Original Article

Evaluation of the Automated Fluorescent Immunoassay System anti-Hepatitis C Virus Assay for the Detection of Hepatitis C Virus Infection

Usman Waheed[1,2], Min-woong Hwang[3], Noor e Saba[4], Sooncheol Hong[3], Muhammad Saeed[5], Akhlaaq Wazeer[6], Muhammad Arshad[6], Javaid Usman[7], Hasan Abbas Zaheer[1,2]

[1]Department of Pathology and Transfusion Medicine, Shaheed Zulfiqar Ali Bhutto Medical University, PIMS, Islamabad, Pakistan, [2]Safe Blood Transfusion Programme, Ministry of National Health Services, Government of Pakistan, [3]R and D Centre BoditechMed Inc., Chuncheon-si, Gangwon, Korea, [4]Regional Blood Centre, Department of Health, Government of Khyber Pakhtunkhwa, [5]Department of Pathology, DHQ Hospital, Mandi Bahauddin, Punjab, [6]Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, [7]Department of Pathology, Army Medical College, Rawalpindi, Pakistan

Abstract

Introduction: Hepatitis C has emerged as a fatal epidemic in the country in the past two decades. One of the major risk factors remains unsafe blood transfusions. **Aims and Objectives:** The present study evaluated the performance of a newly developed automated fluorescent immunoassay system (AFIAS anti-hepatitis C virus [HCV] assay) in comparison with the Architect anti-HCV assay for the detection of anti-HCV antibodies in the blood donor samples. **Methodology:** This cross-sectional study was conducted in the Department of Pathology and Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, PIMS, Islamabad. About 3–5 ml blood was collected from 282 blood donors and was processed for the detection of anti-HCV by the Architect anti-HCV assay and AFIAS anti-HCV assay during the period January–October 2016. All those samples which were indeterminate by the Architect anti-HCV assay (with 1–5 value in the signal-to-cutoff) were further examined by the AFIAS anti-HCV assay. Architect anti-HCV assay was considered as gold standard. We compared the results of individual specimen; discrepant results specimens were further tested by third-party test, the Elecsys anti-HCV II assay on Cobas e411 by Roche. Sensitivity (level of detection [LOD]) of the AFIAS anti-HCV assay with 10 widely used rapid immune-chromatographic diagnostic tests (RIDTs) for anti-HCV was evaluated. We assessed the imprecision of the AFIAS anti-HCV assay. To measure the variation of the intra-assays (within days), 12 replicate tests were performed with known concentrations of anti-HCV. **Results:** The results obtained by the AFIAS anti-HCV assay showed similarity with those obtained by the Architect anti-HCV assay when tested with 282 blood specimens. When examining the results of an individual specimen by these two assays, 126 and 151 specimens showed identical positive and negative results, respectively; overall identity was 98.23%. Only five specimens showed comparatively different results. In comparison to RIDT, the AFIAS anti-HCV assay is 20–40 times more sensitive. **Conclusion:** The AFIAS anti-HCV assay will be useful in small-to-medium-sized laboratories for testing ready to use single sample test. It showed good agreement with the Architect anti-HCV assay and is useful for the detection of HCV infection. It is a superior alternative for low-sensitive RIDT anti-HCV assay.

Keywords: *Architect, automated fluorescent immunoassay system, hepatitis C virus, rapid immune-chromatographic diagnostic test*

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Introduction

Emergence of hepatitis C virus (HCV) infection is a global dilemma; increasing burden is alarming

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Address for correspondence:
Dr. Usman Waheed, E-mail: usman.waheed07@gmail.com

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for developing countries such as Pakistan. The virus is distributed worldwide with varying prevalence in different countries,[1] It has threatened the health-care systems and general community, about 3% of the world inhabitants are chronic HCV infected, and the frequency of deaths is almost 500,000 persons/year.[2] At present, the bulk of the HCV-infected community is present in developing nations. Even though we do not have vaccine for hepatitis C, we have medicine that can cure HCV infection 90% of the time. Major source is infected blood, its products, and the other body fluids.[3,4]

In Pakistan, hepatitis C is the cause of considerable morbidity and mortality. The virus is prevalent in the general population (4.8%) and the blood donor population (8.34%) because regular blood transfusion every 3–4 weeks is the only convenient way of treatment available in thalassemia patients to maintain the hemoglobin level from 9 to 11.5 g/d, therefore, this extensive community is more prone to get HCV infection; previous studies reported up to 21.7%.[4,5]

In human beings, blood is considered as lifeline for the existence.[6] Although blood transfusions save millions of lives, blood transfusion is associated with certain risks, which can cause many adverse consequences such as HCV transmission.[7] Blood safety is a serious issue all over the world because of transfusion-transmitted infections.[8] In Pakistan, the HCV infection risk for blood recipient is at the upper end. The Pakistan blood transfusion system is largely fragmented,[9] as the country has an incipient culture of voluntary donations and a predominant reliance on family/replacement blood donations. Undeniably safe blood is a universal right of all human beings; therefore, every donor should be screened for at least recommended Transfusion Transmitted Diseases (TTDs) for each region.

