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Original Research Article

Comparative evaluation of immunochromatographic card tests with enzyme-linked immune sorbent assay for the detection of hepatitis C virus antibody

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ABSTRACT

Background: Hepatitis C virus (HCV) is a global prevalent pathogen causes both acute and chronic hepatitis and leading to serious liver damage. Correct and rapid diagnosis is pivotal for the management of HCV disease. Rapid card tests are superior alternatives for the large-scale screening of HCV infection.
Methods: The present observational study evaluates analytical performance of four different anti-HCV rapid tests. A total of 200 ELISA confirmed, HCV positive (n=100) and HCV negative (n=100) clinical specimens were selected and re-tested for anti-HCV antibodies by using commercially available four different immunochromatography cards (Meriscreen, Accurate, Oscar and Biolab).
Results: Among all, Biolab rapid card test shown highest (98%) sensitivity. On the other hand, all rapid card test kits showed identical 100% specificity.
Conclusions: Overall BioLab anti-HCV rapid card tests found to be superior in the present study and strongly suggest in house validation of rapid card tests before their diagnostics use on clinical specimens.

Keywords: Hepatitis C, Anti-HCV, Rapid test, HCV disease, ELISA

INTRODUCTION

Hepatitis C virus (HCV) is a global prevalent pathogen and leading cause of morbidity and mortality around the world.[1] HCV infects the liver and may cause acute and/or chronic disease. both acute and chronic hepatitis, ranging in severity from a mild illness to a serious, lifelong illness including liver cirrhosis and cancer. An estimated 170 million people are chronically infected with HCV worldwide and more than 350,000 deaths occur each year.[2-3] In 2016, the World health organization (WHO) has set the target to eliminate viral hepatitis by 2030 with the objectives of 90% reduction in the incidence and 65% reduction in the mortality.[4] Suboptimal diagnosis may obstruct the goal of HCV elimination and therefore, correct and rapid diagnosis of HCV is pivotal for the treatment and management of HCV disease.[5] Various methods for the HCV diagnosis are commercially available which includes rapid immuno-chromatographic technique (ICT), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Rapid point-of-care ICTs are single-use assays which are provided with simple-to-use systems that usually require no extra reagents for the test kits and prove to be superior alternatives as compared to other tests for the large-scale screening of HCV.[6] Primary screening for HCV is based on anti-HCV antibody detection as per WHO guideline.[7]

Sensitivity and specificity of rapid immuno-chromatographic tests are less than ELISA however still in laboratories with less infrastructure and inadequate testing facilities can rapidly diagnose, facilitate treatment and management of the particular disease. Many rapid HCV ICTs are commercially available which can give results within 15-20 minutes. However, many times their performances and analytical sensitivity may vary, which may result in false positive or false negative reporting. To minimize false reporting of hepatitis C, the sensitivity and specificity of HCV diagnostic tests must be constantly monitored and improved. Globally, many studies have been published comparing performance of anti-HCV rapid diagnostic tests, however, very few studies have been done in India.[8-14] Therefore, the present study was designed to evaluate analytical performance and relative sensitivity and specificity of four different anti-HCV rapid tests using clinical samples received in a tertiary care centre in central India.

METHODS

Study design

This observational study was carried out at State Virology Laboratory, Gandhi Medical College, Bhopal, India to evaluate analytical performance and relative sensitivity and specificity of four different commercially available anti-HCV rapid tests. The study protocol was duly approved by the Institutional Ethics Committee, Gandhi Medical College, Bhopal (Letter no. 26801-03/MC/IEC/2016 dated September 24, 2016). The study was carried out during the period of July 2022 to October 2022. The following four ICT kits were included for this study (Table 1) and kits details were MERISCREEN (Meril Diagnostics Pvt. Ltd., Vapi, Gujarat, India); ACCURATE (Aspen laboratories Pvt Ltd, Delhi, India); OSCAR (Oscar Medicare Pvt. Ltd., New Delhi, India) and BIOLAB (Biolab Diagnostics (I) Pvt Ltd., Tarapur, Boisar, India). All kits were stored at respective temperature as recommended by the kit manufacturers. None of the manufacturers were involved in the assessment and interpretation of the study results.

