

Comparison of the Reverse Transcription-PCR with the Branched DNA Assay for Measurement of Human Immunodeficiency Virus Type 1 RNA Levels in Plasma of Korean Patients

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Viral load testing of human immunodeficiency virus (HIV) is an essential tool for initiating and monitoring the antiretroviral therapy for HIV patients. To this end, several methods including polymerase chain reaction (PCR), branched DNA (bDNA), nucleic acid sequence based amplification assay (NASBA) and internally controlled virion PCR (ICV PCR) have become available. Of these methods, the standard reverse transcription-PCR (RT-PCR) assay has been widely used in Korea. However, no comparison study has been performed among the various detection methods currently used in Korean patients.

We evaluated the correlation and agreement between the PCR and the branched DNA (bDNA) assay for measurement of HIV RNA in Korean patients. Eighty randomly selected samples from HIV-1-seropositive patients visiting Yonsei Medical Center Severance Hospital were studied.

We found that these assays show good agreement, have a reliable correlation ($r=0.92$, mean difference in \log_{10} copies/mL ± 2 standard deviation $= 0.098 \pm 0.805$) and produce values whose relationship is given by the following equation: $\log_{10} \text{v3 bDNA} = -0.3405 + 1.0601 \cdot \log_{10} \text{RT-PCR}$. Thus, we conclude that these two methods may allow direct comparison of the results obtained from different assay systems.

Key Words: Human immunodeficiency virus (HIV) type 1, reverse transcription-PCR assay (RT-PCR), branched DNA assay (bDNA)

INTRODUCTION

Although the prevalence of acquired immune deficiency syndrome (AIDS) in Korea is not as

high as observed in Western or other Asian countries, there is growing concern about the disease because it is evident that the incidence of AIDS is increasing in this country too.

Thus accurate detection of HIV in patients is essential for the initiation of therapy as well as for monitoring the patients on therapy and is now regarded as standard medical practice for managing the treatment of HIV-1-infected individuals. Currently, there are several commercial kits for HIV-1 RNA quantitation in the market; Amplicor HIV-1 MONITOR assay (Roche Diagnostics Corporation, Branchburg, New Jergsey, USA), Quantiplex HIV-1 RNA assay (Bayer Corporation, Emeryville, California, USA), nucleic acid sequence based amplification (NASBA) for HIV-1 RNA (Organon Teknika, Durham, North Carolina, USA), internally controlled virion polymerase chain reaction (ICV PCR).

The Amplicor HIV-1 MONITOR assay is an in vitro nucleic acid target amplification (polymerase chain reaction, PCR) test for the quantitation of HIV-1 RNA in human plasma. It uses an internal quantitation standard which is incorporated into each individual specimen to monitor the efficiency of RNA extraction and reverse transcription-PCR (RT-PCR), and eliminates the need for a standard curve. Quantiplex HIV-1 RNA version 3 assay quantitates HIV-1 RNA in plasma by branched DNA (bDNA) signal amplification based on hybridization without target RNA amplification.¹

The limit imposed by statistics of sampling for the presence of 1 copy RNA per actually tested plasma volume (0.025 mL) is 20~50 copies/mL.

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The recent advent of combination drug therapies allows viral load suppression for many patients to a level below the quantitation limit of the currently available HIV-1 RNA assays. Early reports suggest that suppression to below 20 HIV-1 RNA copies/mL compared to 50 to 400 HIV-1 RNA copies/mL is associated with a more sustained response.^{2,3} These considerations support the use of more sensitive methodologies for HIV-1 RNA quantitation.^{4,6} The linear range for the standard MONITOR test is about 400 to 750,000 copies/mL and that for the Quantiplex v3 test is 50 to 500,000 copies/mL. Very recent studies have reported that a modified specimen preparation procedure enhances the sensitivity of the standard MONITOR test and that increased sensitivity is obtained by increasing the input plasma volume, performing high-speed centrifugation to concentrate the virus particles from plasma, and reducing the final resuspension volume for the recovered nucleic acid. This procedure can result in a 10-fold increase in the analytical sensitivity of the MONITOR test (detection limit 50 copies/mL).⁴ But as of the present, only the standard MONITOR kits are supplied in Korea.

