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ORIGINAL ARTICLE

# Comparative evaluation of three rapid immunochromatographic test assays with chemiluminescent microparticle immunoassay for the detection of hepatitis C virus antibody

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**Abstract** Rapid diagnostic tests (RDTs) can serve as good alternatives to standard serological assays for hepatitis C virus (HCV) detection in limited resource settings. Aim of this study was to evaluate performance of three Indian manufactured RDTs with chemiluminescent microparticle immunoassay (CLIA) for screening of HCV infection with further evaluation using HCV RNA. Serum samples tested for anti-HCV by CLIA (Architect i1000SR, Abbott Diagnostics, IL, USA) were retrieved from − 80 °C and retested for anti-HCV by three RDTs: Alere Trueline (SD Bioline; Haryana, India) (RDT 1), Benesphera HCV Rapid card test (Avantor Performance Materials India Limited; Uttarakhand, India) (RDT 2), AccuTest HCV (Accurex Biomedical Pvt. Ltd.; Mumbai, India) (RDT 3). HCV RNA results were obtained from hospital information system and anti-HCV reactive but RNA negative cases without treatment were considered as either 'false positives' or 'spontaneous clearance of HCV RNA'. Among 86 samples, 75 (87.2%), 49 (57%), 58 (67.4%) and 51 (59.3%) were reactive by CLIA, RDT1, RDT2 and RDT3, respectively. Taking CLIA as reference standard, RDT 1, 2 and 3 demonstrated sensitivity of 65.30%, 77.33% and 68% respectively. Specificity of all three RDTs was 100% with sensitivity of 97.6–100% above signal/cut-off ratio (S/Co) of 6 by CLIA and 88–100% in all HCV RNA positive cases. Sensitivity of RDTs increased from 65.30–77.33 to 72–82.4% when RNA negative/anti-HCV reactive results were considered as non-reactive. The three RDTs have acceptable sensitivity and specificity in anti-HCV detection especially in RNA positive patients that would require treatment for HCV.

**Keywords** Hepatitis C virus · Rapid diagnostic tests · Chemiluminescent microparticle immunoassay · Anti-HCV · HCV RNA

## Introduction

Hepatitis C is a global health problem and approximately 2–3% of the world's population is chronically infected with hepatitis C virus (HCV), which amounts to an estimated 170 million people [1]. HCV leads to 27% of cirrhosis, 25% of hepatocellular carcinoma, and causes more than 350,000 deaths each year [2]. Screening of HCV infection is therefore mandatory particularly in high-risk epidemiologic settings [3]. As per WHO recommendations, current screening for HCV is based on anti-HCV antibody detection followed by HCV RNA determination using nucleic acid amplification tests (NAT) for confirmation of active replication [4]. Serological assays are based on the immunoassay principle, and are available in the form of rapid diagnostic tests (RDTs) or laboratory-based enzyme immunoassays (EIAs), chemoluminescent microparticle immunoassays (CLIAs) and electrochemoluminescence immunoassays (ECLs) [4].

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Nowadays, automated immunoasssays like CLIA analyzers are widely used, particularly in high-volume clinical laboratories as these instruments offer excellent precision and reliability, high-speed throughput, random access, and the technical simplicity of full automation [5, 6]. However, need of sophisticated equipment, trained technicians, continuous supply of electricity, and high facility costs make them unsuitable for use in limited resource settings [7].

In settings with limited access to the laboratory infrastructure or testing and where rapid testing can facilitate linkage to treatment and care, RDTs prove to be good alternatives. RDTs are single-use disposable assays that are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and can give a simple qualitative result in 15–20 min. Due to their simplicity, low cost and rapid turnaround time, they can be performed by trained lay providers or health-care workers, without the need for venepuncture [4]. However, several vital uncertainties remain regarding their use, including the variable performance characteristics of different RDTs available in the market [8]. Hence using the most sensitive RDT is important in order to identify true hepatitis C infected individuals.

