Evaluation of the HISCL™ KL-6 Reagent for use on a Fully Automated Immunoassay Analyzer HISCL™

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INTRODUCTION

Sialylated carbohydrate antigen KL-6 (hereinafter referred to as "KL-6"), an MUC-1 molecule recognized by the anti-KL-6 monoclonal antibody established by Kohno, et al., is a transmembrane glycoprotein expressed on the surface of type 2 alveolar epithelial cells.[1)] KL-6 is highly specific to interstitial pneumonia,[2)] particularly to the active form rather than inactive interstitial pneumonia, and is therefore widely used as a marker for the diagnosis of interstitial pneumonia and as an indicator of disease activity.[3)] In this paper, we report the results of a fundamental study of the fully automated immunoassay analyzer HISCL™ (Sysmex Corporation), which employs a chemiluminescent enzyme immunoassay (CLEIA) detection method, and its HISCL™ KL-6 Reagent. In this study, we evaluated their basic performance and usefulness in routine testing. We also compared the reagent with PicoLumi KL-6, a reagent used in conjunction with the electrochemiluminescence immunoassay device PicoLumi.

I. MATERIALS AND METHODS

1. Materials

Human serum samples purchased from overseas were used for the evaluation of precision, dilution linearity, and the hook effect. To evaluate the effect of potentially interfering substances, two different concentrations of control serum samples were used. For other evaluations, EDTA-added plasma (which was included in a plan reviewed and approved by the hospital's ethical committee and was then concurrently collected with human sera) was used. We used the residual serum and plasma samples that had been anonymized in an unlinkable fashion, in accordance with the Japanese Society of Laboratory Medicine's "policy on the use of leftover laboratory test samples for other tests, education, and research."[4)] The study was approved by the hospital's ethical committee.

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2. Devices and Reagents

The device studied was the fully automated immunoassay analyzer HISCL-5000 (CLEIA, Sysmex) with its HISCL KL-6 Reagent. As a control assay, the electrochemiluminescence immunoassay device PicoLumi (ECLIA, EDIA) and its reagent PicoLumi KL-6 were used.

3. Measurement Principle

HISCL KL-6 Reagent (EDIA Co., Ltd.) is a two-step sandwich assay to quantify the level of KL-6 in a serum or plasma sample by CLEIA using the anti-KL-6 monoclonal antibody developed by Kohno, et al. The measurement procedures are shown in ***Fig. 1***.

1) Biotin-binding anti-KL-6 monoclonal antibodies (mouse) in Reagent 1 (R1) specifically react with KL-6 in the sample. They then bind to streptavidin-binding magnetic particles in Reagent 2 (R2).
2) After removing unreacted liquid, add Reagent 3 (R3). ALP-labeled anti-KL-6 monoclonal antibodies (mouse) specifically react with KL-6 on the magnetic particles.
3) After removing unreacted liquid, add Reagents 4 and 5 (R4 and R5). The luminescent substrates CDP-*Star*™ undergo enzymatic degradation via the ALP on the magnetic particles. Measure the resulting luminescence intensity.
4) The luminescence intensity increases as the concentration of KL-6 in the samples increases. By measuring the KL-6 level in the samples containing KL-6 at known concentrations (HISCL KL-6 Calibrators C0 to C4) and preparing calibration curves, the KL-6 concentrations in the samples can be measured.

4. Methods

To determine precision, human serum samples with three different concentrations of KL-6 were used to assess the within-run reproducibility in 10 repeated measurements. The between-run reproducibility was also examined by measuring the KL-6 concentrations in these samples for 20 days.

