Evaluating the Analytical Performance of New Immunoassay System "HISCL-5000"

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Sysmex Corporation developed HISCL-5000 to enhance the lineup of its fully automated immunoassay systems, including HISCL-2000i. We evaluated the basic performance of the new system for HBs antigen (HBsAg) and prostate-specific antigen (PSA). The within-run reproducibility and between-day reproducibility of HISCL-5000 were both found to be good; the coefficient of variation (CV) in within-run reproducibility was determined to be 1.61-1.72% for HBsAg and 1.59-1.78% for PSA, and that in between-day reproducibility as 0.920-2.06% for HBsAg and 1.02-3.59% for PSA. The limit of detection was determined to be 0.008 IU/mL for HBsAg and 0.001 ng/mL for PSA; the requirements for limit of detection shown in the product's package insert were well satisfied. Regarding correlations of HBsAg assay values with those obtained using other products (ARCHITECT i2000, ABBOTT JAPAN Co., Ltd.; LUMIPULSE G1200, Fujirebio Inc.; AIA-1800ST, Tosoh Corporation), the qualitative test results concordance rate was found to be 100% for all products, with the correlation with ARCHITECT i2000 determined to be y = 1.73x - 1061.4, r = 0.988. Likewise, the correlations of PSA assay values were determined to be y = 0.875x + 1.61, r = 0.999 with ARCHITECT i1000 (ABBOTT JAPAN Co., Ltd.), y = 0.853x + 1.78, r = 0.995 with LUMIPULSE G1200, and y = 0.869x + 1.48, r = 0.999 with AIA-1800ST. These findings demonstrate good analytical performance and short measuring time; HISCL-5000 is useful in routine laboratory testing and pre-consultation examination.

Key Words HISCL-5000, Chemiluminescent enzyme immunoassay, HBsAg, PSA, High sensitivity

INTRODUCTION

In recent years, immunoserological testing, like biochemical testing, has faced a new demand for quicker reporting of test results before starting clinical practice. There are few systems meeting this requirement, however, high expectations exist for faster performance in the fully automated immunoassay systems. Against this background, in 2007, Sysmex Corporation launched the HISCL-2000*i* (hereafter also referred to as HS2), which is based on chemiluminescent enzyme immunoassay (CLEIA). HS2 is capable of highly sensitive and quick assays using very small amounts of samples, and has been reported to be useful for routine laboratory testing, including examinations conducted before clinical practice[1-4]. To enhance the lineup of the HS2 series, HISCL-5000 (hereafter also referred to as HS5) has recently been developed with an improved throughput. This paper reports on our study to evaluate the analytical performance of the new system for detection of hepatitis B surface (HBs) antigen and prostate specific antigen (PSA).

INSTRUMENTATION AND REAGENTS

In this study, HS5 was used in combination with dedicated reagents HISCL® HBsAg Assay kit and HISCL® PSA Assay kit. For comparison, ACHITECT i1000 (ABBOTT JAPAN Co., Ltd.; hereafter AR1), ARCHITECT i2000 (ABBOTT JAPAN Co., Ltd.; hereafter AR2), LUMIPULSE G1200 (Fujirebio Inc.; hereafter LU), and AIA-1800ST (Tosoh Corporation; hereafter AIA) were used in combination with respective dedicated reagents: ARCHITECT®·HBsAg QT and ARCHITECT®·PSA (both from ABBOTT JAPAN Co., Ltd.), LUMIPULSE® II HBsAg and LUMIPULSE® PSA-N (both from Fujirebio Inc.), and E-test TOSOH II HBsAg and E-test TOSOH II PSA II (both from Tosoh Corporation).

