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ORIGINAL ARTICLE

Evaluation of performance of human immunodeficiency virus antigen/antibody combination assays in Taiwan[☆]



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Abstract *Background:* The fourth-generation human immunodeficiency virus (HIV) combination assay, which can simultaneously detect the presence of anti-HIV antibody and HIV antigen, has been shown to shorten the window period in HIV diagnosis compared with the third-generation HIV antibody immunoassay. This study was aimed to determine the performance of HIV combination assays in Taiwan, where the HIV-1 seroprevalence is 0.007% and HIV-2 infection has never been reported.

Methods: Performance of three fourth-generation HIV Ag/Ab combination assays (Dia.Pro, Wantai, and Bio-Rad) and one third-generation HIV Ab immunoassay (AxSYM HIV 1/2 gO) was assessed.

Results: A total of 152 specimens, including 86 confirmed HIV-seropositive and 66 HIV-seronegative samples, were used in the study. The sensitivity of four assays varied from 98.8%

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to 100%, and specificity varied from 98.5% to 100%. Performance of the 75 equivocal samples, the HIV status of which was confirmed later, in terms of negative prediction varied from 81.8% to 87.5%. The Bio-Rad and Dia.Pro assays exhibited higher sensitivity for the detection of p24 antigen among the three fourth-generation HIV combination assays.

Conclusion: The three fourth-generation HIV Ag/Ab combination assays exhibited better sensitivity, specificity, and negative prediction than the third-generation HIV Ab immunoassay.

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Introduction

Human immunodeficiency virus (HIV), the causative agent of AIDS, can be transmitted through sexual transmission, intravenous drug use (IDU), blood transfusion, and maternal vertical transmission.¹ According to the Joint United Nations Program on HIV/AIDS, it has been shown that, in 2013, about 35 million adults and children were infected and living with HIV, and 1.5 million people died of AIDS around the world.² By the end of 2014, 29,665 people were living with HIV in Taiwan and about 4600 people died of AIDS.³ The HIV-1 seroprevalence is about 0.007%, and HIV-2 infection has never been reported in Taiwan.⁴ Prior to 2004, sexual transmission was the major route of HIV infection in Taiwan, with subtype B circulating among men who have sex with men (MSM), and subtypes B and CRF01_AE in patients infected through heterosexual transmission.^{5,6} An outbreak of CRF07_BC among intravenous drug users was observed in 2004,^{7,8} and its spread was rapidly controlled by the harm reduction program initiated in 2005 by the Taiwan Centers for Disease Control.⁹ By the end of 2014, the cumulative proportion of HIV transmission routes in Taiwan was 47.37% for MSM, 23.78% for IDU, and 18.53% for heterosexual behavior.³ In addition, an increase of new HIV infections in MSM, accompanied by a decrease of that among IDUs, was observed.^{4,9}

Previous studies have strongly suggested that an early use of combined highly active antiretroviral therapy (HAART) can significantly reduce sexual transmission of HIV, which consequently has a preventive effect on HIV infection.^{10,11} Therefore, an early diagnosis of HIV infection followed by antiretroviral therapy is critical for disease control. The principles of HIV serodiagnosis were mainly based on enzymatic immunoassay, by detecting the presence of anti-HIV antibody. The early first- and second-generation HIV immunoassays were designed to capture human anti-HIV immunoglobulin G (IgG) antibody by HIV viral lysate and HIV recombinant antigens, respectively.¹² The third-generation HIV immunoassay showed improved ability to detect both human anti-HIV IgM and human anti-HIV IgG antibodies.^{12,13} Nevertheless, during acute HIV infection, many patients are asymptomatic, and antibodies against HIV are absent in spite of the presence of HIV viral antigens (e.g., p24) and viral RNA in blood.^{12,14} This period is the so-called "window period" or "seroconversion window." Thus, fourth-generation HIV Ag/Ab combination immunoassays, which can simultaneously detect the presence of anti-HIV

antibodies and HIV antigens, have been expected to shorten the window period.^{15–19} Currently, several commercial fourth-generation combination assays are available and used worldwide.^{19,20} Many studies compared the performance of fourth-generation combination assays with that of third-generation HIV immunoassays, and tried to validate the application of the fourth-generation assays in clinical use.^{13,15,21,22} The reports showed that most fourth-generation assays presented high sensitivity and specificity, and could detect seroconversion more than 2 days earlier than third-generation assays, leading to a reduction of the seroconversion window.^{13,15,23} However, the detection sensitivity of p24 antigen varied significantly between different fourth-generation combination assays.^{13,15,21,22}

