

# Performance Evaluation of the Bioneer AccuPower<sup>®</sup> HIV-1 Quantitative RT-PCR kit: Comparison with the Roche COBAS<sup>®</sup> AmpliPrep/COBAS TaqMan<sup>®</sup> HIV-1 Test Ver.2.0 for Quantification of HIV-1 Viral Load in Indonesia

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## Abstract

**Background:** Measurement of viral load in human immunodeficiency virus type 1 (HIV-1) infected patients is essential for the establishment of a therapeutic strategy. Several assays based on qPCR are available for the measurement of viral load; they differ in sample volume, technology applied, target gene, sensitivity and dynamic range. The Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR is a novel commercial kit that has not been evaluated for its performance.

**Objective:** This study aimed to evaluate the performance of the Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit.

**Methods:** In total, 288 EDTA plasma samples from the Dharmais Cancer Hospital were analyzed with the Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit and the Roche COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 version 2.0 (CAP/CTM v2.0). The performance of the Bioneer assay was then evaluated against the Roche CAP/CTM v2.0.

**Results:** Overall, there was good agreement between the two assays. The Bioneer assay showed significant linear correlation with CAP/CTM v2.0 ( $R^2=0.963$ ,  $p<0.001$ ) for all samples ( $N=118$ ) which were quantified by both assays, with high agreement (94.9%, 112/118) according to the Bland-Altman model. The mean difference between the quantitative values measured by Bioneer assay and CAP/CTM v2.0 was 0.11 Log<sub>10</sub> IU/mL (SD=0.26).

**Conclusion:** Based on these results, the Bioneer assay can be used to quantify HIV-1 RNA in clinical laboratories.

**Keywords:** HIV-1, PCR, viral load, *AccuPower*<sup>®</sup>, COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup>

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## Introduction

HIV is the responsible etiologic agent for the onset of AIDS, a disease characterized by the depletion of CD4<sup>+</sup> helper T-cells.<sup>1</sup> AIDS leaves the host susceptible to opportunistic infections and cancer development, which frequently result in mortality.<sup>2</sup> At the end of 2013, there were 35 million people living with HIV. This number has risen as more people are living longer because of antiretroviral therapy (ART), alongside the number of new HIV infections which, although declining, is still very high.

Measurement of viral load in HIV-1 infected patients is essential for the establishment of a therapeutic strategy. In 2013, the World Health Organization recommended that, with the exception of dried blood spot samples, the threshold for detection of virological failure should be lowered to 1,000

copies/mL.<sup>3</sup> Thus, the commercial HIV-1 quantitative assay should be able to detect and quantify at least 1,000 copies/mL. Several commercial kits utilizing different principles, such as nucleic acid sequence based amplification (NASBA), branched DNA (bDNA) and reverse transcriptase real-time PCR (RT-qPCR) have been developed to detect and quantify HIV-1 RNA.<sup>4</sup> These kits are able to detect and quantify HIV-1 viral load of 1,000 copies/mL, which is within the linear range of viral load assay claimed by manufacturers.<sup>5</sup>

Indonesia, a country with a population of 249.9 million in 2013, has an estimated HIV prevalence of 0.5% among the 15 to 49-year-old age group. The situation in Indonesia is a cause for concern, as new HIV infections have increased by

48% from 2005 to 2013, and the country's share of new HIV infections in the Asian/the Pacific region reached 23% in 2013, second only to India.<sup>6</sup> The high incidence of HIV-1 infection in Indonesia consequently translates into a high demand for HIV-1 viral load measurement. Unfortunately, HIV-1 viral load measurement is still considered expensive, and requires expensive analyzers and skilled operators, and is therefore still underutilized.

The Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit (Bioneer, Daejeon, Korea) is a novel commercial kit which applies TaqMan technology and targets the HIV-1 *pol* (integrase) gene for the quantification of HIV-1 group M, N and O in human EDTA-treated plasma. The dynamic range of *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit is 100~100,000,000 IU/mL. This system uses only 0.4 mL plasma, and has a short turnaround time of 3.5 hours. Thus, the Bioneer kit and *ExiStation*<sup>™</sup> Universal Molecular Diagnostic System offer an affordable alternative for HIV-1 viral load testing for developing countries, but its performance has not been evaluated in Indonesia.