With the launch of National Hepatitis Control Program in India, main focus lies in decentralization of testing facilities to fulfil the gap of delayed diagnosis. Also, limited data is available regarding the performance evaluation of RDTs manufactured in India for the detection of anti-HCV antibodies. Hence, this study aims to compare the diagnostic performance of three different RDTs manufactured by different Indian companies with CLIA (taken as reference standard) for the detection of anti-HCV antibody in patients of suspected HCV infection with further evaluation using HCV RNA.

## Materials and methods

### Study population

In this retrospective study conducted in the department of Clinical Virology in a tertiary liver-care hospital in Delhi from March to May 2018, blood samples from clinically suspected cases of HCV infection with deranged liver function tests had been sent to the Virology laboratory to screen for HCV via CLIA on a routine basis and serum samples were archived at − 80 °C. Once thawed samples retrieved from − 80 °C were only used and retested for anti-HCV on RDTs manufactured by three different Indian companies.

Patients with HIV and/or HBV co-infection, those on immunosuppressive therapy, and insufficient or hemolyzed or lipemic samples were excluded from the study. Details of liver function tests (LFT), kidney function tests (KFT) and clinical records were obtained from the hospital information system (HIS).

### Chemiluminescent microparticle immunoassay (CLIA) (reference assay)

All the samples included in the study were tested for anti-HCV using CLIA (Architect i1000SR, Abbott Diagnostics, IL, USA) as per the manufacturer's protocol. The results were expressed as signal/cut-off ratio (S/Co). Specimens with S/Co of $< 0.90$ were considered as nonreactive, between 0.9 and 1.0 as indeterminate and $\geq 1$ as reactive. CLIA results were retrospectively obtained from the HIS.

A total of 86 samples were taken in this study of which 75 (87.2%) were reactive and 11 (12.8%) non-reactive by CLIA.

### Real-time PCR for HCV RNA quantitation

Wherever HCV RNA results were available in the HIS among the anti-HCV reactive cases included in the study, they were also recorded. HCV RNA test was performed by the Abbott Real Time HCV assay (Abbott, Weisbaden, Germany) on the automated m2000rt platform, as per the manufacturer's instructions. Results were expressed as IU/ml. Lower limit of detection for the assay was 12 IU/ml with a linear range of 12–$10^8$ IU/ml.

### Anti-HCV rapid diagnostic tests

All three RDTs were lateral flow devices in which a membrane strip was used precoated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) on test band region. The protein A-colloid gold conjugate and serum sample moved along the membrane chromatographically to the test region and formed a visible line as the antigen–antibody-protein. A gold particle complex was formed. Interpretation of the results was similar for all three assays. An assay was interpreted as negative if a control line was present (regardless of intensity) with no corresponding test line. Appearance of a control line and a test line indicated a positive result. The following three RDTs were used in the study and their procedural details are described in Table 1: RDT 1 [AlereTrueline (SD Bioline; Haryana, India)]; RDT 2 [Benesphera HCV Rapid card test (Avantor Performance Materials India Limited; Uttarakhand, India)]; RDT 3 [AccuTest HCV (Accurex Biomedical Pvt. Ltd.; Mumbai, India)].

**Table 1** Procedural details of rapid diagnostic tests (RDTs) used in the study

| RDT | Type of specimen | Amount of specimen required (μl) | Amount of assay diluent required (μl) | Time required for interpreting the test results (min) |
|---|---|---|---|---|
| RDT 1 | Whole blood/ serum/plasma | 10 | 120 | 5–20 |
| RDT 2 | Serum/plasma | 25 | 80 | 10–20 |
| RDT 3 | Whole blood/ serum/plasma | 25 | 60 | 20 |

### Statistical analysis

86 samples were retrospectively selected and sample size calculation was done based on comparing the sensitivity of the new test with reference test as follows: Sensitivity of the new test as seen in previous studies [9] (%): 90; Sensitivity of the reference test (%): 100; Difference (%): 10; Power (1-beta) (%): 80; Alpha error (%): 5; 1 or 2 sided: 2; No. of diseased subjects needed: 73; Drop-out rate: 8%; Total no. of samples required: 81.

