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Comparison of Elecsys Anti-HCV II Assay With Other HCV Screening Assays

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Objective: Early detection of hepatitis C virus (HCV) is an important step in preventing progression to cirrhosis and hepatocellular carcinoma. Serologic assays for anti-HCV antibody are valuable first-line tests in the screening and diagnosis of HCV infection. This study's aim was to evaluate the sensitivity and specificity of Elecsys Anti-HCV II assay for HCV screening. *Design and Methods:* A total of 1,044 routine sera, 20 known HCV-positive samples, plus 54 preselected weakly positive samples were tested for anti-HCV with Elecsys Anti-HCV II assay, Elecsys Anti-HCV assays, InTec HCV enzymoimmunoassay (EIA), and Livzon Anti-HCV EIA. Interference test was assessed with additional 423 specimens without clinical evidence of HCV infection: preselected HCV weak reactive samples; dialysis samples; anti-HBc (antibody to HBV core antigen) (+), anti-*Treponema pallidum* (+), and anti-HIV (+) sera; and samples form autoimmune/alcoholic hepatitis or systemic Lupus erythematosus (SLE). Discrepant results were evaluated with recombinant immunoblot assay. The seroconversion panels were evaluated to assess how early each assay could detect HCV infection. *Results:* The specificity (99.81%) of the Elecsys Anti-HCV II assay was less than that with the two EIA comparison methods. However, false-negative results were easily seen in the EIA assays. When serial bleeds of HCV panels were compared with the above-mentioned methods, the assay detected acute HCV infection only 3.5 days after a positive HCV-RNA nucleic acid test and earlier than the comparator assays. *Conclusion:* Sensitivities and specificities of the anti-HCV assays were sufficiently high for use in this study. The Elecsys Anti-HCV II assay is suitable for screening and reliable early detection of HCV infection. J. Clin. Lab. Anal. **30**:451–456, 2016. © 2015 Wiley Periodicals, Inc.

Key words: HCV; Elecsys; evaluation

INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem worldwide. Currently, 130 to 170 million people worldwide are infected with HCV, and the annual increase in the number is approximately 3.5 million (1). About 80% of new infections progress to chronic infection, with cirrhosis developing in about 20% after 20 to 30 years, resulting in an increased risk for liver-related complications and hepatocellular carcinoma (HCC) (2). In China, approximately 40 million people are infected with HCV, and there is considerable geographic and temporal variation in the incidence and prevalence of HCV infection in China; the prevalence of antibodies to HCV (anti-HCV) has been reported to vary considerably, ranging from 1.0% to 3.2% in most areas.

Many patients infected with HCV are asymptomatic, making clinical diagnosis difficult. An estimated 45% to 85% of HCV-infected persons are unaware of their infection in the United States, and only 50% of HCV-infected persons have been tested in France (3, 4). Centers for Disease Control (CDC) recently has expanded its HCV testing guidelines to recommend a one-time HCV

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test for all persons born in and between 1945 and 1965 (5). Consequently, screening assays play an essential role in the diagnostic process (6–8) .In clinical practice, the usual approach is to test for anti-HCV, which indicates that the individuals have been infected with the virus, and then to detect HCV-RNA to confirm whether it is an active infection. However, anti-HCV detection does not secure the first 6 weeks of HCV infection, the so-called "window phase," in which the antibody response against HCV is nonreactive. The HCV infection rate in the HCV seronegative (anti-HCV bodies negative) blood donors between 18 and 60 years of age is around 0.08% in China, twice of 0.041% reported in Western countries (9). False-negative results increased risk for liver-related complications, HCC, and unsecure blood transfusion. Therefore, given the importance of early detection of HCV infection in routine clinical practice and blood donor screening, there is a need for sensitive assays for HCV infection screening, which can bring fewer false-negative results.

The Elecsys Anti-HCV II assay was developed to offer enhanced sensitivity and specificity over the Elecsys Anti-HCV assay. The capillary electrophoresis (CE) performance evaluation study found the Elecsys Anti-HCV II assay to have improved seroconversion sensitivity, as well as improved specificity in samples from European origin, compared with the Elecsys Anti-HCV assay (10). It was also demonstrated to have superior or equal performance compared with the other assays tested (11, 12). This study evaluated the Elecsys Anti-HCV II assay in west China and compared its performance with that of other local HCV assays routinely used.