In this study, two commercially available assays for the measurement of HIV-1 RNA, the standard HIV-1 Amplicor MONITOR assay and the Quantiplex version 3 assay, were compared in order to assess their quantitative relationship.

MATERIALS AND METHODS

Patient population

The study group consisted of 48 randomly selected HIV-1/2-seropositive patients who visited and were treated in Yonsei Medical Center Severance Hospital from December 1999 to May 2000. A total of 80 samples from these 48 patients were analyzed. As for HIV subgroups, forty-five patients were HIV subgroup B, two HIV subgroup C and one HIV subgroup A.

Quantitation of HIV-RNA

Quantitation of HIV-RNA was performed by RT-PCR using the Amplicor HIV-1 MONITOR test

version 1 (Roche Diagnostics Corporation, Branchburg, New Jersey, USA) and by bDNA using the Chiron Quantiplex version 3 bDNA assay (Bayer Corporation, Emeryville, California, USA) according to the manufacturer's instruction.

RESULTS

The results of the 48 samples were within linear range by both methodologies. Twenty-seven samples were below the detection limit of either RT-PCR or bDNA. Five samples were above the analytic linear range. Linear regression and correlation were used to determine the relationship between the v3 bDNA and RT-PCR values.

Values were transformed into common logarithms (\log_{10}) and expressed as \log_{10} v3 bDNA or \log_{10} RT-PCR.

The relationship within linear range

The results were correlated highly ($r=0.92$, $p<0.001$). Fig. 1 shows a plot of \log_{10} v3 bDNA (y) versus \log_{10} RT-PCR (x) with the fitted regression line described by the equation $y=-0.3405+1.0601 \log_{10}x$. The estimate of the intercept is -0.3405 with a standard error of 0.29. A 95% confidence interval for the estimate of the intercept is -0.92 to 0.24. The value of the slope is 1.0601, with a standard error of 0.07. A 95% confidence interval for the

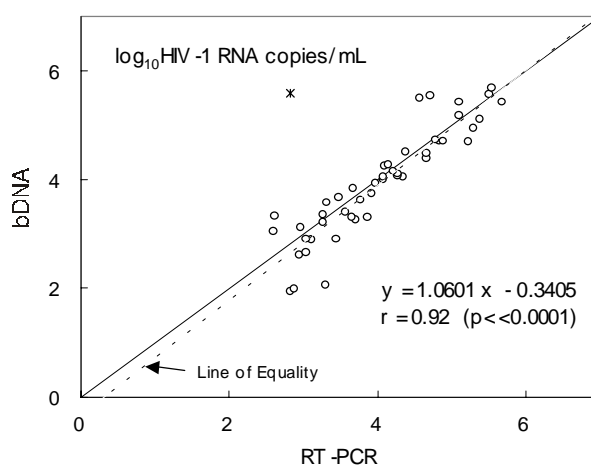


Fig. 1. Scatter plot of \log_{10} v3 bDNA versus \log_{10} RT-PCR with the line of equality (solid) and the fitted regression line (hatched). One outlier (*) was excluded. There is good agreement between the two assays.

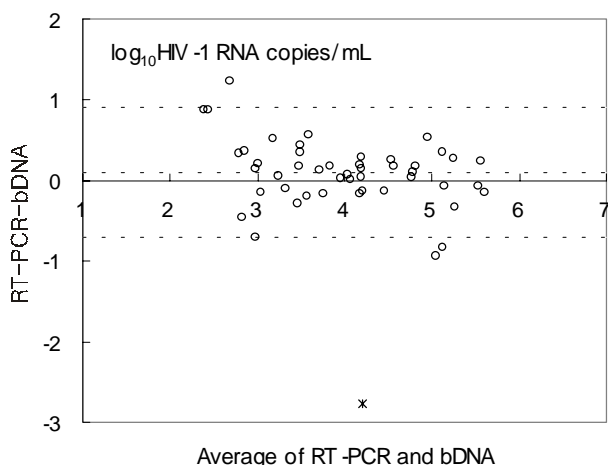


Fig. 2. Difference in \log_{10} assay results versus the average of \log_{10} assay results. The mean difference (middle dashed line) ± 2 standard deviations (upper and lower dashed lines) is shown. One outlier (*) was excluded. The plot shows that the difference values are fairly homogeneously distributed between 2 standard deviations (SD) above and below the mean. All of the values fall between the 2SD except 2 samples, indicating normal distribution.

estimate of the slope is 0.92 to 1.20, a range that includes 1.0, indicating that the assays were in good agreement.

