Modified Boosted - p24 Antigen can Predict HIV-1 RNA Viral Load (Ampiclor HIV-1 Monitor)

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ABSTRACT

Objective: To evaluate the predictive value of HIV-1 RNA viral load (Ampiclor) in log-copies per ml by each level of modified boosted-p24 antigen in log-fg per ml.
Methods: 283 plasma samples were collected and blindly determined HIV-1 RNA Amplicor Monitor, Roche as a standard test with modified boosted p24 antigen assay. Likelihood ratio positive analyses of multiple levels of p24 were calculated as well as the post-test probability in predicting the amount of virus (log-copies/ml)
Results: Subjects were between 18 to 73 years old with the range of virus 1.75 to 5.92 log-copies per ml. By the calculation of likelihood ratio positive and positive predictive value, it was demonstrated that modified boosted p24 antigen (Ag in log-fg per ml) might predict the viral load (VL) in log-copies per ml as follow:

Ag 2.0-3.0 log-fg per ml corresponding to 2.0 or lower log-copies per ml VL
Ag 3.0-3.5 log-fg per ml corresponding to 2.5 or lower log-copies per ml VL
Ag 3.5-4.0 log-fg per ml corresponding to 3.5 or lower log-copies per ml VL
Ag 4.0-over log-fg per ml corresponding to 4.5 or lower log-copies per ml VL

Conclusions: In countries where financial resources are limited, modified p24 antigen may be clinically applied in antiretroviral management program, instead of HIV-1 RNA Amplicor Monitor, Roche.

Keywords: HIV-1; Boosted-p24 antigen; Viral load; Prediction Likelihood ratio positive

HIV-1 RNA viral load was one of the essential laboratory tools to monitor disease progression. Decisions to start antiretroviral therapy (ART) as well as therapeutic responses were based on the amount of viral load.[1-4] Ampiclor HIV-1 monitor was a gold standard test, but it was labor intensive, time consuming and expensive ($100 per test). p24 antigen assay measures viral capsid (core) p24 protein. With the enzyme immunoassay method, only free form of p24 Ag could be detected as early as 2 weeks from the beginning of infection through 3 to 5 months. When the body responded with p24 antibody, antigen-antibody complex was bound and the regular p24 antigen assay was not sensitive enough for clinical application[5]. Even pre-treatment of sample with acid to dissociate the complex the sensitivity remained low.[6]

The modified boosted-p24 antigen assay was a modification of a regular p24 antigen assay (Organon Teknika) by using preheated immune complex dissociation combined with a booster step amplified signal of ELISA with biotinyl tyramide to increase to the lower limit of p24 antigen detection (0.5 pg/ml).

Besides the higher stability of the antigen and the property of being less affected by variation in time and physical conditions (freeze/thaw), the test could be performed with a simple preparation and equipment at a lower cost ($3 per test). Additionally, antigen testing might be less affected by genetic diversity of the virus (B or E). Although p24 antigen did not specifically identify live virus but it did identify viral replication. In the past, before the availability of viral load testing, p24 antigen assay was used extensively for monitoring the development of AIDS and for charting disease progression. Currently, the new and highly improved modified boosted-p24 antigen assay might be nearly sensitive and accurate as HIV-1 RNA viral load.

MATERIALS AND METHODS

Two hundred and eighty-three plasma samples were collected from HIV infected adult subjects between November 1999 and April 2001. In each sample, a blind determination was performed on both the number of virus (by Ampiclor HIV Monitor test, version 1.5 Roche Diagnosis Systems, NJ, USA) and the amount of p24 antigen (by modified boosted p24 antigen assay). The number of HIV virus was ranged from 400 to 750,000 copies/ml.

