Evaluation of HISCL HCV Antibody Reagent and Comparative Examination of HCV Antibody Reagents of Various Manufacturers

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INTRODUCTION

As a routine screening test for hepatitis C virus (HCV) infection, the HCV antibody test has been used widely. Recently, various manufacturers have developed and released HCV antibody reagents that can be measured automatically using a number of proprietary immunoserological assay systems. Sysmex Corporation has also developed and released HCV antibody reagents that are compatible with the Sysmex HISCL Series chemistry analyzer. HISCL, which adopts chemiluminescence enzyme immunoassay (CLEIA) as the measurement principle, is regarded as a relatively high-speed system requiring a reaction period of only 17 minutes. Recently, we had an opportunity to use the HISCL HCV antibody reagent and evaluated its basic performance. This evaluation was important as both HISCL reagents and other HCV antibody reagents used in routine examinations show inconsistent results due to inter-reagent differences [1,3,4]. Sufficient caution should be exercised in interpreting the measurement results because of the differences in the antigens used in the various kits, different performance of reagents themselves, and variability of existing patterns of antibodies in the samples collected from patients. In the present study—in consideration of these actual conditions—we measured panel serum and patient samples using different measurement systems at several facilities. In order to confirm the measurement characteristics of reagents from various manufacturers, we conducted a comparative examination and report the results in this publication.

MATERIALS AND METHODS

1. Materials

1) HISCL System, Reagent

HISCL System and HISCL Anti-HCV Reagent, special reagent for the system, (Sysmex Corporation) were used in the present study.

2) Other systems

Other measurement systems used in the present study included the following eight test reagents and RIVA Test III.

i) ARCHITECT HCV (ARCHI) (Abbott Japan Co., Ltd.)
ii) AxSYM HCV Dynapack II (AxSYM) (Abbott Japan Co., Ltd.)
iii) Vitros HCV Antibody (Vitros) (Ortho-Clinical Diagnostics, Inc.)
iv) ECLusys Reagent Anti-HCV (Elecsys) (Roche Diagnostic K.K.)
v) E Test 'TOSOH' II (Anti-HCV) (AIA) (TOSOH Corporation)
vi) Chemi-Lumi Centaur-HCV Antibody (Centaur) (Siemens Healthcare/Diagnostics K.K.)
vii) Lumipulse II Ortho HCV (FORTE) (measured by LUMIPULSE FORTE) (Fujirebio Inc./ Ortho-Clinical Diagnostics, Inc.)
viii) Lumipulse Presto Ortho HCV (PRESTO) (Fujirebio Inc.)
ix) Chiron HCV RIBA Test III (RIBAIII) (Ortho-Clinical Diagnostics, Inc.)

(***Table 1***)

3) Materials for evaluation of reagents

The manufacturer quality control samples and the negative and high-level panel samples that emerged from routine screening were used for evaluation of the HISCL reagent.

As samples for evaluation of the reagent systems, we used 15 HCV low-titer panel serum samples (PHV106-01 - PHV106-15) from SeraCare Life Sciences, Inc. (SeraCare) (Sample Group 1) and 18 HCV seroconversion panel serum samples (PHV901-01 — PHV901-11; PHV912-01 - PHV912-03; PHV915-01 - PHV915-04) from SeraCare (Sample Group 2), which were collected from a single person infected with HCV in the course of time immediately after infection. According to the data sheets attached to these HCV seroconversion panel serum samples, the origins of PHV901, PHV912 and PHV915 were c100p (NS4) antibody, c22p (Core) antibody and c33c (NS3) antibody respectively. Of the samples collected at participating laboratories, those that provided measurements suggesting low HCV antibody titers were selected for evaluation, and, thus, a total of 121 samples collected from patients who agreed to use of their samples were examined in this study (Sample Group 3).

2. Methods

1) Evaluation of HISCL Anti-HCV Reagent

i) Within-run reproducibility test: The quality control samples at two different concentrations were used to evaluate within-run reproducibility (n = 20).

ii) Between-run reproducibility test: The quality control samples were used at two different concentrations to evaluate the measurement results obtained over 11 days.

iii) Negative distribution test: A total of 168 samples that tested negative during routine screening were used to obtain the distribution of the negative results of the HISCL Anti-HCV Reagent.

iv) Hook effect test: Hook effect testing of HISCL Anti-HCV Reagent was conducted after dilution measurement of the high-level samples.