Fig. 2 shows the differences in \log_{10} assay results ($\log_{10}\text{RT-PCR} - \log_{10}\text{bDNA}$) versus the average of those assay results $[(\log_{10}\text{bDNA} + \log_{10}\text{RT-PCR})/2]$. The plot shows that the difference values are fairly homogeneously distributed between 2 standard deviations above and below the mean (0.098) and are in good agreement (mean difference in \log_{10} copies/mL ± 2 standard deviations = 0.098 ± 0.805). A paired t-test demonstrated that the values by both methods are not significantly different ($p=0.10$).

Relationship outside linear range

On the basis of RT-PCR, twenty-three samples were found to be below the detection limit by RT-PCR (<400 copies/mL), of which eighteen were below the detection limit by bDNA (<50 copies/mL). Another 5 samples tested above the detection limit of bDNA (>50 copies/mL) and below 400 copies/mL.

And otherwise, on the basis of bDNA, twenty-two samples tested below the detection limit by bDNA (<50 copies/mL), of which eighteen also

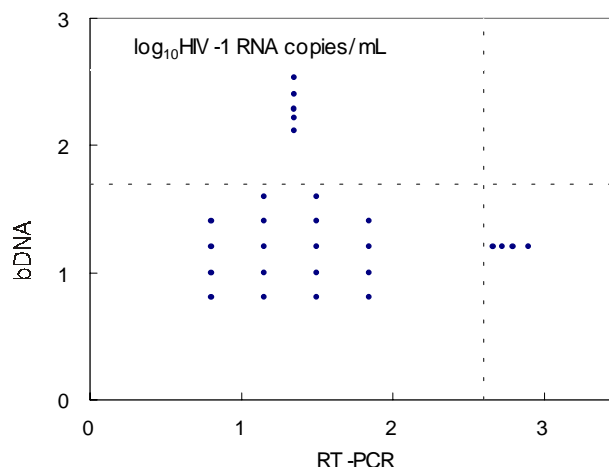


Fig. 3. Comparison of the results of samples tested as being below the detection limit by RT-PCR or bDNA.

tested below the detection limit by RT-PCR (<400 copies/mL). But, another 4 samples tested above 400 copies/mL by RT-PCR. This last result is considered to represent a discrepancy between the two methods. The results are depicted in Fig. 3. Individual values of the 5 samples tested above the linear range ($>500,000$ copies/mL) by bDNA were 247,192, 433,097, 541,913, $>750,000$, and $>750,000$ copies/mL, by RT-PCR.

DISCUSSION

In the follow up of HIV-1 RNA quantitation, current recommendations for clinical testing discourage the comparison of results obtained from different assay systems, because the various systems have not been cross-standardized for accuracy of RNA quantitation. Furthermore, other studies have showed a disparity between bDNA and RT-PCR results.⁷⁻⁹ However, with the improvement of bDNA in its recent 3rd version and also the newly available sensitized version of RT-PCR which features enhanced sensitivity by concentrating samples these two methods have become closely related and have been determined to be in good agreement.⁵

In this study, we performed standard RT-PCR, rather than sensitized RT-PCR, because only the standard MONITOR kits have been supplied in Korea until this study. In linear range, the correlation between the two methods as determined

in this study was high, although lower than that of the study of Highbarger et al. in 1999 ($\log_{10}\text{bDNA} = -0.0915 + 1.0052 \log_{10}\text{RT-PCR}$, $r = 0.98$, $P < 0.0001$). Because patients may be tested with both of these assays at some point during their treatment, confirmation of good agreement overall and a reliable mathematical relationship between these two assays should aid clinicians in their use of both data sets when monitoring a patient's viral load in case. Highbarger et al. reported RT-PCR values that are, on the average, larger than that of v3 bDNA (difference mean \pm 2SD, 0.072 ± 0.371 , $P < 0.0001$) in analyzing 318 samples.⁵ However, in our study we could not observe any significant difference in values between these two methods. The mean difference was smaller and the standard deviation was larger than those of Highbarger et al (mean \pm 2SD, 0.098 ± 0.805), perhaps due to our small sample size. The reason for this discrepancy between our results and those of Highbarger et al. is not immediately apparent.