In this study, the performance of the Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit was evaluated in comparison to the Roche CAP/CTM v2.0 assay, which is the assay currently used at the Dharmais Cancer Hospital, a referral hospital for HIV testing in Indonesia.

## Material and Methods

### Ethical approval

This study has been approved by the Dharmais Cancer Hospital Ethics Committee (approval number: 060/KEPK/IX/2016).

### Clinical samples and WHO panel

For the determination of the limit of detection (LoD), a WHO panel (WHO International Standard Third HIV-1 International Standard, NIBSC code 10/152, subtype B) was used. For the evaluation of HIV-1 subtype detection, the Second WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) (NIBSC code: 12/224) and the First WHO International Reference Preparation for HIV-1 CRFs (NIBSC code: 13/214) were used. The panels included 14 subtypes from Group M, including subtype AE and Circulating Recombinant Form (CRF)01\_AE which were the subtypes reported as the most prevalent in some provinces in Indonesia,<sup>7,8</sup> plus Group N and Group O.

A total of 288 EDTA plasma samples from outpatients requesting HIV-1 RNA viral load testing were collected at the Dharmais Cancer Hospital, Indonesia consisting of fresh samples (N=94) which were stored at 4-8°C before testing and frozen samples (N=194) which were stored at -20°C until testing.

### Measurement of viral load

#### Bioneer *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit

The HIV-1 RNA was quantified using an *ExiStation*<sup>™</sup> Universal Molecular Diagnostic System which consisted of three *ExiPrep*<sup>™</sup> 16 Dx nucleic acid extraction instruments and one *Exicycler*<sup>™</sup> 96 real-time quantitative thermocycler.

*ExiStation*<sup>™</sup> manager software controlled the entire process of nucleic acid preparation, amplification, automatic data acquisition and analysis, and finally delivered a report to the user. The *ExiPrep*<sup>™</sup> 16 Dx automated the extraction and purification of nucleic acids from clinical specimens by utilizing magnetic particle technology. All reagents for extraction were included in the cartridge of the *ExiPrep*<sup>™</sup> Dx Viral RNA kit. Extracted RNA was eluted into diagnostic reaction tubes directly, and thus RT-qPCR on the *Exicycler*<sup>™</sup> 96 was performed without any manual pipetting steps.<sup>9</sup>

HIV-1 RNA was extracted from 0.4 mL of EDTA plasma using the *ExiPrep*<sup>™</sup> Dx Viral RNA kit and the *ExiPrep*<sup>™</sup> 16 Dx of the *ExiStation*<sup>™</sup> system. The extracted HIV-1 RNA was automatically loaded into the PCR tubes of the *AccuPower*<sup>®</sup> HIV-1 Quantitative RT-PCR kit, and RT-qPCR was performed by the *Exicycler*<sup>™</sup> 96 real-time quantitative thermocycler with the following program: 55°C for 15 min; 95°C for 5 min; 45 cycles of 95°C for 5 sec, 55°C for 5 sec and fluorescence scan; 25°C for 1 min. All procedures were conducted according to the manufacturer's instructions. The concentration of HIV-1 RNA was determined based on the threshold cycle (Ct), and the corresponding RNA concentration (IU/mL) was calculated using a standard curve. An internal positive control consisting of RNA sequences unrelated to the HIV-1 target sequence, was preloaded in each sample loading tube from the beginning of RNA extraction from specimens to determine whether the nucleic acids were properly extracted and amplified in each reaction and whether PCR was inhibited by the sample.

#### Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test, v2.0 (CAP/CTM v2.0)

The Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0 (CAP/CTM v2.0) (Roche Diagnostics Ltd, Burgess Hill, UK) uses a fully automated analyzer for the quantification of HIV-1 groups M and O in human plasma. CAP/CTM v2.0 targets both the *gag* and LTR regions using two dual-labeled hybridization probes with a dynamic range of 34-17,000,000 IU/mL and LoD of 34 IU/mL.<sup>10</sup> HIV-1 RNA was obtained from a 0.85 mL of EDTA plasma using the COBAS AmpliPrep system. RNA was extracted using the COBAS AmpliPrep system prior to being automatically transferred to the COBAS TaqMan system for amplification and detection. All procedures were conducted according to the manufacturer's instructions.

### Statistical analysis

The correlation coefficient ( $r$ ) was used to assess the strength of the linear association between the  $\log_{10}$  IU/mL levels in the quantified samples measured by the two assays. The Bland-Altman method was used to assess the level of agreement between the paired measurements.<sup>11</sup> The correlation coefficient, the Bland-Altman plot, mean differences and standard deviation (SD) were generated using SPSS 18.0 software.

## Results

### Limit of detection

The LoD of the Bioneer HIV-1 assay was determined by analyzing a dilution series of the WHO Panel (Third HIV-1 International Standard, subtype B) at concentrations of 100,

50, 25, 12.5, 6.3, and 3.1 IU/mL. The detection rates of each concentration are summarized in **Table 1**. The proportion of positive results from each concentration was analyzed to calculate the 95% hit detection limit, which was 38.0 IU/mL.

**Table 1. Limit of Detection of the Bioneer HIV-1 test using the WHO International Panel.**

Concentration (IU/mL)	Percent detected (Number detected / Number tested)
100	100 (30/30)
50	100 (31/31)
25	93.5 (29/31)
12.5	56.3 (18/32)
6.3	32.3 (10/31)
2.1	29 (9/31)

The detection of HIV-1 subtypes by the assay was evaluated using the WHO subtype panel, which includes 14 subtypes: A, C, D, AE, F, G, AG-GH, CRF\_11AJ, CRF\_02AG, CRF\_01AE, CRF\_01AGJU, CRF\_24BG, J, and CRF\_ADG. The assay detected all 14 subtypes at a concentration of 100, and at 50 IU/mL when four replicates of each concentration were tested. The assay detected positive all of four replicates of Group N at a level of 50 IU/mL and Group O at a level of 100 IU/mL.

**Performance of the AccuPower® HIV-1 Quantitative RT-PCR kit versus CAP/CTM v2.0**

Of the 288 EDTA plasma samples, HIV-1 was not detected in 107 samples, detected below the lower limit of quantification

