RESEARCH ARTICLE

Diagnostic reliability of Architect anti-HCV assay: Experience of a tertiary care hospital in India

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Background & Aims: Anti-HCV assays are prone to false positive results. Thus, accurate detection of HCV infection is critical for the timely therapeutic management. This study ascertained the reliability of Architect anti-HCV assay (Abbott) and to estimate the agreement of this assay with Ortho HCV 3.0 ELISA Test System with Enhanced SAvE (Ortho), HCV Tri-dot (Tri-dot) and HCV-PCR in a tertiary care setting.

Methods: A total of 78 788 consecutive sera were routinely screened for anti-HCV antibodies using Architect. All repeatedly reactive anti-HCV sera (n=1000) and anti-HCV negative sera (n=300) were tested in Ortho and in Tri-dot assays. Representative proportions of sera (n=500) with various signal-to-cut-off (S/Co) ratio were also compared with HCV-PCR.

Results: When Architect was compared with Ortho, Tri-dot, and HCV-PCR, the level of agreement as assessed by kappa were .26, .16, and .27 respectively. Using Latent class analysis (LCA), we found that sensitivity and specificity were 100% and 36.1% for Architect, 93.8% and 100% for Ortho and 63.8% and 100% for Tri-dot respectively. The median S/CO ratio of Architect and Ortho anti-HCV assays were significantly different between HCV-PCR positive and negative results ($P<.0001$). Furthermore, Architect S/CO ratio of >8 showed higher accuracy indices in both anti-HCV assays.

Conclusions: Architect can be used as a screening assay because of its high sensitivity, high throughput, and short turnaround time. However, S/Co ratios of ≥ 1 to <8 in Architect necessitates HCV PCR to identify current infection and or EIA to distinguish true positivity from false biological positivity.

KEYWORDS

chemiluminescent microparticle immunoassay, enzyme immunoassay, HCV diagnosis, hepatitis C virus

1 | INTRODUCTION

Hepatitis C virus (HCV) is one of the leading causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma.¹ HCV infection is diagnosed routinely by screening of anti-HCV antibodies.² Several commercial assays with varying principles are employed for screening of anti-HCV antibodies.³ Reliable anti-HCV screening results with appropriate public health measures can reduce the transmission rate

and incidence of HCV infection.⁴ Furthermore, accurate and early detection of current HCV infection is critical for the timely initiation of currently available anti-viral treatment. Despite the technological improvement, currently available anti-HCV screening assays are prone to generate high rates of false positive results.³ Anti-HCV screening immunoassays can generate 10%-60% of false positive results in populations with no recognizable high risk and low anti-HCV prevalence.^{3,5} Similarly in the immunocompromised populations the occurrence of

false positive rate averages approximately 15%.³ Hence, it is difficult to ascertain whether an individual is infected with HCV based on screening-test-positive results. This undermines the reliability of these assays in routine diagnostic settings.

In India, automated chemiluminescence immunoassay (CLIA) analyzers are increasingly being used in high volume laboratories. Considering several advantages of CLIA, many laboratories use this as the first line assay to screen for anti-HCV.^{6,7} In our assessment thus far, Architect anti-HCV assay generates many repeatedly reactive low signal-to-cut-off (S/Co) ratio results compared to ortho which is an enzyme immunoassay (EIA). Both are FDA-approved anti-HCV screening assays. Currently Centres for Disease Control (CDC) recommends the use of FDA approved assay for initial testing of HCV antibody without emphasizing on the importance of S/Co ratio and HCV-PCR to identify current HCV infection.⁸ However, the earlier CDC guidelines was remarkably different which emphasized on certain S/Co ratios of anti-HCV assays to predict reliably the true antibody positive results regardless of the anti-HCV prevalence.^{3,9} This strategy was adopted earlier by CDC for judicious use of expensive reflex supplemental testing.³ In contrast, United Kingdom Health Protection Agency (UK HPA) has suggested a second EIA as an alternative for confirmation of HCV antibody in routine clinical laboratory.¹⁰ This study was undertaken to identify the reliability of Architect anti-HCV assay and to evaluate the agreement of this assay with Ortho, Tri-dot assay and HCV-PCR. Using Latent Class Analysis (LCA), we have also attempted to establish the accuracy indices of these assays in the absence of gold standard for comparison.