In Taiwan, where the HIV-1 seroprevalence is about 0.007% and the third-generation HIV immunoassays are the major HIV screening tests used in most hospitals and voluntary HIV counseling and testing sites, the performance of the fourth-generation combination assays is unclear and needs to be evaluated. In this study, we evaluated the sensitivity, specificity, and negative predictive value of three fourth-generation combination assays (Dia.Pro, Wantai, and Bio-Rad) and compared with those of one third-generation HIV immunoassay (AxSYM HIV 1/2 gO). Hence, this study was aimed to provide some practical advices on HIV diagnostic setting in Taiwan.

Materials and methods

Specimen collection

The study specimens included 86 HIV-seropositive specimens, 66 HIV-seronegative specimens, and 75 specimens with equivocal results by HIV screening test. The 86 plasma samples, which were confirmed for HIV-1 infection by western blot assay (New LAV Blot I; Bio-Rad, Marnes La Coquette, France), were provided by Centers for Disease Control in Taiwan. Sixty-six HIV-seronegative plasma samples were found to be negative by a routine HIV screening test (AxSYM HIV 1/2 gO; Abbott GmbH & Co. KG, Wiesbaden-Delkenheim, Germany). Sensitivity and specificity were evaluated using these 86 HIV-1-seropositive and 66 HIV-1-seronegative samples. The HIV status of 75 plasma samples with equivocal results in HIV screening test was later confirmed by HIV RNA viral load using the Cobas AmpliCor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics

Corporation, Indianapolis, IN, USA), and/or by HIV western blot assay at later time points with a median follow-up period of 37 days (range 5–247 days).

Third-generation HIV antibody assay

AxSYM HIV 1/2 gO (Abbott GmbH & Co. KG), the third-generation HIV antibody assay used in this study, can detect HIV antibodies against HIV-1 group M, HIV-1 group O, and HIV-2 by a three-step microparticle enzyme immunoassay. Anti-HIV antibodies, including human IgG and IgM antibodies, are captured in samples by recombinant HIV antigen-coated microparticles. Captured antibodies are bound to biotin-labeled recombinant HIV antigens and peptides, and detected by an antibiotin alkaline phosphatase conjugate. All tests were performed and interpreted in accordance with the manufacturer's recommendations. The principles and procedures of this assay have been described previously.¹³

Fourth-generation HIV combination assays

The fourth-generation HIV combination assays evaluated in this study included the following: HIV (1+2) Ag&Ab (Beijing Wantai Bio-pharm, Beijing, China), HIV Combination Ag/Ab enzymatic immunoassay (Bio-Rad Laboratories) and HIV Ab&Ag [Dia.Pro, Sesto San Giovanni (MI), Italy]. These assays use a "sandwich" format technique for the detection of anti-HIV antibodies. Anti-HIV antibodies are captured by recombinant HIV antigen-coated microwells. Captured antibodies are bound to peroxidase-conjugated recombinant HIV antigens and peptides. The simultaneous detection of p24 antigen is achieved by coating anti-p24 antibodies on the microwells. The captured p24 antigen is bound to a second biotin-labeled antibody, and detected by an antibiotin peroxidase conjugate. These assays do not distinguish between anti-HIV antibodies and HIV antigens present in a sample. All tests were performed and interpreted in accordance with the manufacturer's recommendations. The principles and procedures of these assays have been described previously.^{17,24}

Detection of p24 antigen

To evaluate the ability of the fourth-generation HIV combination assays to detect p24 antigen, the p24 positive control of each fourth-generation HIV combination assay was serially diluted and subsequently detected by three fourth-generation combination assays. For each panel of serially diluted p24 antigen, the highest dilution which could be detected by the assays was determined.

Statistical analysis

All statistical analyses were performed using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Categorical variables were compared using χ^2 or Fisher's exact test, whereas noncategorical variables were compared using Student *t* test or Mann–Whitney *U* test. All tests were two tailed and a *p* value of <0.05 was considered significant.

Results

Sensitivity and specificity

The sensitivity and specificity of these four assays were determined using the 86 confirmed HIV-seropositive samples and 66 HIV-seronegative samples (Table 1). The third HIV antibody immunoassay, AxSYM HIV 1/2 gO, showed 100% sensitivity and 100% specificity. Among the three fourth-generation combination assays, the sensitivity varied from 98.8% to 100% and specificity from 98.5% to 100%. The Dia.Pro combination assay showed 100% sensitivity and 100% specificity. The Wantai assay presented lower sensitivity (98.8%), while the Bio-Rad assay presented lower specificity (98.5%). In summary, the Dia.Pro combination assay exhibited better sensitivity and specificity among the three fourth-generation HIV combination assays.

