Original Article

Evaluation of Performance Characteristics of different Commercially available Diagnostic tests for hepatitis C virus antibodies in major Public Laboratories in Baghdad

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Summary:

Background: Immunoassays are one of the oldest techniques used in diagnostic virology where a number of serologic techniques, with different degrees of sensitivity and specificity, for the detection of HCV antigens and their specific antibodies, have been developed.

Materials and methods: One hundred and four sera samples were collected from National Center for Blood Transfusion, Gastroenterology and Liver Diseases Hospital, Central Public Health Laboratories and Teaching Laboratories. According to the manufacturers practical instructions, many available methods for detecting Anti-HCV antibodies, including enzyme linked immunosorbent assay (ELISA), immunochromatographic assay (ICA), recombinant immunobloting assay (RIBA), were applied.

Results: Although RIBA test is expensive and little bit laborious, this technique proved to be a powerful laboratory technique with both very high sensitivity and specificity when compared to ELISA, since the latter gave false negative results that were found by RIBA to be repeatedly positive. The ICA test for anti-HCV Abs was found to be a test with a relatively comparable sensitivity and specificity results to EIA / ELISA.

Conclusion: RIBA is a trustful test in big laboratory centers for anti-HCV Abs screening well with or without ELISA test (if a laboratory personnel are feasible to be available and trained for this purpose).The ICA test for anti-HCV, that need no expensive instrumentation, was found as a rapid, simple and cheaper test that could be used with comparable results to ELISA, for mass screening, at least, in rural laboratory centers.

Key wards: Anti-HCV antibodies, ELISA, RIBA, and ICA.

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Introduction:

Most cases of acute viral hepatitis in children and in adults are caused by one of the following viruses; Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV) and Hepatitis E Virus (HEV) (1).Acute HCV infection could be resolved without sequel. However, chronic liver disease followed 40 % of them and hepatocellular carcinoma developed in1% - 5% chronic HCV infection (2-7).

HCV is the major cause of parenterally transmitted hepatitis. Most HCV infections are transmitted by blood transfusion and other parenteral means such as sharing of needles, occupational exposure to blood and hemodialysis (4).In patients with multiple transfusions, including those with thalassemia or hemophilia, are particularly at high risk (8-10). Prenatal transmission and sexual transmission are relatively infrequent. However, the route of transmission is unknown in about 50% of individuals with HCV infection (11).

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Diagnosing acute hepatitis C is still difficult since the disease is frequently asymptomatic and the presence of HCV-RNA in serum or liver is the first biochemical evidence of exposure to this virus (5).However, the diagnosis of hepatitis C virus infection is most frequently based on anti HCV antibodies seroconversion which is screened by enzyme linked immunosorbent assay (ELISA) and confirmed by recombinant immunobloting assay (RIBA) or Reverse Transcriptase (RT)-PCR (12). More rarely, diagnosis is based on a double serum conversion where initially HCV-RNA undetectable by RT-PCR, subsequently positive and serum conversion for HCV antibodies determined by Enzyme Immunoassay (EIA) and RIBA techniques (13). Recently, it was possible to diagnose acute HCV infection by a rising anti-HCV titer rather than by seroconversion (14).

Routine diagnostic laboratories are confronted with an ever-increasing workload with limited resources. Rapid diagnostic kits and automation, particularly high through-put analyzers, have provided some solutions to these challenges. However, the real panel of criteria of those rapid commercial tests versus high standard qualification of companies producing the approved confirmatory methodologies is behind the

derangement of promotion criteria of many commercial diagnostic kits from practically obtained results (15-17). Throughout the second half of the previous century, a wide range of different serological, immunological and molecular techniques for the diagnosis of hepatitis viruses were introduced. According to feasibility of financial facilities, technical personnel, and advanced laboratory equipments, one should have the choice to select and apply one or more of these laboratory tests.

Materials and Methods:

This study included a total number of (104) sera from patients and blood donors who were repeatedly tested by ELISA and found to be anti HCV- positive and – negative, respectively. They were collected from the following participating medical centers: Gastroenterology and Liver Diseases Hospital, teaching Laboratories / Virology Unit / Baghdad Medical city, hemodialysis and Artificial Kidney Unit in Baghdad Teaching Hospital, national Blood Transfusion Center.and central Public Health Laboratories /Viral Hepatitis Unit.

