Fundamental Evaluation for the HIV Ag+Ab Screening Assay Kit by Automated Chemiluminescent Enzyme Immunoassay Analyzer "HISCL-2000*i*"

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We evaluated HISCL HIV Ag+Ab Assay Kit (hereafter referred to as the assay) developed by Sysmex Corporation using the HISCL-2000i, a fully automated immunoassay system employing the chemiluminescent enzyme immunoassay (CLEIA) process for detection. This assay exhibited competent results in terms of within-run and between-day reproducibility as well as interferent and prozone effect susceptibility. The assay also detected a positive in serum specimens prospectively collected from 300 HIV-1 infected patients who are undergoing treatment at our clinic and who provided informed consent. Specificity for HIV-positive specimens was 100%. In analytical sensitivity verification using a commercially available HIV-1 antigen sensitivity panel, the assay proved its high sensitivity by positively detecting HIV-1 p24 antigens at concentrations of up to approximately 2 pg/mL. In tests using a commercially available performance panel, the assay also positively detected HIV-1 group M subtypes A-G and CRF B/D, HIV-1 group O and HIV-2. In tests using 5 HIV seroconversion panels obtained from commercial vendors, the assay showed satisfactory acute phase sensitivity by appropriately detecting increases in HIV-1 antigens and antibodies in blood samples. Based on these results, we conclude HISCL HIV Ag+Ab Assay Kit that is highly sensitive and specific assay is an effective screening test for HIV infection.

Key Words Antibodies, Antigens, 4th Generation, Screening, HIV, HISCL

INTRODUCTION

The human immunodeficiency virus (HIV) [1] was first discovered in 1983. Since then, two types of the virus have been identified: HIV-1 and HIV-2 [2,3]. HIV-1 has been phylogenetically classified into groups M, N, O, P, and so on. The predominant HIV-1 group M is known to be the cause of the global AIDS pandemic. It has also been found that HIV-1 group M and HIV-2 possess some genetic subtypes as well as circulating recombinant forms (CRFs) of two or more of these subtypes [4-6].

It is estimated that about 33 million people around the world are living with HIV. The number of HIV-positive individuals in Japan has been rising every year since the first infections were recognized in 1985. 1,075 new HIV and 469 new acquired immunodeficiency syndrome (AIDS) diagnoses were made in Japan in 2010, marking the year as the third worst in terms of HIV statistics and the worst in terms of AIDS statistics [7].

Research and development of various treatments to control AIDS has advanced greatly over the years since the discovery of the virus, and HIV-positive individuals in developed countries are living significantly longer. However, a treatment that completely eradicates HIV from the body has yet to be attained; as a result, infected individual need to undergo anti-HIV therapy for their entire lives. Consequently, the most effective method of curbing the spread of HIV is prevention. Because early diagnosis of HIV-positive individuals is one effective means of stopping further infection, development of a highly sensitive and specific assay as a screening test is an urgent priority.

Conventional assays are based on HIV antibody detection. However, these assays have the drawback of not being able to detect HIV during the so-called window period between infection and antibody detection.

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Therefore, it has increased significance of the 4th generation diagnostic test that can simultaneously detect HIV-1 antigens which appear at an earlier stage in the blood with HIV-1/2 antibodies. In its 2008 guidelines, the Japanese Society for AIDS Research recommended use of screening tests enabling simultaneous detection of HIV-1 antigens and HIV-1/2 antibodies [8].

This study describes the results of a basic performance evaluation of HISCL HIV Ag+Ab Assay Kit developed by Sysmex Corporation in light of the current situation of HIV research as described above.

I. ASSAY AND MEASUREMENT PRINCIPLE

1. Assay and instruments

Assays were assessed using HISCL-2000*i* (Sysmex Corporation; Sysmex), a fully automated immunoassay system employing the chemiluminescent enzyme immunoassay (CLEIA) process for detection. This assay has a short detection time of only 17 min. and requires a volume of only 30μL for samples.

2. Measurement principle

The mechanism of the assay is based on the two-step sandwich, chemiluminescent enzyme immunoassay which detect photon emissions that are produced from decomposition of a chemiluminescent agent "CDP-*Star*" by the enzyme alkaline phosphatase (ALP). The HISCL-2000*i* (Sysmex) was used as the measuring instrument.