For the evaluation of the minimum detection sensitivity, a dilution series was prepared using reference standards with the zero and lowest concentrations of KL-6 for

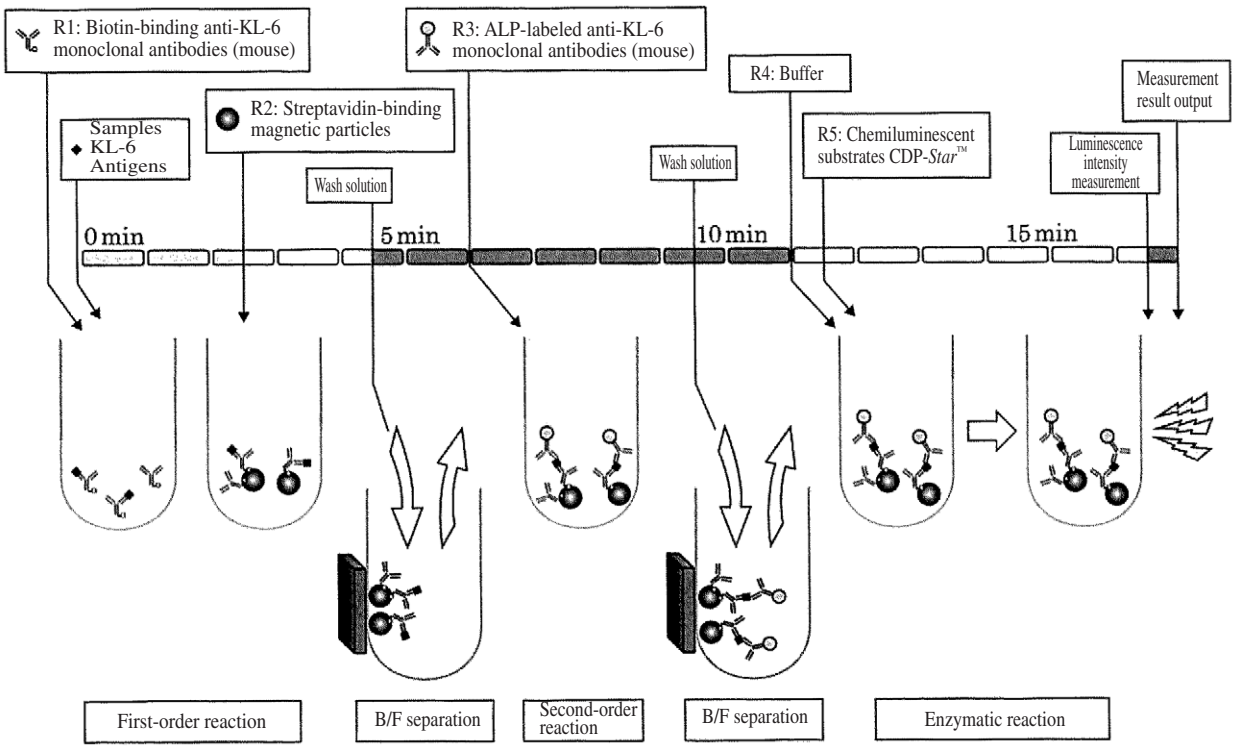

Fig. 1 *Measurement Principle of HISCL KL-6 Reagent*

calibration curve preparation. Using these samples and the reference standard with zero concentration, a total of 10 repeated measurements were made to determine the mean ± 2 standard deviation (SD) of the luminescence intensity (counts). The detection limit was defined as the concentration at which the mean + 2SD of the luminescence intensity for the reference standard and the mean - 2SD of that for each sample in the dilution series do not overlap.

For the evaluation of the dilution linearity, a dilution series was prepared using three samples with high concentrations of KL-6 and HISCL Sample Diluent 2. Based on the test results, the quantitative range of the assay was determined.

To assess the presence or absence of the hook effect, a dilution series was prepared using samples showing abnormally high levels of KL-6 and a reference standard with zero concentration for calibration curve preparation. Based on the test results, it was ascertained if there was any hook effect.

The effect of interfering substances was examined by adding Interference Check A Plus and RF Plus (Sysmex) to control sera L and M at two different concentrations (300 to 800 U/mL and 900 to 2,500 U/mL, respectively) and assessing the variation in test results.

