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Screening for human immunodeficiency virus using a newly developed fourth generation lateral flow immunochromatography assay

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ABSTRACT

Background: High sensitivity for detection of HIV-1 p24 antigen allows for early detection of primary HIV-1 infections.
Objectives: To evaluate the detection sensitivity and specificity of the Daina Screen® HIV Combo assay using clinical specimens in Japan where the pretest probability (prevalence) is low.
Study design: We screened 17,373 preoperative outpatient blood samples using 4th generation lateral flow immunochromatography Daina Screen® HIV Combo assay for simultaneously detecting anti-HIV-1/2 and HIV-1 *p24 antigen*.
Results: Of the samples tested, 24 were positive for HIV-1 p24 antigen and 49 for HIV-1/2 antibody. Of the 49 samples, 36 were WB and HIV-1 RNA negative, 10 were WB and HIV-1 RNA positive, and 3 were WB positive, HIV-1 RNA negative, and in-house HIV-1 proviral DNA positive. RT-PCR revealed that of the 24 samples that were p24 antigen positive, one sample was HIV-1 RNA positive, which was reconfirmed using an in-house HIV-1 provirus DNA assay. From the 17,300 HIV-1 p24 antigen and anti-HIV-1/2 negative samples, pools containing 10 negative samples each were tested for HIV-1 by RT-PCR; all results were negative.
Conclusion: The Daina Screen® HIV Combo assay had a sensitivity and specificity of 100% and 99.7%, respectively, which sufficiently detected HIV infection in the cohort.

1. Introduction

While 4th generation lateral flow immunochromatographic screening assays for the detection of HIV-1 p24 antigen and HIV-1/2 antibodies are used for the rapid detection of HIV infection, their sensitivity for p24 antigen detection has been shown to be inferior to that obtained using automated methods, such as chemiluminescent immunoassay methods (Brennan et al., 2013; Chavez et al., 2011; Masciotra et al., 2017; Pandori et al., 2009), in several previous studies (Brauer et al., 2013; Duong et al., 2014; Rosenberg et al., 2012). HIV screening using immnochromatographic screening assay is possible for the whole blood specimen; however, the ability to detect HIV in whole blood specimen in inferior compared with that in plasma specimen (Masciotra et al., 2017). In light of the limitations of previous p24 antigen testing methods, the Daina Screen® HIV Combo Cat#7D2846 (Daina Screen Combo; Alere Medical Co. Ltd., Tokyo, Japan) was developed with improved sensitivity for p24 antigen detection in combination with the detection of antibodies for both HIV-1 and HIV-2. We used commercial HIV-1 seroconversion panels to examine the reactivity of the early period of infection using the newly developed Daina Screen Combo assay because the prevalence of HIV infection is low in Japan and there are few opportunities to meet with patients having acute HIV infection. When pools containing aliquots of 10 plasma samples were tested by RT-PCR for HIV-1 RNA, a detection sensitivity of 200 copies/mL was found to cover approximately 97.4% of untreated HIV-1-infected patients (Deeks et al., 2004).

In the present study, using 10-sample pools prepared from residual patient samples following routine HIV screening, we aimed to

Abbreviations: LOD, limit of detection; WB, western blot

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Table 1
Sensitivity assessment in the early phase of infection.