This increasing burden of HCV may be attributed to a lack of knowledge about disease transmission, re-use of syringes, poor screening of blood donations, hospitalization, and sharing of razors, etc. The accurate diagnosis of HCV infection coupled with a suitable screening of the blood supply is very important so that appropriate treatment can be carried out.[10] The diagnosis of HCV is based on two types of tests: the serological test, which detects specific antibodies against HCV (anti-HCV), and the molecular test that detects HCV RNA.[11] The detection of HCV antibodies is generally performed using ELISA technique and/or by chemiluminescence immunoassay (CLIA).

Small laboratories or point-of-care laboratories choose rapid diagnostic tests based on immunochromatographic lateral flow immunoassays. These tests are simple with no complex infrastructural requirements. Rapid detection is the key factor behind their widespread use. However, the clinical efficacy of these rapid tests is still limited due to lower sensitivity and specificity.

Automated fluorescent immunoassay system (AFIAS) is a newly introduced assay for the detection of HCV and other viral hepatitis and is highly sensitive and specific technique along with rapid detection.

Aims and objectives

This study was designed to assess the AFIAS anti-HCV assay, a newly introduced point-of-care test for anti-HCV assay, as a valuable addition to testing the presence of antibodies against hepatitis C virus in blood specimens.

Methodology

This cross-sectional study was conducted in the Department of Pathology and Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto (SZAB) Medical University, PIMS, Islamabad, from January to October 2016.

Ethics approval

The Ethical Committee of the SZAB Medical University approved the study protocol. About 3–5 ml blood sample was collected from 282 blood donors and was processed for the detection of anti-HCV by the Abbott's Architect anti-HCV assay and AFIAS (Boditech Med Inc.) anti-HCV assay.

All samples with a cutoff (signal-to-cut-off [S/CO]) value of 1–5 by the Architect anti-HCV assay were regarded as indeterminate and were further examined by the AFIAS anti-HCV assay.

Discrepant results were further tested by third-party assay, the Elecsys anti-HCV II assay on Cobas e411 by Roche. As per the Centers for Disease Control and Prevention (CDC) recommendations, specimens with indeterminate results by the Architect anti-HCV assay were confirmed by a more sensitive assay, recombinant immunoblot assay (RIBA), or nucleic acid test (NAT).

We compared sensitivity (LOD) of the AFIAS anti-HCV assay with 10 widely used rapid immune-chromatographic diagnostic test (RIDT) for anti-HCV. For this purpose, we performed the RIDT assay with two different specimens on 10 RIDTs.

We evaluated the imprecision of the AFIAS anti-HCV assay. To measure the variation of the intra-assays (within days), 12 replicate tests were performed with known concentrations of anti-HCV. For the variation of the inter-assays (between days), the same samples were measured on 10 sequential days, with two runs per day and 12 replicates at each concentration. Two positive controls and one negative sample were prepared for the intra- and inter-assay imprecision tests.

We examined whether the whole blood as a sample will affect the outcome of the test in comparison to serum samples by analyzing the same specimens that had been collected less than a week before the test. For this, we selected five positive specimens and one indeterminate specimen as determined by the Architect anti-HCV assay and performed the AFIAS anti-HCV assay.

The Architect anti-HCV assay is a qualitative immunoassay using chemiluminescent microparticles, which can analyze the presence or absence of anti-HCV antibody in plasma or serum samples. The results are shown as sample relative light units (RLU)/ cutoff RLU (S/CO) value. The S/CO value >1 is considered positive and <1 is negative, respectively. Samples between 1 and 5 were considered indeterminate.

The AFIAS anti-HCV assay uses a sandwich immunodetection method as described previously. The amount of anti-HCV is measured by analyzing the intensity of fluorescence on the test strip induced by laser. The intensity of fluorescence formed is proportional to the concentration of antibodies contained in the sample. The result of the samples is given as positive, negative, or indeterminate in the form of the cutoff index (COI). The samples with COI ≤0.9 are considered negative, COI >0.9 to <1.0 are indeterminate, and COI ≥1.0 are positive. COI was determined by measuring the fluorescence intensity and adjusted mathematically.