MATERIALS AND METHODS

Serological Assays

Four HCV antibody screening assays, two ChIA (chemiluminescence immune assay) assays, and two EIA enzymoimmunoassay assays were compared. The Elecsys Anti-HCV II and Elecsys Anti-HCV assays were tested on the Modular E170 analyzer (Roche Diagnostics, Munich, Germany). Livzon Diagnostic Kit for Antibody to HCV (EIA; Livzon, Zhuhai, China) and Intec Diagnostic Kit for Antibody to HCV (EIA) were tested on TECAN analyzer (Freedom EVOlyzer, Männedorf, Switzerland).

The result of a sample is given in the form of a cut-off index (COI) for ChIA or ratios of specimen signals to the cut-off values (S/CO) for EIA. Samples with a COI or S/CO <0.9 are nonreactive. Samples with a COI or S/CO ≥ 0.9 and <1.0 are considered borderline. Samples with a COI or S/CO ≥ 1.0 are reactive. All initially reactive (IR) or borderline samples should be redetermined in duplicate. If no reactivity is found in both cases, the sample is negative for anti-HCV. If the result from either of the two measurements is reactive or borderline, then the sample is repeatedly reactive (RR). Samples (COI or S/CO ≥ 5) do not have to be retested, which is deemed as RR or positive directly in our study.

Familiarization Process

The appropriate function of E170 and the correct reagent handling had to be verified by performing an intermediate precision experiment (between run) with PreciControls 1 and 2 (Roche Diagnositics); this process was called familiarization before the main trial. Single determinations in ten different runs were performed within 3 days from each PreciControls 1and PreciControls 2. SD for PreciControls 1 must be below 0.12 S/CO and the CV for the PreciControls 2 must be below 10%.

Samples

A total of 1,044 routine hospitalized samples, 20 known HCV-positive samples, and 54 preselected HCV weakly reactive or borderline samples (with COI < 20 of Elecsys Anti-HCV assay) were collected from West China Hospital of Sichuan University. Furthermore, assay interference was assessed with additional 423 specimens: anti-HBs (antibody to Hepatitis B Surface Antigen, HBsAb) positive ($n = 50$), anti-*Treponema pallidum* (TP) positive ($n = 25$), anti-HIV ($n = 50$), anti-HAV (antibody to Hepatitis A Virus) or HEV (antibody to Hepatitis E Virus) ($n = 10$), HbsAg positive ($n = 100$), rheumatoid factor (RF) positive ($n = 20$); samples from patients with SLE ($n = 19$), immnuohepatitis ($n = 12$), high immunoglobulin ($n = 17$), dialysis ($n = 100$); HCV patients infected with HBV (Hepatitis B Virus) or HIV ($n = 10$); and patients infected with other virus, such as cytomegalovirus, herpes simplex virus, and others ($n = 10$).

Every sample should be measured in parallel with Elecsys Anti-HCV II, Elecsys Anti-HCV, Intec, and Livzon assays. A minimum of 600 μl should be guaranteed and stored at –80°C for subsequent analysis. Total sample volume should be sufficient to enable a repetition of experiments or further methods (in case discrepancies are found).

Four different commercially available HCV seroconversion panels representing a maximum of 21 samples were used. Panels were purchased from SeraCare Life Sciences (Milford, CT; panels PHV912) and ZeptoMetrix Corporation (New York; panels 6212, 9058, and 6224). The panels were evaluated to assess how early each assay could detect infection and data were calculated using the Paul Ehrlich Institute model, as described previously (13).

TABLE 1. Precision Result of Four Assays

	Familiarization		Main trail	
	Negative control (NC) Mean (SD)	Positive control (PC) Mean (CV%)	Negative control (NC) Mean (SD)	Positive control (PC) Mean (CV%)
Elecsys HCV II[a]	0.06 (0.001)	4.75 (0.7)	0.059 (0.00112)	4.72 (2.38)
Elecsys HCV[b]	0.14 (0.0288)	12.94 (2.4)	0.158 (0.0357)	13.95 (4.31)
Intec	/	/	0.001	2.75 (21.45)
Livzon	/	/	0.003	2.05 (15.8)

[a]QC target range for Elecsys HCV II: NC (0.00, 0.30), PC (3.23, 5.99).
[b]QC target range for Elecsys HCV: NC (0.00, 0.30); PC (9.59, 17.8).