Performance of HIV immunoassays in terms of early prediction

Interpretation of test results in most HIV immunoassays is based on the ratio of signal to cutoff (*S/CO*). Samples tested with *S/CO* values ≥ 1.00 are considered reactive, and samples with *S/CO* values <1.00 are considered negative. However, there usually are some samples with *S/CO* values very close to 1.00. Samples with equivocal results pose some difficulty in clinical interpretation, and HIV infections with a low antibody titer, which can probably present an equivocal *S/CO* value of <1.00, may be regarded as seronegative HIV infections.

Therefore, the ability of these four assays in distinguishing 75 samples with equivocal results was evaluated. Furthermore, since the HIV status of these 75 samples was confirmed by HIV viral load or antibody responses at later time points, we were able to compare the performance of these four assays on early prediction. The results of 75 equivocal samples detected by four assays are summarized in Table 2. The samples tested by AxSYM HIV 1/2 gO included 57 reactive and 18 negative samples, with the mean *S/CO* values of 1.30 and 0.94, respectively. More than 70% of 75 equivocal samples showed negative results when tested by the fourth-generation combination assays: 57 samples (mean *S/CO* = 0.16) in Dia.Pro, 56 samples (mean *S/CO* = 0.01) in Wantai, and 58 samples (mean *S/CO* = 0.34) in Bio-Rad (Table 2). In contrast to AxSYM HIV 1/2 gO, the fourth-generation combination assays seemed to unambiguously differentiate between reactive and negative samples, since the *S/CO* values of reactive and negative samples were significantly discriminated and far from 1.00 (Figure 1). In the 2×2 χ^2 analysis, the difference between the fourth-generation assays and the third-generation assay was statistically significant by the McNemar's test (supplementary Table 1.)

Among these 75 equivocal samples, seven were confirmed for HIV infection by HIV western blot assay or HIV RNA viral load at later time points (median follow-up period 34 days, range 22–114 days), and 50 samples were confirmed as HIV negative at later time points (median follow-up period 40 days, range 5–247 days). The remaining 18 samples for which the HIV status was not confirmed later

Table 1 Performance of four assays on 86 HIV-1-positive samples and 66 HIV-1-negative samples

	Third-generation Ab assay		Fourth-generation Ag/Ab assay					
	AxsYM HIV 1/2 gO		Dia.Pro		Wantai		Bio-Rad	
	R	N	R	N	R	N	R	N
HIV positive (<i>n</i> = 86)	86	0	86	0	85	1	86	0
HIV negative (<i>n</i> = 66)	0	66	0	66	0	66	1	65
Sensitivity (95% CI), %	100 (95.8–100)		100 (95.8–100)		98.8 (93.7–99.9)		100 (95.8–100)	
Specificity (95% CI), %	100 (94.6–100)		100 (94.6–100)		100 (94.6–100)		98.5 (91.8–99.9)	

Ab = antibody; Ag = antigen; CI = confidence interval; HIV = human immunodeficiency virus; N = negative; R = reactive.

were excluded from subsequent analyses. Performance, in terms of early prediction, of these four assays on the 57 equivocal samples was evaluated (Table 3). The positive prediction values the four assays ranged from 5.6% to 11.8%. All three fourth-generation combination assays showed better negative prediction than AxsYM HIV 1/2 gO (81.8%), and the Bio-Rad assay showed the best performance (87.5%). These results suggested that the fourth-generation combination assay had better performance on early negative prediction, which might lead to less false-negative interpretation.

Detection of p24 antigen

The most distinct difference between the third-generation HIV antibody immunoassay and fourth-generation HIV combination assay is the detection of p24 antigen in the fourth-generation HIV combination assay.¹⁷ With this characteristic design, the fourth-generation combination assay is expected to shorten the window period, since p24 antigen is released prior to the production of anti-HIV antibody in acute HIV infection.^{12,14} Owing to the detection of both viral antigens and antibodies by fourth-generation combination assays, several previous studies evaluated the p24 detection sensitivity of these assays and showed that the sensitivity in detecting p24 antigen varied significantly between different combination assays.^{13,15,21,22} Here, the capability of these three fourth-generation combination assays to detect p24 antigen was also evaluated (Table 4). The p24 positive control of each combination assay was twofold serially diluted. Each panel of serially diluted p24 positive control was tested by the three combination assays, and the end point was determined by the highest detectable dilution. Bio-Rad and Dia.Pro combination assays showed better performance than Wantai in two panels of diluted p24 antigen. The three combination assays

exhibited the same ability to detect Wantai p24 antigen. In summary, Dia.Pro and Bio-Rad showed better performance in detecting p24 antigen.