2 | METHODS

2.1 | Samples

A total of 78 788 consecutive sera were screened routinely for anti-HCV in the Department of Clinical virology, Christian Medical College, Vellore, India. This study was approved by ethics committee. All sera were stored at 4°C for anti-HCV testing and at -60°C for HCV PCR testing.

2.2 | Anti-HCV assays

1. Architect anti-HCV assay (Abbott, Wiesbaden, Germany) is a two-step immunoassay which uses chemiluminescent microparticle immunoassay (CMIA) technology for the qualitative detection of anti-HCV antibodies in human serum/plasma. It uses HCr43 protein composed of two non-contiguous coding regions of the HCV genome (33c and core) and c100-3 (putative non-structural-NS3 and NS4).
2. Ortho HCV 3.0 ELISA Test System with Enhanced Save (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) is a qualitative assay for the detection of antibody to HCV in human serum/plasma. It uses the following three recombinant antigens: (i) c22-3 a putative core region (ii) c200 a putative NS3 and NS4 regions (iii) NS5.
3. HCV TRI-DOT (J. Mitra & Co. Pvt. Ltd., New Delhi, India) is a rapid visual test for the qualitative detection of antibody to HCV in

human serum/plasma using four modified HCV antigens (core, NS3, NS4 & NS5). Briefly HCV antigens are immobilized on a porous immunofiltration membrane. When serum/plasma passes through membrane HCV antibodies bind to the immobilized antigens if present. After washing, protein-A conjugate containing gold colloid is added resulting in appearance of distinct pink color dot at the test region (T1 and/or T2). The result is valid only if the built-in-quality control dot appears pink.

2.3 | HCV-PCR

Abbott RealTime HCV (Abbott Molecular Inc., Des Plaines, IL, USA) is an in vitro RT-PCR assay for the quantification of HCV RNA. It works on TaqMan principle, the amount of target sequence at each amplification cycle is measured by fluorescent-labeled oligonucleotide probes. The cycle at which the amplification is detected by the m2000rt is proportional to the log of the HCV concentration of the sample.

2.4 | Laboratory testing

All sera were prospectively tested in i2000SR using Architect anti-HCV assay. Sera that were negative (S/CO<1.0) were considered negative for anti-HCV. One thousand and twenty-four sera that were initially reactive were retested in duplicate according to manufacturer's guidelines. Sera that were repeatedly reactive (n=1000) were further tested in duplicate in Ortho HCV and singleton in fourth generation HCV Tri-dot. The results of the Tri-dot assays were independently read by two personnel. Representative proportions of sera (n=500) from this panel with various S/Co ratios were further tested for HCV-RNA in the Abbott real-time PCR. Additional three hundred sera that were initially negative in Architect were subsequently tested in Ortho HCV and HCV Tri-dot assays.

2.5 | Statistical analysis

The concordance between pairs of assays and all three screening assays were calculated. Agreement between pairs of assays was calculated using Kappa statistics. LCA was performed for all three assays to determine the diagnostic accuracy measures for the respective assays. The distribution of S/Co ratios for anti-HCV screening assays was compared with HCV PCR results using medians with interquartile range (IQR) by Mann-Whitney *U* test. A *P*-value of <.05 was considered statistically significant. Receiver operating characteristic (ROC) curve was plotted to determine the optimal cut-offs for S/Co ratios in diagnosing HCV infection. All statistical analysis was performed using STATA 11.0 (StataCorp, College Station, TX, USA).

3 | RESULTS

3.1 | Concordance and level of agreement

Architect HCV reactive (n=1000) and additional non-reactive sera (n=300) were compared with Ortho HCV and HCV Tri-dot assays. The

concordance rate for sera that were reactive in Architect HCV was 29.8% for all three assays, 31.2% for Architect HCV and HCV Tri-dot and 45.2% for Architect HCV and Ortho HCV. We found higher concordance rate (80.7%) between Ortho HCV and HCV Tri-dot assay. Irrespective of the assay, the concordance rate for negative sera ($n=300$) that were initially anti-HCV negative in Architect HCV was 99.7%. The level of agreement as assessed by kappa is shown in Table 1.

3.2 | Latent class analysis

LCA revealed that the sensitivity and specificity were 100% and 36.1% for Architect HCV, 93.8% and 100% for Ortho HCV and 63.8% and 100% for HCV Tri-dot respectively (data not shown).