2) Evaluation of measurement systems

According to their package inserts, a total of nine reagents for HCV antibody measurement adopted by nine

Table 1 *Characteristics of eleven assays used to detect Anti-HCV.*

Reagent	Analysis system	Principle	HCV antigen protein used					B/F separation	Conjugate reagent	Unit	Cutoff value
			core	E1～NS2	NS3	NS4	NS5				
ARCHITECT HCV (ARCHI)	ARCHITECT i2000SR (connected)	CLIA	HC-43			c100-3		Magnetic particle	Acridinium labeled human immunoglobulin (monoclonal)	S/CO	1.00
AxSYM HCV Dynapack II (AxSYM)	AxSYM	EIA	HC-34			c100-3		Resin particle	Alkaline phosphatase-labeled anti-human IgG antibody (polyclonal)	S/CO	1.00
						C200					
Vitros HCV Antibody (Vitros)	Vitros 5600	CLEIA	c22-3			C200	NS5	Well	Peroxidase labeled anti-human IgG antibody (mono)	C.O.I.	1.0
ECLusys Reagent Anti-HCV (Elecsys)	COBAS e601 COBAS e411	ECLIA			c33-c			Magnetic particle (biotin)	Ruthenium-labeled HCV antigen	C.O.I.	1.0
HISCL Anti-HCV Reagent (HISCL)	HISCL	CLEIA		?				Magnetic particle (biotin concomitantly used)	Alkaline phosphatase labeled anti-human IgG antibody (mono)	C.O.I.	1.0
E Test 'TOSOH' II (Anti-HCV) (AIA)	AIA-1800	EIA	c50 antigen					Magnetic bead	Alkaline phosphatase labeled antibody	Index	1.0
Chemi-Lumi Centaur-HCV Antibody (Centaur)	ADVIA Centaur XP	CLIA			c200		NS5	Magnetic particle	Acridinium	Index	1.0
Lumipulse II Ortho HCV (FORTE)	Lumipulse f	CLEIA	c25		c200		NS5	Ferrite particle	Alkaline phosphatase labeled anti-human IgG antibody (mono)	C.O.I.	1.0
					c33c	c100-3					
Lumipulse Presto Ortho HCV (PRESTO)	Lumipulse Presto II	CLEIA	c25		c200		NS5	Ferrite particle	Alkaline phosphatase labeled anti-human IgG antibody (mono)	C.O.I.	1.0
					c33c	c100-3					
Chiron HCV RIBA Test III (RIBAIII)	Manual method	Immunoblot	c22p		c33c	c100p	NS5	Immunoblot	Peroxidase labeled anti-human IgG antibody (poly)		Positive line

laboratories were used to measure the above sample groups. The samples of Sample Group 1 and 2 were divided into smaller quantities and delivered to the participating laboratories for measurement. The samples of Sample Group 3 were kept refrigerated for delivery among the participating laboratories and thus about two months were needed to complete these measurements. Moreover, an HCV RIVA Test III was conducted on all the samples.

RESULTS

1. Basic performance of HISCL Anti-HCV Reagent

1) Within-run reproducibility test
With the accuracy control samples at two different concentrations, 20 consecutive measurements were conducted and the coefficients of variation (CVs) were 2.20% and 2.25% (***Table 2***).

2) Between-run reproducibility test
With the accuracy control samples at two different concentrations, measurements were conducted for 10 days and the CVs were 6.40% and 5.58% (***Table 2***).

3) Hook effect test
A high-level sample (cutoff index (COI): 114.3) was diluted serially with the HISCL sample diluent for measurement with HISCL. All the original serum and diluted samples tested positive. Up to a COI of 114.3, no Hook effect phenomenon was observed on testing using the HISCL Anti-HCV Reagent (***Fig. 1***).

Table 2 *Within-run reproducibility and between-run reproducibility of HISCL Anti-HCV reagent*

Within-run reproducibility

Measurement sample	1	2
Measurement frequency	20	20
MEAN	3.88	18.06
SD	0.09	0.41
CV (%)	2.20	2.25

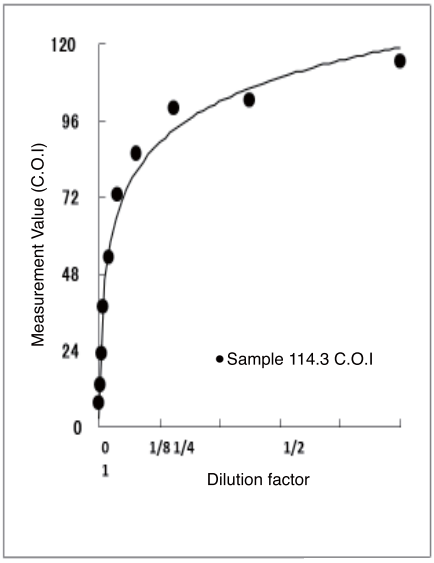
CV of within-run reproducibility ≤ 2.25%

Between-run reproducibility

Measurement sample	1	2
Measurement frequency	10	10
MEAN	3.79	17.33
SD	0.24	0.97
CV (%)	6.40	5.58

CV of between-run reproducibility ≤ 6.40%

* Units: COI
* Viratrol (Sysmex) was used for measurement.