Device		Daina Screen Combo assay			Seracare Data	
Time (min)		20		Interpretation	COBAS TaqMan method	Abbott HIV Ag/Ab ARCHITECT
Bar Window		Ab	Ag		HIV-1 RNA copies/ml	Ag/Ab
Panel	No. of days after HIV infection					S/CO***
PRB961-01	0	(−)	(−)	(−)	< 50*	0.1
PRB961-02	5	(−)	(−)	(−)	< 50*	0.1
PRB961-03	7	(−)	(−)	(−)	< 50*	0.1
PRB961-04	12	(−)	(−)	(−)	< 50*	0.1
PRB961-05	14	(−)	(−)	(−)	< 50*	0.1
PRB961-06	19	(−)	(−)	(−)	< 50*	0.1
PRB961-07	21	(−)	(−)	(−)	**4.8×10^2***	0.1
PRB961-08	27	(−)	(+)	(+)	**1.5×10^5**	**8.8**
PRB961-09	29	(−)	(+)	(+)	**2.5×10^5**	**28.7**
PRB962-01	0	(−)	(−)	(−)	< 50*	0.2
PRB962-02	2	(−)	(−)	(−)	< 50*	0.2
PRB962-03	7	(−)	(−)	(−)	**7.6×10^2**	0.2
PRB962-04	9	(−)	(−)	(−)	**7.7×10^3**	0.3
PRB962-05	14	(−)	(+)	(+)	**7.0×10^5**	**28.4**
PRB962-06	17	(−)	(+)	(+)	**1.2×10^7****	**189.6**
PRB963-01	0	(−)	(−)	(−)	< 50*	0.1
PRB963-02	2	(−)	(−)	(−)	< 50*	0.1
PRB963-03	7	(−)	(−)	(−)	< 50*	0.1
PRB963-04	9	(−)	(−)	(−)	< 50*	0.2
PRB963-05	14	(−)	(−)	(−)	**7.8×10^3**	0.3
PRB963-06	17	(−)	(+)	(+)	**9.6×10^4**	**5.9**
PRB963-07	21	(−)	(+)	(+)	**6.2×10^5**	**20.1**
PRB966-01	0	(−)	(−)	(−)	< 50*	0.1
PRB966-02	2	(−)	(−)	(−)	< 50*	0.2
PRB966-03	20	(−)	(−)	(−)	< 50*	0.2
PRB966-04	22	(−)	(−)	(−)	< 50*	0.1
PRB966-05	30	(−)	(−)	(−)	< 50*	0.2
PRB966-06	35	(−)	(−)	(−)	**3.4×10^2***	0.2
PRB966-07	37	(−)	(−)	(−)	**1.9×10^3**	0.3
PRB966-08	44	(−)	(−)	(−)	**2.8×10^5**	**2.1**
PRB966-09	48	(+)	(−)	(+)	**4.8×10^4**	**2.1**
PRB966-10	51	(+)	(−)	(+)	**8.2×10^4**	**9.6**
PRB972-01	0	(−)	(−)	(−)	**1.5×10^2**	0.3
PRB972-02	3	(−)	(−)	(−)	**1.6×10^2**	0.3
PRB972-03	11	(−)	(−)	(−)	**2.0×10^4**	0.4
PRB972-04	14	(−)	(−)	(−)	**3.5×10^4**	0.6
PRB972-05	18	(+)	(−)	(+)	**2.2×10^5**	**3.7**
PRB972-06	21	(+)	(−)	(+)	**6.8×10^5**	**26.7**
PRB974-01	0	(−)	(−)	(−)	BLD	0.1
PRB974-02	7	(−)	(−)	(−)	**1.1×10^4**	0.4
PRB974-03	9	(−)	(+)	(+)	**9.1×10^4**	**3.4**
PRB974-04	16	(−)	(+)	(+)	**9.2×10^5**	**53.6**
PRB975-01	0	(−)	(−)	(−)	BLD	0.1
PRB975-02	2	(−)	(−)	(−)	BLD	0.1
PRB975-03	7	(−)	(−)	(−)	**2.2×10^2**	0.1
PRB975-04	9	(−)	(−)	(−)	**3.4×10^3**	0.1
PRB975-05	14	(−)	(+)	(+)	**2.8×10^6**	**27.1**
PRB976-01	0	(−)	(−)	(−)	**1.6×10^4**	0.4
PRB976-02	2	(−)	(−)	(−)	**3.0×10^4**	0.6
PRB976-03	7	(−)	(+)	(+)	**6.5×10^5**	**40.7**
PRB976-04	9	(−)	(+)	(+)	**2.3×10^6**	**72.6**

(−): Negative; (+): Positive.
Bold text indicates positive data (levels greater than the cut-off value).
BLD: below the limit of detection.

