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HIV-2 Infection in Malaysia: Current situation and the use of in-house real-time reverse transcription PCR for HIV-2

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Abstract. HIV-2 surveillance has been carried out in Malaysia for more than 25 years ago. Tests to discriminate HIV-1 and HIV-2 are available but the options of test are limited and the need to develop a new in-house HIV-2 real-time reverse transcription PCR (RT-PCR) is crucial. A study was done on 29 samples from hospitals in Malaysia which were found to be positive screening for HIV-2 antibodies by the commercial Western Blot assay. These samples were further tested by a Western Blot assay that detects specific antibodies to HIV-2. Detection of HIV-2 genome was then performed by using a commercial kit. Fifteen samples were evaluated by using in-house real-time RT-PCR for HIV-2. Ninety-three percent (27/29) of samples have positive results for HIV-2 on HIV-2 Western Blot with only 2 samples showing indeterminate results. All samples showed negative results for HIV-2 genomes by using a PCR commercial kit and the 15 samples that were subjected to our in-house real-time RT-HIV-2 PCR were also tested negative for HIV-2 RNA. Results of HIV-2 Western Blot did not reflect the actual positivity as both HIV-1 and HIV-2 antibodies may cross-react with either viral proteins. None of the samples was confirmed positive for HIV-2 by the commercial and in-house real-time RT-PCR. In-house real-time RT-HIV-2 PCR assay can be further used to confirm the presence of HIV-2 genome. Up to the year 2015, Malaysia is still free from HIV-2 infection.

INTRODUCTION

The mainstay of HIV diagnosis in adults in many parts of the world is through the detection of HIV antibodies by using enzyme-linked immunosorbent assay (ELISA) test and the Western blot test to confirm the presence of antibodies. However, the discrimination between HIV-1 and HIV-2 infection cannot be done by detecting the HIV antibodies alone as there is always a cross reaction between HIV-1 and HIV-2 antibodies on Western Blot assays and this might give indeterminate results. The confirmatory test to differentiate these infections is to perform HIV-1 or HIV-2 PCR which specifically targets the nucleic acid of HIV-1 or HIV-2.

HIV-2 infection has been detected in many parts of the world. It is endemic in West Africa and is sporadically detected outside the African countries such as Portugal, Spain, France, Brazil and the United Kingdom (Maueia *et al.*, 2011). Despite its existence since 1966 (Kawamura *et al.*, 1989) which was based on the serologic data, HIV-2 infection remains rare. In Asia, most reported cases came from India (Kannangai *et al.*, 2010) and its prevalence varies according to regions. While up till 2004, Korea has reported 10 cases of HIV-2 infections with 9 of the infected individual having lived in the southern part of Korea (Nam *et al.*, 2006).

The National AIDS Reference Laboratory (NARL) in the Virology Unit of the Institute for Medical Research, established since 1986 assists the Ministry of Health in combating problems related to HIV infections. Establishment of the NARL has also resulted in the build-up of local expertise in the diagnosis of AIDS and it also complements

the surveillance operations. It has become the main reference centre for HIV in Malaysia.

Since 2005, a network of regional laboratories for HIV confirmatory testing were established. These laboratories had the capability for confirming results at regional level, without the need to refer routine blood samples to NARL. Five of these laboratories are in Peninsular Malaysia and the two others are in East Malaysia. These laboratories also function as the HIV viral load laboratory for their region. Currently, NARL performs test for HIV 1 and HIV-2 discordance results, confirms HIV infection for infants and conducts the National External Quality Assurance Scheme (NEQAS) for HIV antibodies screening centres in Malaysia.

HIV-2 infection surveillance was started by NARL in November 1992. Detection of HIV-2 is crucial as its medical management is different as compared to HIV-1 infection. Many antiretroviral drugs used to treat HIV-1 infection are not effective against HIV-2 infection (Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. 2015, Reeves & Doms 2002 & Peterson *et al*., 2011). As with the new current technologies in HIV screening, healthcare providers and laboratories should consider HIV-2 testing if tests for HIV-1 are inconsistent or inconclusive. Often HIV-2 has been misdiagnosed as HIV-1 infection due to cross reactivity between HIV-1 and HIV-2 antibodies. Hence, there is a possibility that HIV-2 cases may be under reported (Maueia *et al*., 2011) (Ruelle, 2012). The current study aimed to determine the presence of HIV-2 infection in Malaysia by using commercial and in-house real-time reverse transcriptase PCR (RT-PCR) methods.

