIT FOR ANTIBODY-ANTIGEN RECOGNIZATION FOR HUMAN IMMUNODEFICIENCY VIRUS

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Abstract: Human immunodeficiency virus type 1 (HIV-1) capsid p24 protein is one of the most important components in viral pathogenesis. It is highly secreted in the blood serum during early stage of HIV-1 that act as a biomarker for early diagnosis. Enzyme immunoassays (EIAs) is a conventional test to detect human immunodeficiency virus (HIV) by incorporating p24 antigen detection with current HIV antibody detection. However, this method requires many steps and time-consuming. Therefore, it is necessary to develop a new diagnostic approach that is easier and faster than this conventional test. In this study, a rapid detection kit using colloidal gold was developed to detect the presence of p24 antigen in blood serum. The detection kit strip that has been developed composed of a sample pad, an absorbent pad, conjugate pad, and a nitrocellulose membrane containing control and test line. The nanocolloidal gold, with an average particle diameter of 30 nanometres (nm) was labelled with anti-HIV-1 p24 antibody as the detection reagent. An antibody colloidal gold probe was applied on the conjugate pad. Anti-HIV-1 p24 antibody was immobilized to test line as the capture reagent and HIV-1 p24 antigen on the control line at the nitrocellulose membrane in order to prepare this detection kit. The commercialized p24 antigen was added onto the sample pad and reacts with anti-HIV-1 p24 antibody conjugated with colloidal gold particle. The mixture then migrates to the nitrocellulose membrane and react with anti-HIV-1 p24 in the detection zone which resulting in red marks within this detection zone with an intensity proportional to the concentration of the p24 antigen in the sample. The result can be observed within less than 10 minutes.

Keywords: Human immunodeficiency virus type 1, p24 protein, rapid detection kit, colloidal gold probe.

1. Introduction

HIV infection has changed from an acute disease to chronic manageable condition for the last two decades (Bertoldi et al., 2017). There are more than 700 infants diagnosed with HIV every day (Penazzato et al., 2014). The infected infants need virological testing that is very complex and expensive (Penazzato et al., 2014). About 50% of infants that affected with the HIV died at age 2 because of the absence of early treatment and diagnosis or else the treatment itself is expensive for the patient (Penazzato et al., 2014).

According to The Joint United Nations Program on HIV/AIDS (UNAIDS), more than 90% of people were infected with HIV in 12 countries which are China, Cambodia, Malaysia, India, Papua New Guinea, Indonesia, Pakistan, Myanmar, Nepal, Philippines, Thailand and Vietnam with additional of 90% of new HIV infections in Asia. Some countries showed decrement of new HIV infection such as Philippines, Pakistan, Malaysia, and Indonesia in which Malaysia was ranked 8th in Asia (UNAIDS, 2013).

1.1 HIV Test Generation

With improvement of the immunological technique, anti-HIV antibodies detection has developed and divided into several generations (Paschale et al., 2018). First generation of HIV detection used HIV lysate which act as antigen source to capture the antibody that is present in the sample. For the second generation test, the time taken between infection and HIV biomarker detection (window period) was reduced to 42 days as compared to the first generation which was 56 days. The second and third test generation have a similarity in which both are able to detect anti-HIV-1 antibodies and anti-HIV-2 antibodies. Besides, the third test generation also can detect immunoglobulin M (IgM) antibodies which is the first antibody that will be produced during acute infection. Thus, the window period for this generation will be reduced to 28 days after infection. Last but not least is the fourth test generation, which combines the detection of p24 viral antigen and HIV 1 and 2 antibodies (Chappel et al., 2009; Ly, Laperche & Couroucé 2001).

1.2 Life Cycle of HIV

Replication is the process of reproducing the virus. It requires Cluster of Differentiation 4 (CD4) cells. This process is known as HIV life cycle (Paschale et al., 2018). In HIV life cycle, CD4 cells are destroyed by HIV. Basically, HIV life cycle is consisted of seven stages which are binding, fusion, reverse transcription, integration, replication, assembly, and budding as shown in Figure 1.1 (AIDSinfo, 2018).