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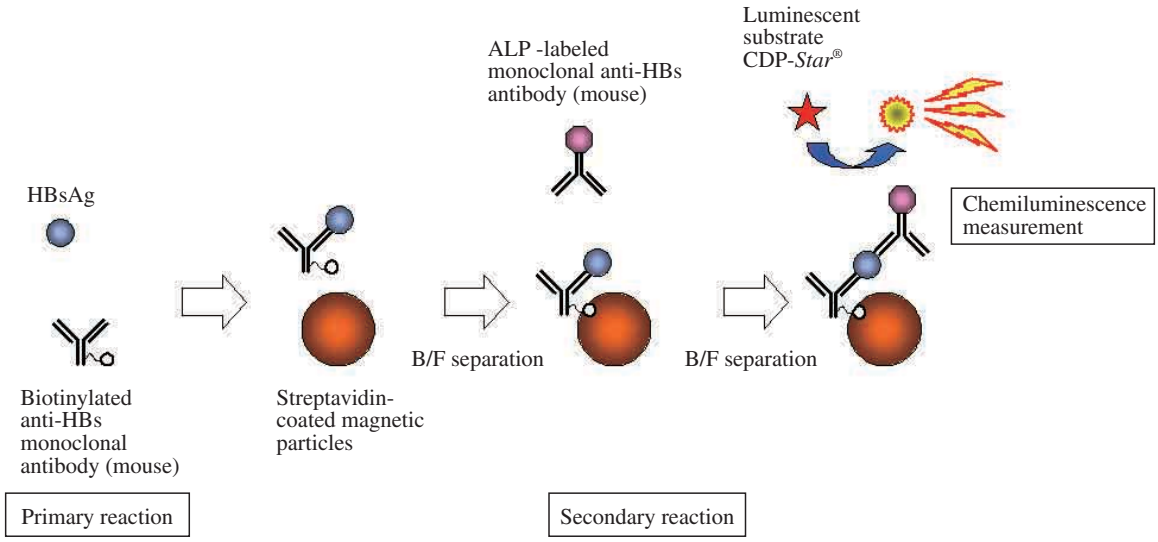

Fig. 1 *Measuring principle of HBsAg reagent*

MEASURING PRINCIPLE

The measuring principle of the HBsAg reagent used in this study is shown in ***Fig. 1***. This reagent works under the principle of CLEIA using the 2-step sandwich chemiluminescent enzyme immunoassay. Specifically, after a primary reaction is carried out in a liquid phase, the resulting immune complex is bound to magnetic particles by means of streptavidin-biotin reaction. After B/F separation, the complex is reacted with an ALP-labeled secondary anti-HBs antibody, and this is followed by B/F separation again. Finally, CDP-*Star*® (1,2-dioxetane derivative), a chemiluminescent substance, is added, and the intensity of its luminescence is converted to a concentration of the analyte.

COMPARISON OF HISCL-5000 AND HISCL-2000*i*

Instrumental specifications for HS5 and HS2 are shown in ***Table 1***. Compared with HS2, HS5 possesses an improved throughput from 180 to 200 tests per hour, and a maximum number of simultaneously analyzable parameters increased from 12 to 24. Also, the maximum sample loading capacity has been increased from 50 samples (standard function of HS2, optionally up to 100 samples loadable) to 100 samples (possible with standard function). HS5 has been developed for use at medium- and large-scale facilities, where there is demand for quick assays of a larger number of samples for a larger number of parameters than those for HS2.

SUBJECTS AND METHODS

1. Subjects

Commercially available control samples (Sysmex Corporation) were used for within-run reproducibility and between-day reproducibility evaluation. Specifically, the HBsAg used were Viratrol Level 1 (low concentration; hereafter VL1) and Viratrol Level 2 (high concentration; hereafter VL2), and the PSA used were Sero-trol I·X (high concentration; hereafter Sero-I) and Sero-trol II·X (low concentration; hereafter Sero-II). For determination of limit of detection, respective dedicated calibrators were used. Correlations with other analytical systems were examined using residual serum (151 specimens for HBsAg, 115 specimens for PSA) that had

Table 1 *Instrument specifications for HISCL-5000 and HISCL-2000i*

	HISCL-5000	HISCL-2000*i*
Measuring principle	Chemiluminescent enzyme immunoassay (CLEIA)	
Reaction time	17 minutes	
Throughput (test/h)	200	180
Number of simultaneously analyzable parameters	UP to 24	UP to 12
Sample loading capacity	100 samples	50 samples (optionally up to 100 samples)

been obtained after HBsAg or PSA assays at Aichi Medical University Hospital (August-November 2012). These serum were immediately frozen and stored at –80°C and then used as the assay samples after being thawed just before use. This study was conducted after approval by the ethical committee of Aichi Medical University.