HIV-1 p24 antigens in the sample react with biotinylated anti-HIV-1 p24 antibodies, and then anti-HIV antibodies in the sample react with HIV-1 gp41 recombinant antigens, HIV-1 gp41 peptide antigens, HIV-2 gp36 peptide antigens on the magnetic particles. Concurrently, HIV-1 p24 antigens and biotinylated anti-HIV-1 p24 antibody complexes bind with strepavidin on the magnetic particles. After bound/free (B/F) separation, ALP-labeled HIV-1 p24 antibodies, ALP-labeled HIV-1 gp41 recombinant antigens, ALP-labeled HIV-1 peptide antigens and ALP-labeled HIV-2 gp-36 antigens are added. These react with either the HIV-1 p24 antigens or the anti-HIV antibodies bound to the magnetic particles to subsequently form sandwich complexes. Chemiluminescent CDP-*Star* is added following the second B/F separation. The photon emissions resulting from the decomposition of CDP-*Star* by ALP are then measured (***Fig. 1***).

As this assay is a qualitative test, results are obtained through comparison with a cutoff value preliminarily established with a calibration assay. Specimens with cutoff Index (C.O.I.) equal to or greater than 1.0 are considered HIV-positive. Likewise, specimens with C.O.I. less than 1.0 are considered negative.

3. Test samples

As control serum samples, pooled serum negative for HIV antibodies and antigens, serum positive for HIV-1 p24 antigen and serum positive for HIV-1 antibodies were used to evaluate within-run and between-day reproducibility.

Control serum and the Interference Check.A Plus (Sysmex) were used to evaluate the effects of interferents. Each sample was adjusted to five concentration levels.

To test for prozone effects, 4 ng/mL samples of HIV-1 p24 recombinant antigen and serum positive for either HIV-1 subtype B or HIV-2 selected from a performance panel (WWRB302) were employed. We used samples that were diluted 4^n-fold with pooled serum negative for HIV antigens and antibodies.

We evaluated specificity using frozen serum prospectively collected from 300 HIV-1 infected patients who attended our clinic and provided informed consent. They were diagnosed with asymptomatic carrier (AC) of HIV or AIDS.

HIV-1 p24 sensitivity panel PRA801, Worldwide HIV Performance Panel WWRB302, and seroconversion panels PRB944, PRB951, PRB952, PRB953 and PRB959 used in this study were purchased from SeraCare Life Science.

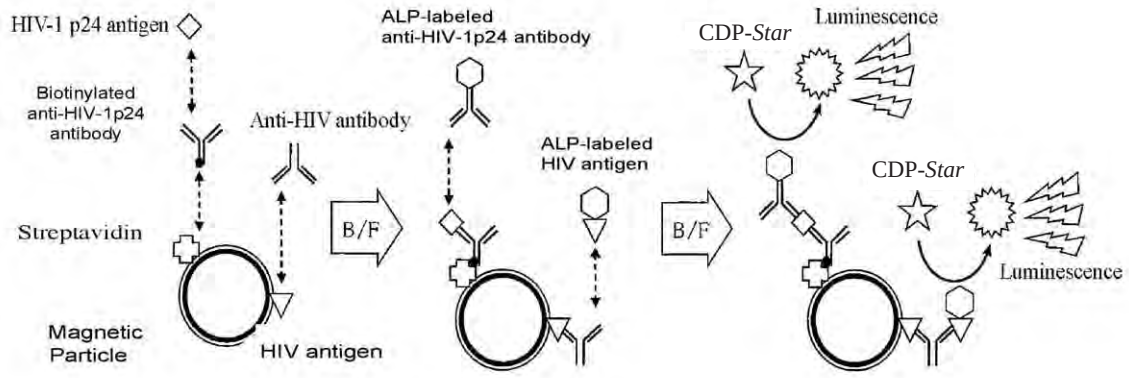

Fig. 1 *Measurement Mechanism*

II. RESULTS

1. Within-run reproducibility

Ten within-run tests were repeatedly performed on serum negative samples for both HIV antigens and antibodies, positive for HIV antigens and positive for HIV antibodies (***Table 1***). Serum samples negative for both HIV antibodies and antigens were all negative. Serum samples positive for HIV antigens and antibodies satisfactorily exhibited CV of 1.9% and 2.0% respectively,

2. Between-day reproducibility

Between-day tests were repeatedly performed for ten days on samples of serum negative for both HIV antigens and antibodies, positive for HIV antigens and positive for HIV antibodies (***Table 2***). Serum samples negative for both HIV antibodies and antigens were all negative. Serum samples positive for HIV antigens and antibodies satisfactorily exhibited CV of 2.4% and 1.6% respectively.