3.3 | Receiver operating characteristic curve analysis

ROC-curve analyses were performed for Ortho HCV and HCV Tri-dot assays to determine optimal S/Co ratios of Architect HCV for the prediction of anti-HCV positive results in two screening assays. When Architect HCV was compared with Ortho HCV, the maximum diagnostic sensitivity (82.3%) and specificity (82.3%) was observed at Architect HCV S/Co ratio of 2.78 (data not shown). Similarly, when Architect HCV was compared with HCV Tri-dot assay, the maximum diagnostic sensitivity (90.6%) and specificity (91.2%) was seen at S/Co ratio of 4.7 (data not shown). When this analysis was performed for HCV-PCR testing, an optimal S/Co ratio of 6.28 predicted the viremic status with the diagnostic sensitivity and sensitivity of 94% and 91.1% respectively (Figure 1).

3.4 | Estimation of accuracy indices

Accuracy indices including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated for

FIGURE 1 ROC curve for HCV-PCR and Architect assay. At ARCHITECT S/Co ratio of 6.28 the maximum diagnostic sensitivity and specificity are 94% and 91.1% respectively. The area under ROC curve (95% CI) is 0.95 (0.9273-0.9724)

Architect HCV at various S/Co ratios by comparing with Ortho HCV and HCV Tri-dot assays. Architect HCV S/Co ratio of >8 showed higher accuracy indices in both the assays to reliably diagnose HCV infection (Tables 2, 3). We also found that 39.8% of the sera were positive by Architect HCV and HCV-PCR (data not shown).

3.5 | HCV antibody levels and viremia

The Architect median S/Co ratio was 1.93 (IQR 1.39-3.03) for PCR-negative sera and the median S/Co ratio was 13.20 (IQR 11.41-14.40) for PCR-positive sera ($P<.0001$, Figure 2). The Ortho HCV median S/Co ratio was 0.45 (IQR 0.05-2.18) for PCR-negative sera and the median S/Co ratio was 6.63 (IQR 6.60-6.64) for PCR-positive sera ($P<.0001$, Figure 3).

4 | DISCUSSION

Accurate diagnosis of HCV infection is a key for effective management of HCV infection. To achieve this, several screening assays with various principles are routinely used.¹¹ HCV screening assays are known to generate high rate of false positive results.¹² Resolving false positive results is one of the critical challenges of HCV diagnosis. In our experience, Architect generated lot of repeatedly reactive low S/Co ratio results among low risk individuals. In our tertiary care setting, samples are received from patients with assorted risk groups often without adequate clinical information to aid HCV diagnosis. In such circumstances, it is difficult to establish the accuracy of the screening test positive results. Furthermore, in patients with high pre-test probability (eg liver disease patients) false positives occurred infrequently. On the contrary, the occurrence of false positive results was high for individuals who are screened for anti-HCV in pre-operative settings (data not shown).

The principle of Architect, Ortho and Tri-dot anti-HCV assays are chemiluminescent microparticle immunoassay (CMIA), enzyme

TABLE 1 Estimation of level of agreement for anti-HCV assays and with HCV PCR

	Level of agreement (κ -value)
Anti- HCV antibody assays ($n=1000$)	
Architect anti-HCV ^a vs HCV TRI-DOT ^b	.16
Architect anti-HCV vs ORTHO HCV ^c	.26
ORTHO HCV vs HCV TRI-DOT	.68
Anti-HCV antibody assays vs HCV PCR ^d ($n=500$)	
Architect anti-HCV	.27
ORTHO HCV	.62
HCV TRI-DOT	.81

^aARCHITECT anti-HCV (Abbott, USA) works on the principle of chemiluminescent microparticle immunoassay (CMIA).

^bHCV TRI-DOT (J. Mitra, New Delhi, India)—Rapid visual test works on the principle of immunofiltration.

^cORTHO HCV 3.0 ELISA Test (Ortho Clinical Diagnostics, Raritan, NJ, USA) works on the principle of enzyme immunoassay.

^dAbbott real-time HCV-PCR (Abbott Molecular Inc., Des Plaines, IL, USA).