* Ultrasensitive; Quantity of HIV-1 RNA was measured using the Roche Amplicor HIV-1 Monitor Ultrasensitive method with a detection sensitivity of 50 copies/mL. In case no viruses were present, we considered it as > 50 copies/mL (by attaching the seroconversion panel).

** Calculated from 1:100 dilution value.

*** Assay results are presented as ratios of specimen signals to the cutoff values (S/CO), where an S/CO ratio of ≥1.00 indicates a positive reaction. We measured all panels simultaneously.

Table 2
Sensitivity assessment in the early phase of infection.

	Daina Screen Combo assay	ARCHITECT Combo assay	COBAS TaqMan method (HIV-1 RNA)
Mean duration until positive conversion with eight panels	19.3 days	18.8 days	11.4 days

determine the sensitivity and specificity for detection using the Daina Screen Combo assay in comparison with RT-PCR testing.

2. Material and methods

2.1. Plasma specimens from seroconversion panels

Eight types of commercial HIV-1 seroconversion panels were purchased from SeraCare Life Sciences, Inc. Milford, USA for the diagnosis of acute HIV-1 infection.

2.2. Plasma specimens used in the clinical study

From April 2016 to December 2017, 17,373 blood specimens (5 mL) were collected in EDTA 2Na blood collection tubes from preoperative outpatients for HIV screening at Kawasaki Medical School Hospital (Okayama, Japan). Of these, 152 samples were from patients who visited us for suspected HIV infection or voluntary HIV testing.

HIV screening examinations were performed using plasma specimens. In addition, plasma specimens were used for all other confirmation examinations except for HIV-1 proviral DNA assay, which used whole blood specimens.

This study was conducted after informed consent was obtained from all subjects and was performed in compliance with the World Medical Association's International Code of Medical Ethics.

2.3. Detectivity examination using seroconversion panels

Other than the Daina Screen assay, ARCHITECT® HIV Ag/Ab Combo (ARCHITECT Combo; Abbott Diagnostics, Wiesbaden, Germany) assay was used for seroconversion panels. In addition, HIV-1 RNA viral load of seroconversion panels used concentrations listed in the panel attachment.

2.4. HIV screening and confirmatory testing

HIV screening was performed using the Daina Screen Combo assay developed in Japan in 2015 and not the Determine® Combo assay (Brauer et al., 2013; Duong et al., 2014; Rosenberg et al., 2012). The Daina Screen Combo assay is based on the sandwich immunoassay technique. The assay uses a nitrocellulose strip with a sample dripping site containing biotin-labeled p24 antibody and a conjugate site containing colloid-labeled p24 antibody and colloid-labeled HIV-1 and HIV-2 antigens.

When an HIV-1 p24 antigen or HIV antibody-positive plasma specimen is dropped in the sample dripping site, in response to biotin-labeled anti-p24, colloid-labeled anti-p24, or colloid-labeled p24 antigen, the complex of "biotin-labeled anti-p24 antibody–p24 antigen–colloid-labeled anti-p24 antibody" or "colloid-labeled HIV antigen–HIV antibody" is individually formed. Furthermore, the complex of "biotin-labeled anti-p24 antibody–p24 antigen–colloid-labeled anti-p24 antibody" gets trapped in solid-phase avidin in the antigen detection site, and a red line resulting from the colloid is formed. On the contrary, the complex of "colloid-labeled HIV antigen–HIV antibody" gets trapped in solid-phase HIV antigen in the antibody detection part; this results in a red line that is derived from the colloid. In addition, the required quantity of the plasma specimen is 50 µL, and the measurement duration is 20 min.