Comparison of the results obtained from RDTs was done with respect to CLIA results (taken as reference standard) to find out the diagnostic parameters of the three assays. Results obtained from CLIA and RDTs were further evaluated with HCV RNA results. Cases that were anti-HCV reactive and HCV RNA negative without any prior treatment were considered as either 'false positive for active infection' or 'spontaneous clearance of HCV RNA' in this study. The diagnostic parameters of the three RDTs were re-evaluated after ruling out RNA negative and anti-HCV reactive cases. Besides this, data has been represented as median (range) or in frequencies. 95% confidence interval (CI) of diagnostic parameters was seen by applying Wilson score. The analysis was carried out using SPSS version 22.0 (IBM Corp, Armonk; NewYork, USA) and Open Epi (version 3.01).

## Results

### Baseline characteristics of the study group

A total of 86 patients with a median age of 47.5 (12–79) years were included in the study; out of which 75 (87.2%) were reactive and 11 (12.8%) non-reactive by CLIA. Baseline characteristics of the study group are depicted in Table 2.

### Comparative analysis of three RDTs with CLIA for detection of anti-HCV

Anti HCV was detected by RDT 2 in 58 (67.4%) cases followed by RDT 3 [51 (59.3%)] and RDT 1 [49 (57%)] (Table 3).

### Evaluation of RDTs in relation to different ranges of S/Co on CLIA

The comparative analysis of sensitivity of the three HCV RDTs to different ranges of S/Co ratios obtained from CLIA is depicted in Table 4. Overall, sensitivity of the 3 RDTs was approximately 100% above S/Co of 6 by CLIA. Out of 34 CLIA reactive cases with a low S/Co range of 1–6, RDT 2 was able to detect 17 (50%) anti-HCV reactive cases whereas RDT 3 and RDT 1 were able to detect 11 (32.3%) and 9 (26.5%) reactive cases respectively.

**Table 2** Baseline characteristics of the study group

| | |
|---|---|
| Total (n) | 86 |
| Age (years); median (range) | 47.5 (12–79) |
| Male [n (%)] | 54 (62.8%) |
| Female [n (%)] | 32 (37.2%) |
| Male: female | 1.7:1 |
| Bilirubin (total) (mg/dl); median (range) | 2.1 (1.2–30.7) |
| (Normal range: 0.1–0.2 mg/dl) | |
| ALT (IU/L); median (range) | 151 (70–1021) |
| (Normal range: 7–56 IU/ml) | |
| AST (IU/L); median (range) | 127 (62–8886) |
| (Normal range: 10–40 IU/ml) | |
| Serum urea (mg/dl); median (range) | 40.15 (1.27–266.3) |
| (Normal range: 7–20 mg/dl) | |
| Serum creatinine (mg/dl); median (range) | 0.91 (0.31–97) |
| (Normal range: 0.6–1.2 mg/dl) | |

**Table 3** Comparative analysis of three RDTs with CLIA for detection of anti-HCV (n = 86)

| Test results based on CLIA | RDT 1[a] [n (%)] | RDT 2[b] [n (%)] | RDT 3[c] [n (%)] |
|---|---|---|---|
| Reactive; n = 75 | 49 (57) | 58 (67.4) | 51 (59.3) |
| Non-reactive; n = 11 | 37 (43) | 28 (32.5) | 35 (40.7) |

[a]RDT 1: AlereTrueline (SD Bioline); Haryana, India

[b]RDT 2: Benesphera HCV Rapid card test; Avantor Performance Materials India Limited; Uttarakhand, India