Statistical analysis

In this study, Cohen's kappa coefficient was used to assess the agreement between the two assays, using Statistical Product and Service Solution (SPSS) version 22.0 (IBM, USA).

Results

Of 282 specimens, anti-HCV positivity was 128 (45.3%) and 129 (45.7%) by Architect anti-HCV assay and the AFIAS anti-HCV assay, respectively [Table 1].

When we compared the results of the individual specimen by these two assays, 126 and 151 specimens showed identical positive and negative results, respectively. Only five specimens of 282 showed discrepant results between them with 98.23% identity. We further examined those five specimens with the third-party test, the Elecsys anti-HCVII assay on Cobas e411 by Roche [Table 2]. Two specimens that showed positivity with the Architect anti-HCV assay but negative with the AFIAS anti-HCV assay turned one positive and one negative result with the Elecsys anti-HCVII assay. Three specimens that were negative with the Architect anti-HCV assay but positive with the AFIAS anti-HCV assay revealed one positive and two negative results with the Elecsys anti-HCVII assay. This showed that conflicts found between two assays, the Architect anti-HCV assay and the AFIAS anti-HCV assay, are not just differences between two assays and it can happen in other well established CLIAs.

Table 1: Automated fluorescent immunoassay system anti-hepatitis C virus assay versus Architect anti-hepatitis C virus assay for the detection of anti-hepatitis C virus

Results	Architect (%)	AFIAS (%)
Negative	154 (54.6)	153 (54.2)
Positive	128 (45.3)	129 (45.7)
Total	282 (100)	282 (100)

AFIAS: Automated fluorescent immunoassay system

Table 2: Results of the test by the Elecsys anti-hepatitis C virus II on specimens that do not show the same results when performed by the Architect anti-hepatitis C virus assay and by the automated fluorescent immunoassay system anti-hepatitis C virus assay

Sample number	Architect	AFIAS	Elecsys
1	+	−	+
2	+	−	−
3	−	+	+
4	−	+	−
5	−	+	−

+= Reactive, −= Non-Reactive. AFIAS: Automated fluorescent immunoassay system

We examined 32 specimens that were indeterminate by the Architect anti-HCV assay. Those specimens have a S/CO between 1 and 5. The CDC of America recommended those specimens with low S/CO being examined again by a more sensitive assay, such as a RIBA or NAT. Oh *et al*.[12] showed that the Architect anti-HCV assay at S/CO of 7.5 showed 94.9% sensitivity and 96.6% specificity. Therefore, it is very difficult to judge whether it is positive or negative for anti-HCV on specimens when their S/CO values are in the range of 1–5 by the Architect anti-HCV assay. With the AFIAS anti-HCV assay, 14 of 32 specimens considered positive with higher than COI 01. Specimens with higher S/CO with the Architect anti-HCV assay had higher COI value with the AFIAS anti-HCV assay [Table 3], even though it is not a perfect match. Fourteen samples with higher than COI, one with an average of 4.10, were therefore considered positive by the AFIAS anti-HCV assay which showed S/CO 2.77 by the Architect anti-HCV assay. On the other hand, 18 anti-HCV negative samples by the AFIAS anti-HCV assay (below COI 01 with an average of COI 0.13) had an average S/CO 1.30 by the Architect anti-HCV assay.

We next compared sensitivity (LOD) of the AFIAS anti-HCV assay with 10 widely used rapid

immunochromatographic diagnostic test (RIDT) for anti-HCV. To do that, we performed RIDT assay with two different specimens on 10 RIDTs. All 10 RIDT assays showed a similar pattern of sensitivity [representative data are shown in Figure 1]. They all showed positive signals with undiluted specimens but not with diluted specimens; positive signals can be seen in 1:10 diluted but not in 1:100 diluted samples with all RIDTs tested. However, we were able to detect the positive signal with COI 3.2 with the AFIAS anti-HCV assay. In another specimen, the AFIAS anti-HCV assay was able to detect anti-HCV among samples that were diluted 1,000 times with COI 10.1. On the other hand, RIDT did not show any signal at that dilution. These two sets of experiments suggested that all 10 RIDTs cannot detect specimens with COI 10 or lower.

