Introduction of Products

Outline of HISCL Reagents

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INTRODUCTION

In recent years, high sensitivity measurements that use chemiluminescence in the detection system have become the mainstay for immunoassay of infection markers, tumor markers, hormones, etc. Considerable shortening of the measurement time has also been achieved to make the testing efficient.
Sysmex launched an automated immunoassay system HISCL-2000*i* (Sysmex Corporation, Kobe, Japan) that comprises an analyzer and reagents capable of rapid high sensitivity measurements with very small sample volumes. I shall describe here an outline of the HISCL reagents, referring to the principle and characteristics of the measurement.

DEVELOPMENT CONCEPT

The name HISCL is used for the new immunoassay system of Sysmex. It stands for high sensitivity chemiluminescence enzyme immunoassay.
As the name suggests, HISCL was developed with the aim of achieving the world's top level sensitivity for parameters like HBsAg and TSH for which there is a demand for high sensitivity measurements. We could achieve minimum detection levels of 0.03 IU/mL for HBsAg and 0.002 μIU/mL for TSH.
In the development of this system, we also aimed to achieve this sensitivity with the small sample volume of 10-30 μL to reduce the burden on the patient, and to complete the measurement of various parameters, each in a short time (about 17 minutes), to meet the demand for rapid testing.

TECHNOLOGY

1. Principle of measurement (common part)

With the HISCL reagents, magnetic particles are used as the solid phase on which the reaction occurs. This is a crucial technology for realizing high sensitivity through reduction of background interference, as the magnetic particles dispersed in the reaction mixture facilitate rapid reaction in a near-liquid phase and magnetic B/F separation (separation by washing the unreacted components from those that have undergone the antigen-antibody reaction).
Alkaline phosphatase (ALP), which is used as the labeling enzyme, in combination with high sensitivity chemiluminescent substrate CDP-*Star* helps realize high sensitivity measurements. CDP-*Star* is a dioxetane derivative with the structure shown in ***Fig. 1***, which becomes luminescent in the presence of ALP through the reaction shown in ***Fig. 2***. CDP-*Star* is more sensitive than other detection substrates (***Fig. 3***), and has made a major contribution to the high sensitivity measurement capability of HISCL reagents.

Note: This article is translated and republished from Sysmex Journal 31, 126-131, 2008.