[c]RDT 3: AccuTest HCV; Accurex Biomedical Pvt. Ltd.; Mumbai, India

**Table 4** Comparative evaluation of the RDTs with different S/Co ratios for anti-HCV by CLIA

| CLIA S/Co (n) | RDT1 reactive [n (%)] | RDT2 reactive [n (%)] | RDT3 reactive [n (%)] |
|---|---|---|---|
| < 0.9 (11) | 0 (0) | 0 (0) | 0 (0) |
| 1–2 (15) | 1 (6.7) | 3 (20) | 1 (6.7) |
| 2–4 (12) | 4 (33.3) | 8 (66.7) | 6 (50) |
| 4–6 (7) | 4 (57) | 6 (85.7) | 4 (57) |
| 6–10 (7) | 7 (100) | 7 (100) | 7 (100) |
| 10–14 (30) | 29 (96.7) | 30 (100) | 29 (96.7) |
| > 14 (4) | 4 (100) | 4 (100) | 4 (100) |
| Total (86) | 49 (57) | 58 (67.4) | 51 (59.3) |

Interpretation of S/Co by CLIA

< 0.9—non-reactive

> 1—reactive

### Evaluation and interpretation of results of the three RDTs and CLIA in relation to HCV RNA

Both CLIA and the three RDTs were evaluated using HCV RNA as gold standard as shown in supplementary Fig. 1. Data on HCV RNA was available for 40/75 (53.3%) cases of which 7 (17.5%) were negative and 33 (82.5%) showed a significant viral load with a median value of 5.66 (1.08–6.30) $\log_{10}$ IU/ml. CLIA showed positivity in all 7 RNA negative cases but showed reactive results in all RNA positive cases. All 7 RNA negative and anti-HCV reactive cases via CLIA who were treatment naïve were considered as either 'false positive for active infection' or 'spontaneous clearance of HCV RNA' in this study. For verification of results, second aliquots of the same serum and plasma samples of the 7 RNA negative cases were retrieved from − 80 °C and retested for anti-HCV via CLIA and the 3 RDTs as well as for HCV RNA; but results were similar as before (depicted in supplementary Fig. 1). Overall, among the four assays (CLIA and the three RDTs), RDT 2 (Benesphera assay) showed the best results with no false negative results and 2 false positive results (supplementary Fig. 1).

Keeping in mind HCV RNA as the gold standard for confirmation of infection, individual S/Co ratios of the CLIA results were also evaluated and it was found that all the 7 RNA negative cases were anti-HCV reactive via CLIA and had S/Co ratio of < 6. The three RDTs demonstrated variable and low sensitivity for anti-HCV detection (when compared with CLIA results) in cases where S/Co ratio via CLIA was < 6 (supplementary Fig. 2). In contrast, among cases with S/Co ratio of > 6; there were no RNA negative cases, all RNA positive cases demonstrated reactive anti-HCV via CLIA and all RDTs also demonstrated almost 100% sensitivity in these cases (supplementary Fig. 3).

Table 5 shows the performance characteristics of the three RDTs before and after considering the 7 HCV RNA negative cases as Anti-HCV non-reactive. Out of 86 samples, there were 75 (87.2%) CLIA anti-HCV reactive cases; of which 7 (9.3%) turned out to be HCV RNA negative. Hence, true anti-HCV reactive and non-reactive cases in the study were 68/86 (79%) and 18/86 (21%) respectively. Taking 75 CLIA reactive cases as the reference standard, the performance characteristics of the 3 RDTs have been depicted under the 'before' category of Table 5 and taking 68 CLIA reactive cases as the reference standard, the performance characteristics of the 3 RDTs have been depicted under the 'after' category of Table 5.