Discussion

In this study, we compared the sensitivity and specificity of three fourth-generation HIV combination assays with one third-generation HIV antibody immunoassay. In addition, performance on early prediction and p24 antigen detection sensitivity of the three fourth-generation combination assays were evaluated. Our results suggested that among the three fourth-generation HIV combination assays, the Dia.-Pro combination assay exhibited both better sensitivity and better specificity, whereas Wantai and Bio-Rad assays showed lower sensitivity and lower specificity, respectively. Nevertheless, the fourth-generation combination assay had better performance on early negative prediction, which might result in a reduction of false-negative interpretation.

During acute HIV infection, anti-HIV antibodies are often produced during the first 3–5 weeks after infection.^{13,25,26} In most HIV antibody immunoassays, low anti-HIV antibody response at the initiation of acute HIV infection might cause equivocal results, which often leads to an ambiguity in data interpretation and difficulty in clinical diagnosis. Hence, acute HIV infections with low or developing antibody response might be missed by HIV antibody immunoassays due to the results that are equivocal but <1.00. Consequently, this could probably lead to a delayed treatment and an increased risk of transmission of HIV.¹¹ Therefore, with the combined detection of p24 antigen, which is a viral core protein often detectable prior to antibodies, the fourth-generation combination assays may exhibit better ability in reducing the false-negative interpretation. As it has been shown in this study, the fourth-generation combination assays significantly discriminated

Table 2 Results of 75 equivocal samples when tested by four assays

	Third-generation Ab assay		Fourth-generation Ag/Ab assay		
	AxsYM HIV 1/2 gO		Dia.Pro	Wantai	Bio-Rad
Reactive	57		18	19	17
Mean S/CO (95% CI)	1.30 (1.23–1.36)		10.81 (7.46–14.16)	4.17 (3.21–5.14)	5.08 (3.74–6.42)
Negative	18		57	56	58
Mean S/CO (95% CI)	0.94 (0.92–0.95)		0.16 (0.14–0.18)	0.05 (0.01–0.09)	0.34 (0.27–0.41)

Ab = antibody; Ag = antigen; CI, confidence interval; HIV = human immunodeficiency virus; S/CO = ratio of signal to cutoff.

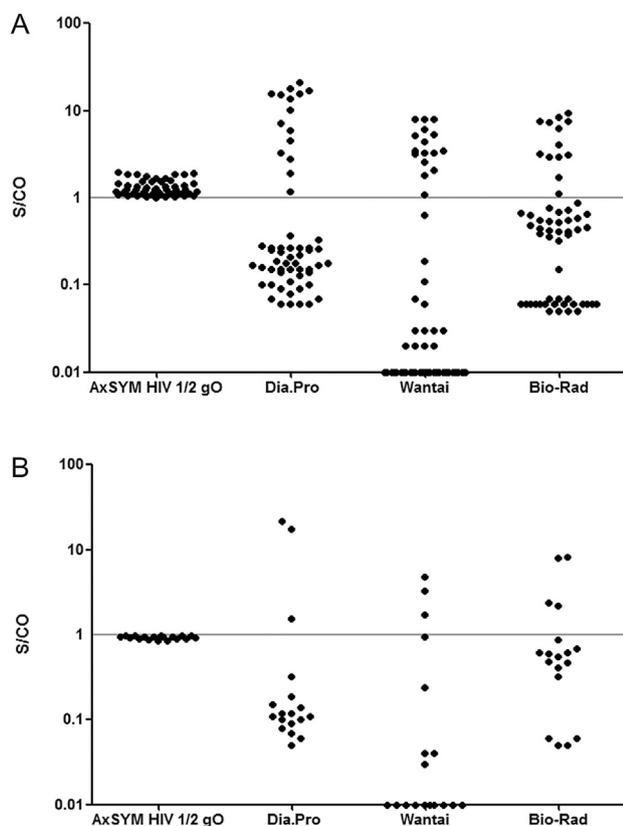


Figure 1. Distinct S/CO distribution of 75 equivocal samples by the fourth-generation combination assays. (A) S/CO distribution of samples ($n = 57$), which showed S/CO values ≥ 1.00 by AxSYM HIV 1/2 gO, using the fourth-generation combination assays. (B) S/CO distribution of samples ($n = 18$), which showed S/CO values < 1.00 by AxSYM HIV 1/2 gO, using the fourth-generation combination assays. HIV = human immunodeficiency virus; S/CO = signal to cutoff ratio.