Confirmatory Testing

RR samples must be investigated by Mikrogen recomline HCV IgG assay. But unanimously positive samples in all methods at initial test (S/CO > 5) do not necessarily have to be repeated nor have to be confirmed in this study.

Discrepancy Resolution

If individual results do show discrepancies with those of comparative methods, the procedure should be as follows: (1) Any failure due to handling (e.g., sample mix-up) during the data comparison should be definitely excluded. (2) If there is no obvious explanation for the discrepancy such as a handling error or an instrument problem, the sample should be rerun. (3) If the discrepancy is confirmed in the repeated measurement check which result is plausible regarding the results of other parameters. (4) Finally, discrepancy samples must be investigated by Mikrogen recomline HCV IgG assay.

Quality Assurance

In all experiments, controls were used to monitor the appropriate function of the instrument. Controls are handled according to the instructions stated in the package insert. Every run must be checked by measuring appropriate controls ahead of samples for all assays. Measurements are valid only if the results of the corresponding control materials are within target ranges. QC (Quality control) measurements must be performed once a day and at Rack Pack change.

Statistical Analyses

The statistical software package SPSS 11.0 (SPSS, Chicago, IL) was used for data analysis. Statistical comparisons were made using the chi-square test to assess the difference between two proportions. Tests of statistical significance included the 95% confidence intervals of unadjusted relative risks. Values less than 0.05 were considered statistically significant. The data were reviewed by laboratory quality-control staff, and data entry was performed by two independent persons. Most important, confirmed indeterminate samples were not enrolled into calculation in each equation.

RESULTS

Precision of Assays

The precision of the four assays was evaluated by using the commercial negative and positive control materials. In Table 1, it can be seen that SDs for Negative control(NC)

TABLE 2. Seroconversion Sensitivity of the Assays Tested

	Elycsys HCV II	Elycsys HCV	Intec	Livzon
Number of panels tested	4	4	4	4
Number of HCV positive bleeds detected/total number of bleeds	18/21 (85.7%)	15/21 (71.4%)	1/21 (4.76%)	2/21 (9.52%)
Days since the first positive bleed				
PHV912 (2b/3)	1	1	8	8
HCV6212 (1)	1	13	>54	54
HCV6224[a]	8	12	>23	>23
HCV9058 (1a)	4	8	>15	>15
Mean number of days	3.5	8.5	>25	>25

Day of bleeding with first positive results (last negative sample plus 1 day) compared with PCR data provided by the panel suppliers. The first positive bleed with the PCR assay was considered to be day 0.
[a]No genotype available.

TABLE 3. Assay Specificity Determined Using Routine Hospital Samples ($n = 1,044$)

	Elecsys HCV II	Elecsys HCV	Intec	Livzon
IR% (COI ≥ 5)	10 (0.96%)	11 (1.05%)	7 (0.67%)	7 (0.67%)
IR% (COI < 5)	4 (0.38%)	17 (1.63%)	2 (0.19%)	6 (0.57%)
RR% (COI > 1)	14 (1.34%)	27 (2.59%)	9 (0.86%)	13 (1.24%)
Confirmed positive[a]	8 (0.77%)	8 (0.77%)	7 (0.67%)	8 (0.77%)
Confirmed indeterminate[b]	4	5	2	3
No. of false positives	2	14	0	2
Specificity	99.81% (99.54%, 100.07%)	98.65% (97.94%, 99.35%)	100%	99.81% (99.54%, 100.07%)

were less than 0.12 S/CO and the CVs for the Positive control (PC) were less than 10% in two ChIAs. In the main trail, CVs for positive controls of Elecsys HCV II were 2.38%, nearly half of the CV of Elecsys HCV (4.31%).