MATERIALS AND METHODOLOGY

Prior to August 2010, the NARL had used a third generation enzyme immunoassay (EIA) (AxSym, Abbott Laboratories, Abbott Park, Illinois) for the detection of the HIV antibodies. As to keep abreast with the advances in diagnosing HIV infection, the NARL started to use the fourth generation EIA in August 2010. Currently, all samples are screened for HIV antigen and antibodies by using Cobas e411(Roche Diagnostics, GmbH, Germany). All HIV 1/2 antibodies positive samples will be subjected to further confirmation by using particle agglutination (Serodia, Fujirebio, Inc., Tokyo, Japan) and a supplementary test, HIV Blot 2.2 Western Blot assay (MP Diagnostics, France). While for HIV antigen positive samples, repeat testing on duplicate samples will be carried out. With repeated positive results for HIV antigen, the HIV antigen confirmatory test will be done. Our current testing algorithm for HIV-1 and HIV-2 is shown in Figure 1. All samples with a positive screening of HIV-2 band on the supplementary testing would be further tested for HIV-2 infection by using particle agglutination for HIV-2 and MPD HIV-2 Blot 1.2 Western Blot assay (MP Diagnostics, France). HIV-2 PCR for genome detection will be performed on samples with positive HIV-2 antibodies.

A total of 29 archived samples were obtained from the Virology Unit, Institute for Medical Research, Kuala Lumpur over a period of 9 years (2007-2015). All these sera or plasma samples were previously received either from the government or private hospitals in Malaysia and kept at -80°C. None of the samples were from Sabah or Sarawak. These samples were found, on screening to be positive for HIV-2 antibodies as per WHO criteria (MP Diagnostics HIV 1/2 Blot 2.2).

HIV RNA was extracted from 140 µl of serum by spin-column purification method by using QIAamp Viral RNA Mini Kit (Qiagen, Germany), according to manufacturer's instructions. These samples were tested for Human Immunodeficiency Virus type 2 (HIV-2) Genomes by real-time PCR using genesig advanced kit (PrimerDesign™ Ltd, USA), according to the manufacturer's instructions. The amount of extracted RNA required for the test was 5 µl. All 29 samples were subjected to one step RT-PCR protocol for HIV-2 genome detection (Genesig Standard Kit Quantification of Human Immunodeficiency Virus type 2 genomes genesig Standard kit handbook 2016).