2. Methods

1) Within-run reproducibility
Control samples (VL1 and VL2 for HBsAg, Sero-I and Sero-II for PSA) were each analyzed in 20 consecutive runs.

2) Between-day reproducibility
Control samples (VL1 and VL2 for HBsAg, Sero-I and Sero-II for PSA) were each analyzed once daily for 10 consecutive days.

3) Limit of detection
The dedicated calibrators were used as they were (0 concentration) and also used after being serially diluted to 6 concentrations (0.008-0.250 IU/mL for HBsAg, 0.0005-0.016 ng/mL for PSA). They were each analyzed in 10 runs, and the limit of detection was determined using the 2SD method.

4) Correlations with other analytical systems
For HBsAg, samples were analyzed using HS5, AR2, LU, and AIA, and the qualitative test results concordance rate of HS5 with each system was determined. In addition, HS5 and AR2 were examined for assay value correlation using the least- square method. For PSA, samples were analyzed using HS5, AR1, LU, and AIA, and correlations were examined in the same way as with the HBsAg assays. For the samples found to contain the analyte at a level exceeding the upper limit of each reagent, the automatic dilution function, if any, was used. For the analytical systems lacking such function and for the samples required to be diluted to levels not achievable by the automatic dilution function, final concentrations were determined after manual dilution.

5) Comparison of assay times for HBsAg
While operating each of HS5, AR2, LU, and AIA, 20 samples were continuously analyzed for HBsAg, and time to completion of output of all results after sample injection was measured.

RESULTS

1. Within-run reproducibility

The CV in HBsAg values was determined to be 1.61% for VL1 and 1.72% for VL2. The CV in PSA values was determined to be 1.59% for Sero-I and 1.78% for Sero-II (***Table 2***).

2. Between-day reproducibility

The CV in HBsAg values was determined to be 0.920% for VL1 and 2.06% for VL2. The CV in PSA values was determined to be 1.02% for Sero-I and 3.59% for Sero-II (***Table 3***).

3. Limit of detection

Limit of detection (the minimum concentration that produced a -2SD of intensity of its luminescence not overlapping the +2SD from 0-concentration calibrator) was determined to be 0.008 IU/mL for HBsAg and 0.001 ng/mL for PSA (***Figs. 2 and 3***).

Table 2 *Within-run reproducibility (n = 20)*

Parameter	HBsAg (IU/mL)		PSA (ng/mL)	
Sample	Viratol Level 1	Viratol Level 2	Sero-trol I·X	Sero-trol II·X
Mean	2.07	11.3	25.6	3.24
Standard deviation	0.0334	0.195	0.406	0.0577
CV	1.61%	1.72%	1.59%	1.78%

Table 3 *Between-day reproducibility (n = 10)*

Parameter	HBsAg (IU/mL)		PSA (ng/mL)	
Sample	Viratol Level 1	Viratol Level 2	Sero-trol I·X	Sero-trol II·X
Mean	2.31	11.7	26.0	3.28
Standard deviation	0.0212	0.240	0.264	0.118
CV	0.920%	2.06%	1.02%	3.59%