The detection limit is defined as the minimal concentration which tests positive in more than 90% of repeated measures. The two commercial kits used in this study have detection limits similar to the lower limit of their linear range. Because RT-PCR uses an internal quantitation standard of each individual sample, the minimal detectable concentration is different in each sample. The HIV-1 RNA quantitation test is not intended to be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of HIV-1 infection. Hence, a result of 'undetectable' does not necessarily mean that HIV-1 RNA is not present in plasma. But, according to the MONITOR test package, among 495 anti-HIV-1 negative blood donors, none was found to be reactive with the RT-PCR.¹⁰ And the mean \pm 2SD of absorbance at 450 nm (OD_{450}) was 0.053 ± 0.024 and the maximum was 0.130. The expected background OD_{450} is specified as 0.070 in the MONITOR test package insert. The samples with OD_{450} readings greater than background may actually contain HIV-1 RNA levels that are not quantifiable by the standard RT-PCR assay. One study has reported that although 10 samples chosen by these criteria contained quantifiable HIV-1 RNA levels as determined by the ultrasensitive assay, the viral load level was not associated with OD_{450} of the

samples.¹¹ The MONITOR test package insert indicates that samples which have OD_{450} values less than 0.20 should be regarded as having undetectable HIV-1 RNA levels.¹⁰ The samples that tested as having an undetectable HIV-1 RNA level by RT-PCR also tested undetectable or with a quantifiable level less than the detection limit of RT-PCR by bDNA. But some of the samples which tested undetectable by bDNA actually tested as being detectable by RT-PCR. It is not clear which test is more accurate, nor how this might be determined, but if this discrepancy was due to the false negativities of bDNA, these results highlight/emphasize the disadvantage of bDNA which uses signal amplification without DNA amplification. In one study, the ultrasensitive RT-PCR was able to measure HIV-1 RNA in 10 of the 10 samples tested which were quantified as less than the detection limit of the Quantiplex HIV RNA 2.0 assay (detection limit 500 copies/mL).⁶ In 7 of these 10 samples, the values were greater than 500 HIV-1 RNA copies/mL. It was suggested that at low levels of HIV, different methods may produce different values. In the 5 samples which were above the linear range by bDNA, one sample was quantified with a much smaller value within the linear range by RT-PCR.

HIV-1 has been divided into three divergent phylogenetic groups. Group M is the prevalent group and is subdivided into at least nine subtypes (subtypes A to H and J). HIV-1 groups O and N are both highly divergent and rare. First-generation viral RNA quantitation tests were designed for optimal performance with Group M subtype B, which initially predominated in North America and Europe. Evidence for increasing geographic intermixing of HIV-1 subtypes makes equivalent quantitation of all subtypes essential.¹² HIV-1 subtype variation can have a major influence on viral load quantitation as determined by different methods.¹³ Previous studies showed subtypes B, C, and D were quantified with similar efficiencies by v2 bDNA and v1 RT-PCR; however, v1 RT-PCR was found to be less efficient in quantitating subtypes A, E, and F.⁷ The HIV-1 RNA quantitation tests recently developed or modified are capable of quantifying a broader range of HIV-1 subtypes than the original tests which were developed earlier when less infor-

mation was available on the diversity of HIV-1 nucleic acid sequences.¹² Detection of subtypes A and E has been significantly improved with v1+ and 1.5 RT-PCR compared to that with v1 RT-PCR, whereas detection of subtypes B, C, D, and G was found to be equivalent among all three versions.¹⁴ In Korea subtype B is predominant and the HIV-1 subtype variation seems to have had little influence on this study.

In this study, we observed that both assays show good agreement and have a reliable correlation ($r=0.82$, mean difference in \log_{10} copies/mL ± 2 standard deviation $= 0.038 \pm 1.150$) of determined values as given by the equation: $\log_{10} \text{v3 bDNA} = 0.1228 + 0.9600 \cdot \log_{10} \text{RT-PCR}$. Thus, these two methods may allow direct comparison of the results obtained from different assay systems.