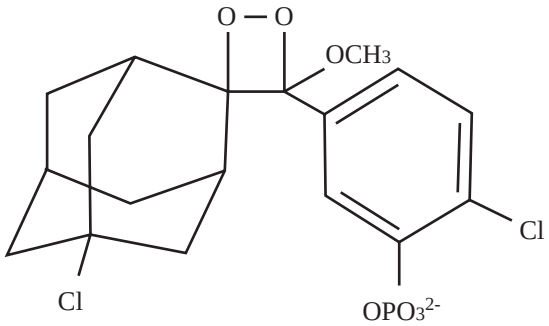


Fig. 1 *Structural formula of CDP-Star*

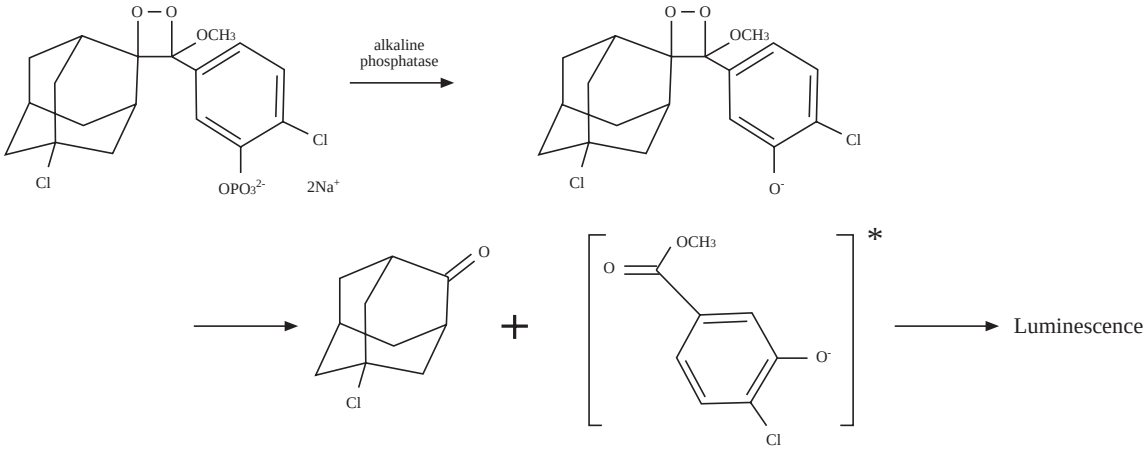


Fig. 2 *Reaction equation of chemiluminescence caused by CDP-Star*

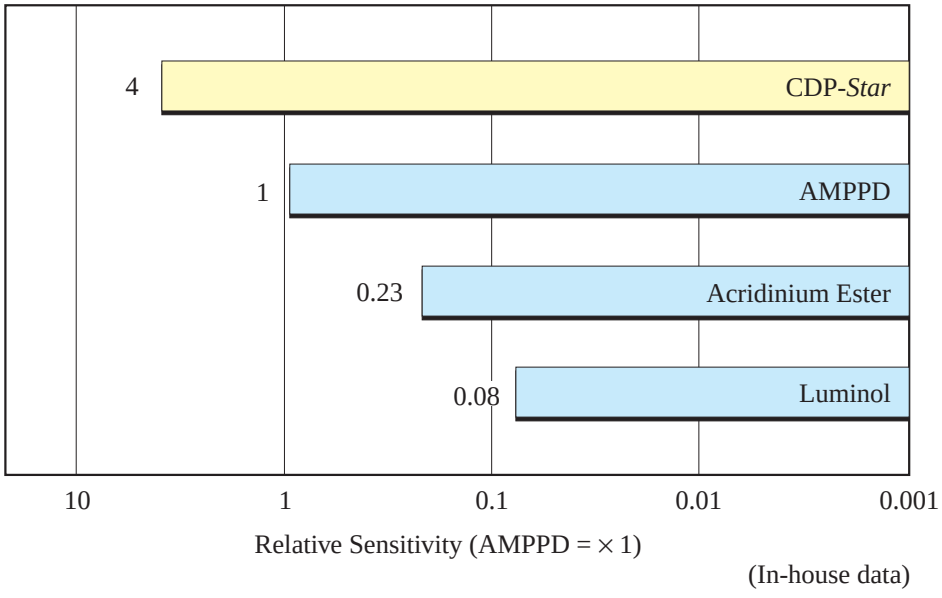


Fig. 3 *Comparison of sensitivity of different detection reagents*

2. Reagent composition

The reagent composition for each parameter consists of a calibrator and R1 to R3 reagents, which differ from one parameter to another, and R4 and R5 reagents (the luminescent substrate reagent set) and the washing solution, which are common for all the parameters.
The details of the R1 to R3 reagents differ depending on the parameter and are shown in ***Table 1***.

3. Principle of measurement

The reaction principle of each reagent differs from parameter to parameter because of the adoption of the optimal reaction for each parameter. I have shown here the principle of measurement for HBsAg and HCVAb, two typical analysis parameters (***Fig. 4***).

Table 1 *Details of R1 to R3 reagents for different analysis parameters*

Parameter	R1 reagent	R2 reagent	R3 reagent
HBsAg	Biotinylated anti-HBs monoclonal antibody	Streptavidin-coated magnetic particles	ALP-labeled anti-HBs monoclonal antibody
HBsAb	ALP-labeled HBsAg	HBsAg-coated magnetic particles	-
HCVAb	Biotinylated HCVAg	HCVAg-coated magnetic particles	ALP-labeled anti-human IgG monoclonal antibody
TSH	ALP-labeled anti-TSH monoclonal antibody	Streptavidin-coated magnetic particles	Biotinylated anti-TSH monoclonal antibody
FT3	Biotinylated anti-T_3 monoclonal antibody	Streptavidin-coated magnetic particles	ALP-labeled T_3
FT4	Biotinylated anti-T_4 monoclonal antibody	Streptavidin-coated magnetic particles	ALP-labeled T_3