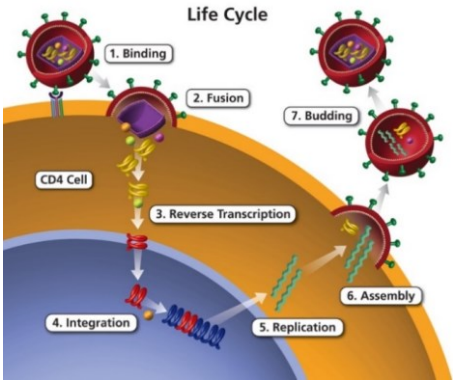

Figure 1.1 HIV replication cycle (AIDSinfo, 2018).

The first stage is binding of HIV to CD4 cell that will trigger a conformational change in the viral surface envelope protein which leads to binding to the chemokine receptors, either on the Cysteine-Cysteine Chemokine Receptor 5 (CCR5) or on the C-X-C Chemokine Receptor 4 (CXCR4). This triggers second conformational change that exposes the membrane fusion domain and leads to cell entry. In this stage, the viral capsids uncoat and the genetic materials and enzymes are released inside CD4 cell (Maartens et al., 2014).

The third stage is reverse transcription. This is when the genetic material is converted from RNA to deoxyribonucleic acid (DNA). This conversion allows the virus to enter CD4 cell nucleus and combines with cell DNA (AIDSinfo, 2018). Next stage is the integration of viral DNA with DNA of the host cell with the help of integrase. Then this integrated DNA material will be transcribed and translated into chains of amino acids.

In the next stage, more protein chains will be replicated and assembled. The assembly process will result in immature virus and move on to the membrane surface to bud out and becomes matured. This virion will continue to infect the other CD4 cells by repeating the same cycle.

1.3 P24 Protein

P24 protein is one of the primary structural components that plays essential role during early and late stages of infection. This antigen can be found in the infected cell and also the virus particle that is released by the infected cell (Wehrly & Chesebro, 1997). It usually presents one or two weeks earlier than HIV antibody. Thus, it can reduce the window period of detection (Workman et al., 2009). The common method used is enzyme- linked immunosarbent assay (ELISA) as antigen capture and demonstrate virus replication (Wehrly & Chesebro, 1997).

1.4 Immunogold Labelling

Immunogold labelling, also known as immunogold staining, is a technique used in electron microscopy. It follows the same pattern of the indirect immunofluorescence which employs two antibodies. The first antibody will specifically bind to the target molecule, whereas the second antibody which carry the marker and bind to the primary antibodies (Hermann et al., 1996). The most often particle attached to secondary antibodies is the colloidal gold particles. Gold can increase electron scatter and give high contrast to dark spot with its high electron density. Immunogold labelling can be used to visualize one or more target simultaneously by using two different sizes of gold particles.

1.5 HIV Test Kits Comparison

There are four types of HIV test kit that have been approved by the United State (US) Food and Drug administration (FDA) which are OraQuick Advance Rapid HIV-1/2 Antibody test, Reveal G-2 rapid HIV-1 Antibody test, Uni- Gold Recombigen HIV test and Multispot HIV-1/HIV-2 Rapid test (Greenwald et al., 2006).

Based on Table 1.1, the result from all HIV detection kit can be obtained within 10-40 minutes except for Reveal G-2 rapid HIV-1 Antibody test which take shortest time to process the result however longer time is required for preparing the blood serum. Thus, it increases the complexity to the user. Sgp41 and Sgp120 is commonly used as detection of HIV in all kits which differ from this project that used p24 antigen for the detection of HIV-1 infection. P24 is chosen because it is more stable and highly expressed during virus replication (Wehrly & Chesebro, 1997).

Table 1.1 Comparison between four HIV rapid tests (Wehrly & Chesebro, 1997).

Rapid test	Test type	Specimen type	Specimen volume required	HIV-1 antigen peptide	Duration	Ref
OraQuick Advance Rapid HIV-1/2 Antibody test	Lateral flow	Oral fluid	5 µL	Sgp41	20- 40 minutes	(Greenwald et al., 2006
Reveal G-2 rapid HIV-1 Antibody test	Flow through	Serum plasma	50 µL	Sgp41 and Sgp120	Result in 3 minute. Preparation of blood serum required several step. Only available in laboratory.	(Use, n.d. ; Greenwald et al., 2006)
Uni- Gold Recombigen HIV test	Lateral flow	Whole blood, serum and plasma	50 µL	Rgp41 and Rgp120	10-20 minute	(Greenwald et al., 2006)
Multispot HIV-1/HIV-2 Rapid test	Flow through	Fresh frozen serum and plasma	50 µL	Sgp41 and Rgp 41	15 minutes	(Greenwald et al., 2006; Almazini, 2011)