For the evaluation of the correlation between assays, test results from 84 human serum samples were compared between HISCL KL-6 and PicoLumi KL-6. In addition, test results were obtained for 83 concurrently collected EDTA-added plasma samples to examine the effect of the difference in the sample type.

II. RESULTS

Table 1 shows results for the within-run reproducibility. The mean and coefficient of variation (CV) of the within-run reproducibility were 488.7 U/mL and 1.0% for Level 1, 956.0 U/mL and 1.0% for Level 2, and 3,958.1 U/mL and 2.2% for Level 3, respectively. The mean and CV of the between-run reproducibility determined using the same samples were 503.5 U/mL and 1.7% for Level 1, 985.0 U/mL and 2.0% for Level 2, and 4,157.5 U/mL and 2.0% for Level 3, respectively (***Table 2***).

The minimum detection sensitivity was observed for the sample with a theoretical KL-6 concentration of 0.5

Table 1 *Within-Run Reproducibility*

(U/mL)

	Level 1	Level 2	Level 3
1	494	973	3,993
2	493	947	4,076
3	488	960	3,962
4	488	960	3,895
5	495	941	4,017
6	489	949	4,099
7	488	950	3,948
8	488	952	3,854
9	487	965	3,855
10	477	963	3,882
Mean	488.7	956.0	3,958.1
S.D.	5.0	9.8	88.1
C.V. (%)	1.0	1.0	2.2

U/mL, which yielded the mean - 2SD (11,197 counts) for the dilution series not overlapping the mean + 2SD (4,253 counts) of the luminescence intensity of the zero concentration sample (***Table 3***).
When the dilution linearity was examined using three samples with a KL-6 concentration of 2,000 to 4,500 U/mL (Samples A, B, and C), all of these samples showed linearity converging on the origin up to 4,450 U/mL (***Fig. 2***).
Regarding the hook effect, the measured values increased in a semi-linear fashion up to 56,811,876 counts of luminescence intensity (the theoretical KL-6 concentration of 6,307 U/mL). After this peak, no further increase or hook effect was observed (***Fig. 3***).
When examining the effect of potentially interfering substances, no change in measurements was shown for bilirubin F up to 19 mg/dL, for bilirubin C up to 21 mg/dL, for hemolyzed hemoglobin up to 500 mg/dL, for chyle up to 1,410 FTU (formazinturbidity unit), and for rheumatoid factor (RF) up to 500 IU/mL, as compared with KL-6-free samples (***Fig. 4***).

Table 2 *Between-Run Reproducibility*

(U/mL)

	Level 1	Level 2	Level 3
1	499	1,011	4,227
2	502	981	4,164
3	497	995	4,282
4	503	975	4,201
5	497	990	4,221
6	502	976	4,149
7	500	959	4,127
8	489	963	4,012
9	500	961	4,219
10	502	974	4,019
11	503	960	4,195
12	489	956	4,130
13	527	996	4,174
14	514	988	4,223
15	503	1,008	4,228
16	510	997	4,040
17	507	1,018	4,247
18	513	978	4,109
19	505	1,018	4,016
20	507	995	4,167
Mean	503.5	985.0	4,157.5
S.D.	8.5	19.8	81.9
C.V. (%)	1.7	2.0	2.0

Table 3 *Minimum Detection Sensitivity*

	HISCL KL-6 (U/mL)				
	0	0.5	2.5	5	25
1	3,769	12,016	45,524	85,967	399,389
2	3,544	11,460	44,349	85,544	401,062
3	3,742	11,855	43,272	82,723	396,792
4	3,747	11,456	43,605	84,970	400,777
5	3,568	11,820	45,074	88,417	393,882
6	3,746	11,460	44,149	86,034	405,797
7	4,063	11,603	44,998	85,876	408,715
8	3,619	11,754	43,762	85,776	410,427
9	3,710	11,386	43,469	87,263	387,907
10	4,313	11,484	44,306	83,655	402,873
Mean (counts)	3,782	11,629	44,251	85,623	400,762
S.D.	236	216	753	1,619	6,805
Mean − 2SD		11,197	42,744	82,385	387,152
Mean + 2SD	4,253				