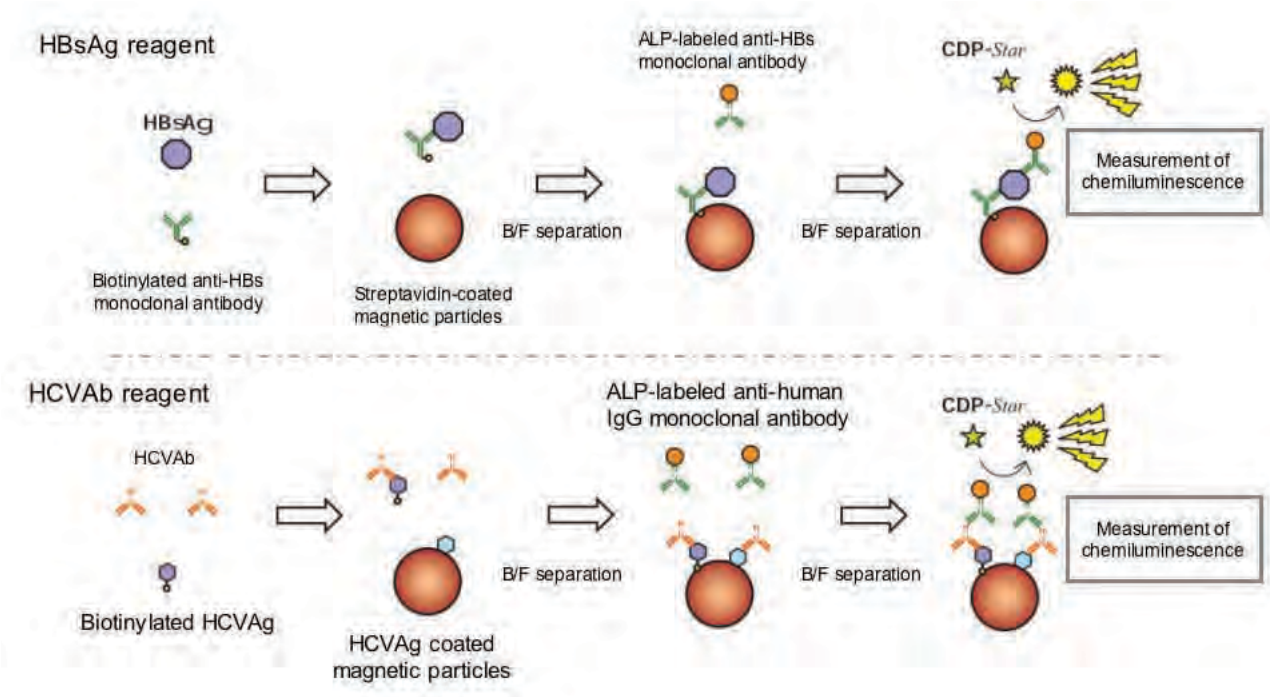


Fig. 4 *Principle of measurement*

1) Reagents for HBsAg

The 2-step sandwich assay is the principle of measurement. In this method, HBsAg in the serum (or plasma) is first made to react with biotinylated anti-HBs monoclonal antibody in R1 reagent to form antigen-antibody complexes. Streptavidin-coated magnetic particles in R2 reagent are added in the next step, which causes strong reaction between the biotin and streptavidin, and immobilizes the HBsAg on the magnetic particles.

After washing with HISCL washing solution (B/F separation) ALP-labeled anti-HBs monoclonal antibody in R3 reagent is added to couple the ALP with the HBsAg of Immunocomplex on the magnetic particles through the antigen-antibody reaction.