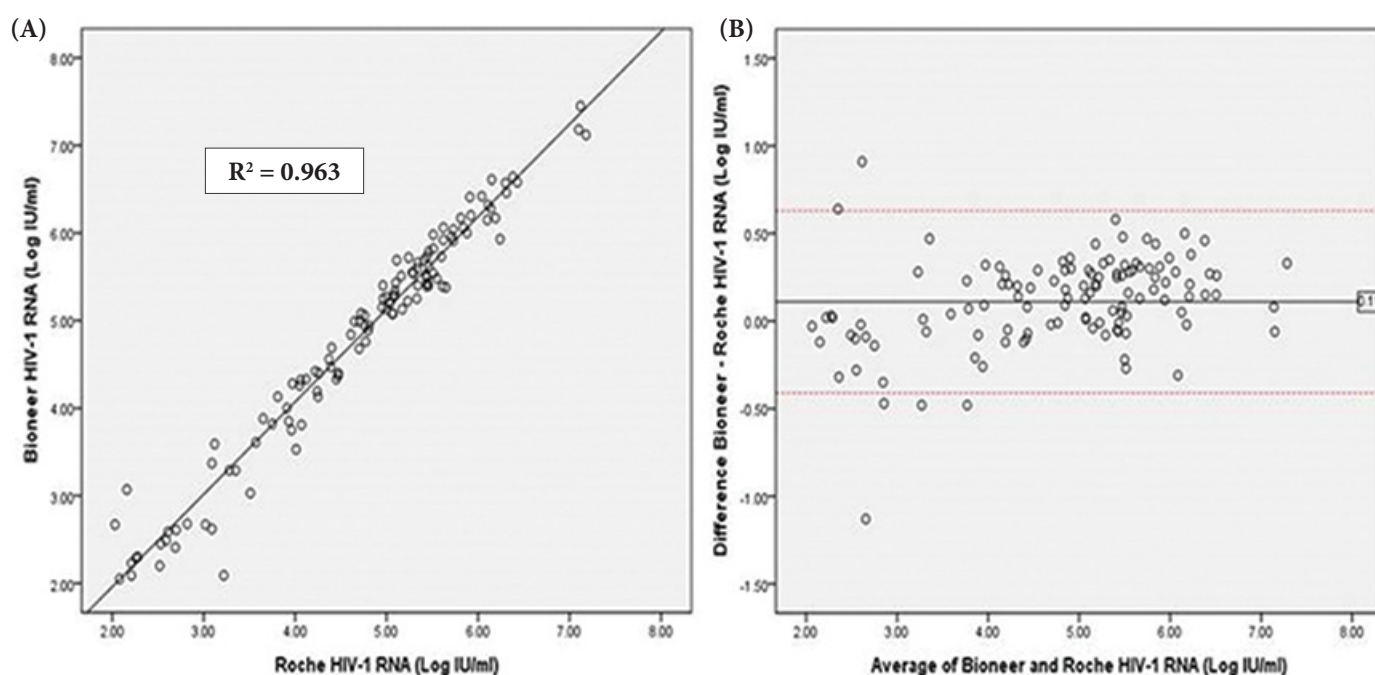
(LLoQ) in 16 samples, and quantified in 118 samples by both assays (**Table 2**). Among the 118 quantified samples, 17 samples were quantified between the LLoQ and 1,000 copies/mL. The average viral loads ( $\log_{10}$  IU/mL; the conversion factor for HIV RNA was 1.74 copies/IU) in the 17 samples measured by the Bioneer assay and CAP/CTM v2.0 were 2.44 (SD=0.27) and 2.53 (SD=0.36), respectively.

**Table 2. Comparison of HIV-1 viral load determinations by both assays.**

		No. of samples with the following result with CAP/CTM v2.0			
		Undetected	<LloQ	Quantified	Total
Results with Bioneer assay	Undetected	107	14	4	125
	<LloQ <sup>a</sup>	5	16	19	40
	Quantified	4	1	118	123
	Total	116	31	141	288

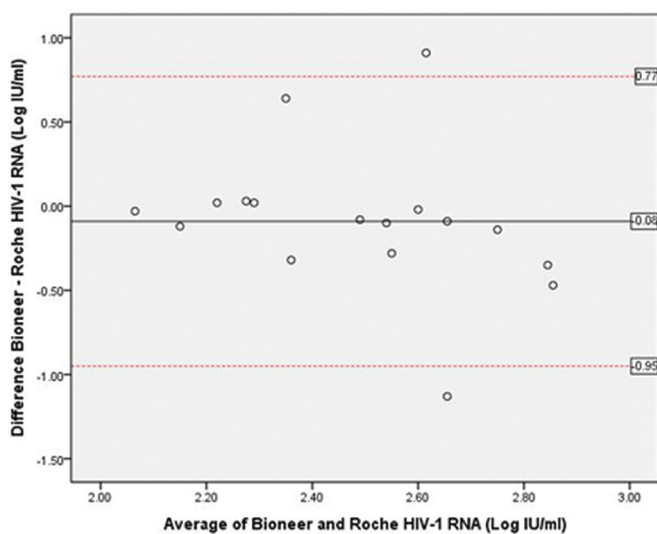
<sup>a</sup>LloQ: Lower limit of quantification

The Bioneer assay showed a significant linear correlation with the CAP/CTM v2.0 assay ( $R^2=0.963$ ,  $p<0.001$ ) for all samples ( $N=118$ ) that were quantified by both assays (**Figure 1A**), with high agreement (94.9%, 112/118) according to the Bland-Altman model (**Figure 1B**). The fitted regression equation was: Bioneer assay =  $1.0563 \times \text{CAP/CTM v2.0} - 0.1549$ . The mean difference between the quantitative values measured by the Bioneer assay and the CAP/CTM v2.0 assay was 0.11  $\log_{10}$  IU/mL (SD=0.26).