Table 1 *Within-run Reproducibility*

HIV Ag and Ab negative serum			HIV Ag positive serum			HIV Ab positive serum		
	C.O.I.	Int.		C.O.I.	Int.		C.O.I.	Int.
1	0.0	-	1	4.3	+	1	5.4	+
2	0.0	-	2	4.3	+	2	5.3	+
3	0.1	-	3	4.4	+	3	5.4	+
4	0.0	-	4	4.3	+	4	5.2	+
5	0.1	-	5	4.4	+	5	5.4	+
6	0.0	-	6	4.3	+	6	5.3	+
7	0.0	-	7	4.3	+	7	5.3	+
8	0.1	-	8	4.5	+	8	5.4	+
9	0.1	-	9	4.3	+	9	5.6	+
10	0.0	-	10	4.2	+	10	5.3	+
Ave	0.0		Ave	4.3		Ave	5.4	
SD	—		SD	0.082		SD	0.107	
CV (%)	—		CV (%)	1.9		CV (%)	2.0	

Table 2 *Between-day Reproducibility*

HIV Ag and Ab negative serum			HIV Ag positive serum			HIV Ab positive serum		
Day	C.O.I.	Int.	Day	C.O.I.	Int.	Day	C.O.I.	Int.
1	0.0	-	1	4.3	+	1	5.4	+
2	0.0	-	2	4.5	+	2	5.5	+
3	0.0	-	3	4.5	+	3	5.6	+
4	0.1	-	4	4.3	+	4	5.5	+
5	0.0	-	5	4.4	+	5	5.4	+
6	0.0	-	6	4.3	+	6	5.4	+
7	0.0	-	7	4.2	+	7	5.3	+
8	0.0	-	8	4.4	+	8	5.4	+
9	0.0	-	9	4.4	+	9	5.4	+
10	0.0	-	10	4.5	+	10	5.5	+
Ave	0.0		Ave	4.4		Ave	5.4	
SD	—		SD	0.103		SD	0.084	
CV (%)	—		CV (%)	2.4		CV (%)	1.6	

3. Interferents

In test of serum negative for both HIV antigens and antibodies, serum positive for HIV antigens and serum positive for HIV antibodies, the assay did not show interference at the confirmed concentration of the following, unconjugated bilirubin level ≤ 18.7 mg/dL, conjugated bilirubin level ≤ 20.9 mg/dL, hemoglobin level ≤ 484 mg/mL, turbidity ≤ 1460 FTU, rheumatoid factor ≤ 500 IU/mL (***Fig. 2***).

4. Prozone effects

Samples of diluted HIV-1 p24 antigen, diluted HIV-1 antibody samples and diluted HIV-2 antibody samples did not exhibit prozone effects such as decreased or negative detection in high concentration ranges (***Table 3***).

5. Specificity

The assay reacted positively to all 300 serum specimens from HIV-positive patients who are undergoing treatment at our institute and who are diagnosed with AC of HIV or AIDS (***Table 4***). The assay reacted particularly strongly when identifying CRFs AC, AE, AG, and subtypes B, C, and G in 21 specimens (***Table 5***).

6. Sensitivity panel

In tests using HIV-1 p24 sensitivity panel PRA801 purchased from SeraCare Life Science, the DuPont Standard on the documentation accompanying the panel confirmed HIV-1 p24 reactivity up to concentrations of approximately 2 pg/mL (***Table 6***).

7. Performance panel

In tests using the Worldwide HIV Performance Panel WWRB302 purchased from SeraCare Life Science, the assay was able to detect HIV-1 group M subtypes A-G and CRF B/D, HIV-1 group O and HIV-2 as a positive, consequently confirming a high level of specificity (***Table 7***).