between positive and negative samples among the 75 samples with equivocal readings when tested by the third-generation assay. Furthermore, with the HIV status being confirmed at later time points, we could determine the performance of these assays for early prediction. As shown in Table 3, the fourth-generation combination assays showed better negative prediction, leading to a reduction of false-negative interpretation. On the other hand, the low positive prediction (5.6–11.8%) presented by the four

Table 3 Performance of four assays on 57 equivocal samples

	AxSYM HIV 1/2 gO	Dia.Pro	Wantai	Bio-Rad
Sensitivity	71.4	14.2	28.6	28.6
Specificity	18	66	66	70
Positive prediction	10.9	5.6	10.5	11.8
Negative prediction	81.8	84.6	86.8	87.5

Data are presented as percentage.
HIV = human immunodeficiency virus.

Table 4 Ability of three fourth-generation HIV combination assays to detect p24 antigen

	Dia.Pro	Wantai	Bio-Rad
Highest dilution detectable			
Dia.Pro p24 Ag	2 \times	1 \times	2 \times
Wantai p24 Ag	32 \times	32 \times	32 \times
Bio-Rad p24 Ag	8 \times	2 \times	8 \times

HIV = human immunodeficiency virus.

assays demonstrate that additional tests are essential for the confirmation of diagnosis and reduction of false-positive interpretation.

Due to the variation in p24 antigen detection sensitivity of different combination assays, which was reported by several previous studies,^{13,15,21,22} we also compared the p24 detection sensitivity of the three combination assays by detecting serially diluted p24 antigen. Both Dia.Pro and Bio-Rad assays showed better performance in detection of p24 antigen. It is interesting that each combination assay exhibited a different ability to detect different panels of p24 antigen, in which the highest dilution detectable ranged from one- to 32-fold. This might result from, in different combination assays, the varied design of the anti-p24 antibodies or the recombinant p24 antigen used as an antigen positive control, leading to different affinities.

Since the availability of the first fourth-generation HIV combination assay in the late 2000s,^{17,27} several studies have investigated the performance of diverse combination assays and tried to validate their applications in clinical use. The results of some previous studies that evaluated these assays are briefly summarized in Table 5. In general, the fourth-generation combination assays showed sensitivity as high as 100%. Early reports indicated that, with higher complexity than single marker antigen or antibody assays, the fourth-generation combination assays might compromise their specificity.^{17,18} Nevertheless, among previous studies summarized in Table 5, the specificity between different combination assays could vary from 98.0% to 99.9%, which was similar to that of third-generation immunoassays (varied from 98.0% to 100%).^{13,15} The three fourth-generation combination assays evaluated in this study also showed specificity within the range described above, with the exception of the Wantai combination assay presenting lower sensitivity (98.8%). The lower sensitivity of the Wantai combination assay could be correlated with its lower sensitivity for p24 antigen detection.¹⁵ However, in spite of the significantly varied sensitivity for p24 antigen detection and seroconversion between different combination assays, the fourth-generation combination assays were still proven to reduce the seroconversion window by early detection of HIV infection.^{13,21,28} Thus, standards for comparison of different combination assays were recommended for validation on site.

In Taiwan, the HIV-1 seroprevalence is about 0.007%, which is relatively low in comparison with other high-prevalence areas in the world. On the other hand, HIV-2 infection has never been reported in Taiwan.⁴ Prior to 2004,

Table 5 Summary of studies evaluating the performance of fourth-generation combination assays