TABLE 2 Accuracy indices of Architect assay at various S/Co ratios with comparison to Ortho HCV

S/Co values of ARCHITECT anti-HCV assay (positive ^a n=1000 & negative ^b n=300)	Sensitivity %	Specificity %	PPV %	NPV %
<2 (n=375)	100	48	14.7	100
2-4 (n=215)	100	70.1	40.5	100
4-6 (n=55)	100	92.9	58.2	100
6-8 (n=25)	100	98	76	100
8-16 (n=330)	100	98.4	98.5	100

PPV, positive predictive value; NPV, negative predictive value.

^aSera those were positive in duplicate after retesting in ARCHITECT anti-HCV (Architect, Abbott, USA).

^bSera those were negative in ARCHITECT anti-HCV (Architect, Abbott, USA), ORTHO HCV 3.0 ELISA Test (Ortho Clinical Diagnostics, Raritan, NJ, USA) and Fourth generation rapid visual test (HCV TRI-DOT, J. Mitra, New Delhi, India).

TABLE 3 Accuracy indices of Architect assay at various S/Co ratios with comparison to HCV Tri-Dot

S/Co values of ARCHITECT anti-HCV assay (positive ^a n=1000 & negative ^b n=300)	Sensitivity %	Specificity %	PPV %	NPV %
<2 (n=375)	91.7	45.1	2.9	99.7
2-4 (n=215)	95.5	60.6	9.8	99.7
4-6 (n=55)	90	86.7	16.4	99.7
6-8 (n=25)	93.3	96.5	56	99.7
8-16 (n=330)	99.7	92.9	93	99.7

PPV, positive predictive value; NPV, negative predictive value.

^aSera those were positive in duplicate after retesting in ARCHITECT anti-HCV (Architect, Abbott, USA).

^bSera those were negative in ARCHITECT anti-HCV (Architect, Abbott, USA), ORTHO HCV 3.0 ELISA Test (Ortho Clinical Diagnostics, Raritan, NJ, USA) and Fourth generation rapid visual test (HCV TRI-DOT, J. Mitra, New Delhi, India).

FIGURE 2 Distribution of Architect S/Co ratio compared with HCV-PCR results **FIGURE 3** Distribution of ORTHO S/Co ratio compared with HCV-PCR results

immunoassay (EIA) and immunofiltration respectively. The three antibody HCV screening assays are claimed to have similar accuracy indices by manufacturers. In reality these assays had significant variation in performance. Furthermore, the level of agreement between Architect and HCV-PCR was also inadequate. A recent study independently reaffirmed our findings which showed that significant variations in diagnostic performance exist among anti-HCV assays and false positive results are not infrequent.¹³ Apart from variation in assay principle, the poor agreement could be attributed to low S/Co ratio

(<5.0) in Architect.⁹ Furthermore Ortho and Tri-dot use additional NS5 antigens and host organisms for the expression of HCV antigens are also different between Architect and Ortho assays. We speculate that these factors could have contributed to the variation in diagnostic performance of anti-HCV assays.

Since there is no gold standard assay available for comparison, LCA was performed to find out the accuracy indices of three anti-HCV assays. The sensitivity was highest for the Architect and the specificity was highest for Ortho and Tri-dot. Based on these analyses, Architect

could be used as a screening assay and Ortho can be used as a second assay for distinguishing true positivity from biologic false positivity. And Tri-dot assay can play a similar role in diagnosis of HCV infection which should be judiciously used in resource poor settings. Good agreement between Ortho and Tri-dot in the present study reaffirms our previous study findings.¹⁴ Furthermore, the specificity of Architect was 36.1% indicating false positivity associated with this assay. Kesli et al.¹⁵ had also shown that the specificity of Architect HCV was 26.9% which independently validates our finding. Hence, it is important to test sera repeatedly reactive in Architect in second EIA to obtain reliable anti-HCV results.

ROC curve analyses were performed to determine a specific Architect S/Co ratio that would predict maximum sensitivity and specificity in Ortho, Tri-dot and HCV-PCR. The Architect was compared with Ortho on the premise that the currently available Ortho assays are known to have exquisite specificity.¹⁶ At Architect HCV S/Co ratio of ≥ 2.78 , Ortho HCV showed maximum diagnostic sensitivity and specificity. Furthermore, this comparison was extended to HCV Tri-dot assay because this assay was comparable to Ortho HCV in anti-HCV diagnosis in our setting.¹⁴ In addition, HCV Tri-dot assay is used widely in India and in many low resource settings. At Architect HCV S/Co ratio of ≥ 4.7 , HCV Tri-dot assay showed maximum sensitivity and specificity (data not shown).