Then, after washing off the unreacted ALP-labeled antibodies, the magnetic particles are dispersed in the R4 reagent after which the R5 reagent (CDP-*Star*) is added, and the intensity of the luminescence generated by the chemical reaction is measured.

The HBsAg concentration is then determined using the calibration curve prepared with the calibrator. The sample is assessed as HBsAg positive if the measured value ≥ 0.03 IU/mL, and as HBsAg negative if < 0.03 IU/mL. HBsAg concentration in the positive samples can be quantified from the intensity of the luminescence measured.

2) Reagent for HCVAb

A 2-step sandwich assay that employs HCVAg and anti-human IgG monoclonal antibody is used as the principle of measurement for detection of HCVAb.

In the first reaction, the biotinylated HCVAg in the R1 reagent and HCVAb in the serum (or plasma) react. After that HCVAg coated magnetic particles in R2 reagent is added to allow reaction between the HCVAg and unreacted HCVAb and to capture the biotinylated HCVAg by the streptavidin on the magnetic particles.

After washing, ALP-labeled anti-human IgG monoclonal antibody is added to couple the ALP to the HCVAb on the magnetic particles. Then, the intensity of chemiluminescence is measured following the same steps as in the HBsAg test for the rest of the procedure.

The cut-off index (C.O.I.) is then calculated using the calibrator, and a C.O.I. of ≥ 1.0 is taken as a positive and < 1.0 as negative.

The flow of reactions in HISCL-2000*i*, i.e., the HISCL analyzer system, is shown in ***Fig. 5***. The result can be obtained in about 17 minutes from dispensing the sample.

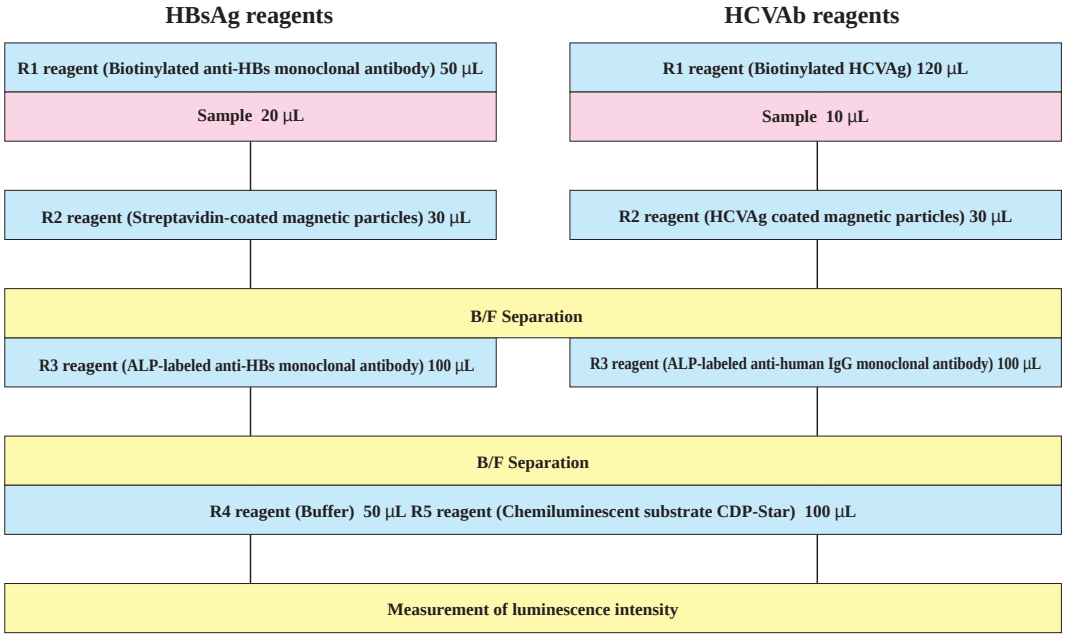
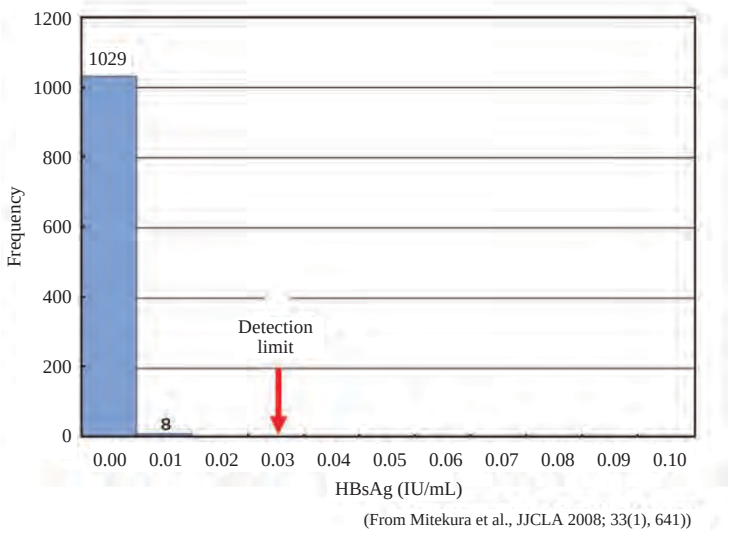