Sensitivity Evaluation

The ChIA assays demonstrated more sensitivity than the other two EIA assays with respect to early detection of HCV ($P < 0.05$, Table 2). Using HCV-RNA PCR (the most sensitive assay) as a reference, there were significant differences overall between the assays with regard to the mean number of days before a first positive test was recorded (Table 2). In comparisons with the Elecsys Anti-HCV assays, Elecsys HCV II assay showed more number of positive bleeds 18/21 (85.5%, $P < 0.05$), with one panels (PHV912) time identical and three panels detected earlier. In comparisons with the two EIA assays, the ChIA showed all panels were detected earlier ($P < 0.05$). The value of mean days since the first positive bleeds of Elecsys HCV II was 5 days earlier than Elecsys HCV, and at least 20 days earlier than the EIA assays.

Clinical Specificity

Table 3 provides a summary of results in 1,044 routine samples. At the first glance, the clinical specificities of two ChIA assays were lower than those of two EIA assays. Two false-positive results in routine samples (Table 4) and five false-positive results in preselected weakly reactive samples (Table 5) were found in the Elecsys HCV II assays. The overall specificity of the Elecsys Anti-HCV II assay in 1,044 samples from routine samples was 99.81% and a good discrimination between negative and positive samples was observed in 54 preselected weakly reactive samples (AUC = 0.932). Another test of specificity was assessed using samples from patients with potentially cross-reacting factors (Table 6). Dialysis and anti-TP may produce false-positive results in ChIA assays, and HIV infectors could also have false reactive results in the Elecsys HCV II assays. False-negative results can be found in RF patients tested by two EIA assays Intec and Livzon.

Discussion

With the development of newer generations of ELISA, sensitivity and specificity were greatly improved. However, a residual risk still exists due to the seroconversion period of approximately 56 days, and high false-positive rates were not resolved (14). The Elecsys Anti-HCV II assay showed excellent sensitivity and specificity in a wide range of samples, and compared well with existing assays in a Europe multicenter study (10). In addition, it also showed improved sensitivity and specificity over the

TABLE 4. All Routine Positive Samples Using Four Assays

					Immunoblot		
Number	Elecsys HCV II	Elecsys HCV	Intec	Livzon	Negative	Indeterminate	Positive
3	Negative	Negative	Negative	Positive < 5	2	1	0
16	Negative	Positive < 5	Negative	Negative	14	2	0
1	Positive < 5	Negative	Negative	Negative	1	0	0
1	Positive < 5	Positive < 5	Negative	Negative	0	1	0
1	Positive < 5	Positive > 5	Negative	Negative	0	1	0
1	Positive < 5	Positive > 5	Negative	Positive < 5	0	0	1
1	Positive > 5	Negative	Negative	Negative	1	0	0
2	Positive > 5	Positive > 5	Positive < 5	Positive < 5	0	2	0
7	Positive > 5	Positive > 5	Positive > 5	Positive > 5	0	0	7[a]

[a]Samples with COI >5 in all assays were deemed as true HCV infection.

TABLE 5. Assay Evaluation Using 54 Preselected Samples Weakly Reactive to Elecsys HCV (0.9 < COI < 20)

	Elecsys HCV II	Intec assay	Livzon assay	Confirmatory test
RR	12/54 (22.2%)	2/54 (3.70%)	4/54 (7.40%)	/
Confirmed indeterminate	5/54 (9.26%)	2/54 (3.70%)	1/54 (1.85%)	8
Confirmed positive	2/54 (3.70%)	0/54 (0.00%)	00/54 (0.00%)	2
Number of false positives	5	0	3	/
Number of false negatives	0	2	2	/
Area under ROC	0.932 (0.854, 1.009)	0.989 (0.957, 1.020)	0.562 (0.04, 1.08)	/

previous version of the assay (Elecsys Anti-HCV) (12). Here, we investigated the performance of Elecsys HCV II, Elecsys HCV, and two local assays Intec and Livzon assays in west China.