Materials:These include many ready-to-use commercial kits as shown in Table (1).

Table1: Ready -to-use commercial kits and their manufacturing companies used in this study.

	Trading Names of Kits	Manufacturing Company	The country Of origin
1-	Anti HCV (ELISA)	Bio Kit	Spain
2-	HCV (RIBA)	CHIRON	USA
3-	HCV(Rapid Test Device)	Atlas Medical	England

The following laboratory techniques were done in the Virology unit of the Teaching Laboratories / Medical City and applied according to the detailed instructions of the manufacturing companies were :. Enzyme–Linked Immunosorbent Assay (ELISA), Recombinant Immunobloting Assay (RIBA) and immunochromatographic Assay (ICA).

Statistical Analysis:

The suitable statistical methods (18) were used in order to analyze and assess the results, these method include: Descriptive statistics: Statistical tables including observed frequencies with their percentage, summary statistic of the readings distribution (mean, SD, S.E, minimum & maximum) and graphical presentation by (bar, Pie & ROC curve - charts).

Inferential statistics:

These were used to accept or reject the statistical hypotheses, they include the followings: Binomial test, kruskal Wallis test, student test (t- test) and mann-Whitney U test.

Results:

The validity of Enzyme–Linked Immunosorbent Assay versus Recombinant Immunobloting Assay for testing anti-HCV Antibodies:

The current study included (74) anti HCV- positive sera by ELISA criteria that were tested in referring to recombinant immunobloting assay (RIBA). Table(2) and figure(1) show that out of the total number of these sera, (11) (14.9 %) were completely-negative for anti-HCV antibodies on RIBA strips (i.e. false-positive by ELISA) and the rest (63) samples show positive-RIBA results, and this confirms the true-positive anti-HCV antibodies results of ELISA test.

When examining (30) sera, that had proved to be repeatedly negative for anti-HCV antibodies by ELISA testing, it was found that (12) (40%) of them were anti-HCV positive sera on RIBA testing (i.e. false-negative sera by ELISA test). On statistical analysis, ELISA, when compared to the referred technique of RIBA for anti-HCV antibodies detection, had (84 %) sensitivity and (62 %) specificity (with accuracy in detection real positive and negative samples of 77.9%).

Table2: The validity of Enzyme–Linked Immunosorbent Assay versus Recombinant Immunobloting Assay for testing anti-HCV Antibodies.

Validity		RIBA		Total
		Positive	Negative	
ELISA	Positive	63	11	74
	Negative	12	18	30
Total		75	29	104

Sensitivity = 84.0 %.
Specificity = 62.06 %.
PPV = 85.13 %.
NPV = 60.0 %.
Accuracy = 77.88 %.

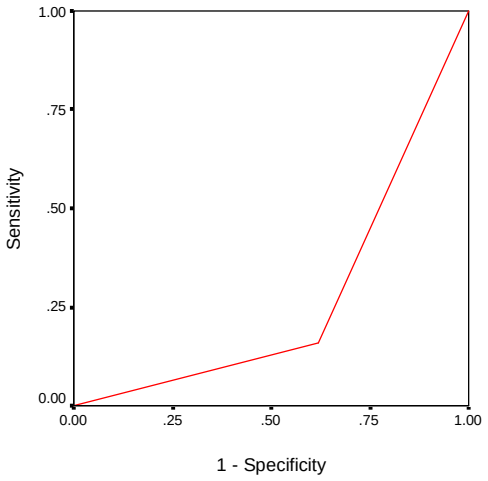

Figure1: ROC Curve for validity of ELISA as compared to RIBA for testing anti-HCV antibodies.

Immunochromatographic Assay versus Enzyme Linked Immunosorbent Assay for anti-HCV antibodies detection:

For comparing immunochromatographic assay (ICA) to ELISA technique, (79) sera samples proved to be positive for anti-HCV antibodies by criteria of this rapid test device were included in the current study. It was that found (62) samples had positive compatible results to ELISA; while the rest (17) sera had been determined to have false-positive results. In addition, among those (25) negative sera samples (tested by rapid device too) (48 %) (12/25) had shown false negative results on testing them by ELISA technique (table 3 and figure 2).