8. Acute phase sensitivity

In tests using seroconversion panels PRB944, PRB951, PRB952, PRB953 and PRB959 purchased from SeraCare Life Science, the assay reacted positively after two days

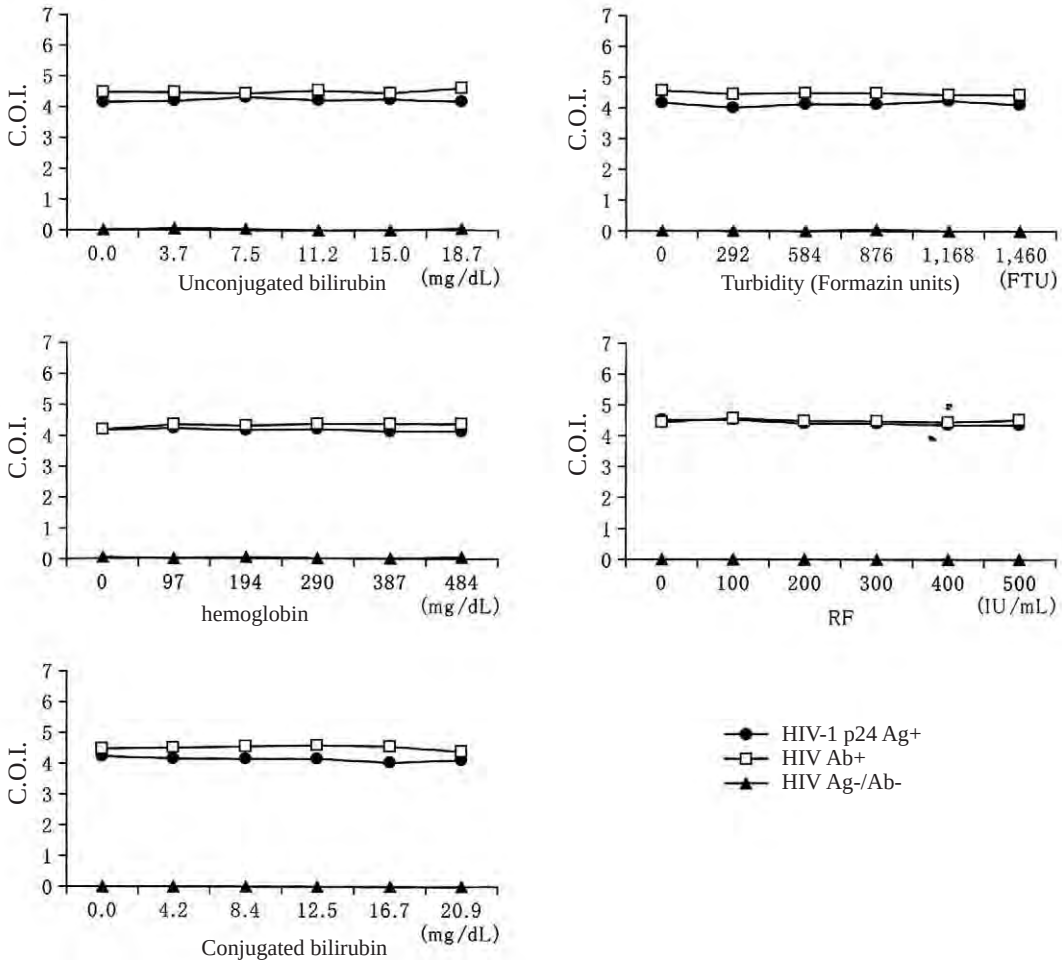

Fig. 2 *Interferents*

Table 3 *Prozone effects*

Dilution rate	HIV-1 p24 Ag		Anti-HIV-1 Ab		Anti-HIV-2 Ab	
	C.O.I.	Int.	C.O.I.	Int.	C.O.I.	Int.
4^0	> 100.0	+	> 100.0	+	> 100.0	+
4^1	> 100.0	+	> 100.0	+	56.2	+
4^2	> 100.0	+	> 100.0	+	19.5	+
4^3	30.8	+	82.4	+	5.8	+
4^4	7.8	+	49.5	+	1.5	+
4^5	1.9	+	21.8	+	0.4	-
4^6	0.6	-	6.6	+	0.2	-
4^7	0.3	-	1.9	+	0.1	-
4^8	0.2	-	0.5	-	0.0	-
4^9	0.1	-	0.2	-	0.1	-