We evaluated the repeatability of the AFIAS anti-HCV assay. To measure the variation of the intra-assays (within days), 12 replicate tests were performed with known concentrations of anti-HCV. For the variation of the inter-assays (between days), the same samples were measured on 10 sequential days, with two runs per day and 12 replicates at each concentration. Two positive controls and one negative sample were prepared for the intra- and inter-assay imprecision tests. The CVs for the AFIAS anti-HCV assay were 3.52% and 3.32% with sample number 1 and 7.13% and 7.35% with sample number 2, respectively, in both the intra- and inter-assays at each tested concentration [Table 4].

We examined whether the whole blood as a sample will affect the outcome of the test in comparison to serum samples by analyzing the same specimens that had been collected less than a week before the test. For this, we selected five positive specimens and one indeterminate specimen as determined by the

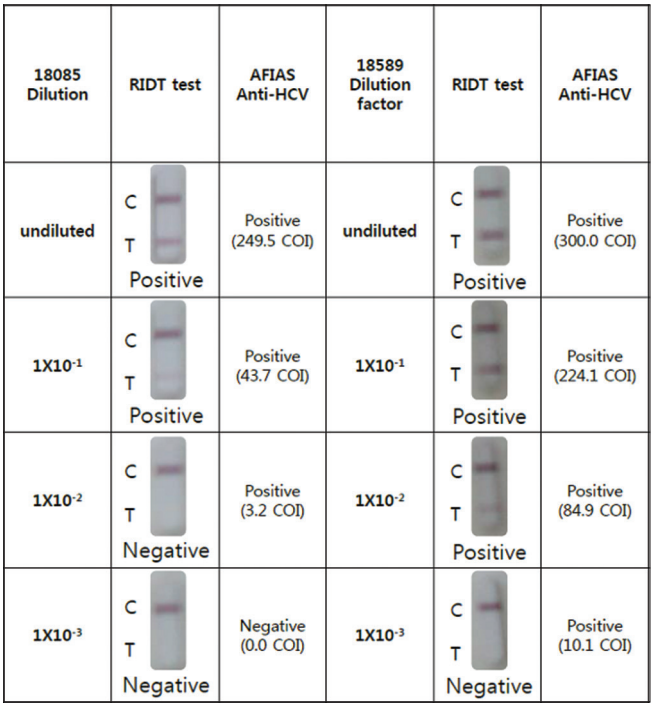

Figure 1: The representative figure for the rapid immunodiagnostic test for anti-hepatitis C virus assay

Table 3: Comparison of the signal-to-cutoff value obtained by the Architect anti-hepatitis C virus assay and cutoff index value obtained by the automated fluorescent immunoassay system anti-hepatitis C virus assay

Sample ID	Architect		AFIAS		Sample ID	Architect		AFIAS	
	S/CO	Result	COI	Result		S/CO	Result	COI	Result
C-010	1.01	Indeterminate	<0.01	Negative	C-055	3.06	Indeterminate	14.48	Positive
C-023	1.04	Indeterminate	0.06	Negative	20338	1.73	Indeterminate	1.34	Positive
C-029	0.95	Indeterminate	<0.01	Negative	18580	2.69	Indeterminate	4.08	Positive
C-040	1	Indeterminate	0.23	Negative	16372	3.46	Indeterminate	3.35	Positive
C-042	1.04	Indeterminate	0.1	Negative	20317	2.56	Indeterminate	1.46	Positive
C-051	1.04	Indeterminate	0.14	Negative	19626	1.07	Indeterminate	2.95	Positive
21071	1.5	Indeterminate	0.21	Negative	19248	1.25	Indeterminate	7.34	Positive
20320	1.83	Indeterminate	<0.01	Negative	20283	4.63	Indeterminate	3.44	Positive
20184	1.1	Indeterminate	0.03	Negative	180	3.61	Indeterminate	8.43	Positive
19804	1.02	Indeterminate	0.09	Negative	9787	3.47	Indeterminate	1.08	Positive
20181	1.84	Indeterminate	<0.01	Negative	8051	1.85	Indeterminate	4.98	Positive
16520	1.15	Indeterminate	0.13	Negative	8198	3.09	Indeterminate	1.06	Positive
16988	1.62	Indeterminate	<0.01	Negative	8494	3.94	Indeterminate	1.02	Positive
18188	1	Indeterminate	<0.01	Negative	9524	2.33	Indeterminate	2.39	Positive
17638	1.5	Indeterminate	0.23	Negative	Average	2.77		4.10	
2685	1.39	Indeterminate	<0.01	Negative					
765	1.02	Indeterminate	0.05	Negative					
21172	1.16	Indeterminate	< 0.01	Negative					
Average	1.23		0.13						

COI: Cutoff index, AFIAS: Automated fluorescent immunoassay system, S/CO: Signal-to-cutoff

Architect anti-HCV assay and performed the AFIAS anti-HCV assay. As shown in Table 5, almost identical data were obtained between whole blood samples and serum samples. All positive samples were shown as the positive and indeterminate samples as negative. In some cases, some of the COI values were higher in whole blood samples, without affecting outcome of the decision.