The modified boosted p24 antigen assay was employed by a modification of Vironostika® HIV-1 Antigen assay (Organon Teknika, NJ, USA) and ELAST ELISA.[4-5]

The heat-denatured immune complex dissociation p24 antigen and amplified signal of ELISA assay with biotinyl tyramide were able to increase the lower limit of p24 antigen detection. The plasma was diluted with 0.5% Triton X-100 to a concentration of 1:6. The diluted plasma was heated at 100°C for 5 minutes. A known concentra-

TABLE 1. Sample calculation of the likelihood ratio positive for several levels of p24 antigen against a viral load over or under 4.

→VL Ampiclor log-copies/ml / p24 antigen log-fg/ml	Viral Load (Ampiclor) log-copies/ml > 5.5 Number	> 5.5 Proportion	< 5.5 Number	< 5.5 Proportion	Likelihood ratio positive	95% CI
>4	4	4/26=0.15	5	5/257=0.02	0.15/0.02=7.91	1.27→14.54
3.5→4	2	2/26=0.08	19	19/257=0.07	0.08/0.07=1.04	-4.02→6.10
3.0→3.5	11	11/26=0.42	51	51/257=0.20	0.42/0.20=2.13	1.25→3.02
2.5→3.0	9	9/26=0.35	108	108/257=0.42	0.35/0.42=0.82	-0.10→1.75
<2.5	0	0/26=0	74	74/257=0.29	0.0/0.29=0	0
Total No.	26		257			

tion of p24 antigen varying from 0-80 pg/ml was used as a positive control. All samples (100 microliters in each well) were incubated at 37°C for 60 minutes. After washing with a diluted phosphate buffer (PBS), 100 microliters of anti HIV-1 (human) conjugate labeled with horseradish-peroxidase was added to each well, and incubated at 37°C for 60 minutes. The S-HRP conjugate (Streptavidin-horseradish peroxidase) was used at a dilution of 1:500. After washing, 100 microliters of Biotynyl-Tyramide (B-T) working solution from the ELAST ELISA Amplification System® was added to each well and incubated at room temperature for 60 minutes. At that point 100 microliters of diluted S-HRP from the ELAST ELISA Amplification system® was diluted to 1:500 using 1% bovine serum albumin phosphate between 20 (1% BSA-PBS-T) which was added to each well after washing step and incubated at room temperature for 30 minutes. Then, 100 microliters of tetramethyl benzidine substrate in urea peroxide solution was added to each well after the washing step and incubated at room temperature for 30 minutes. Finally, the color reaction was stopped by adding 100 microliters of 1 M sulfuric acid. The absorbance of each well was read at 450 mm within 15 minutes by the ELISA reader and resulted in an optical density (OD) value. The OD value was converted to log p24 antigen in fg/ml by a standard curve with a range of 500 to 80,000 fg/ml.

The likelihood ratio positive of 5 different levels of modified boosted p24 antigen (<2.5, 2.5→3.0, 3.0→3.5, 3.5→4.0 and >4.0 log-fg /ml) against the different levels of the amount of virus (< 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 log-copies/ml) were calculated with a 95% confidence interval in the same method which has been described by David L. Sackett and collegues.[7] Table 1 is an example of the likelihood ratio positive calculation of p24 antigen predicting viral load (Ampiclor) of over or under 4.

RESULTS

The 283 sera were collected from HIV-1 subjects with an age ranging from 18 to 73 years old. The amount of viral load was between 1.75 and 5.92 log-copies/ml. The correlation coefficient between modified boosted p24 antigen and viral load Ampiclor was 0.5. Linear regression analysis resulted in the equation of viral load in log-copies/ml = 1.14+1.05 (boosted p24 antigen in log-fg/ml). The likelihood ratio positive of modified boosted p24 antigen at each level is displayed in Table 2 and Table 3.