According to CDC recommendation, HCV-PCR is a gold standard assay to diagnose current HCV infection. At Architect S/Co ratio of ≥ 6.28 , HCV-PCR showed maximum sensitivity and specificity. The median S/Co ratios of Architect anti-HCV and Ortho anti-HCV were significantly different between PCR positive and negative results. This is critical because identifying individuals with current HCV infection would be helpful for timely initiation of antiviral treatment and management of HCV diseases. Architect HCV being the first line HCV screening assay, estimation of PPV and NPV is essential to improve the diagnostic accuracy of HCV infection. The S/Co ratio of Architect was categorized and accuracy indices were estimated to establish optimal S/Co ratio that could predict the anti-HCV positive result in Ortho HCV and HCV Tri-dot assays. The Architect S/Co of >8 showed highest sensitivity, specificity, PPV, and NPV for both assays. This S/Co ratio is higher than S/Co ratio established by CDC which was ≥ 5.0 for Architect.^{3,9} A similar S/Co ratio (≥ 5.0) was also shown in the Chinese Population.¹⁷ Therefore, further studies are warranted to establish the S/Co ratio to identify true anti-HCV positive result in Architect.

In conclusion, Architect anti-HCV is a highly sensitive screening assay, however false positive results are not infrequent. Thus, S/Co ratio of ≥ 1 to <8 in Architect requires HCV-PCR to identify current HCV infection for timely management and or EIA to distinguish true positivity from false biologic positivity.

REFERENCES

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med*. 2001;345:41-52.
2. Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. *Int J Med Sci*. 2006;3:35-40.
3. Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 2003;52:1-13, 5; quiz CE1-4.
4. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology*. 2009;49:1335-1374.
5. Schroter M, Feucht HH, Schafer P, Zollner B, Polywka S, Laufs R. Definition of false-positive reactions in screening for hepatitis C virus antibodies. *J Clin Microbiol*. 1999;37:233-234.
6. Kim S, Kim JH, Yoon S, Park YH, Kim HS. Clinical performance evaluation of four automated chemiluminescence immunoassays for hepatitis C virus antibody detection. *J Clin Microbiol*. 2008;46:3919-3923.
7. Alborino F, Burighel A, Tiller FW, et al. Multicenter evaluation of a fully automated third-generation anti-HCV antibody screening test with excellent sensitivity and specificity. *Med Microbiol Immunol*. 2011;200:77-83.
8. Hepatitis C Guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology*. 2015;62:932-954.
9. Kamili S, Drobeniuc J, Araujo AC, Hayden TM. Laboratory diagnostics for hepatitis C virus infection. *Clin Infect Dis*. 2012;55(Suppl 1):S43-S48.
10. Vermeersch P, Van Ranst M, Lagrou K. Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. *J Clin Virol*. 2008;42:394-398.
11. Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. *Semin Liver Dis*. 1999;19(Suppl 1):3-15.
12. Raghuraman S, Subramaniam T, Daniel D, Sridharan G, Abraham P. Occurrence of false positives during testing for antibodies to hepatitis C virus among volunteer blood donors in India. *J Clin Microbiol*. 2003;41:1788-1790.
13. Maasoumy B, Bremer B, Raupach R, et al. How to interpret borderline HCV antibody test results: a comparative study investigating four different anti-HCV assays. *Viral Immunol*. 2014;27:7-13.
14. Daniel HD, Abraham P, Raghuraman S, Vivekanandan P, Subramaniam T, Sridharan G. Evaluation of a rapid assay as an alternative to conventional enzyme immunoassays for detection of hepatitis C virus-specific antibodies. *J Clin Microbiol*. 2005;43:1977-1978.
15. Kesli R, Polat H, Terzi Y, Kurtoglu MG, Uyar Y. Comparison of a newly developed automated and quantitative hepatitis C virus (HCV) core antigen test with the HCV RNA assay for clinical usefulness in confirming anti-HCV results. *J Clin Microbiol*. 2011;49:4089-4093.
16. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J Viral Hepat*. 2001;8:87-95.
17. Wu S, Liu Y, Cheng L, Yin B, Peng J, Sun Z. Clinical evaluation of the signal-to-cutoff ratios of hepatitis C virus antibody screening tests used in China. *J Med Virol*. 2011;83:1930-1937.

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