All three RDTs demonstrated increased sensitivity and negative predictive values (NPV) after clearing out the 7 RNA negative and anti-HCV reactive results via CLIA; but there was a slight decrease in the specificity and positive predictive values (PPV) of RDTs 2 and 3 (Table 5) as both showed two anti-HCV reactive results respectively among these 7 cases as shown in supplementary Fig. 1. Overall,

**Table 5** Performance characteristics of the three RDTs for the detection of anti-HCV antibody before and after considering the 7 HCV RNA negative cases as Anti-HCV non-reactive

| Characteristics | RDT 1 (Before) (%) | RDT 1 (After) (%) | RDT 2 (Before) (%) | RDT 2 (After) (%) | RDT 3 (Before) (%) | RDT 3 (After) (%) |
|---|---|---|---|---|---|---|
| Sensitivity | 65.30 | 72 | 77.33 | 82.4 | 68 | 72 |
| Specificity | 100 | 100 | 100 | 89 | 100 | 89 |
| Positive predictive value | 100 | 100 | 100 | 96.5 | 100 | 96 |
| Negative predictive value | 29.73 | 48.6 | 39.39 | 57 | 31.43 | 45.7 |

Before: Previous results considering the 7 HCV RNA negative cases as anti-HCV reactive (n = 75)

After: New results after considering the 7 HCV RNA negative cases as anti-HCV non-reactive (n = 68)

RDT 2 demonstrated the best performance characteristics with CLIA followed by RDT 3 and then RDT 1 (Table 5).

## Discussion

This study evaluated and compared the results and diagnostic characteristics of three RDTs manufactured by different Indian companies with CLIA (taken as reference standard) as screening assays for the detection of anti-HCV antibody in patients with suspected HCV infection. CLIA results were also evaluated using HCV RNA as the gold standard in order to further analyse the results of the three RDTs. To the best of our knowledge, this is the first study which evaluates the diagnostic efficacy of RDTs manufactured by Indian companies for the screening of Hepatitis C infection.

Overall, sensitivity of the RDTs in the study ranged from 65.30 to 77.33% which increased to 72–82.4% when 7 RNA negative results were excluded. This is in contrast with other two studies where sensitivity of 86.8–97.8% and 97.1% respectively were reported on performance evaluation of RDTs which were not manufactured in India with CLIA [9, 10]. Specificity of the RDTs in the study ranged from 89 to 100% which is similar to other studies [9, 10]. Five other published studies have compared RDTs to EIA as reference and demonstrated the pooled RDT sensitivity and specificity as 99% and 100% respectively [11–15].

In this study, when HCV RNA was taken as the gold standard, RDTs 2 and 3 demonstrated two anti-HCV reactive results among the 7 RNA negative cases. Although WHO guidelines have recommended that NAT should be used only as a confirmatory test for detection of current viremic infection and is not an appropriate reference to assess RDT diagnostic performance [4]; NAT results were still preferred in this study only to rule out the RNA negative and anti-HCV reactive cases via CLIA so as to get the true anti-HCV reactive cases against which the RDT results can be compared in a more accurate manner. Several studies have compared RDT results to NAT or immunoblot in which the pooled sensitivity and specificity of RDTs were 93% and 98%, respectively [9, 16–27].

Other RDTs, such as Anti-HCV Ab rapid test (TemaRicerca, Bologna, Italy), SMHCV Rapid Test (SEROMedLaborSpezialitaten, Pollenfeld,Germany), Multiplo Rapid HIV/HCV Antibody Test (MedMira, Halifax, Nova Scotia, Canada) were previously studied, with sensitivity and specificity ranging from 78.9–100% to 83.3–100%, respectively [14, 24, 28]. In this study, RDT 2 (Benesphera assay) demonstrated comparable diagnostic performance as the above mentioned RDTs made by manufacturers from outside India. A recent meta-analysis on diagnostic performance of RDTs for hepatitis C diagnosis concluded that inspite of excellent specificity, significant variation in sensitivity (22–100%) had been observed [3] which also holds true in this study where all the three RDTs demonstrated varied performance characteristics with RDT 2 (Benesphera assay) showing the best diagnostic efficacy followed by RDT 1 (AlereTrueline assay) and then RDT 3 (AccuTest assay).