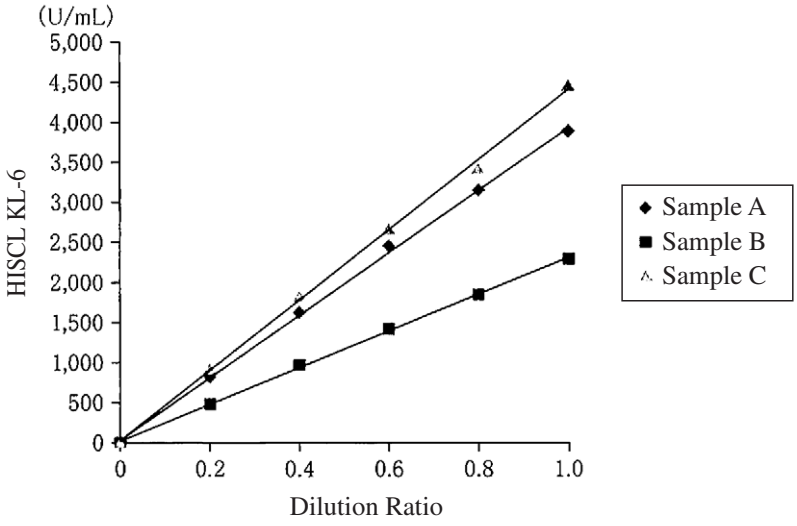

Fig. 2 *Dilution Linearity*

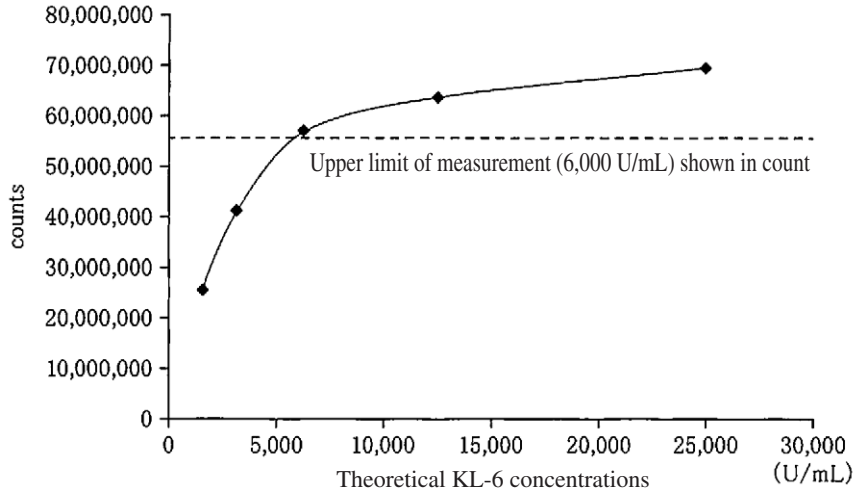

Fig. 3 *Hook Effect*

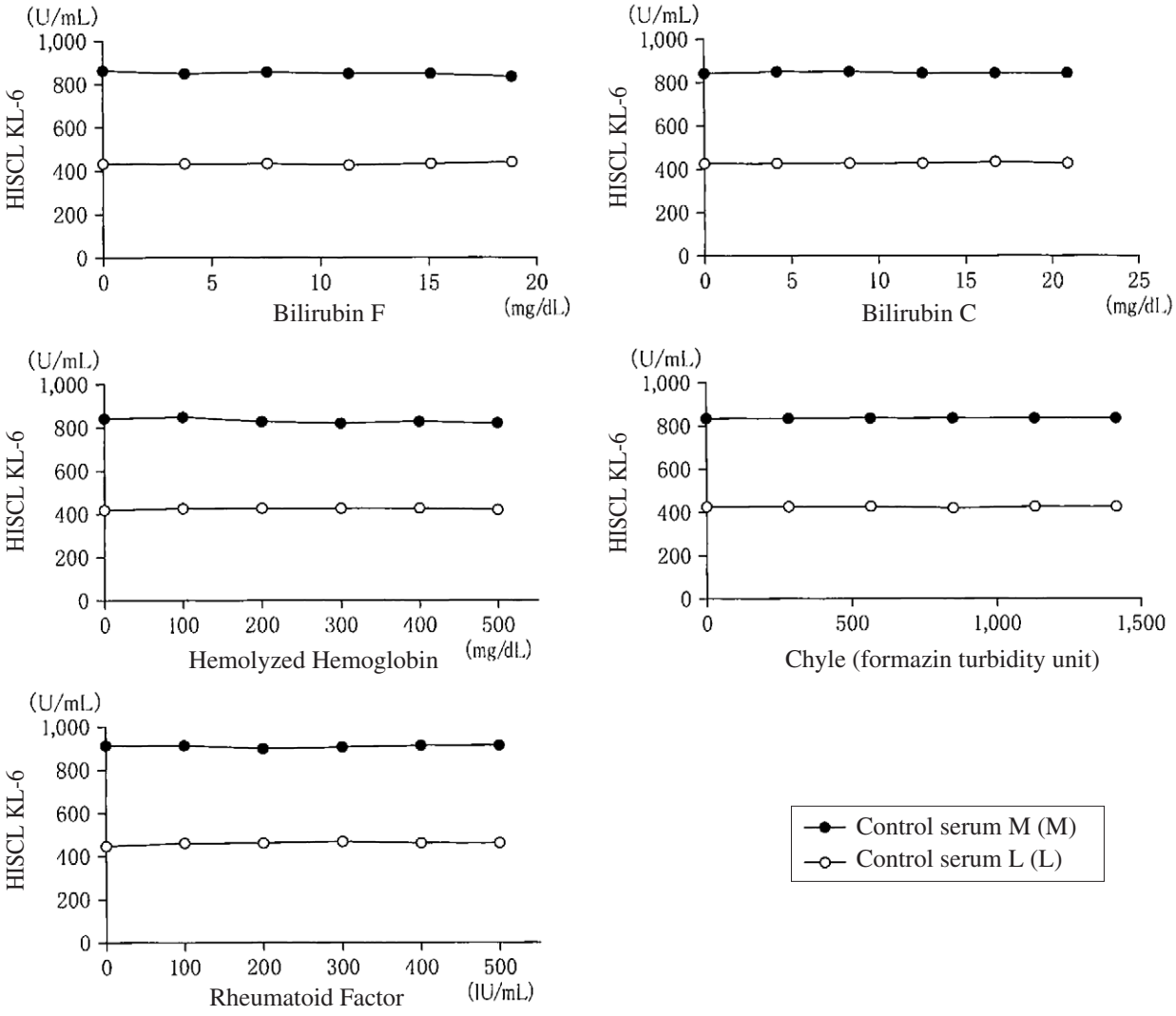

Fig. 4 *Effect of Potentially Interfering Substances*

For the correlation between PicoLumi-KL-6 (x) and HISCL-KL-6 (y) in 84 samples with concentrations of PicoLumi-KL-6 ranging from 121 to 5,268 U/mL, the regression equation was y = 1.02x - 52.31 and the correlation coefficient was r = 0.997 (***Fig. 5a***). In 53 samples with KL-6 concentrations at the cutoff value[5] of 500 U/mL or lower, the regression equation was y = 0.98x - 20.70 and the correlation coefficient was r = 0.958 (***Fig. 5b***).