**Figure 1. Correlation between HIV-1 RNA levels from all quantified samples obtained using the Bioneer assay and the CAP/CTM v2.0,  $R^2 = 0.963$  (A). Agreement between the Bioneer assay and CAP/CTM v2.0 calculated using the Bland-Altman model (B). Solid horizontal lines indicate the mean values, and red-dashed horizontal lines indicate the  $\pm 2$  SD values.**

The Bland-Altman model of the 17 low copy samples which were quantified between the LLoQ and 1,000 copies/mL showed good agreement between the two assays (88.2%, 15/17). A difference of  $>0.3 \log_{10}$  IU/mL was observed in two samples (11.8%), in which the concentrations ( $\log_{10}$  IU/mL) were 2.62 and 2.67 by Bioneer (3.09 and 3.02 by CAP/CTM v2.0, respectively); a difference of  $>0.5 \log_{10}$  IU/mL was observed in three samples (17.6%), in which the concentrations ( $\log_{10}$  IU/mL) were 2.09, 3.07, and 2.67 by Bioneer (3.22, 2.16, and 2.03 by CAP/CTM v2.0, respectively). The mean difference in 17 low copy samples between the quantitative values measured by the Bioneer assay and the CAP/CTM v2.0 assay was  $-0.08 \log_{10}$  IU/mL (SD=0.43) (Figure 2). Overall, the quantification of low copy samples was similar by both assays.



**Figure 2. Bland-Altman model of 17 low copy samples with VL between LLoQ and 1,000 copies/mL.** Solid horizontal line indicates the mean value, and red-dashed horizontal lines indicate the  $\pm 2$  SD values.

The average viral loads ( $\log_{10}$  IU/mL) in the 118 samples measured by the Bioneer assay and CAP/CTM v2.0 were 4.81 (SD=1.31) and 4.70 (SD=1.21), respectively. There were four samples that were quantified by CAP/CTM v2.0, but not detected by the Bioneer assay. The viral loads were 57, 86, 118, and 130 IU/mL by CAP/CTM v2.0. In contrast, four samples were quantified by the Bioneer assay, but not detected by CAP/CTM v2.0. The viral loads determined by the Bioneer assay were 584, 404, 135, and 204 IU/mL.

## Discussion

HIV-1 viral load is the most important indicator of initial and sustained response to ART and should be measured in all HIV-infected patients at entry into care, at the initiation of therapy, and on a regular basis thereafter.<sup>12</sup> Current international guidelines and clinical trials define virological suppression as the achievement and maintenance of an HIV-1 RNA level of 50 copies/mL or to levels below assay limits as the virologic endpoint of successful ART. In contrast, the HIV RNA threshold for defining virological failure differs

between guidelines. The WHO guidelines define virological failure as a viral load that is persistently above 1,000 copies/mL after  $\geq 6$  months on ART.<sup>3</sup> The British HIV Association (BHIVA),<sup>13</sup> International Antiviral Society (IAS),<sup>14</sup> European AIDS Clinical Society (EACS),<sup>15</sup> and Department of Health and Human Services (DHHS) guidelines consider virological failure to be a confirmed viral load of more than 400, 200, 50, or 48 copies/mL after suppression, respectively.<sup>12,16</sup>