Absence of prozone phenomenon before reaching COI 114.3 confirmed

* Of the samples sent for Anti-HCV measurement, Anti-HCV positive samples were used for measurement.
* HISCL sample diluent was used for dilution.

Fig. 1 *Hook effect test*

4) Negative distribution test

A total of 175 HCV antibody negative samples obtained during routine screening were used to examine the negative sample part. All the samples showed a level below 1.0 (< 1.0); the mode was 0.1, and the percentage of relevant samples was 46.3% (n = 81). A total of 164 samples showed levels less than 0.2 (≤ 0.2), accounting for 93.7% of all negative samples (***Fig. 2***).

2. Comparison of assay systems of various manufacturers

Sample Group 1 was measured with ARCHI, AxSYM, FORTE, AIA and HISCL. Of 15 samples, one (PHV106-11) tested negative using all measurement systems. Two samples (PHV106-8 and PHV106-10) tested positive for all measurement systems. The remaining 12 samples tested negative on measurement with some reagents. Fourteen samples that tested positive on measurement with all or some reagents were selected to arrange the reagents in the order of the number of samples tested positive. These were placed in the following order and only five samples tested positive on measurement with HISCL: AxSYM (13/14), ARCHI (12/14), FORTE (11/14), AIA (6/14) and HISCL (5/14). Six samples showed positive results in the recombinant immunoblot assay (RIBA-III) documented in the data sheet of SeraCare's panel serum, and ARCHI, AxSYM and FORTE showed consistent results for all the samples, while AIA and HISCL showed inconsistent results for three samples. Three samples showed positive results on HCV polymerase chain reaction (PCR) analysis documented in the data sheet of SeraCare's panel serum, and ARCHI, AxSYM and FORTE showed consistent results for all the samples, while HISCL showed inconsistent results for two samples and AIA showed inconsistent results for all the samples (***Table 3***).

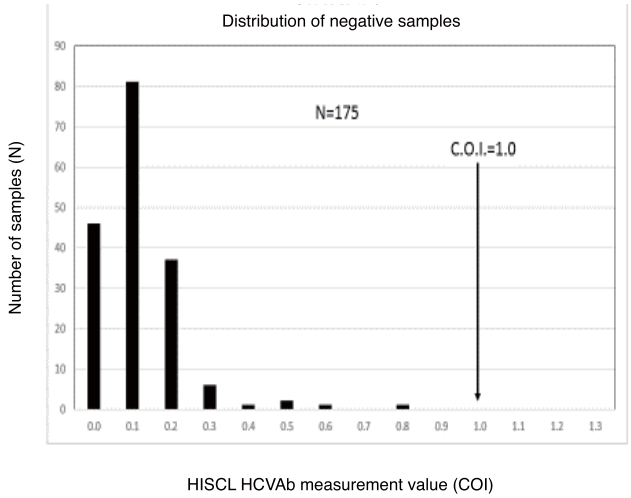


Fig. 2 *Distribution of negative results on measurement with HISCL Anti-HCV Reagent*

Table 3 *Comparison between seven different Anti-HCV and HCV RNA assays using low titer Anti-HCV Group 1 positive panels*

	ARCHI		AxSYM		FORTE		AIA		HISCL		RIBA Ⅲ (Seracare)					HCVRNA(Seracare)
	S/CO	Assessment	S/CO	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	c100p (NS4)	c33c (NS3)	c22p (Core)	NS5	Assessment	IU/ml
PHV106-01	2.55	+	2.74	+	0.1	-	0.0	-	0.0	-	-	1+	-	-	Undetermined	<600
PHV106-02	3.84	+	2.52	+	5.7	+	0.0	-	2.2	+	-	2+	-	-	Undetermined	<600
PHV106-03	1.02	+	1.16	+	0.7	-	3.9	+	1.6	+	+/-	+/-	1+	-	Undetermined	<600
PHV106-04	13.81	+	63.33	+	36.5	+	0.0	-	2.0	+	3+	4+	-	-	Positive	3.8E+03
PHV106-05	2.00	+	2.45	+	1.0	+	1.5	+	0.6	-	-	+/-	1+	+/-	Undetermined	<600
PHV106-06	3.87	+	3.97	+	2.6	+	0.0	-	0.2	-	1+	1+	-	-	Positive	<600
PHV106-07	5.50	+	7.61	+	2.4	+	1.5	+	0.4	-	2+	1+	+/-	-	Positive	<600
PHV106-08	6.67	+	7.43	+	2.9	+	5.5	+	3.7	+	1+	2+	2+	1+	Positive	<600
PHV106-09	7.61	+	12.31	+	6.1	+	0.0	-	0.1	-	+/-	2+	-	-	Undetermined	1.4E+03
PHV106-10	4.34	+	3.91	+	1.5	+	9.5	+	3.6	+	1+	+/-	3+	+/-	Positive	<600
PHV106-11	0.12	-	0.41	-	0.2	-	0.0	-	0.1	-	-	-	-	-	Negative	<600
PHV106-12	12.74	+	52.42	+	27.8	+	0.0	-	0.7	-	3+	4+	-	-	Positive	7.4E+03
PHV106-13	0.31	-	1.05	+	1.4	+	0.0	-	0.0	-	3+	-	-	-	Undetermined	<600
PHV106-14	0.37	-	1.28	+	1.6	+	0.0	-	0.0	-	3+	-	-	-	Undetermined	<600
PHV106-15	1.27	+	0.99	-	0.2	-	21.4	+	0.3	-	-	-	2+	-	Undetermined	<600