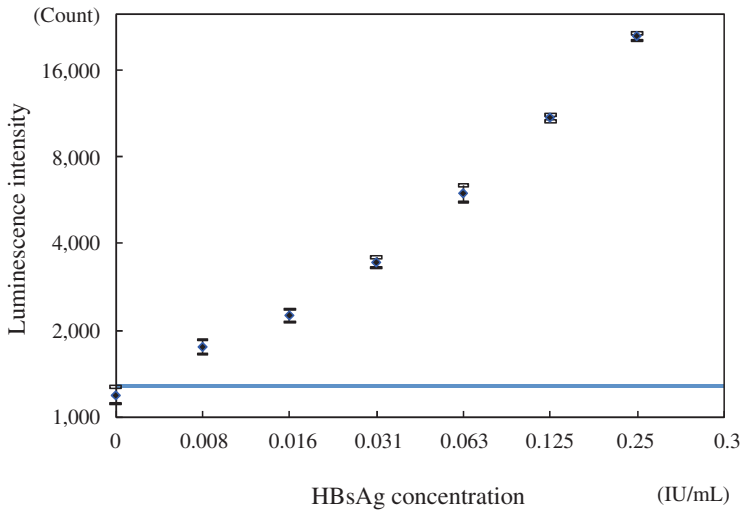

Fig. 2 *Limit of detection for HBsAg*

4. Correlations with other analytical systems

Qualitative test results for HBsAg were found to be 100% consistent among all of AR2, LU, and AIA (***Tables 4-6***). The assay value correlation with each analytical system was determined to be y = 1.73x - 1061.4, r = 0.988 for AR2 (***Fig. 4***) for all samples. The upper limits of measurement range are 2,500 IU/mL for HS5 and 250 IU/mL for AR2. The correlation was determined to be y = 1.08x + 82.5, r = 0.976 (***Fig. 5***) for the samples containing the analyte at 2,500 IU/mL or less, including those required to be diluted for AR2 only, and y = 1.80x - 2915.2, r = 0.988 (***Fig. 6***) for the samples that contained the analyte at levels exceeding 2,500 IU/mL and were required to be diluted, for both systems.

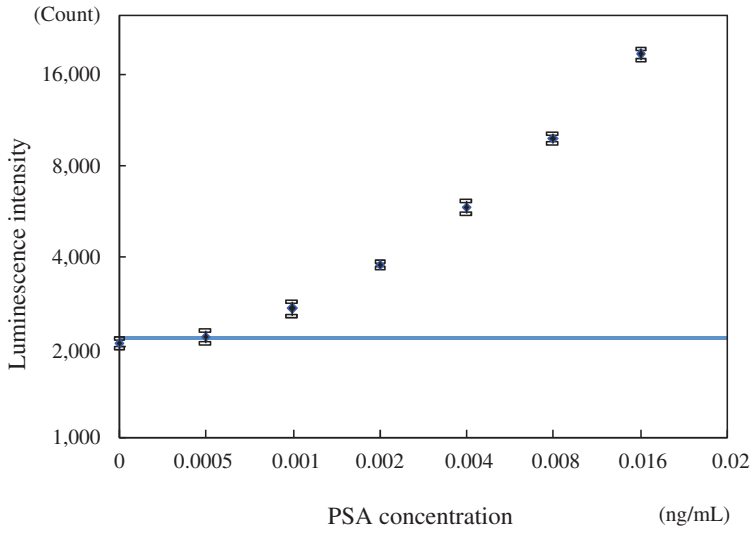

Fig. 3 *Limit of detection for PSA*

Table 4 *Comparison of qualitative test results between HISCL-5000 and ARCHITECT i2000 (n = 151)*

HBsAg		ARCHITECT i2000	
		+	-
HISCL-5000	+	101	0
	-	0	50

Concordance rate: 100%

Table 5 *Comparison of qualitative test results between HISCL-5000 and LUMIPULSE G1200 (n = 151)*

HBsAg		LUMIPULSE G1200	
		+	-
HISCL-5000	+	101	0
	-	0	50

Concordance rate: 100%

Table 6 *Comparison of qualitative test results between HISCL-5000 and AIA-1800ST (n = 151)*