Five different pretest probabilities (5, 25, 50, 75 and 95) were constructed for calculating post-test probabilities of each level of modified boosted p24 antigen, and displayed in Fig 1. (a to e) below. From those figures, for each pretest probability, the prediction of almost 100%

TABLE 2. 95% confidence interval (CI) of likelihood ratio positive of modified boosted p24 antigen in predicting viral load. (≥ 5.5 - ≥ 4.0)

→VL Ampiclor log-copies/ml / ↓modified boosted p24 Ag log-fg/ml	≥5.5 LR+	≥5.5 95% CI	≥5.0 LR+	≥5.0 95% CI	≥4.5 95% CI	≥4.5 LR+	≥4.0 95% CI	≥4.0 LR+
>4	7.91	1.27→14.54	25.29	-3.44?54.03	∞	∞	∞	∞
3.5→4	1.04	-4.02→6.10	1.95	-0.92→4.81	1.35	-1.70→4.40	2.22	-2.71→6.62
3.0→3.5	2.13	1.25→3.02	2.6	1.71→3.50	3.00	1.66→4.34	3.61	1.55→5.67
2.5→3.0	0.82	-0.10→31.75	0.82	0.23-1.41	0.89	0.44→1.33	0.87	0.44→1.30
<2.5	0	0	0	0	0.29	-1.24→1.81	0.35	0.64→1.34

TABLE 3. 95% confidence interval (CI) of likelihood ratio positive of modified boosted p24 antigen in predicting viral load. (≥ 5.5 - ≥ 4.0)

→VL Ampiclor log-copies/ml / ↓modified boosted p24 Ag log-fg/ml	≥3.5		≥3.0		≥2.5		≥2.0	
	LR+	95% CI	LR+	95% CI	95% CI	LR+	95% CI	LR+
>4	∞	∞	∞	∞	∞	∞	∞	∞
3.5→4	∞	∞	∞	∞	∞	∞	∞	∞
3.0→3.5	6.44	1.95→10.92	17.83	2.26→33.39	∞	∞	∞	∞
2.5→3.0	0.76	0.34→1.18	0.63	0.23-1.03	1.11	0.05→2.16	∞	∞
<2.5	0.38	-0.43→1.18	0.54	-0.17→1.25	0.38	-0.27→1.03	∞	∞