A comparison of test results between the serum (x) and plasma (y) samples (83 cases) produced a regression equation of y = 0.92x + 3.92 and a correlation coefficient of r = 0.999 (***Fig. 6***).

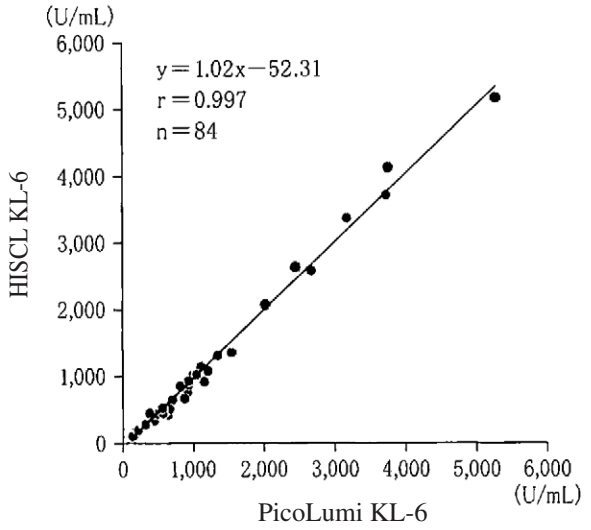

Fig. 5a *Correlation between HISCL KL-6 and PicoLumi KL-6 (Serum)*

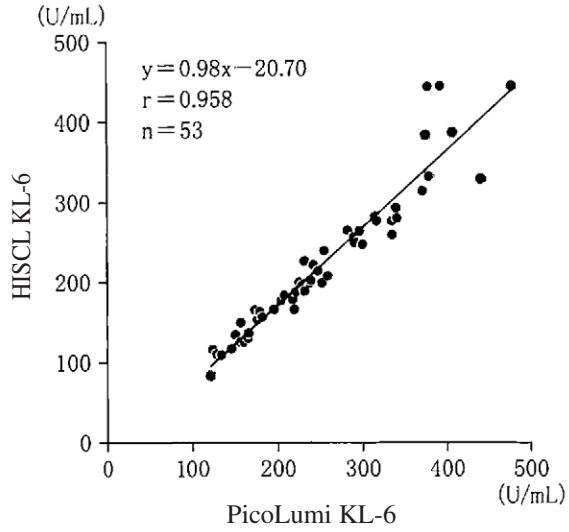

Fig. 5b *Correlation between HISCL KL-6 and PicoLumi KL-6 in Samples Containing KL-6 at 500 U/mL or Lower (Serum)*

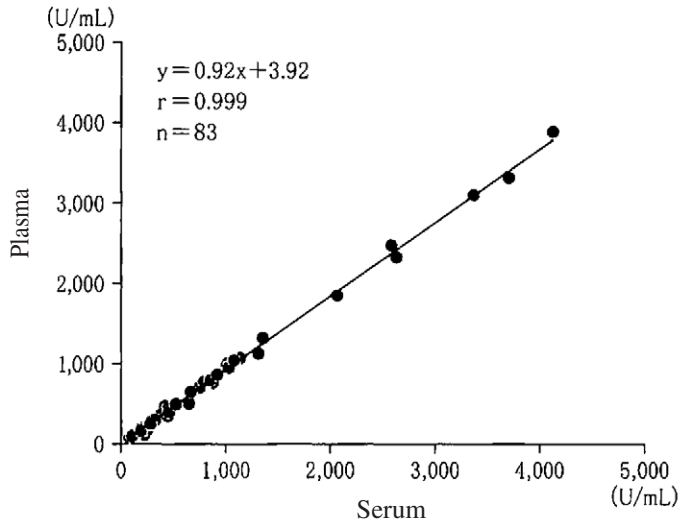

Fig. 6 *Correlation between Plasma and Serum Samples in HISCL KL-6 Reagent*

III. DISCUSSION

Commercially available KL-6 assay reagents include the widely used latex agglutination assay[6] (LIA)-based reagents, reagents for ECLIA, which was used as the control in the present study, and reagents for CLEIA.[7] The reagent studied, HISCL KL-6 Reagent, is a CLEIA-based reagent.