HBsAg		AIA-1800ST	
		+	-
HISCL-5000	+	101	0
	-	0	50

Concordance rate: 100%

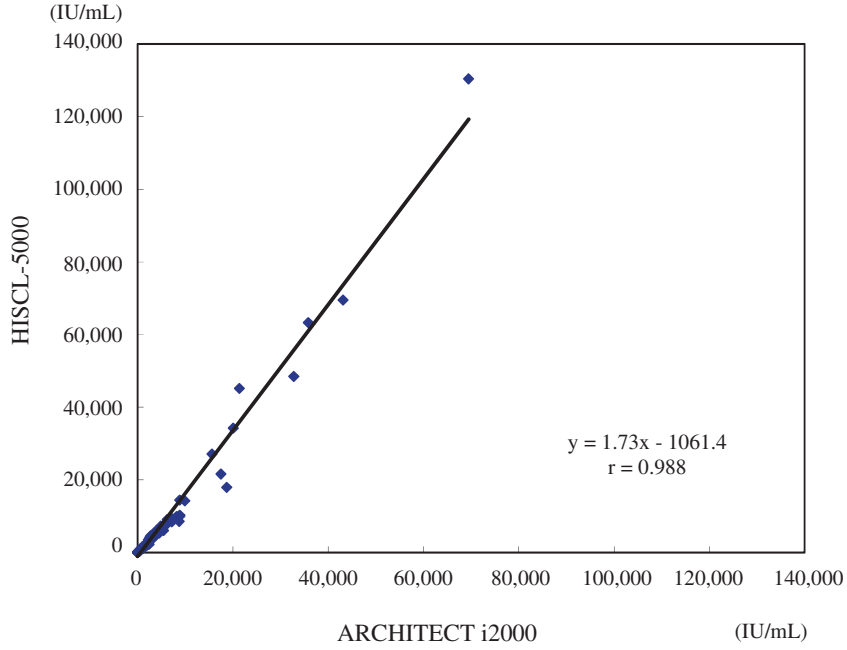

Fig. 4 *Correlation of HBsAg assay values between HISCL-5000 and ARCHITECT i2000 (all samples)*

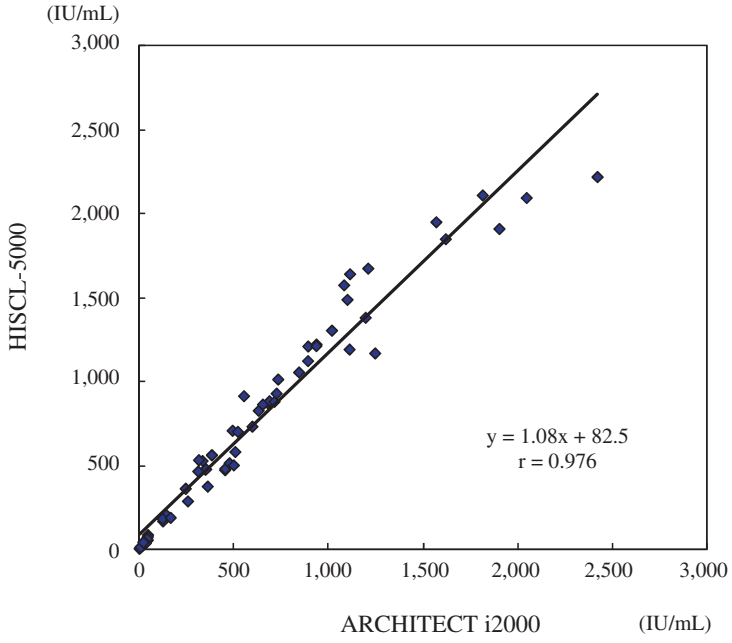

Fig. 5 *Correlation of HBsAg assay values between HISCL-5000 and ARCHITECT i2000 (samples containing the analyte at 2,500 IU/mL or less)*

The correlations of PSA assay values with each other analytical system was determined to be y = 0.875x + 1.61, r = 0.999 for AR1 (***Fig. 7***), y = 0.853x + 1.78, r = 0.995 for LU (***Fig. 8***), and y = 0.869x + 1.48, r = 0.999 for AIA (***Fig. 9***).

5. Comparison of assay times for HBsAg

Time to completion of continuous runs of 20 samples for HBsAg under the operating conditions for each system was found to be 23 minutes for HS5, 35 minutes for AR2, 40 minutes for LU, and 29 minutes for AIA.

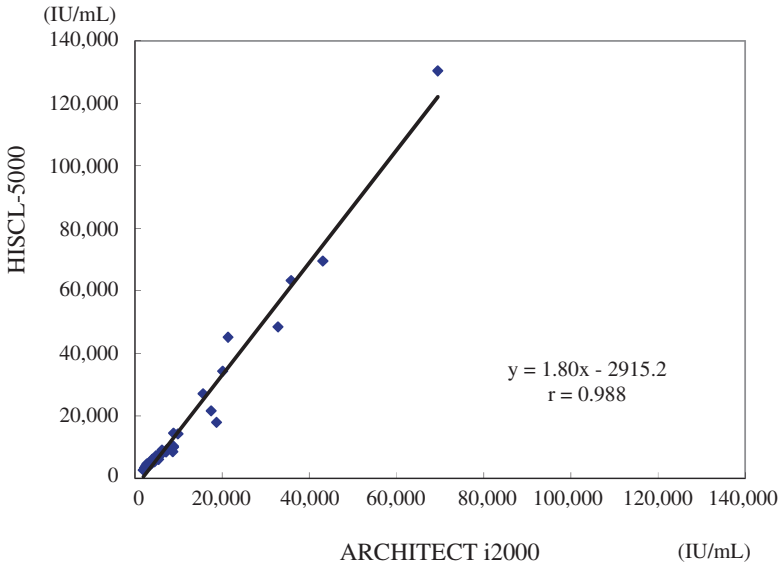

Fig. 6 *Correlation of HBsAg assay values between HISCL-5000 and ARCHITECT i2000 (samples containing the analyte at levels exceeding 2,500 IU/mL)*