Table 4 *Results of Serum Collected by the AIDS Clinical Center*

	HISCL HIV Ag+Ab Assay Kit	
	AC	AIDS
Positive	248	52
Negative	0	0

Table 5 *Results for Domestically Identified HIV Subtypes*

HISCL HIV Ag+Ab Assay Kit			
No.	subtype	C.O.I.	Int.
1	B	> 100.0	+
2	AE	> 100.0	+
3	B	> 100.0	+
4	AE	> 100.0	+
5	AE	> 100.0	+
6	B	> 100.0	+
7	B	> 100.0	+
8	AE	> 100.0	+
9	B	> 100.0	+
10	G	> 100.0	+
11	B	> 100.0	+
12	B	> 100.0	+
13	C	> 100.0	+
14	AG	> 100.0	+
15	AE	> 100.0	+
16	AE	> 100.0	+
17	G	> 100.0	+
18	AE	> 100.0	+
19	AE	47.9	+
20	AC	21.1	+
21	AC	70.6	+

Table 6 *Sensitivity Panel Results*

	HISCL HIV Ag+Ab Assay kit		* DuPont Standard
	C.O.I.	Int.	pg/mL
PRA801-1	> 100.0	+	> 200
PRA801-2	67.1	+	140
PRA801-3	48.3	+	85
PRA801-4	17.8	+	42
PRA801-5	8.8	+	21
PRA801-6	4.6	+	10
PRA801-7	2.2	+	5
PRA801-8	1.2	+	2
PRA801-9	0.5	-	< 2
PRA801-10	0.1	-	Negative

* Data from panel data sheet

Table 7 *Performance Panel Results*

		HISCL HIV Ag+Ab Assay kit		* Abbott AxSym HIV-1/HIV-2	
	subtype	C.O.I.	Int.	S/CO	Int.
WWRB302-01	O	15.8	+	2.7	+
WWRB302-02	A	69.0	+	28.4	+
WWRB302-03	G	> 100.0	+	34.9	+
WWRB302-04	G	> 100.0	+	40.1	+
WWRB302-05	A	> 100.0	+	45.3	+
WWRB302-06	G	86.5	+	30.4	+
WWRB302-07	2	> 100.0	+	26.1	+
WWRB302-08	G	> 100.0	+	39.3	+
WWRB302-09	A	> 100.0	+	32.2	+
WWRB302-10	Neg	0.0	-	0.3	-
WWRB302-11	2	> 100.0	+	30.2	+
WWRB302-12	C	> 100.0	+	36.4	+
WWRB302-13	A	71.0	+	35.4	+
WWRB302-14	D	84.9	+	19.0	+
WWRB302-15	D	90.4	+	24.7	+
WWRB302-16	D	> 100.0	+	19.0	+
WWRB302-17	D	> 100.0	+	26.8	+
WWRB302-18	C	> 100.0	+	36.7	+
WWRB302-19	C	53.8	+	22.0	+
WWRB302-20	C	> 100.0	+	27.4	+
WWRB302-21	B	> 100.0	+	29.1	+
WWRB302-22	E	67.0	+	28.1	+
WWRB302-23	E	> 100.0	+	31.7	+
WWRB302-24	E	90.6	+	25.4	+
WWRB302-25	2	> 100.0	+	27.2	+
WWRB302-26	B	> 100.0	+	41.5	+
WWRB302-27	B/D	83.2	+	26.0	+
WWRB302-28	F	> 100.0	+	43.4	+
WWRB302-29	B	> 100.0	+	335.4	+
WWRB302-30	Neg	0.0	-	0.4	-

* Data from panel data sheet

since 1st bleed for PRB944, three days since 1st bleed for PRB953, eight days since 1st bleed for PRB951, ten days since 1st bleed for PRB952, and since 1st bleed for PRB959 (***Table 8***).

III. Discussion

This study establishes that HISCL HIV Ag+Ab Assay Kit newly developed by Sysmex Corporation has a superior level of within-run and between-day reproducibility. Susceptibility to interferents and prozone effects were not observed within the range of this evaluation. The assay reacted positively to serum our clinic collected from Japanese HIV-1 infected patients who were diagnosed with AC of HIV or AIDS. Furthermore, specificity for individual HIV types was 100%. Among the 21 positive specimens identified as CRFs AC, AE, AG and subtypes B, C and G, 18 specimens reached a cutoff index (C.O.I.) of more than 100.0 and the remaining three reached strong C.O.I. of 47.9, 21.1 and 70.6. This verifies the assay has the adequate sensitivity and specificity to detect CRFs and subtypes identified in Japan. In tests using HIV-1 p24 sensitivity panel PRA801, the Du Pont Standard on the documentation accompanying the panel confirmed HIV-1 p24 reactivity up to concentrations of approximately 2 pg/mL, affirming the assay's high sensitivity. In tests on