Sample Group 2 was measured with ARCHI, AxSYM, FORTE, PRESTO, AIA, HISCL, Elecsys and Centaur. PHV901 tested positive at the same time on measurement with ARCHI, AxSYM, FORTE, PRESTO, Elecsys and Centaur. This timing was consistent with the time of the positive c100p (NS4) antibody test with RIBA-III. The samples tested positive later on measurement with AIA and HISCL. According to the RIBA-III results described in the data sheet of SeraCare's panel serum, PHV912 is the sample in which c22p (Core) antibody is first detected as positive. All the samples tested positive on measurement with Elecsys; PHV912-1 and PHV912-3 tested positive on measurement with AxSYM; and PHV912-3 alone tested positive on measurement with ARCHI, FORTE, PRESTO, AIA, HISCL and Centaur. According to the RIBA-III results described in the data sheet of SeraCare's panel serum, PHV915 is the sample that tests positive using c33c (NS3) antibody. On measurement with AxSYM and PRESTO, positive results were obtained from PHV915-2, while, on measurement with ARCHI, FORTE, Elecsys and Centaur, positive results were obtained from PHV915-3. On measurement with AIA and HISCL, all the samples tested negative (***Table 4***).

Sample Group 3 was measured with ARCHI, AxSYM, Vitros, Elecsys, HISCL, AIA, Centaur, FORTE, PRESTO and RIBA-III. Sample Group 3 (n = 121) included 43 samples that could be measured with all the measurement systems and showed inconsistent results on measurement with one or more systems. The data here obtained are displayed in ***Table 4***. In Sample Group 3, a total of 13 samples tested positive on measurement with one system alone, and the number of samples for each reagent was as follows: ARCH (n = 2), AxSYM (n = 4), Vitros (n = 1), ELecsys (n = 1), HISCL (n = 4) and RIBA-III (n = 1). A total of two samples tested negative on measurement with one system alone, and the number of samples for each reagent was as follows: HISCL (n = 1) and AIA (n = 1). There were 15 samples that tested positive on measurement using more than six of 10 reagents. In Sample Group 3, more than 22 of 43 samples showed positive results for three reagents: ARCHI, AXSYM and Vitros; while fewer than 14 samples showed positive results for four reagents: Centaur, FORTE, PRESTO and RIBA-III. Five samples tested positive for RIBA-III and these included one independent positive sample. For 21 samples or approximately half of all Group 3 samples, the results were undetermined. When one sample that tested independently positive for RIBA-III was excluded, the remaining four samples tested negative with the following reagents: AIA (n = 1), HISCL and AIA (n = 1), FORTE and PRESTO (n = 1), Elecsys, HISCL, Centaur, FORTE and PRESTO (n = 1). When the positive samples and the undetermined samples were included in the analysis, eight samples (19%) tested positive for c22p (Core) antibody alone and five samples (12%) tested positive for c33c (NS3) antibody alone. The former samples showed higher positive rates on measurement, particularly with ARCHI, AxSYM, Vitros, Elecsys and AIA; while the latter samples showed similar

Table 4 *Comparison between ten different Anti-HCV assays using three Anti-HCV Group 2 Seroconversion panels*