Statistically, immunochromatographic assay (ICA) for anti-HCV Abs testing showed 83.8 % sensitivity; 43.3 % specificity and 83.8 % accuracy (in detection true positive as well as true negative samples). The ability of this rapid device for detecting and predicting positive sample (tested and proved by ELISA) was found to be (78.5 %) whereas its ability in predicting ELISA- negative samples was only (52 %).

Table3: Validity of Anti- HCV Antibodies testing by Immunochromatographic assay (ICA) versus Enzyme–Linked Immunosorbent Assay (ELISA).

Validity		ELISA		Total
		Positive	Negative	
Rapid ICA test	Positive	62	17	79
	Negative	12	13	25
Total		74	30	104

Sensitivity = 83.78 %.
Specificity = 43.33 %.
PPV = 78.48 %.
NPV = 52.0 %.
Accuracy = 72.11 %.

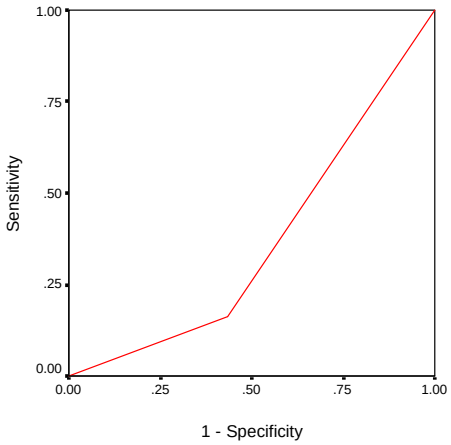

Figure2: ROC Curve for validity of ICA as compared to ELISA for Anti- HCV antibodies testing.

The validity of Immunochromatographic Assay (ICA)as compared to Recombinant Immunobloting Assay in testing anti-HCV antibodies. In the current study, the application of RIBA on (79) positive sera for anti-HCV antibodies by criteria of rapid ICA test device had revealed compatible positive results with (65) sera samples whereas the rest (14) (17.7 %) samples were negative for any band indicative of anti-HCV Abs (i.e. False-positive by this rapid ICA device).In addition, RIBA testing results of (25) negative samples for these antibodies (by rapid test device) showed that (40 %) (10/25) of these samples were falsely- positive and the rest (60 %) (15/25) were negatively-reacted for any band on RIBA strips (i.e. true negative) (table 4 and figure 3). The validity analysis results of ICA in comparison to RIBA testing results had showed that this rapid test device had (86.7 %) sensitivity; (51.7 %) specificity; (76.9 %) accuracy; (82.3 %) positive- predictive value and finally (60 %) negative- predictive value.

Table4: The validity of Immunochromatographic assay (ICA) versus Recombinant Immunobloting Assay (RIBA) for testing anti-HCV Antibodies.

Validity		RIBA		Total
		Positive	Negative	
Rapid ICA test	Positive	65	14	79
	Negative	10	15	25
Total		75	29	104

Sensitivity = 86.66 %.
Specificity = 51.72 %.
PPV = 82.27 %.
NPV = 60.0 %.
Accuracy = 76.92 %.

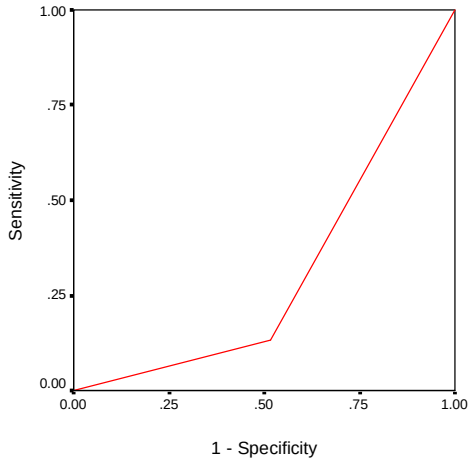

Figure3: ROC Curve for validity of Immunochromatographic assay (ICA) as compared to Recombinant Immunobloting Assay (RIBA) for anti-HCV Antibodies testing.