Residual specimens from the screening tests were used for confirmatory RT-PCR testing with a COBAS® TaqMan® HIV-1 Test, v2.0 (COBAS TaqMan; Roche Diagnostics, Tokyo, Japan), with a COBAS® TaqMan® 48 Analyzer (Roche) (Pyne et al., 2010). Additionally, HIV-1 proviral DNA was determined using an in-house HIV-1 DNA PCR assay (Lynch et al., 1992) with a limit of detection of 10 copies/µg DNA. Positive HIV-specific antibody detection was confirmed using the western blot kits LAV Blot I and II (WB ; Bio-Rad Laboratories, Inc., Hercules, USA) as described previously (Davey et al., 1992; Jackson et al., 1997). For HIV negative screened samples, pools consisting of 100-µL aliquots from 10 negative samples each were used for RT-PCR testing. The COBAS TaqMan is originally an HIV-1 RNA fixed-quantity method but was used under the implication of qualitative nucleic acid test in this study because we measured a plasma pool. All assays were performed using standard molecular techniques to avoid cross-contamination. This study was reviewed and approved by the Kawasaki Medical School Internal Review Board.

3. Results

3.1. Seroconversion

Table 1 shows the eight types of seroconversion panels. With reference to the data sheet provided by SeraCare Life Science Inc., HIV Ag/Ab measurements by the ARCHITECT Combo assay and HIV-1 RNA viral load by COBAS TaqMan method were shown.

The result of the Daina Screen Combo assay accorded with the result of the ARCHITECT Combo assay in seven of the eight panels on positive conversion day on each day blood was taken.

The positive conversion days of the average of each method are shown in Table 2.

In the sensitivity test performed using seroconversion panels in the early phase of infection, the mean duration until positive conversion was 19.3 days, whereas in the ARCHITECT Combo assay, mean duration was 18.8 days.

3.2. HIV screening test performance

Fig. 1 shows the performance results of the screening test compared with the confirmatory test. Of the 17,373 plasma samples screened, 73 (0.42%) were HIV positive, of which 24 were positive only for p24 antigen and 49 were positive only for HIV antibodies; no samples were positive for both p24 antigen and HIV antibodies. Ten-sample pools were prepared from the 17,300 samples that tested negative by the HIV screening test. All 1730 pooled samples were confirmed negative using RT-PCR.

3.3. Confirmatory testing

Reportedly, the antibody detection sensitivity of HIV screening is higher than that of WB law. Furthermore, the WB law of the confirmatory screening may not be essential for only p24 antigen of the HIV screening.

The new HIV screening reagent just after the release enforced a WB law about the plasma specimen with positive only p24 antigen purposely to inspect the specificity of HIV antibody reaction.

Of the 73 samples positive for HIV screening by Daina Screen Combo assay, 23 of the 24 samples positive for p24 antigen but negative for HIV antibody were WB/HIV-1 RNA negative and considered to have false-positive p24 antigen results. The remaining one p24-positive