	ARCHI		AxSYM		FORTE		PRESTO		AIA		HISCL		ELecsys		Centaur		RIBA Ⅲ (Seracare)				
	S/CO	Assessment	S/CO	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	C.O.I.	Assessment	c100p (NS4)	c33c (NS3)	c22p (Core)	NS5	Assessment
PHV901-01	0.04	-	0.52	-	0.1	-	0.1	-	<0.1	-	0.1	-	0.1	-	0.11	-	-	-	-	-	Negative
PHV901-02	0.02	-	0.30	-	0.1	-	0.1	-	<0.1	-	0.0	-	0.1	-	0.05	-	-	-	-	-	Negative
PHV901-03	9.90	+	67.08	+	12.7	+	16.5	+	<0.1	-	0.4	-	944.4	+	>11.0	+	3+	-	-	-	Undetermined
PHV901-04	8.66	+	63.36	+	11.8	+	15.9	+	<0.1	-	0.4	-	851.5	+	>11.0	+	3+	4+	-	-	Positive
PHV901-05	8.51	+	67.93	+	13.1	+	16.9	+	<0.1	-	0.7	-	970.6	+	>11.0	+	3+	4+	-	-	Positive
PHV901-06	9.91	+	69.26	+	12.0	+	16.3	+	<0.1	-	0.6	-	856.7	+	>11.0	+	3+	4+	-	-	Positive
PHV901-07	10.77	+	94.36	+	29.6	+	34.3	+	2.2	+	10.7	+	904.7	+	>11.0	+	4+	4+	-	-	Positive
PHV901-08	11.11	+	85.28	+	25.0	+	29.6	+	2.1	+	9.6	+	858.3	+	>11.0	+	4+	4+	-	-	Positive
PHV901-09	10.84	+	99.28	+	44.5	+	46.6	+	15.7	+	30.9	+	723.4	+	>11.0	+	4+	4+	-	-	Positive
PHV901-10	11.20	+	88.26	+	39.5	+	44.7	+	15.3	+	27.0	+	807.8	+	>11.0	+	4+	4+	-	-	Positive
PHV901-11	12.26	+	133.49	+	71.8	+	61.4	+	73.7	+	39.8	+	591.5	+	>11.0	+	4+	4+	±	-	Positive
PHV912-01	0.27	-	1.01	+	0.2	-	0.3	-	<0.1	-	0.1	-	11.7	+	0.33	-	-	-	-	-	Negative
PHV912-02	0.26	-	0.76	-	0.2	-	0.3	-	<0.1	-	0.1	-	10.1	+	0.28	-	-	-	-	-	Negative
PHV912-03	9.66	+	20.65	+	2.7	+	3.0	+	84.9	+	36.5	+	262.8	+	>11.0	+	±	-	4+	-	Undetermined
PHV915-01	0.11	-	0.78	-	0.2	-	0.3	-	<0.1	-	0.3	-	0.1	-	0.20	-	-	±	-	-	Negative
PHV915-02	0.53	-	1.59	+	0.8	-	1.2	+	<0.1	-	0.4	-	0.6	-	0.93	-	-	1+	-	-	Undetermined
PHV915-03	1.70	+	3.88	+	1.6	+	2.8	+	<0.1	-	0.5	-	1.9	+	2.91	+	-	2+	-	-	Undetermined
PHV915-04	4.12	+	12.57	+	3.6	+	6.5	+	<0.1	-	0.9	-	16.0	+	7.22	+	-	3+	-	-	Undetermined

findings on measurement with ARCHI and AxSYM. No sample tested positive for c100p antigen alone or NS5 antigen alone (***Table 5***).

In Sample Group 3, the 43 samples where testing disagreed were selected for further analysis. For both positive and negative results, higher agreement rates, ranging from 83.7% to 88.4%, were recognized among the measurements with Centaur, FORTE and PRESTO. The agreement rates among the measurements with ARCHI, AxSYM and Vitros ranged from 74.4% to 76.7%. The agreement rates between the measurements obtained with Elecsys and the measurements obtained with other reagents (excluding RIBA-III) exceeded 60% (67.4%-74.4%). The agreement rates (excluding the assessment of "undetermined") between RIBA-III, and ARCHI, Centaur, FORTE or PRESTO exceeded 40% (41.9%-46.5%) (***Table 6***).

The samples were moved among the participating laboratories for measurement. To assure absence of any influence of a change in the samples over time, or any difference between the former measurement results and the latter measurement results, of 121 samples, 62 samples where sufficient quantities remained for measurement were remeasured with ARCHI, which was used in the initial measurement. Disagreement was recognized in measurements obtained from one sample alone (1.00 → 0.98). The agreement rate was 98.4% and difference in measurement timing did not affect the results.