Discussion

In the current study, we examined a newly introduced automatic anti-HCV test, the AFIAS anti-HCV assay, that can be used around the world without substantial monetary and infrastructural investment, such as setting up a major CLIA equipment. Our data supported that the AFIAS anti-HCV assay is sensitive enough to substitute one of the CLIA assays, the Architect anti-HCV assay, run by expensive equipment and could be a rational substitute for much less sensitive RIDTs for the diagnosis of HCV infection. One of the significant points arising from our data was that the AFIAS anti-HCV assay is as good as the current gold standard for the diagnosis of HCV, the Architect anti-HCV assay with 98.3% identity. Assuming that the results obtained by the Architect anti-HCV assay are 100% correct, the sensitivity and specificity of the AFIAS anti-HCV assay would be 98.5% and 98%, respectively.

Table 4: Estimating intra- and inter-assay results of automated fluorescent immunoassay system anti-hepatitis C virus

Test sample	Intra-assay			Inter-assay		
	Negative control	Positive #1	Positive #2	Negative control	Positive #1	Positive #2
Test number	12	12	12	12	12	12
Mean	0.10	48.24	12.03	0.08	47.76	12.03
SD	0.09	1.70	0.86	0.07	1.58	0.88
Percentage CV	-	3.52	7.13	-	3.32	7.35

SD: Standard deviation, CV: Coefficient of variation

Table 5: Comparison of results with serum samples and those with whole blood as determined by the automated fluorescent immunoassay system anti-hepatitis C virus assay

Samples ID	Architect		AFIAS			
			Serum		WB	
	S/CO	Result	COI	Result	COI	Result
21219	12.01	Positive	242.83	Positive	242.31	Positive
21228	13.14	Positive	95.02	Positive	107.41	Positive
21349	1.12	Indeterminate	0.04	Negative	0.1	Negative
21320	4.75	Positive	13.51	Positive	11.69	Positive
21347	12.69	Positive	22.1	Positive	41.47	Positive
21321	13.86	Positive	12.86	Positive	57.89	Positive

COI: Cutoff index, AFIAS: Automated fluorescent immunoassay system, S/CO: Signal-to-cutoff, WB: Whole Blood

There is a gray zone in the diagnosis of anti-HCV. This can happen when anti-HCV antibody concentration in specimens is too low to be readily picked up by an assay or when HCV antigens used by an assay cannot recognize the antibody in the specimen. When HCV titer is low or epitope recognized by a specific set of antibodies has a low affinity, it will generate lower than 5 S/CO by the Architect anti-HCV assay and 5 COI by the AFIAS anti-HCV assay. Even though there are some trends between those values [Table 3], it will be practical to resort to another higher sensitive assay for the final decision.

RIDT anti-HCV assays, widely used worldwide due to their affordability and convenience, are much less sensitive than the AFIAS anti-HCV assay. This is alarming for many blood banks where screening for HCV is performed on rapid devices. By using a sensitive enough assay, these blood banks can eliminate the number of dubious specimens that need to be re-examined by more sensitive assay. Without major monetary and infrastructure investment, the AFIAS anti-HCV assay could be a very reasonable substitute.

Speed and convenience are one of the good merits of the AFIAS anti-HCV test. Since only 30 µl of sample volume is required for the test, blood drawn from fingertip pricking can be directly used as well as serum, plasma, and whole blood samples stored in a tube. The use of whole blood did not affect the outcome of the result as shown in Table 5. The result runs on a single sample and can be obtained within 15 min with an actual running time of 12 min per assay. Those speeds and convenience cannot be mimicked with large equipment in clinical laboratory. However, it will be convenient to use large CLIA equipment when you have a large number of samples that can be run at the same time, as in large commercial clinical laboratories or in big university hospital blood banks and clinical laboratories.

Conclusion

The AFIAS anti-HCV assay is useful in small-to-medium-sized laboratories. It showed good agreement with the Architect anti-HCV assay and is useful for the detection of HCV infection. It is a superior alternative for low-sensitive RIDT anti-HCV assay.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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