Discussion:

The validity of Enzyme–Linked Immunosorbent Assay versus Recombinant Immunobloting Assay for testing anti-HCV Antibodies:The third generation of enzyme immunoassays (EIA-3) used today has more sensitivity and specificity than previous generations of this technique. However, as with all EIA s, false-positive results are occasionally a problem with EIA-3. Additional or confirmatory tests are often helpful. In this respect, immunobloting assays (such as Recombinant Immunobloting Assay, RIBA) as well as HCV RNA- PCR are used to confirm such anti- HCV reactivity (19, 20).

The ELISA-positive sera were collected from different testing sites that have used an anti-HCV sequential 3 rd-generation immunoassay testing strategy whereby blood samples that were reactive on the primary screening immunoassay were tested on a secondary immunoassay and if reactive on both assays, were included in the present study for further testing by recombinant immunobloting assay. Out of these (74) ELISA-positive sera, (11) samples (14.9 %) were shown to be completely-negative for anti-HCV antibodies on RIBA strips (i.e. false-positive) and the rest (63) samples showed positive-RIBA results so that confirming the true-positive anti-HCV results of ELISA test. The results of the present study is consistent with those of Koreas and co-workers(21)who showed that 30 % of their sera samples using current 3 rd- generation of ELISA tests gave false-positive results.

If the immunobloting test for anti-HCV is positive, the patients had most likely recovered from hepatitis C and had persistent antibody without virus. If the immunobloting test is negative, the EIA- anti-HCV reactivity could also represent a biologic false-reactive (BFR) results (i.e. false-positive reactions) or could be cases during recovery from hepatitis C infection, or continued viral infection with levels of virus too low to be detected by ELISA where the last occurred only rarely when sensitive PCR assays are used. Our analysis and other researchers suggest that a combination of indicators can be used to help clarify RIBA-3-indeterminate results, specifically donors with high assay signal -to- cutoff (S/CO) ratios on a screening immunoassay as well as RIBA-3 reactivity to c22p or c33c with band intensity of 2+ or greater, with an identifiable risk factor, have a high probability of representing true anti-HCV rather than nonspecific reactivity (20, 22).

Surprisingly, when examining (30) sera that proved to be repeatedly- negative for anti-HCV antibodies by ELISA testing, it was found that (12) of them (40%) were anti-HCV positive sera on RIBA testing (i.e. false-negative sera).

Early in HCV infection, optical-density readings near or little bit lower than the cut-off value of ELISA technique for anti-HCV antibodies could be obtained where the assessing technicians passed them loosely as negative without subjecting such critical samples for another chance of repeating ELISA testing. The researchers have documented that ELISA test could have such false-negative results that were latterly-confirmed by RIBA technique to have positive reactivity. Such false-negatives in EIA are more frequent in immunocompromised patients and renal failure patients on hemodialysis (23).The finding of low EIA- sensitivity was also noticed among oncology patients who have shown lower sensitivity than that previously reported among immunocompetent persons. Impaired antibody production related to cancer and/or chemotherapy might explain the reduced sensitivity (24).

In such respects, these findings indicate that a RIBA and nucleic acid tests should be routinely considered in addition to EIA Also it is possible to utilize a new, rapid and specific as well as sensitive enzyme immunoassay that has been developed for detecting and quantifying total hepatitis C virus (HCV) core antigen in anti-HCV positive or negative sera as an additional laboratory diagnostic marker of viremia (25).

Finally, on statistical analysis, ELISA for HCV detection had (84 %) sensitivity and (62 %) specificity (with accuracy in detection real positive and negative samples of (77.9%). When compared to the referred technique (i.e. RIBA), these results are agree with Bhardwaj and colleagues (26) who found that their results of immunobloting assay were, in general, more specific than the corresponding version of EIA but disagree with them since they were slightly less sensitive and therefore, it should be used as an additional or as confirmatory test for the presence of anti-HCV antibodies.