2. Optimization of Antibody-Gold Conjugation

2.1 Optimization of pH value for conjugation

The absorbance measurement results of antibody- gold conjugated solution with different pH shown in Figure 2.1 The optimal pH value for conjugation is when the sample have the maximum absorbance. 0.1 mol/L K_2CO_3 was added in order to change the pH value of the colloidal gold. From Figure 2.1 we can conclude that the colloidal gold solution in tube 2 had the maximum absorbance with 10 µL amount of 0.1 mol/L K_2CO_3.

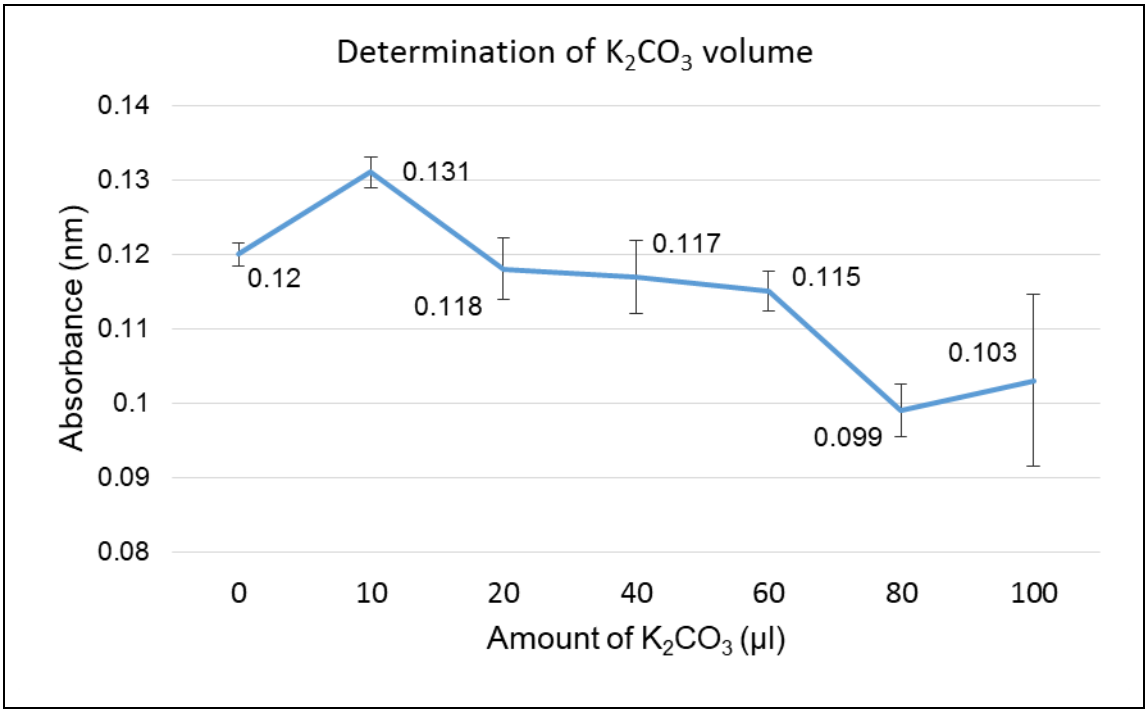

Figure 2.1 Maximum absorbance with different volume of K_2CO_3.

2.2 Optimization for amount of Antibody for the Conjugation

A minimum amount of anti-HIV-1 p24 antibody is required in order to stabilize the colloidal gold particles. The absorbance measurement results of antibody- gold conjugated solution with different concentration of antibodies were listed in Table 2.1 below. Based on the data in Table 2.1, a line graph as shown in Figure 2.2 was drawn by using the absorbance value for the vertical coordinate and different concentration of anti-HIV-1 p24 used as the horizontal coordinate. The result suggested that antibody- gold conjugated was synthesized successfully when the absorbance measurement became stable. From the absorbance result obtained in Figure 2.2, the amount of antibody corresponding to the point that are mostly close to the x-axis was selected as the minimum antibody value in order to stabilize the colloidal gold. Therefore 10 µg/ml of antibody was confirmed to be the best concentration of antibody for the conjugation process.