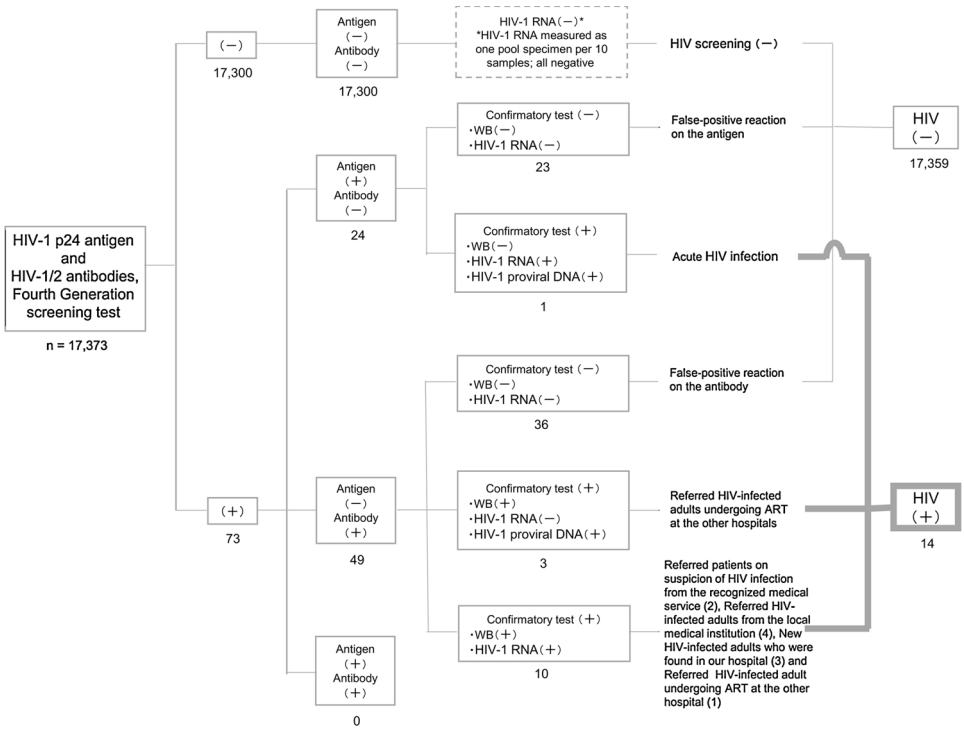

Fig. 1. Diagnostic test results from the HIV screening test compared with those from the confirmatory test.

sample was contradictorily negative for WB and positive for HIV-1 RNA (995,415 copies/mL). This sample tested positive for HIV-1 proviral DNA and was considered a true positive consistent with an acute HIV infection based on the identified transmission route and clinical characteristics of the patient.

Of the 49 samples that were p24 antigen negative/HIV antibody positive, 36 were both WB and HIV-1 RNA negative, 10 were WB and HIV-1 RNA positive, and 3 were WB positive, HIV-1 RNA negative, and HIV-1 proviral DNA positive. Based on these results, the 36 HIV antibody screen positive but WB/HIV-1 RNA negative samples were considered to have false-positive HIV antibody screen results, consistent with the clinical history of the patients. The 10 patients with HIV antibody screen positive samples that were also WB/HIV-1 RNA positive samples were diagnosed with HIV infection. Of these 10 patients, two were referrals from a public health center and blood center, respectively, 4 were referrals from other healthcare institutions after being diagnosed with HIV, 3 were diagnosed with HIV at our institution, and one was receiving antiretroviral therapy (ART) and was a referral from another healthcare institution. The 3 patients that were WB positive, HIV-1 RNA negative, and HIV-1 proviral DNA positive were diagnosed with HIV infection and had received ART prior to referral to our institution. No samples were both p24 antigen and HIV antibody positive with the Daina Screen Combo assay. It was three cases of HIV antibody only positivity in 73 with positive HIV screening that characteristic clinical manifestations of HIV were seen. These three patients were WB/HIV-1 RNA positive. Symptoms of esophagomycosis and Kaposi's sarcoma were seen in two HIV-infected patients who were referred from the local medical institution. Stomatomycosis was observed in one new HIV-infected patient who accidentally underwent oral surgery consultation at our hospital.

Of the 73 patients, four were previously diagnosed to be HIV-infected via ART.

Of the 17,373 patient samples tested, results of HIV screening and confirmatory testing generated an overall HIV positivity rate of 0.08% (14/17,373). The false positivity rate from HIV screening was 0.34% (59/17,373), with 23 (0.13%) false-positive p24 antigen and 36 (0.21%) false-positive antibody results.

4. Discussion

We conducted performance assessment of a newly developed rapid diagnostic reagent for HIV infection using a fourth-generation immunochromatography assay.

Using eight types of seroconversion panels, we determined the positive conversion day in the Daina Screen Combo and ARCHITECT Combo assays. The mean number of days until positive conversion was 19.3 using the Daina Screen Combo assay and 18.8 using the ARCHITECT Combo assay. Positive conversion was slower in the Daina Screen Combo assay than that in the ARCHITECT Combo assay by approximately 0.5 days. However, the detection ability of both the assays was similar.