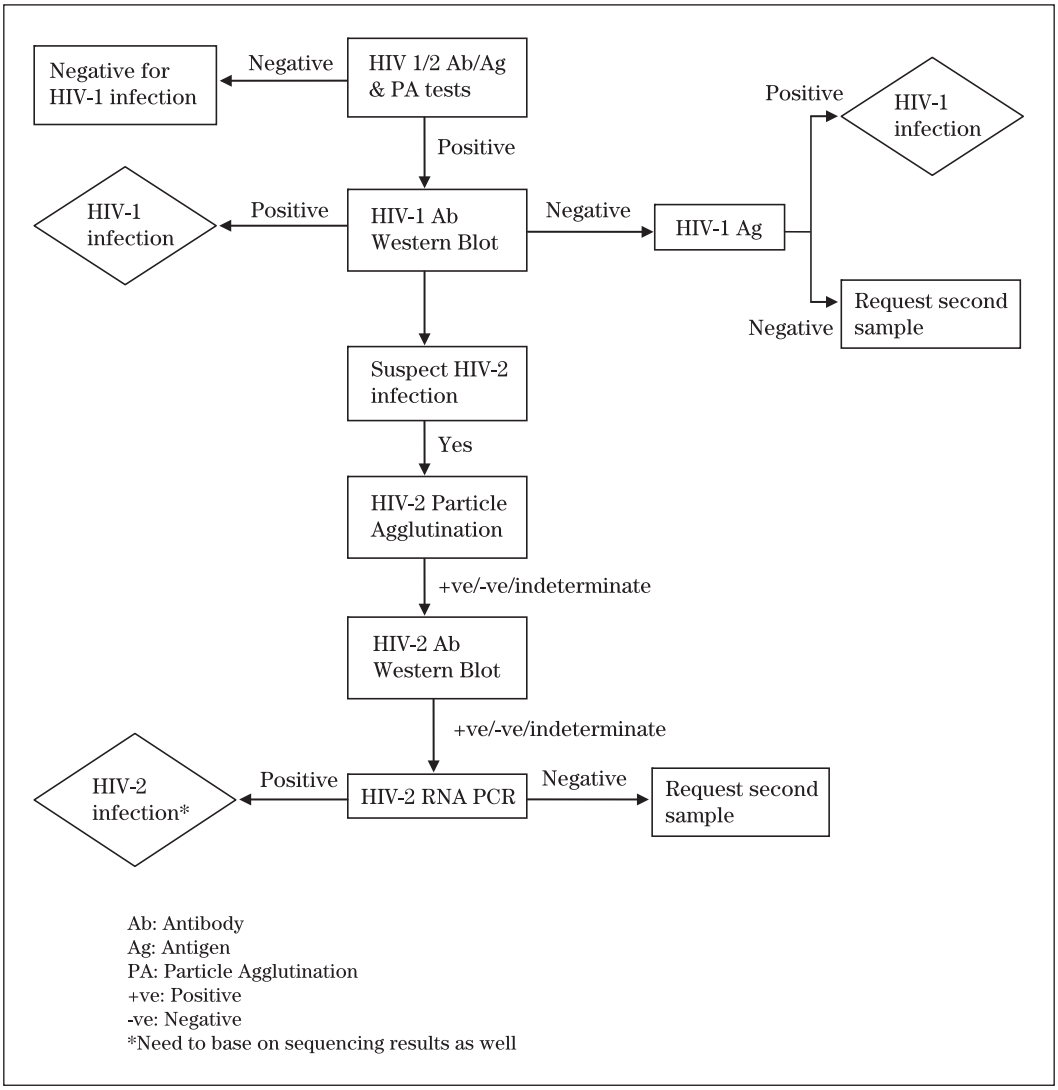

Figure 1. Current HIV-1 and HIV-2 testing algorithm in Virology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia.

A total of 15 samples were further subjected to in-house protocol for detecting HIV-2 genome. HIV-2 RNA standard, primers and probe were designed based on the sequences previously published (Delarue *et al.*, 2013). The remaining 14 samples were insufficient in sample volume for further testing. HIV-2 positive control from the HIV-2 commercial kit was used in the assay. The External Quality Assurance Programme (EQAP) that is specific for the detection of HIV-2 RNA was not available at the time of this study in all the laboratories. Therefore, the inter-laboratory comparison of the test results was not carried out.

RESULTS

Out of the 29 samples tested, 27 (93.0%) samples were found to be HIV-2 positive by Western Blot. All these positive samples had at least 2 of the 3 envelope bands (gp36, gp80 and gp125) present. Only 2 samples showed indeterminate results in which only the gp80 band was present. Results of HIV-2 Western

Table 1. Details of the tested samples with intermediate or positive HIV-2 Western Blot results

Samples/ Controls	Bands
Positive Control	p26, gp36, p53, p56, p68, gp80, gp125
Negative Control	No Band
MY01	p26, gp36, gp80
MY02	p26, gp36, gp80
MY03	p26, gp36, gp80
MY04	p26
MY05	p26, p53, gp36, gp80
MY06	p26, p68, gp36, gp80
MY07	p26, gp36, gp80
MY08	p26, p68, gp36, gp80
MY09	p26, gp36, gp80
MY10	p26, p56, p53, p68, gp36, gp80, gp125
MY11	p26, gp36, gp80
MY12	p26, p53, gp36, gp80
MY13	p26, p68, gp36, gp80
MY14	p26, gp36, gp80
MY15	p26, gp36, gp80
MY16	gp36, gp80
MY17	p26, gp36, gp80
MY18	p26, gp36, gp80
MY19	p26, p68, gp36, gp80
MY20	p26, gp36, gp80
MY21	p26, p68, gp36, gp80
MY22	p26, gp36, gp80
MY23	p26, gp36, gp80
MY24	p26, gp80
MY25	p26, p68, gp36, gp80, gp125
MY26	p26, gp80
MY27	p26, p68, gp36, gp80
MY28	p26, p68, gp80, gp125
MY29	p26, p68, gp80, gp125