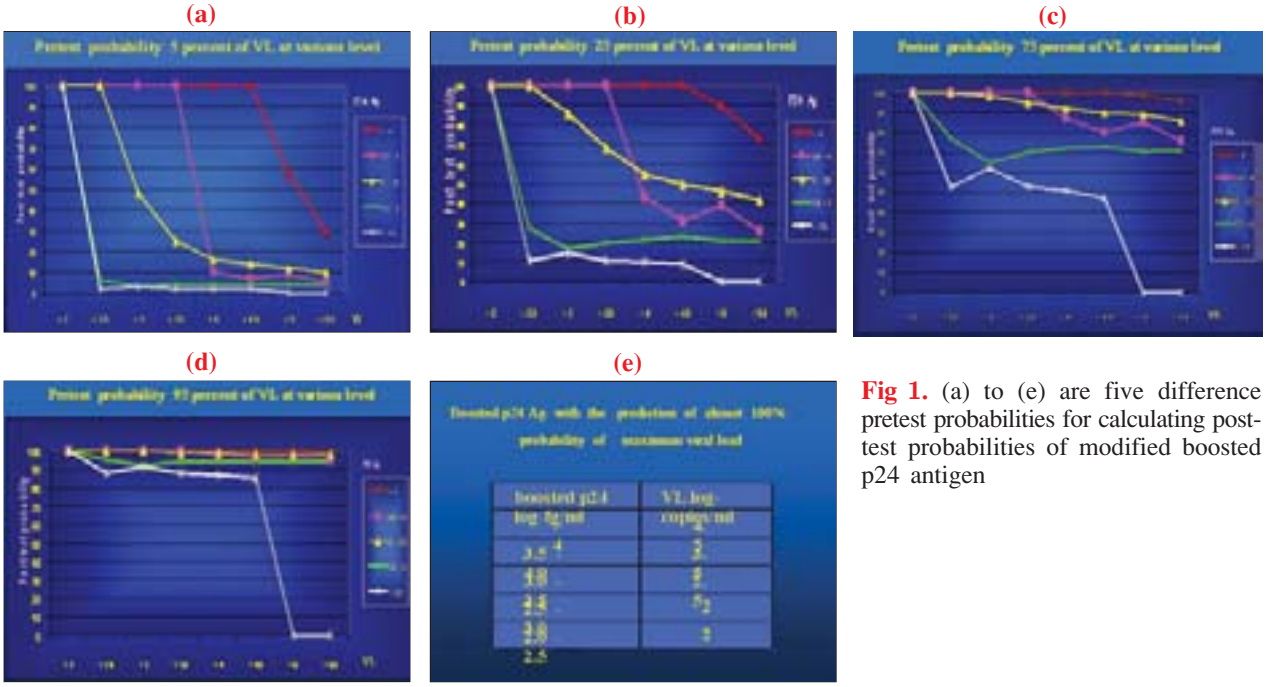


Fig 1. (a) to (e) are five difference pretest probabilities for calculating post-test probabilities of modified boosted p24 antigen

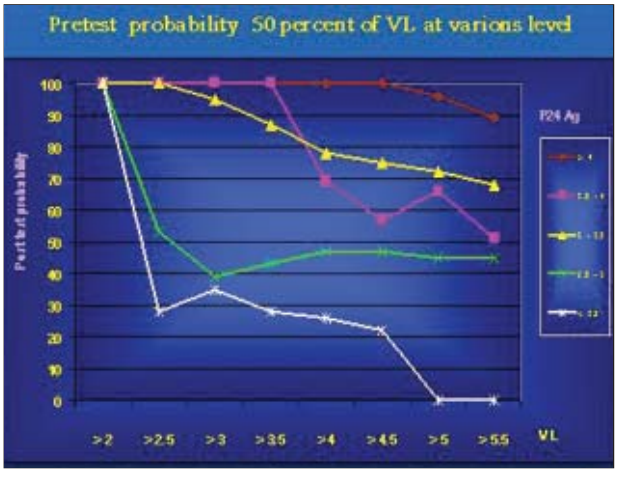


Fig 2. Modified boosted p24 antigen with the prediction of almost 100% probability of corresponding viral load

probability of viral load by modified boosted p24 antigen was shown, as displayed in Fig 2.

DISCUSSION

HIV-1 RNA quantification is an assay used to determine the diagnosis, and it helps in the decision to start or change antiretroviral therapy. In Thailand, the adult guidelines for initiating antiretroviral drugs recommended that the amount of virus be over 30,000 to 50,000 copies per milliliter of plasma. The AIDS Clinical Trial Group (ACTG) has recommended that a sustained 50% drop in p24 antigen concentration be considered a response to the antiretroviral drug. Conversely persistent elevation of p24 antigen may indicate a lack of antiretroviral drug efficacy.[8]

Unfortunately, HIV-1 RNA quantitative assay (Amplicor HIV-1 Monitor, Roche) is an expensive test for countries with limited financial resources, such as Thailand. It costs around $100 per test in addition to the cost of expensive antiretroviral drugs.

Physicians in Thailand are only able to request the test for research projects, and not for their actual clinical service.

Modified boosted p24 antigen costs only $3 per test. However, the correlation coefficient between the modified boosted p24 antigen and viral load was not perfect. Therefore the prediction of viral load by regression equation would not be appropriate by modified boosted p24 antigen. However, the analysis by likelihood ratio positive of each level of p24 antigen is able to predict the amount of virus, because this method does not depend on linearity. The results are shown in Table 2. Clinical application of this test, instead of HIV-1 RNA Ampiclor, appears to be justified in countries like Thailand, with the expectated consequences being more appropriate use of antiretroviral drugs, which ultimately leads to patient benefits.