Table 5 *Comparison between ten different Anti-HCV assays using Low Titer Anti-HCV Group 3 positive samples tested at multiple labs*

	ARCHI		AxSYM		Vitros		ELecsys		HISCL		AIA		Centaur		FORTE		PRESTO		RIBAIII	Details of RIBA-III				
																				c100p (NS4)	c33c (NS3)	c22p (Core)	NS5	SOD
1	7.00	+	5.85	+	17.10	+	185.5	+	7.4	+	23.3	+	4.52	+	2.5	+	2.3	+	Undetermined	-	-	4+	-	-
2	5.78	+	5.68	+	18.70	+	452.3	+	6.9	+	16.3	+	6.99	+	1.8	+	2.5	+	Undetermined	±	±	4+	-	-
3	2.73	+	2.59	+	7.33	+	144.3	+	3.4	+	3.6	+	5.67	+	1.1	+	1.4	+	Undetermined	±	±	3+	±	-
4	7.34	+	7.75	+	9.43	+	877.8	+	4.2	+	0.1	-	9.33	+	5.5	+	7.2	+	Positive	1+	4+	±	±	-
5	5.90	+	6.81	+	6.54	+	603.1	+	0.0	-	7.6	+	7.71	+	3.7	+	6.2	+	Undetermined	-	2+	-	-	-
6	5.46	+	10.78	+	4.85	+	272.9	+	0.7	-	0.1	-	11.00	+	2.9	+	3.7	+	Positive	2+	2+	±	±	-
7	3.70	+	2.90	+	7.62	+	283.3	+	2.4	+	3.6	+	1.92	+	1.0	+	1.0	+	Undetermined	-	±	3+	-	-
8	2.80	+	2.60	+	6.56	+	345.8	+	3.9	+	9.3	+	1.88	+	1.0	+	0.7	-	Undetermined	-	-	3+	-	-
9	4.46	+	3.03	+	17.90	+	74.4	+	1.4	+	4.5	+	1.42	+	0.6	-	0.9	-	Positive	±	±	3+	1+	-
10	2.10	+	2.42	+	3.30	+	223.8	+	2.6	+	3.8	+	1.75	+	0.9	-	0.9	-	Undetermined	-	-	2+	-	-
11	1.10	+	1.53	+	1.53	+	166.7	+	0.4	-	0.1	-	1.32	+	1.2	+	2.2	+	Undetermined	-	-	1+	-	-
12	1.49	+	1.90	+	3.98	+	141.0	+	0.9	-	3.1	+	1.10	+	0.5	-	0.8	-	Undetermined	-	-	3+	-	-
13	1.40	+	1.33	+	1.08	+	19.0	+	2.7	+	3.5	+	0.46	-	0.9	-	0.8	-	Negative	-	±	±	-	-
14	1.17	+	1.56	+	2.34	+	65.2	+	1.4	+	2.5	+	0.73	-	0.5	-	0.6	-	Undetermined	±	-	2+	-	-
15	1.04	+	1.39	+	2.71	+	41.8	+	1.0	+	1.9	+	0.56	-	0.4	-	0.5	-	Undetermined	-	-	±	2+	-
16	1.00	+	1.24	+	1.67	+	54.7	+	1.0	+	2.9	+	0.34	-	0.4	-	0.3	-	Undetermined	-	±	3+	-	-
17	3.90	+	3.15	+	1.59	+	0.1	-	2.0	+	0.1	-	0.68	-	3.1	+	0.7	-	Undetermined	-	3+	-	-	-
18	2.02	+	1.06	+	2.55	+	0.1	-	0.3	-	36.2	+	0.85	-	0.5	-	0.1	-	Positive	-	1+	2+	-	-
19	1.90	+	0.93	-	19.80	+	0.1	-	0.1	-	1.9	+	1.66	+	2.8	+	0.1	-	Undetermined	-	-	2+	-	-
20	1.01	+	0.88	-	0.59	-	52.1	+	8.0	+	0.1	-	0.10	-	1.6	+	0.4	-	Undetermined	-	2+	±	±	-
21	1.47	+	0.97	-	4.68	+	44.3	+	0.8	-	1.4	+	0.56	-	0.3	-	0.3	-	Undetermined	-	-	3+	-	-
22	1.30	+	1.61	+	6.81	+	0.1	-	0.3	-	3.6	+	0.51	-	0.5	-	0.3	-	Undetermined	-	-	3+	-	-
23	1.10	+	0.88	-	0.68	-	0.1	-	0.0	-	0.1	-	1.72	+	1.2	+	0.3	-	Undetermined	-	2+	-	-	-
24	3.68	+	1.67	+	0.60	-	0.4	-	0.9	-	0.1	-	0.17	-	0.5	-	0.2	-	Undetermined	-	3+	-	-	-
25	1.30	+	1.70	+	0.61	-	0.1	-	0.0	-	0.1	-	0.14	-	0.9	-	0.4	-	Undetermined	-	1+	-	-	-
26	1.20	+	1.15	+	0.24	-	0.1	-	0.1	-	0.1	-	0.00	-	0.3	-	0.2	-	Undetermined	-	1+	-	±	-
27	0.68	-	0.92	-	1.55	+	0.1	-	0.1	-	3.1	+	0.00	-	0.3	-	0.2	-	Negative	±	±	-	±	-
28	0.17	-	1.69	+	1.04	+	0.1	-	0.0	-	0.1	-	0.37	-	0.3	-	0.2	-	Negative	-	-	-	-	-
29	0.14	-	0.69	-	0.16	-	1.7	+	19.2	+	0.1	-	0.10	-	0.4	-	0.1	-	Negative	-	±	±	-	-
30	0.06	-	0.50	-	0.32	-	35.9	+	1.3	+	0.1	-	0.10	-	0.3	-	0.1	-	Negative	-	±	±	±	-
31	1.26	+	0.54	-	0.11	-	0.1	-	0.1	-	0.1	-	0.00	-	0.2	-	0.1	-	Negative	-	-	-	-	-
32	1.10	+	0.75	-	0.13	-	0.4	-	0.7	-	0.1	-	0.00	-	0.7	-	0.1	-	Negative	-	±	-	-	-
33	0.72	-	0.45	-	2.40	+	0.1	-	0.1	-	0.1	-	0.00	-	0.2	-	0.1	-	Negative	-	±	-	±	-
34	0.71	-	1.36	+	0.26	-	0.1	-	0.1	-	0.1	-	0.17	-	0.2	-	0.4	-	Negative	±	±	±	±	±
35	0.16	-	0.79	-	0.05	-	0.2	-	1.1	+	0.1	-	0.00	-	0.1	-	0.1	-	Negative	-	-	-	-	-
36	0.15	-	1.15	+	0.87	-	0.1	-	0.1	-	0.1	-	0.48	-	0.4	-	0.2	-	Negative	-	-	-	-	-
37	0.11	-	0.52	-	0.71	-	8.0	+	1.0	-	0.1	-	0.23	-	0.5	-	0.1	-	Negative	-	±	±	±	-
38	0.10	-	1.00	+	0.16	-	0.1	-	1.0	-	0.1	-	0.00	-	0.2	-	0.2	-	Negative	-	-	-	-	-
39	0.08	-	0.82	-	0.07	-	0.3	-	1.3	+	0.1	-	0.00	-	0.1	-	0.1	-	Negative	-	-	-	-	-
40	0.08	-	0.60	-	0.08	-	0.4	-	1.6	+	0.1	-	0.00	-	0.1	-	0.1	-	Negative	-	-	-	-	-
41	0.05	-	1.28	+	0.54	-	0.1	-	0.0	-	0.1	-	0.82	-	0.5	-	0.3	-	Negative	-	-	-	-	-
42	0.05	-	0.48	-	0.08	-	0.2	-	4.7	+	0.1	-	0.20	-	0.1	-	0.1	-	Negative	-	-	-	-	-
43	0.55	-	0.64	-	0.12	-	0.1	-	0.2	-	0.1	-	0.00	-	0.3	-	0.2	-	Positive	±	2+	±	1+	-
No. of positive samples	28		27		24		21		20		18		14		13		8		5	2	11	15	3	0
Percentage of positive samples	65.1		62.8		55.8		48.8		46.5		41.9		32.6		30.2		18.6		11.6	4.7	25.6	34.9	7.0	0.0
No. of undetermined samples																			21	7	13	10	10	1
Percentage of undetermined samples																			48.8	16.3	30.2	23.3	23.3	2.3