Immunochromatographic Assay versus Enzyme–Linked Immunosorbent Assay for anti-HCV antibodies detection: Cloning the viral genome has made possible to use recombinant antigens to develop recent serologic assays (27).Compared to the first generations of HCV-EIA s that using single recombinant antigen, new EIA s have added multiple antigens that have been originated from such recombinant proteins and/or synthetic peptides so as to avoid non-specific cross-reactivity and to increase the sensitivity of the HCV antibody tests (28). ELISA (also referred to as enzyme immunoassay, EIA) detects antibodies against recombinant HCV antigens. First generation-ELISA used a single antigen but later versions (second and third generation tests) added additional antigens (29, 30). The third generation tests (EIA-3), used today, is more sensitive and specific than the previous ones (4). However, as with all enzyme immunoassay, false

positive results are occasionally a problem with the EIA-3 (19).

In the current study, and as mandatory by the Ministry of health, all ELISA tests for anti-HCV Abs, that were done by laboratories of all participated medical centers, must be done by a 3 rd ELISA generation (EIA-3). The presence or absence of anti-HCV Abs in the samples analyzed was determined by relating the absorbance value of each sample to cut-off value of the technique. If the initial test result absorbance value is equal to or greater than the cut-off, it should be re-testing in duplicate (31).

In addition, this HCV- immunochromatographic assay (ICA) is a rapid, one step-test which is used for the qualitative detection of antibodies to hepatitis C virus in serum or plasma, with a claimed sensitivity and specificity comparable to new EIA generations. The test utilized a combination of protein A coated particles and recombinant HCV protein to selectively detect antibody to HCV in serum or plasma. The recombinant HCV proteins used in the test kit were encoded by the genes for both structural (nucleocapsid) and non-structural proteins (32).

When comparing immunochromatographic assay (ICA) to ELISA technique in the current study, it was found that immunochromatographic assay for anti-HCV antibodies testing has statistically showed 83.8 % sensitivity; 43.3 % specificity and 83.8 % accuracy (in detection true-positive as well as true-negative samples). In addition, the ability of this rapid device for detecting and predicting positive sample (tested and proved by ELISA) was found to be (78.5 %) whereas its ability in predicting ELISA negative samples was only (52 %). The present results are agreed with the results of Wilber (32) regarding sensitivity but in disagreement with their results in respect to specificity. In front of the above mentioned results, the present study recommends the use of such rapid ICA-assay in suitable utilization in our country for screening anti-HCV antibodies where the infra-structure and laboratory expertise are limited, since EIA and ICA were shown to have comparable sensitivities. However, confirmatory RIBA as well as RT-PCR tests are necessary and recommended following applying each of them (25).

The validity of Immunochromatographic Assay (ICA) as compared to Recombinant Immunobloting Assay in testing anti-HCV antibodies:

In the present study, the statistical analysis of validity results of ICA in comparison to their counterpart RIBA results had showed that this rapid test device had (86.7 %) sensitivity; (51.7 %) specificity; (76.9 %) accuracy; (82.3 %) positive- predictive value and finally (60 %) negative- predictive value.

The sensitivity results of ICA in this research work agree with the results of Van der Poet et al. (28) and relatively agree with those results announced by the manufacturing company (Atlas medical) (33) who have determined the precision of this test to be (98%) of the time, by correct identification of these samples, regarding their grades of positivism from low to high positive anti-HCV antibodies-containing sera samples. However, the specificity of ICA in reference to RIBA was moderately high when our results of RIBA referred to the results of this commercially introduced HCV-ICA kit. Therefore, this rapid HCV test device will only indicate the presence of antibodies to HCV in the specimen and should not be used as sole criteria for the diagnosis of HCV infection (27).

Conclusions:

Among RIBA, ELISA and ICA techniques for detecting anti-HCV Abs, RIBA proved to be a powerful laboratory technique with both very high sensitivity and specificity when compared to ELISA (since ELISA gave false negative results that were found by RIBA to be repeatedly positive). Although RIBA test is (in comparison to ELISA) is expensive, little bit laborious in time and effects, it could be raised to be a trustful and well test in big laboratory centers for anti-HCV antibodies screening with or without ELISA test (if a laboratory personnel are feasible to be available and trained for this purpose).

The ICA test for anti-HCV antibodies was found to be a rapid, simple, cheaper test with relatively comparable results to EIA / ELISA. In addition, it needs no expensive instrumentation and that in this respect could be raised to be used, at least, in rural laboratory centers.

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