Table 2.1 Average absorbance for optimization of antibody concentration for conjugation.

		Absorbance				
Tube	Final concentration of conjugated antibody	Experiment 2(A)	Experiment 2(B)	Experiment 2(C)	Average	Standard Deviation
1	8	0.134	0.135	0.134	0.134	0.0006
2	9	0.142	0.143	0.139	0.141	0.0021
3	10	0.137	0.138	0.137	0.137	0.0006
4	12	0.139	0.138	0.139	0.139	0.0006
5	14	0.140	0.139	0.140	0.140	0.0006

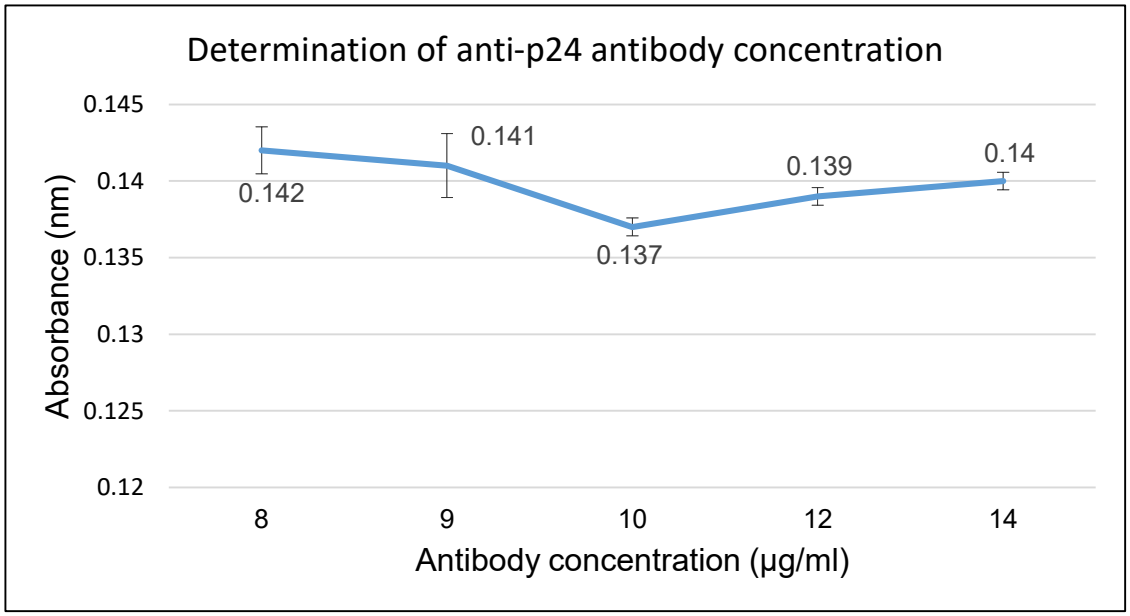

Figure 2.2 Determining the minimum proactive amount of antibody.

2.3 Testing of p24 Antigen on the Strip

The test strip was arranged as shown in Figure 2.3. P24 antigen was used to test the detection kit strip. The result obtained was recorded in Figure 2.4. Both test and control lines were indicating red for positive result. The use of higher concentration of p24 antigen has darken the color of the test line. The negative result was consisting of only control line turning red. There will be no reaction on the test line due to the absence of the p24 antigen in the sample and only control line appears red. The test result was obtained in less than 5 minutes. Therefore the HIV-1 detection kit strip test has shown to detect the p24 antigen effectively and rapidly.

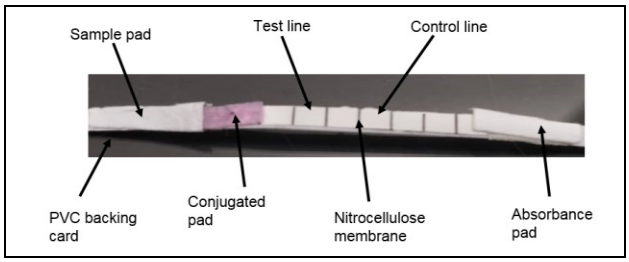

Figure 2.3 Detection Kit Strip.

Figure 2.4 Test strip results for positive p24 antigen (a) and without p24 antigen (b).

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