Table 6 *The Data concordance rate by eleven assay kits*

(%)

	RIBA	PRESTO	FORTE	Centaur	AIA	HISCL	ELecsys	Vitros	AxSYM
ARCHI	41.9	53.5	60.5	67.4	72.1	51.2	69.8	76.7	74.4
AxSYM	34.9	55.8	53.5	60.5	65.1	51.2	62.8	74.4	
Vitros	39.5	62.8	60.5	72.1	86.0	58.1	74.4		
ELecsys	37.2	69.8	67.4	74.4	74.4	74.4			
HISCL	27.9	55.8	60.5	58.1	62.8				
AIA	39.5	62.8	55.8	72.1					
Centaur	46.5	83.7	83.7						
FORTE	44.2	88.4							
PRESTO	44.2								

Bold:>60%
Bold and Thick frame:>80%

DISCUSSION

Many immunoassay methods are used to measure HCV antibody, whether measurement is conducted using the manual method or an immunoassay system. These reagents differ in solid-phase support, labeled form and use of the bound/free (B/F) separation method. The capture antigens used to trap HCV antibody include recombinant antigens and synthetic peptides. The types and number of antigens differ from reagent to reagent. The basic performance of Sysmex's HISCL reagent was examined in the present study. Satisfactory results were obtained in the examination of HISCL reagent alone. On comparison of various types of reagents including the HISCL reagent, however, measurement results were different for different types of reagents.