When the results of CLIA and RDTs in this study were compared with NAT results, it was found that all the 7 RNA negative cases were anti-HCV reactive via CLIA and had S/Co ratio of $< 6$. The three RDTs demonstrated variable and low sensitivity for anti-HCV detection (when compared with CLIA results) in cases where S/Co ratio via CLIA was $< 6$. Previous published studies [9] and CDC (Testing for HCV infection: An update of guidance for clinicians and laboratorians. MMWR 2013; 62: 18) have stated that low antibody titres might be suggestive of resolved past infection or these may be biological false positives in HCV seen due to cross-reactive circulating antigens and antibodies as in cases of pregnancy, autoimmune diseases, nephrotic syndrome, Human immunodeficiency virus, hepatitis B Virus, herpes simplex virus infection, portal cirrhosis, etc. [29–32]. Besides, previous studies have also demonstrated that HCV infections spontaneously clear in approximately 15–45% of infected

individuals without treatment [33]. Hence, all 7 RNA negative and anti-HCV reactive cases via CLIA who were treatment naïve in this study were considered as either 'false positive for active infection' or 'spontaneous clearance of HCV RNA'. Although spontaneous clearance of HCV RNA can be the cause of RNA negativity and anti-HCV reactivity in these 7 cases; however these cases depicted a very low S/Co ratio ranging from 1.61 to 3.08 via CLIA along with lack of concordance with the results of the 3 RDTs used in the study (as depicted in supplementary Fig. 1) and hence these 7 patients were more likely to be 'false-positive cases'. This is in accordance with a study by Gupta et al. [34] which showed that S/Co ranging from 1 to 4 is associated with false positive results in HCV. Another study by Kesli et al. [35] showed that a cut-off index of $\leq 5$ by CLIA is suggestive of false positive anti-HCV result on comparing with HCV RNA results. CDC has also recommended that S/CO ratio of $\geq 5.0$ via CLIA is predictive of a true positive $\geq 95\%$ of the time [Signal-to-Cut–Off Ratios for Commercially Available Assays". https://www.cdc.gov/hepatitis/hcv/labtesting.htm (last reviewed on 15th October, 2015)].

This study was done to evaluate the performance of RDTs as a screening test for HCV diagnosis and since screening test should be highly sensitive, CLIA was chosen as the reference standard but the disadvantage of higher false positive results with CLIA as compared to ELISA had already been reported [35]. Since all three RDTs in this study demonstrated low sensitivity in detecting low antibody titres, hence the overall sensitivity of RDTs was lower when compared with CLIA results but increased by 5–7% when the 7 RNA negative results were ruled out.

In cases with a high S/Co of 6 and above, all three RDTs were able to detect 97.6–100% of anti-HCV reactive cases. The three RDTs in the study also demonstrated 88–100% sensitivity for anti-HCV detection in all 33 HCV RNA positive cases; thus proving that they were able to detect most of the currently viremic patients who required treatment.

To the best of our knowledge, this is the first study which has determined S/Co of 6 regarding the sensitivity of RDTs in comparison with CLIA for the detection of anti-HCV antibody. This is in accordance with a study by Kesli et al. [35] which showed that a cut-off index of $\leq 5$ by CLIA is suggestive of false positive anti-HCV result on comparing with HCV RNA results but no RDT was included in that study. Since, all three RDTs in this study were able to detect almost 100% of anti-HCV reactive cases with S/Co ratio of $> 6$ via CLIA (suggestive of true-positives); hence they can be used as fairly efficient screening assays for the diagnosis of Hepatitis C.

*Limitations of this study*: The patients could not be followed up as well as fresh samples and HCV RNA results could not be obtained for all included patients in the study for authenticity of results as this was a retrospective study.

Expanded use of RDTs in resource-limited settings may mitigate the challenges of specimen collection, processing and transportation to laboratory services, and allow for the simplification of testing. Overall, despite variable performance characteristics of different RDTs evaluated in the study, they have acceptable sensitivity and specificity in anti-HCV detection especially in patients requiring treatment for Hepatitis C.

**Compliance with ethical standards**

**Ethical statement** Institutional Ethics Committee (IEC) of ILBS approved the study protocol.

**Conflict of interest** The authors declare that they have no conflict of interest.

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