Blot for all samples are shown in Table 1. All 29 samples showed negative results for HIV-2 genomes when screened with a commercial kit. Fifteen samples which were subjected to the in-house HIV-2 PCR also were found to be negative for the HIV-2 genome.

DISCUSSION

As the HIV-2 infection is not limited to West African countries, a discrimination of HIV-1 and HIV-2 infection is really important especially in managing the patient clinically. Laboratory tests so far, have played a role in confirming the diagnosis of HIV. The use of HIV-2 Western Blot as a supplementary test in diagnosing HIV-2 is favourable as it is more specific and utilizes native HIV-2 viral proteins. However, HIV-2 positivity on HIV-2 Western Blot does not reflect the actual positivity of HIV-2 in the sample as both HIV-1 and HIV-2 antibodies may cross-react with either viral proteins. This cross-reactivity between HIV types in serology is mainly due to the use of conserved regions of *gag* and *pol* genes in the assay as HIV-2 shows high genetic diversity (Ruelle, 2012). Thus, the HIV-2 infection can be overestimated especially in the region with very low prevalence (Qiu *et al*., 2009 & McKellar *et al*., 2008) and it is strongly recommended for confirmation by using PCR.

The commercial assay for the diagnosis of HIV-2 is not widely available and is very expensive. PrimerDesign™ genesig advanced kit for HIV-2 is designed for detecting HIV-2 GAG genomes, a highly conversed region of HIV-2 as the target sequence region for both HIV-2 subtype A and subtype B. Many studies have shown GAG region to be a good genetic marker for HIV-2 detection using real-time PCR (Genesig Standard Kit Quantification of Human Immunodeficiency Virus type 2 genomes genesig Standard kit handbook 2016).

Developing an in-house assay for detecting HIV-2 has become a priority due to the current era of globalisation with an ease of air travel across the globe. As the test is highly needed and the lack of commercial kits available in the market, NARL has developed the test by adopting the protocol from published article (Delarue *et al*., 2013). The adopted method is comparable with the commercial kit and can be used to detect HIV-2 RNA in suspected patients. In this

analysis, none of the samples was positive for HIV-2 by real time PCR, despite 27 samples being positive for HIV-2 based on the Western Blot assay. Similar finding was reported in Maputo City, Mozambique where only 3 out of 30 (10%) patients were positive for HIV-2 based on INNO-LIA immunoblot assay. They were also shown to be positive for HIV-2 subtype A by PCR (Maueia *et al.*, 2011, McKellar *et al.*, 2008 & Ciccaglione *et al.*, 2010). These results also demonstrated that a highly sensitive PCR assay is crucial and required for the accurate diagnosis of HIV-2 infection. This is further supported by a study done by Ciccaglione *et al.* (2010) which confirmed that a diagnostic strategy based on immunoblot and real time PCR for diagnosing HIV-2 allowed for a discrimination of 84% between HIV-1 and HIV-2.

CONCLUSION

Continuous HIV-2 screening is paramount as HIV-2 cases have been reported in many parts of the world. Based on results of the current virological surveillance, there is still no HIV-2 infection case detected in Malaysia for more than 25 years ago. All the inconclusive samples for HIV-1 and HIV-2 need to be further investigated as the commercial assay for HIV antigen and antibody detection have lower sensitivity to HIV-2 antigen. As the commercial assay for HIV-2 PCR is not easily available in the market, there is a need for us as a reference laboratory to have in-house PCR assay for HIV-2. With the availability of the in-house assay, it is expected that the laboratories will be able to increase the sensitivity of the test to detect HIV-2 and thereby also increase the diagnosis rate of HIV-1 and HIV-2 infected patients in Malaysia.

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