Table 8 *Seroconversion Panel Results*

	Days Since 1st Bleed	HISCL HIV Ag+Ab Assay kit		* Abbott HIV Ag		* Abbott Anti-HIV 1/2		* PCR
		C.O.I.	Int.	S/CO	Int.	S/CO	Int.	copies/mL
PRB944-01	0	0.1	-	0.5	-	0.1	-	7×10^3
PRB944-02	2	1.3	+	1.0	+	0.1	-	8×10^4
PRB944-03	7	24.6	+	6.6	+	0.1	-	4×10^5
PRB944-04	9	5.3	+	7.0	+	0.6	-	$> 8 \times 10^5$
PRB944-05	14	27.2	+	5.8	+	11.7	+	6×10^4
PRB944-06	16	26.6	+	3.2	+	14.4	+	2×10^5
PRB951-01	0	0.0	-	0.5	-	0.2	-	BLD
PRB951-02	2	0.0	-	0.5	-	0.2	-	BLD
PRB951-03	8	4.5	+	1.0	+	0.3	-	2×10^5
PRB951-04	11	54.1	+	5.7	+	0.2	-	1×10^6
PRB951-05	15	> 100.0	+	> 22.0	+	0.4	-	5×10^6
PRB951-06	19	> 100.0	+	14.9	+	8.4	+	2×10^6
PRB952-01	0	0.0	-	0.4	-	0.2	-	BLD
PRB952-02	7	0.4	-	0.5	-	0.2	-	5×10^3
PRB952-03	10	12.5	+	2.3	+	0.2	-	3×10^5
PRB952-04	14	6.0	+	1.2	+	1.0	+	1×10^5
PRB952-05	17	7.5	+	0.9	-	6.2	+	1×10^5
PRB952-06	21	7.6	+	0.5	-	5.4	+	5×10^4
PRB953-01	0	0.1	-	0.3	-	0.2	-	4×10^3
PRB953-02	3	1.8	+	0.5	-	0.1	-	8×10^4
PRB953-03	7	27.4	+	2.2	+	1.1	+	7×10^5
PRB953-04	10	62.8	+	4.8	+	> 16.5	+	1×10^6
PRB959-01	0	1.9	+	0.6	-	0.1	-	2×10^5
PRB959-02	7	> 100.0	+	> 35.1	+	0.3	-	$> 8 \times 10^5$
PRB959-03	9	78.8	+	> 35.1	+	1.7	+	$> 8 \times 10^5$
PRB959-04	14	23.0	+	5.3	+	16.6	+	8×10^5
PRB959-05	19	17.6	+	0.5	-	9.1	+	5×10^5
PRB959-06	21	18.9	+	0.5	-	8.6	+	3×10^5
PRB959-07	26	19.3	+	0.6	-	8.5	+	$> 8 \times 10^5$

* Data from panel data sheet

various HIV genotypes and subtypes from commercially available performance panels, the assay positively detected HIV-1 group M subtypes A-G and CRF B/D, HIV-1 group O, and HIV-2, consequently confirming a high level of specificity. In tests using seroconversion panels, the assay reacted positively after two days since 1st bleed for PRB944, three days since 1st bleed for PRB953, eight days since 1st bleed for PRB951, ten days since 1st bleed for PRB952, and since 1st bleed for PRB959. Comparing the assay's performance to the data provided with the panel on singular HIV antigen and HIV-1/2 antibody detection, we noted that PRB952 and PRB959 could be useful in detecting the significant rise and fall of HIV antigens in the acute phase and the increase of HIV antibodies before and after that period. However, this assay was able to maintain reactivity without any false negatives during the entire period from the fall of antigens to the rise of antibodies. This is evidence of the assay's versatility in being highly sensitive to both HIV antigens and antibodies.

Using the HISCL-2000*i* specially designed for this assay, detection for a 30μL sample takes only 17 min., and 180 tests can be run per hour. The system can also handle emergency specimens and can change the reagent during measurement, which is an extremely beneficial feature for hospitals demanding immediate results and commercial laboratories having a large number of specimens.

From the results of this study, this assay is believed to perform highly as an HIV screening test and to be extremely versatile for use in a wide range of applications such as emergency and high-volume testing.

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