In the present study, 288 clinical samples were tested for the evaluation of the Bioneer assay in comparison with Roche CAP/CTM v2.0. Of 288 samples, 118 samples were quantified by the Bioneer assay and CAP/CTM v2.0. All 107 negative samples were negative in both assays, indicating that the Bioneer assay has a good specificity for the test. There were 19 samples that were undetected by one assay and determined below the LLoQ by the other assay. Five samples were detected below the LLoQ in the Bioneer assay while these were not detected by CAP/CTM v2.0, and 14 samples were detected below the LLoQ in CAP/CTM v2.0 but not detected in the Bioneer assay. There were 20 samples below the LLoQ by one assay and quantified by the other assay. Of the 20 samples, one sample was quantified by the Bioneer assay (238 IU/mL) but detected below the LLoQ by CAP/CTM v2.0, and 19 samples were quantified by CAP/CTM v2.0 and detected below the LLoQ by the Bioneer assay. The average HIV-1 RNA concentration of the 19 samples was 109 IU/mL (SD=80.6). These results were likely caused by different LLoQ of each assay. The LLoQ of CAP/CTM v2.0 is 34 IU/mL, whereas the LLoQ of the Bioneer assay is 100 IU/mL.

A total of eight samples were discordant. The Bioneer assay quantified four samples in which the average concentration of HIV-1 RNA was 331 IU/mL, all of which were undetected by the CAP/CTM v2.0 assay. CAP/CTM v2.0 quantified four samples in which the average concentration of HIV-1 RNA was 98 IU/mL, all of which were undetected by the Bioneer assay. This result may be caused by the different primers. In a 2004 case report in Thailand, a seven-year-old child with HIV-1 subtype (CRF)01\_AE, which is the most prevalent subtype in Indonesia, had undetectable HIV-1 RNA and DNA using env/pol primers, but was positive using gag/pol.<sup>17</sup> Bioneer uses a pol primer, while CAP/CTM v2.0 uses gag/LTR primers. Confirmatory tests for the discordant results of both assays were not performed due to the lack of samples.

A significant correlation ( $R^2=0.963$ ,  $p<0.001$ ) between the two assays was found for the 118 quantified samples, even though the Bioneer assay uses 0.4 mL of plasma while CAP/CTM v2.0 uses 0.85 mL of plasma for the test. Bland-Altman analysis revealed that the differences for 112 samples (94.9%) among the 118 samples were within the 2SD value, while those for the other six samples were outside the 2SD value, thus indicating good agreement between the two assays for the 118 samples assessed as quantified by both assays.

Most guidelines recommend the use of a single quantification assay for therapeutic monitoring of HIV-1 infected patients. Furthermore, several current guidelines advise against switching ART in HIV-1 infected patients when the HIV-1 viral load remains at 200 to 1,000 copies/mL, taking into account the possibility that it may represent a blip.<sup>3,12-15,18</sup> On the other hand, during ART, a careful assessment of

persistent HIV-1 RNA in the range of 200 to 1,000 copies/mL is recommended, since viral evolution and drug resistance mutations might accompany low level viral replication, subsequently leading to virological failure.<sup>19-22</sup> The Bioneer assay showed good agreement and significant quantification of the HIV-1 viral load with CAP/CTM v2.0 using low copy samples. These results indicate that the Bioneer assay is able to quantify low copy HIV-1 viral load in HIV-1 infected patients on ART.

## Conclusion

In conclusion, a good agreement was observed between the two assays. The Bioneer assay met the performance criteria as an alternative for HIV-1 RNA quantification in clinical laboratories.

## Acknowledgements

We thank Bioneer Corp. for providing the AccuPower HIV-1 Quantitative RT-PCR kit for this study.