A limitation of this study was the variability of p24 antigen and the amount of virus. The number of specimens with extreme upper or lower values was too small. No data of very high viral loads up to log 6 copies/ml that is usually seen in infected children, was available in this study. Extrapolation may not be guaranteed the prediction of p24 antigen at very high viral loads. A second limitation is that all the data were cross-sectional. Extrapolation in the change of p24 antigen to predict the change in viral load may be inadequate. Longitudinal data and infected children with high viral loads are needed for further study.

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บทคัดย่อ

การวัดค่าพี 24-แอนติเจน โดยวิธีดัดแปลง สามารถทำนายปริมาณเชื้อไวรัสอาร์-เอ็น-เอ ของโรคภูมิคุ้มกันบกพร่อง

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จุดประสงค์: เนื่องจากการตรวจหาเชื้อไวรัส-อาร์-เอ็น-เอ ของโรคภูมิคุ้มกันบกพร่อง ยังมีราคาแพง ค่าพี-24-แอนติเจน โดยดัดแปลงจากวิธีธรรมดา ทำได้ง่าย และราคาถูกกว่า จะสามารถนำมาใช้ทดแทนกันได้หรือไม่

วิธีการ: เป็นการศึกษาแบบตัดขวาง (cross-sectional) โดยตัวอย่างพลาสมาของผู้ป่วยโรคภูมิคุ้มกันบกพร่องในผู้ใหญ่ 283 ราย นำมาหาค่า ปริมาณไวรัส อาร์-เอ็น-เอ โรคภูมิคุ้มกันบกพร่อง วัดโดยวิธีแอมปลิคลอของบริษัท โรช มีหน่วยเป็น ล็อก-ค็อบปี้ ต่อมิลลิตร เปรียบเทียบกับค่า พี-24-แอนติเจน วัดโดยวิธีดัดแปลงจากธรรมดา มีหน่วยเป็น ล็อก-แฟมโตกรัมต่อมิลลิลิตร โดยผู้ทำการวัดหาค่าปริมาณไวรัส-กับ-ปริมาณแอนติเจนไม่ทราบผลของการประเมินของแต่ละกระบวนการ

ผล: ผู้ป่วยมีอายุระหว่าง 18 ถึง 73 ปี มีปริมาณ อาร์-เอ็น-เอ ไวรัส 1.75-5.92 ล็อก-ค็อบปี้ ต่อมิลลิลิตร

ผลการวิเคราะห์แบบสมการทดถอยเป็นเส้นตรงโดยปริมาณไวรัสเป็นตัวแปรอิสระ และปริมาณแอนติเจนเป็นตัวแปรตาม จะได้สมการ

ปริมาณไวรัส = 1.14+1.05 (พี-24-แอนติเจน)

การประเมินโดยคำนวณ likelihood ratio positive และหาค่า positive predictive value พบว่า

ค่า พี-24-แอนติเจน 2.0-3.0	จะทำนายไวรัส 2.0 หรือต่ำกว่า
ค่าพี-24-แอนติเจน 3.-3.5	จะทำนายไวรัส 2.5 หรือต่ำกว่า
ค่าพี-24-แอนติเจน 3.5-4.0	จะทำนายไวรัส 3.5 หรือต่ำกว่า
ค่าพี-24-แอนติเจน 4.0-มากกว่า	จะทำนายไวรัส 4.5 หรือต่ำกว่า

สรุป: ค่า พี-24 แอนติเจน สามารถทำนายปริมาณ อาร์-เอ็น-เอ ไวรัส ได้ดี แต่การติดตามการเปลี่ยนแปลงของค่า พี-24 แอนติเจน เมื่อได้รับยาต้านไวรัสแล้ว จะทำนายการเปลี่ยนแปลงของปริมาณ อาร์-เอ็น-เอ ไวรัสได้หรือไม่ ยังคงต้องมีการศึกษาแบบติดตาม (longitudinal study) เพื่อยืนยันต่อไป