Regarding precision, HISCL KL-6 Reagent had a CV (%) of the within- and between-run reproducibility at each concentration of 2% or lower, showing similar or better performance compared with other assay reagents.[6,7]

The minimum detection sensitivity was 0.5 U/mL, indicating that HISCL KL-6 Reagent can detect KL-6 at concentrations that are significantly lower than the cutoff value of 500 U/mL for the diagnosis of interstitial pneumonia and the concentration range in healthy individuals[5] of 100 to 300 U/mL. With the reported minimum value being 10 U/mL, HISCL KL-6 Reagent demonstrated superior performance for detection of low levels of KL-6 compared with other assay reagents.[6]

Dilution linearity was observed up to 4,450 U/mL. Due to its measurement principle (a two-step sandwich assay) and double B/F separation process, HISCL KL-6 Reagent provided a measurement system producing less hook effect, with measurement values increasing in a semi-linear fashion up to approximately 6,000 U/mL. The results confirmed that KL-6 can be measured up to the highest concentration in the reference solution for calibration curve preparation (approximately 6,000 U/mL) without diluting the samples.

None of the following coexisting substances had any effect on the results: bilirubin F, bilirubin C, hemolyzed hemoglobin, chyle, rheumatoid factor.

The correlation with PicoLumi KL-6 was shown to be favorable, with no major divergence observed between the two reagents. In samples containing KL-6 at the cutoff value of 500 U/mL or lower, measurement values were slightly lower for HISCL KL-6 than that for PicoLumi KL-6. However, there were no divergent samples, showing a good correlation.

Using serum and EDTA-added plasma samples, we examined the effect of sample type on the test results. An 8% decrease was observed in the KL-6 levels in the plasma samples compared with the serum samples but with a favorable correlation at r = 0.999. Therefore, both types of samples were considered appropriate for HISCL KL-6.

CONCLUSION

We evaluated the basic performance of the HISCL KL-6 Reagent for the fully automated immunoassay analyzer HISCL and its usefulness in routine testing. Results showed that the reagent is useful in the diagnosis and follow-up of interstitial pneumonia when it is used in laboratory tests.

References

1) *Kohno N: Commentary, Basic Research on KL-6 (in Japanese). Respiration Research 18 (3): 391-397, 1997.*
2) *Hattori N and Kohno N: [Disease Markers] With a Focus on KL-6 (in Japanese). Mebio 28 (8): 38-46, 2011.*
3) *Kohno N: Diagnosis of Interstitial Pneumonia and Markers for the Disease (in Japanese). Medical Clinics of Japan 24: 2412-2415, 1998.*
4) *Hayashi S: Informed Consent for the Use of the Remaining Portion of Laboratory Specimens for Education, Research, and Quality Control of Laboratory Tests (in Japanese). The Official Journal of Japanese Society of Laboratory Medicine, 49:273-277, 2001.*
5) *Iwata R: The Interstitial Pneumonia Marker KL-6 (in Japanese). Journal of Analytical Bio-Science 37 (5): 346-351, 2014.*
6) *Takamatsu K, Abe M, Hyoki M, et al.: Evaluation of Nanopia® KL-6 Edia Assay Using Latex Agglutination Turbidimetry (in Japanese). Japanese Journal of Medicine and Pharmaceutical Science 62 (3): 491-499, 2009.*
7) *Sakuraba N and Togami Y: Basic and Clinical Evaluation of the KL-6 Assay Reagent "Lumipulse Presto KL-6 Eisai" Using Chemiluminescent Enzyme Immunoassay (in Japanese). Japanese Journal of Medicine and Pharmaceutical Science 61 (4): 629-635, 2009.*