HCV is a common, parentally transmitted viral infection that is often asymptomatic and difficult to be diagnosed clinically. Early diagnosis is, however, important to prevent progression to chronic HCV and its adverse clinical squeal. Four seroconversion panels were used to compare the sensitivity or the early screen ability of four assays to detect HCV infection. Here, there was a question that why PHV 912, HCV6212, HCV6224, and HCV9058 were chosen in this study. The genotypes of panels, PHV 912, HCV6212, HCV6224, and HCV9058, include genotype 1 and genotype 3, which are major genotypes prevalent in southwest China. Until now, four HCV genotypes, including genotype 1, 2, 3, and 6, were identified in China (15). In south-western China, the HCV subtype 1b was the most prevalent (32.9%), followed by subtypes 3b (18.9%), 6a (18.0%), 3a (12.8%), and 2a (10.4%) (16). Previous studies have suggested that the Elecsys Anti-HCV assay is one of the most sensitive assays for the early detection of HCV in seroconversion samples (17). The Elecsys Anti-HCV II assay detected more positive bleeds than the comparator assays, especially two EIA assays, which were more sensitive in recognizing early HCV infection (Table 2), and correctly identified all 20 samples known to be HCV positive (Table 3).

Based on an analysis of more than 1,044 unselected routine serum samples, the specificity of the Elecsys Anti-HCV assay in our study was 98.8%, which was similar to previous reports (11, 12). It was equivalent to the rates obtained with the Architect Anti-HCV, ADVIA Centaur Anti-HCV, and Vitros Eci aHCV assays. These findings are consistent with the results from other studies in which the specificity of the Elecsys Anti-HCV assay was compared with the Architect Anti-HCV, ADVIA Centaur HCV, and Vitros Eci aHCV assays (17). More false-positive samples were found in the two ChIA assays, especially in Elecsys Anti-HCV, than the two EIA assays. However, one false-negative result was found in Intec assays in 1,044 samples (Table 4), and two false-negative results were found in Intec and Livzon assays. TP infection and HIV infection may produce false-positive results in both ChIA assays and EIA assays. RF-positive patients may show in false-negative results in two EIA assays (Table 6). False-positive or false-negative problems in HCV antibody screening have been discussed in patients with SLE, HIV, TP, and so on (18–22). The diagnosis of HCV infection is defined according to the results

TABLE 6. Sample Testing in Patients With Potential Interfering Factors ($n = 423$)

	Elecsys HCV II	Elecsys HCV	Intec	Livzon
IR% (COI> = 5)	19 (4.49%)	19 (4.49%)	17 (4.02%)	14 (3.31%)
IR% (COI<5)	2 (0.47%)	3 (0.71%)	1 (0.24%)	3 (0.71%)
RR% (COI>1)	21 (4.96%)	22 (5.20%)	18 (4.25%)	17 (4.02%)
Confirmed positive	16 (3.78%)	16 (3.78%)	16 (3.78%)	15 (3.55%)
Confirmed Indeterminate	3 (0.71%)	4 (0.95%)	1 (0.24%)	2 (0.47%)
Number of false positives	2 (0.47%)	2 (0.47%)	1 (0.24%)	0
Number of false negative	0	0	1 (0.24%)	1 (0.24%)
Specificity	99.52% (98.86%, 100.18%)	99.52% (98.86%, 100.18%)	99.76% (99.29%, 100.22%)	100%
Sensitivity	100%	100%	99.76% (99.29%, 100.22%)	99.76% (99.29%, 100.22%)
False results caused by interfering factors (RR/confirmed positive)				
TP(+) ($n = 25$)	1/0	0/0	0/0	0/0
RF(+) ($n = 20$)	1/1	1/1	0/1	0/1
Dialysis ($n = 100$)	7/6	8/6	6/6	6/6
HIV ($n = 50$)	3/3	3/3	4/3	3/3

obtained from screening assays, and confirmation made by supplemental tests, such as HCV immnuoblot or HCV RNA, in order to exclude the possibility of false-positive results. False-positive results may not cause misdiagnosis, although they increase economic burden.

In conclusion, the ElecsysAnti-HCV II assay is a sensitive and specific assay with good precision and is suitable for routine use for the reliable and earlier detection of anti-HCV antibodies. However, supplemental tests, such as HCV immunoblot or HCV nucleic acid test(NAT), were needed to confirm the results.

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