Fig. 5 *Flow of reactions*

Table 2 *Sample volume*

Parameter		HISCL	Manufacturer A	Manufacturer B	Manufacturer C
HBsAg		20µL	100µL	70µL	140µL
Total for 3 thyroid parameters		50µL	160µL	170µL	363µL
Individual thyroid parameters	TSH	30µL	70µL	100µL	178µL
	FT_3	10µL	20µL	45µL	55µL
	FT_4	10µL	70µL	25µL	130µL



(From Mitekura et al., JJCLA 2008; 33(1), 641))

Fig. 6 *Distribution of negative samples in analysis with HISCL reagent for HBsAg (n = 1,037)*

Table 3 *The results of HBs-antigen on HBV seroconversion panel serum.*

No.	Sample	days	HISCL		Manufacturer A		Manufacturer B		PCR
			IU/mL	Int.	IU/mL	Int.	C.O.I.	Int.	Int.
1	PHM931-01	0	0.00	-	0.01	-	0.2	-	-
2	PHM931-02	5	0.00	-	0.01	-	0.3	-	-
3	PHM931-03	12	0.02	-	0.03	-	0.5	-	+
4	PHM931-04	14	0.03	+	0.03	-	0.6	-	+
5	PHM931-05	19	0.08	+	0.09	+	1.2	+	+
6	PHM931-06	21	0.15	+	0.36	+	2.0	+	+
7	PHM931-07	26	0.64	+	0.66	+	9.2	+	+
8	PHM931-08	28	1.24	+	1.21	+	18.2	+	+

(From data collected by Kawasaki Medical School and Saitama Medical Center Jichi Medical University)

Note: HISCL ≥ 0.03 IU/mL Positive
Manufacturer A ≥ 0.05 IU/mL Positive
Manufacturer B ≥ 1.0 C.O.I. Positive

4. Sample volume

One advantageous feature of HISCL reagents is that the sample volume required is very small.
In ***Table 2*** are compared sample volumes required for HISCL reagent system and current reagent systems of different manufacturers, in analysis of HBsAg and three thyroid-related parameters.
The sample volumes required with HISCL reagents are very small, being 20 µL for HBsAg and a total of 50 µL for three thyroid-related parameters, compared to the existing reagent systems.

5. High sensitivity

Detection of a level as low as 0.03 IU/mL with the HBsAg reagent, as can be seen in the distribution of negative samples shown in ***Fig. 6***. This enables detection of infection at an earlier stage than with existing reagents (***Table 3***).

CONCLUSION

High sensitivity and short measurement time have been demanded for immunoassays, and there have been many attempts to improve assay performance by using chemiluminescence detection. With HISCL reagents, all the parameters can be assayed with high sensitivity, with small sample volumes, and within a short time of about 17 minutes. Therefore, the HISCL reagents would be useful for routine testing.