Reference	Fourth-generation assay evaluated	Sensitivity/specificity (%)	p24 detection limit	Reduction of seroconversion window	Performance of early prediction
Ly et al ¹⁵	AxSYM HIV Ag/Ab Combo Enzygnost HIV Integral Genscreen Plus HIV Ag/Ab Murex HIV Ag/Ab Combo VIDAS HIV DUO Vironostika HIV Uniform II Ag/Ab	Specificity: 99.5–99.9	3.3–>25 pg/mL	2.0–2.35 d earlier than third-generation assay	NA
Bourlet et al ⁴²	VIDAS HIV DUO Ultra VIDAS HIV DUO AxSYM HIV Ag/Ab Combo	Sensitivity: 100 Specificity: 99.03–99.86	NA	VIDAS HIV DUO Ultra detected seroconversion earlier	NA
Kwon et al ¹³	Architect HIV Ag/Ab Combo AxSYM HIV Ag/Ab Combo Elecys 2010 HIV Combi	Specificity: 98.0–99.6	5–>25 pg/mL	4–26 d earlier than third-generation assay	NA
Miedouge M et al ²¹	Architect HIV Ag/Ab Combo AxSYM HIVAg/Ab Combo VIDAS HIV DUO Quick VIDAS HIV DUO Ultra	NA	0.43–2.25 U/mL	NA	NA
Song et al ²²	Architect HIV Ag/Ab Combo AxSYM HIVAg/Ab Combo Elecys 2010 HIV Combi Elecys HIV combi PT	Sensitivity: 100 Specificity: 99.0–99.6	1.13–4.50 U/mL	Architect HIV Ag/Ab Combo and Elecys HIV combi PT showed earlier seroconversion (7 d)	NA
Present study	Wantai HIV (1+2) Ag&Ab Bio-Rad HIV Combo Ag/Ab EIA Dia.Pro HIV Ab&Ag 4th Gen.	Sensitivity: 98.8–100 Specificity: 98.5–100	Bio-Rad and Dia. Pro showed relatively lower detection limit	NA	Better negative prediction than third-generation assay

Ab = antibody; Ag = antigen; EIA = enzyme immunoassay; HIV = human immunodeficiency virus; NA = not available.

sexual transmission was the major route of most HIV-1 infections in Taiwan. HIV-1 subtype B and CRF01_AE are two predominant virus strains, which account for about 70% and 25% of HIV-1 infections, respectively.^{5,6} However, CRF07_BC was responsible for the outbreak of HIV-1 among intravenous drug users between 2004 and 2005 in Taiwan.^{7,8,29} In spite of the low HIV prevalence rate in Taiwan, voluntary HIV counseling and testing sites were launched in 1985.³⁰ Currently, the third-generation HIV antibody immunoassay is the major HIV screening method used in Taiwan. It has been reported that the presence of various infections, autoimmune diseases, vaccinations, and hepatitis diseases can interfere with the detection capability of HIV antibody immunoassays and is likely to lead to false-positive results.^{12,13,31–33} In Taiwan, a nationwide HBV vaccination program was implemented in July 1984, and the prevalence of chronic HBV infection among adults born after 1984 was estimated to drop from 15–20% to 0.06%.^{34–36} Nevertheless, the prevalence of chronic HBV infection among MSM, the major risk group of HIV-1 infection in Taiwan, was estimated to be 19.1%.³⁷ Thus, the specificity of an HIV screening test is still an important parameter for its application in clinical diagnosis in Taiwan. This study demonstrated that two of the three fourth-generation combination assays had 100% specificity, as high as that of the third-generation immunoassay evaluated, which suggested the feasible application of the fourth-generation combination assays in Taiwan.

In addition, p24 antigen is often detectable in the blood when HIV RNA viral load is higher than 4 log₁₀ copies/mL,^{38,39} which might limit the application of the fourth-generation combination assays in early detection of infection in some acutely infected patients.^{25,40,41} It has been reported that, in the high-risk population, as compared with the fourth-generation combination assays, nucleic acid tests can reduce more false-negative results and could detect HIV acute infection earlier, resulting in a decrease of HIV transmission and subsequent cost saving on screening tests.²⁵ Nevertheless, the HIV-1 seroprevalence is low in Taiwan; nucleic acid tests as the general screening test is relatively expensive and might not be practical to use. Hence, the fourth-generation combination assays with relatively less cost displayed potential as a general screening test in Taiwan.

In conclusion, this study evaluated the performance of three fourth-generation combination assays and compared them with one third-generation HIV antibody immunoassay. All three fourth-generation combination assays showed better performance for early negative prediction than the third-generation immunoassay, and the Bio-Rad combination assay had the best negative prediction. Among the three combination assays, the Dia.Pro combination assay showed both better sensitivity and better specificity. In addition, Dia.Pro and Bio-Rad assays presented better ability to detect p24 antigen.

Conflicts of interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.jmii.2015.07.009>.