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REFERENCES

1. Natarajan V, Waters D, Dewar RL. Quantitation of viremia in human immunodeficiency virus infection by viral RNA estimation. In: Rose NR, de Macario EC, Folds JD, Lane HC, Nakamura RM editors. *Manual of clinical laboratory immunology*. 5th ed. Washington DC: ASM press; 1997. p.773-80.
2. Izopet J, Salama G, Pasquier C, Sandres K, Kuhlein E, Blancher A, et al. Ultrasensitive detection of plasma HIV-1 RNA for predicting the durability of 3-drug antiretroviral therapy, abstr. 326. In *Program and Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections*. Chicago, Ill., 2 to 4 February 1998.
3. Raboud JM, Rae S, Hogg RS, Yip B, Sherlock CH, Harrigan PR, et al. Suppression of plasma virus load below the detection limit of a human immunodeficiency virus kit is associated with longer virologic response than suppression below the limit of quantitation. *J Infect Dis* 1999;180:1347-50.
4. Sun R, Ku J, Jayakar H, Kuo JC, Brambilla D, Herman S, et al. Ultrasensitive reverse transcription-PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 1998;36:2964-9.
5. Highbarger HC, Alvord WG, Jiang MK, Shah AS, Metcalf JA, Lane HC, et al. Comparison of the Quantiplex version 3 assay and a sensitized Amplicor monitor assay for measurement of human immunodeficiency virus type 1 RNA levels in plasma samples. *J Clin Microbiol* 1999;37:3612-4.
6. Nishida Y, Moriya K, Nagaizumi K, Inaba H, Amano K, Fukue H, et al. Quantitation of human immunodeficiency virus type 1 RNA by ultrasensitive method in the analysis of samples with low viral load. *Rinsho Byori* 2000;48:282-3.
7. Nolte FS, Boysza J, Thurmond C, Clark WS, Lennox JL. Clinical comparison of an enhanced-sensitivity branched-DNA assay and reverse transcription-PCR for quantitation of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 1998;36:716-20.
8. Prud'homme IT, Kim JE, Pilon RG, Minkus T, Hawley-Foss N, Cameron W, et al. Amplicor HIV monitor, NASBA HIV-1 RNA QT and quantiplex HIV RNA version 2.0 viral load assays: a Canadian evaluation. *J Clin Virol* 1998;11:189-202.
9. Segondy M, Izopet J, Pellegrin I, Montes B, Dumon B, Pasquier C, et al. Comparison of the QUANTIPLEX HIV-1 RNA 2.0 assay with the AMPLICOR HIV-1 MONITOR 1.0 assay for quantitation of levels of human immunodeficiency virus type 1 RNA in plasma of patients receiving stavudine-didanosine combination therapy. *J Clin Microbiol* 1998;36:3392-5.
10. Roche Diagnostic Systems. AMPLICOR HIV-1-MONITOR test package insert. Brachburg; Roche Diagnostic Systems; 1996. p.9.
11. Erali M, Hillyard DR. Evaluation of the ultrasensitive Roche Amplicor HIV-1 monitor assay for quantitation of human immunodeficiency virus type 1 RNA. *J Clin Microbiol* 1999;37:792-5.
12. Jagodzinski LL, Wiggins DL, McManis JL, Emery S, Overbaugh J, Robb M, et al. Use of calibrated viral load standards for group M subtypes of human immunodeficiency virus type 1 to assess the performance of viral RNA quantitation tests. *J Clin Microbiol* 2000;38:1247-9.
13. Parekh B, Phillips S, Granade TC, Baggs J, Hu DJ, Respass R. Impact of HIV type 1 subtype variation on viral RNA quantitation. *AIDS Res Hum Retroviruses* 1999;15:133-42.
14. Triques K, Coste J, Perret JL, Segarra C, Mpoudi E, Reynes J, et al. 1999. Efficiencies of four versions of the AMPLICOR HIV-1 MONITOR test for quantification of different subtypes of human immunodeficiency virus type 1. *J Clin Microbiol* 1999;37:110-6.