An HIV screening test should not generate false negative results. In contrast, an emphasis on sensitivity at the expense of specificity will lead to an increase in confirmatory testing, resulting in the need for repeat blood collection as well as increased patient anxiety. Thus, at our institution, only 5 mL of blood is drawn for screening, and confirmatory testing is performed using residual blood samples.

Confirmatory testing of HIV screen positive samples usually involves WB for the detection of HIV-1- and HIV-2-specific antibodies as well as RT-PCR for the detection of HIV-1 RNA to confirm if the patient has viremia. However, discrepancies between screening and confirmatory testing results can occur when HIV-specific antibodies are not detected in an acute HIV infection or when ART has lowered HIV-1 RNA copies/mL below the lower limit of detection (LOD). For this reason, we followed up with HIV-1 proviral DNA testing when WB and RT-PCR results contradicted each other, and samples that were positive by HIV-1 proviral DNA testing were considered true positives. Of the 73 samples confirmed to be HIV positive, 24 and 49 samples were positive for only p24 antigen and HIV antibody, respectively. Of the 24 samples positive for only p24 antigen, one sample was subsequently confirmed as a case of acute HIV infection. As Japan has a low prevalence rate of HIV infection (Rahman et al., 1998), this supports the use of p24 antigen testing in the Japanese population. Regarding the 49 samples positive only by WB, these included patients from other healthcare institutions who were receiving ART. ART led to the inability to confirm

positive WB results using RT-PCR for HIV-1 RNA detection as copy numbers fell below detectable levels. Of the 73 HIV screen positives generated from the 17,373 samples tested, the false-positive rates based on 23 false-positive p24 antigen and 36 false-positive antibody results were 0.13% and 0.21%, respectively, resulting in an overall false-positive rate of 0.34%. The false-positive rate in the present study compares favorably with the 0.1%–2.2% rate reported in countries other than Japan (Duong et al., 2014; Rosenberg et al., 2012).

The suitability of Daina Screen Combo assay as a screening test was confirmed by RT-PCR testing for HIV-1 RNA using pools containing 10 negative samples each. As the sensitivity of HIV RT-PCR is 20 copies/mL, pooling of 10 specimens results in an RT-PCR LOD of 200 copies/mL. This sensitivity appears to be adequate for general screening assays to detect patients with acute HIV infection who have not commenced ART. As HIV-1 RNA as measured by RT-PCR was below the sensitivity of detection in all 1730 pools prepared from 17,300 samples that tested HIV negative in the screening, negative results with this method support the absence of viremia.

In Japan, HIV screening is generally conducted using fully automated methods. In this study, we assessed the utility of the DNA-linked inhibitor antibody assay (Daina Screen Combo), a fourth generation immunochromatography method with high sensitivity for application in rapid HIV screening, for simultaneous measurement of HIV-1 p24 antigen and HIV-1/2 antibodies. In an HIV-positive patient, the reactivity of this immunochromatography method can distinguish p24 antigen from HIV-1/2 antibodies. The results can roughly identify the HIV infectious period, thereby providing an estimate of the stage of HIV infection. To approximately estimate the progress of HIV infection, we expect the development of the automated method so that the identification of HIV-1 p24 antigen and HIV-1/2 antibodies are possible.

5. Conclusions

Using Daina Screen Combo assay to screen 17,373 blood samples for HIV, we found that 73 samples were positive. Confirmatory testing led to the detection of 14 patients infected with HIV, including one case of acute HIV infection. The overall sensitivity of HIV screening was 100% (14/14), whereas the specificity was 99.7% (17,300/17,359). Our results demonstrate the effectiveness of a lateral flow IC immunoassay for rapid HIV screening. Therefore, this assay can be considered a useful laboratory procedure in HIV diagnosis using plasma specimens.

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Ethical approval

Not required.

Declaration of Competing Interest

None.

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