## References

- O'Brien WA, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S, et al. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. *N Engl J Med.* 1996;334:426-31.
- Naidoo K, Naidoo K, Padayatchi N, Abdool Karim Q. HIV-associated tuberculosis. *Clin Dev Immunol.* 2011. doi:10.1155/2011/585919.
- WHO [Internet]. March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach [cited 2016 Oct 1]. Available from: [http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013supplement\\_march2014/en/](http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013supplement_march2014/en/)
- Xu S, Song A, Nie J, Li X, Meng S, Zhang C, et al. Comparison between the automated Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 assay and its version 1 and Nuclisens HIV-1 EasyQ version 2.0 assays when measuring diverse HIV-1 genotypes in China. *J Clin Virol.* 2012;53:33-7.
- Sollis KA, Smit PW, Fiscus S, Ford N, Vitoria M, Essajee S, et al. Systematic review of the performance of HIV viral load technologies on plasma samples. *PLoS One* [Internet]. 2014 Feb [cited 2016 Oct 1];9(2):[about 9 p]. Available from: <http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0085869&representation=PDF>
- UNAIDS [Internet]. The GAP Report [cited 2016 Oct 1]. Available from: [http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS\\_Gap\\_report\\_en.pdf](http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf)
- Roselinda, Jekti RP. Genotype and transmission Human Immunodeficiency Virus-1 in seven provinces in Indonesia. *Health Science Indones* [Internet]. 2012 Dec [cited 2016 Oct 1];3(2):[about 5 p]. Available from: <http://ejournal.litbang.depkes.go.id/index.php/HSJI/article/view/3070/3039>
- Ismail YS, Soetjipto, Wasito EB, Nasronudin. HIV Genotype Analysis from HIV Infected Patients in East Java Area. *Jurnal Natural* [Internet]. 2012 [cited 2016 Oct 1];12(23):[about 7 p]. Available from: <http://jurnal.unsyiah.ac.id/natural/article/view/833/771>
- Cha YJ, Yoo SJ, Sohn YH, Kim HS. Performance evaluation of ExiStation HBV diagnostic system for hepatitis B virus DNA quantitation. *J Virol Methods.* 2013;193:492-7.
- Wall GR, Perinpanathan D, Clark DA. Comparison of the QIAGEN artus HIV-1 QS-RGQ test with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0. *J Clin Virol.* 2012;55:62-6.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-10.
- Panel on Antiretroviral Guidelines for Adults and Adolescents [Internet]. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents [cited 2016 Oct 1]. Available from: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>
- British HIV Association. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy. *HIV Med.* 2014;15(Suppl 1):1-85.
- Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA Panel. *JAMA.* 2012;308:387-402.
- European AIDS Clinical Society [Internet]. Guidelines, version 7.1, November 2014 [cited 2016 Oct 1]. Available from: <http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html>
- Amendola A, Marsella P, Bloisi M, Forbici F, Angeletti C, Capobianchi MR. Ability of two commercially available assays (Abbott RealTime HIV-1 and Cobas AmpliPrep/Cobas TaqMan HIV-1 version 2.0) to quantify low HIV-1 RNA levels (<1,000 copies/milliliter): Comparison with clinical samples and NIBSC working reagent for nucleic acid. *J Clin Microbiol.* 2014;52:2019-26.
- Ananworanich J, Pancharoen C, Sirivichayakul S, Buranapraditkun S, Apateerapong W, Ubolyam S, et al. Undetectable plasma HIV-1 RNA with strong gag-pol specific interferon-gamma ELISPOT response in an HIV-1 clade A/E-infected child untreated with antiretroviral therapy. *Asian Pac J Allergy Immunol.* 2004;22:165-9.
- Laprise C, De Pokomandy A, Baril JG, Dufresne S, Trottier H. Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: Results from 12 years of observation. *Clin Infect Dis.* 2013;57:1489-96.
- Aleman S, Söderbärg K, Visco-Comandini U, Sitbon G, Sönnnerborg A. Drug resistance at low viraemia in HIV-1-infected patients with antiretroviral combination therapy. *AIDS.* 2002;16:1039-44.
- Delaugerre C, Gallien S, Flandre P, Mathez D, Amarsy R, Ferret S, et al. Impact of low-level-viremia on HIV-1 drug-resistance evolution among antiretroviral treated-patients. *PLoS One* [Internet]. 2012 [cited 2016 Oct 1];7:[about 6 p]. Available from: <http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0036673&representation=PDF>
- Karlsson AC, Younger SR, Martin JN, Grossman Z, Sinclair E, Hunt PW. Immunologic and virologic evolution during periods of intermittent and persistent low-level viremia. *AIDS.* 2004;18:981-9.
- Taiwo B, Gallien S, Aga E, Ribaudo H, Haubrich R, Kuritzkes DR. Antiretroviral drug resistance in HIV-1-infected patients experiencing persistent low-level viremia during first-line therapy. *J Infect Dis.* 2011;204:515-20.