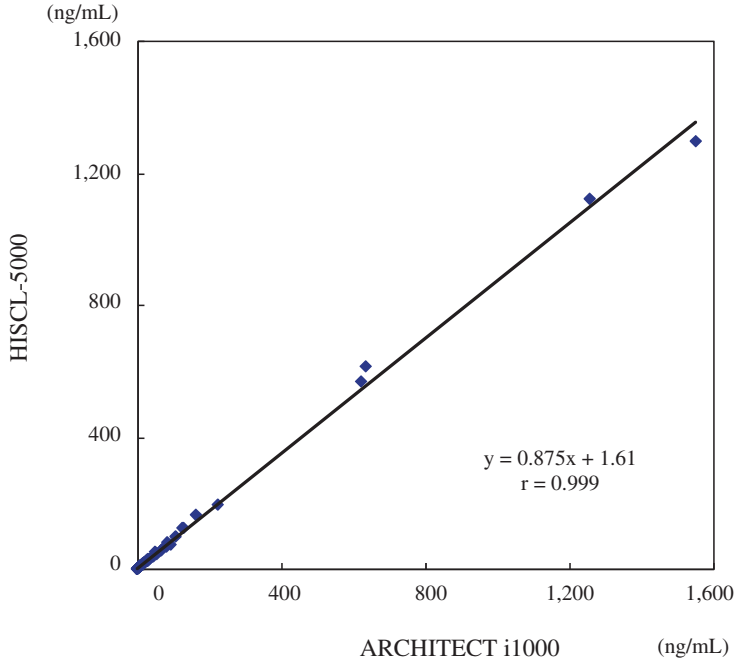

Fig. 7 *Correlation of PSA assay values between HISCL-5000 and ARCHITECT i1000*

DISCUSSION

In addition to screening for hepatitis B virus infections, HBsAg assays have recently been reported to be useful in determining the effects of treatment [5,6], and there is demand for assays obtained at higher sensitivity [7]. PSA assays are important to prostate cancer screening [8] and early detection and early treatment of postoperative recurrences [9]; quick and highly-sensitive assays are needed [10].