Nine reagents including the measurement reagents for the systems including HISCL and RIBA-III were used to measure the panel serum and the patient samples, and the difference in measurement values for different types of reagent was examined. Five measurement systems were used to conduct a comparative measurement of the low HCV antibody titer panel (Sample Group 1). The positive rate was below 50% on measurement with AIA and HISCL. On measurement of the HCV seroconversion panel (Sample Group 2) with AIA and HISCL, the reactions with c100p (NS4) antibody and c33c (NS3) antibody were weak.

Three HCV seroconversion panels measured in the present study were Genotype 1a, 2b/3 and 2b. For AIA, the data on the sequence of antigens used have been released, and C50 antigen synthesized by combination of Genotype 1b and 2a genes was used for this reagent. Therefore, different genotypes seemed to be one of the causes of the low detection rate5). For HISCL, the data on the sequence of antigens have not been released and the cause of differences in reactivity remains unknown. For the 121 samples collected from the participating laboratories in Japan (Sample Group 3), the positive rates in the tests using HISCL and AIA were not particularly low. For the reagents that showed higher positive rates on measurement of Sample Groups 1 and 2, some failed to show positive results on measurement of patient samples. ARCHI and AxSYM, however, showed higher positive rates on measurement of all sample groups. Among the measurements made on Sample Group 3, reagents from each single manufacturer showed similar reactions. Some reagents showed clearly different positive rates for different measurement systems or different principles. One sample (***Table 4***, No. 43) alone was regarded as a RIBA-III independent positive sample. The sample tested positive for c33c (NS3) antibody and NS5 antibody, while it tested positive/negative for c100p (NS4) antibody and c22p (Core) antibody. In the light of change over time following infection, measurements obtained with all of the reagents concerned should be reexamined if samples can be obtained from the same patient some months later. The samples in this group may include those collected from patients who will undergo surgery, underwent screening before treatment or a complete medical workup, suffer from early-stage infection, have a history of infection or are being treated. All samples are unlikely to have been collected from patients with viremia. Because the blood was collected for screening, the quantity of sampled blood was limited and PCR analysis could not be conducted for confirmation. As in the case of former studies [1,3,4], the present study clearly showed that use of different reagents inevitably resulted in disagreement between HCV antibody test results. The sensitivity of reagents for detecting HCV antibodies should be high enough to detect all relevant antibodies. However, excessive effort to detect antibodies poses the

problem of more false-positive results. The current HCV antibody test, which generally serves as a screening examination for HCV infection, poses a more serious problem of generation of false-negative results.

The results of the present study clarify the characteristics of each reagent in terms of reactivity and demonstrate that some reagents actively trapped antibodies, while others did not. Because many reagents with different characteristics are used in the clinical setting, healthcare professionals should have sufficient knowledge about the characteristics of the reagents used at their laboratories. Because the HCV antibody test is a qualitative test, the measurements around cutoff values considerably affect the results of qualitative judgment. Although differences between reagents cannot be avoided, efforts should be made to minimize errors at laboratories. For this purpose, we should always conduct daily quality control, accurately determine the conditions of systems and reagents and the differences between reagent lots, and control accuracy, particularly around the cutoff values. The samples used for quality control around cutoff values should have low titers and should be able to be used in obtaining measurements with many reagents. Although laboratory technicians understand these characteristics, inappropriate results may lead healthcare professionals and patients to misunderstand the test results. Laboratory technicians should be responsible for the accuracy of reported test results. When they encounter a dubious sample, they should check the clinical background of the patient concerned and confirm the results using a different reagent that works via a different mechanism, as needed. In this manner, appropriate information should be provided for clinicians. Moreover, we believe it appropriate to request that manufacturers improve the systems/reagents and minimize the differences between reagent lots.

What was particularly significant in the present joint study was that we were able to establish a network that permitted the exchange of opinions between members working at various laboratories that used assay systems and reagents that differed in terms of working principles. We can enhance the reliability of data if we can establish an environment in which accumulated information can be shared to promote cooperation. Ideally, the present study will contribute to standardization of HCV antibody testing and reporting of consistent judgment results irrespective of laboratories and reagents used.

CONCLUSION

Because HCV measurement reagents have different characteristics, measurement values differ for different reagents. These characteristics cannot be clarified by the methods used for routine reagent evaluation alone. Although the characteristics of reagents used at each laboratory should be determined, these characteristics sometimes cannot be evaluated at a single laboratory. In this case, in collaboration with other laboratories, technicians should conduct a detailed evaluation. The present study demonstrated the importance of monitoring daily quality control, collection of data on the differences between reagent lots, and proper understanding of the conditions of systems and reagents.

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