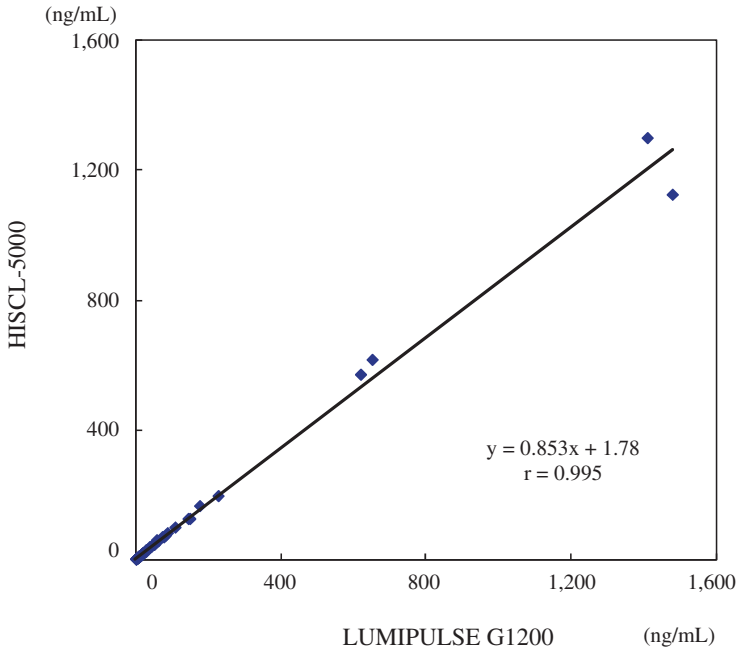

Fig. 8 *Correlation of PSA assay values between HISCL-5000 and LUMIPULSE G1200*

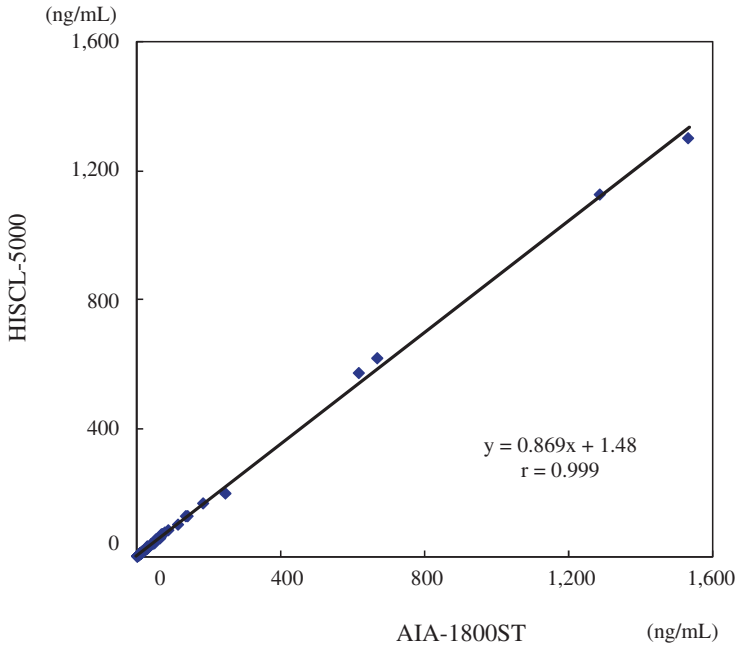

Fig. 9 *Correlation of PSA assay values between HISCL-5000 and AIA-1800ST*

In the present study, we evaluated the analytical performance of HS5, a new immunoassay system developed to enhance the lineup of the HS2 series, which is capable of assaying very small amounts of samples quickly at high sensitivity, focusing on the detection of HBsAg and PSA. Results demonstrated good performance for both parameters; the CV of within-run reproducibility was determined to be less than 2.00%, and the CV of between-day reproducibility to be less than 4.00%. The limit of detection was determined to be 0.008 IU/mL for HBsAg and 0.001 ng/mL for PSA; these results fully satisfied the detection concentration

requirements shown in the package inserts for the respective reagents (0.03 IU/mL for HBsAg, 0.003 ng/mL for PSA). The limit of detection for HBsAg, in particular, was found to be lower by one digit than the concentrations indicated in the package insert, confirming that HS5 possesses extremely high sensitivity.

The qualitative test results concordance rate for HBsAg was found to be excellent, 100%, for all control systems. However, regarding assay value correlations with AR2, the correlation curve slope differed widely depending on the range of concentration: 1.73 for all samples, 1.08 for the samples containing the analyte at 2,500 IU/mL or less, and 1.80 for the samples containing the analyte at levels exceeding 2,500 IU/mL. Mitekura et al. [2] and Ishida et al. [4] reported that the slope of the correlation of HS2 (which uses the same reagent as with HS5) with AR2 was 1.03-1.04; this finding was inconsistent with ours. However, these reports dealt with samples containing the analyte at levels up to the upper limit of measurement of AR2 (250 IU/mL), and did not include high-concentration samples requiring diluted retesting. In the present study, samples requiring diluted retesting for AR2 accounted for 85.1% of positive samples (86/101 samples); the differences from the aforementioned reported data were attributable to the different viral concentrations in the samples analyzed.

The shortest time to completion of measurement of 20 consecutive samples for HBsAg was 23 minutes for HS5. This time was equivalent to 79.3%, 65.7%, and 57.5% compared with AIA, AR2, and LU, respectively; HS5 was proven to be excellent in assay speed.

Regarding PSA value correlations with other analytical systems, HS5 produced good results with all control systems, and was judged to be useful for routine laboratory testing.

CONCLUSION

The present study confirmed that HS5 possesses improved analytical performance and better operability while retaining the favorable features of HS2. Hence, HS5 is expected to make great contributions to the speed of immunoserological testing.

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