Clinical specimens

A total of 200 (ELISA confirmed) clinical specimens were selected and tested for anti-HCV antibodies by using four different rapid card tests. Out of 200 specimens, 100 were known HCV positive and 100 were known HCV negative specimens. Hepatitis B and co-infection specimens were excluded from the study. Insufficient specimens, haemolysed blood samples and specimens with incomplete data were also not included in this study. Archived serum samples were retrieved from a -20 °C deep freezer and they were used for this comparative evaluation study. ELISA test was used as the reference gold standard method for this study and sensitivity, specificity, positive predictive value and negative predictive value of all four rapid card tests were evaluated accordingly.

ELISA test (Gold standard reference test)

All specimens included in this study were earlier tested for IgG antibodies of HCV virus by using the Erba-Lisa HCV Gen3 (v2) anti-HCV ELISA kit (ERBA diagnostics Mannheim GmbH, Germany) as per kit manufacturer's protocol. Results were interpreted according to the kit instructions. The results were expressed as optical density (OD) and cut-off value. Specimens with OD value lower than the Cut-off were considered as non-reactive and OD values equal to or higher than the Cut-off were considered as reactive.

Anti-HCV rapid diagnostic tests

For the detection of Anti-HCV antibodies, one step rapid card tests were performed using four different rapid card test kits as per the kits manufacturers' instruction. The method for testing and result interpretation was similar for all tests. All assays are single use, *in-vitro* qualitative, immune-chromatographic tests which give visual anti-HCV detection results within 15-20 minutes. All these tests were performed by trained lab personnel and results were interpreted as per the kits instruction and interpreted as negative if only the control line was appearing with no parallel test line. Simultaneous emergence of a control line and a test line indicated a positive result.

Statistical analysis

Data of all rapid card tests was recorded, calculated and analysed using Microsoft Excel 2022 and statistical software MedCalc Version 20.011. Anti-HCV ELISA test was considered as the gold standard and each rapid card test results were compared with ELISA test and percentage of sensitivity, specificity, positive predictive value, negative predictive value and accuracy with their 95% confidence intervals (CIs) were calculated.

Table 1: Summary of rapid card test kit evaluated in this study.

Kit name	Manufacturer / Country	Rapid Card Test	
		Sensitivity (%)	Specificity (%)
Meriscreen HCV Meril	Meril Diagnostics Pvt. Ltd., Vapi, Gujarat, India	99.5	99
HCV rapid test accurate	Aspen Laboratories Pvt Ltd, Delhi, India	100	99.9
HCV test, OSCAR	Oscar Medicare Pvt. Ltd., New Delhi, India	100	99.8
Rapid HCV, BIOLAB	Biolab Diagnostics (I) Pvt Ltd., Tarapur, Boisar, India	99	99

RESULTS

A total of 200 clinical specimens (ELISA test confirmed 100 HCV positive and 100 HCV negative) were included for this study and used to assess the performance of four different commercially available RDTs (Meriscreen, Accurate, Oscar and Biolab). Demographic details of the study subjects are summarized in Table 2. The Anti-HCV ELISA test was used as a gold standard reference. The results of different Anti-HCV rapid card tests were compared based on sensitivity, specificity and other parameters and are depicted in Table 3 & 4. The overall performance of all rapid card tests kits was rationally well. Out of 100 HCV positive samples, BioLab kit was able to detect maximum 98 positive samples followed by 97, 95 and 93 positive samples by Meriscreen, Accurate and Oscar rapid card test, respectively (Table 3). Among all rapid card tests investigated in the present study, Biolab found to have the highest (98%) sensitivity followed by Meriscreen (97%), Accurate (95%), and Oscar (93%). On the other hand, for 100 Anti-HCV negative samples detection, all rapid card test kits detected all negative samples and showed identical 100% specificity.

Table 2: Summary of demographic and virology characteristics of clinical specimens included in this study.

Features		HCV Reactive (n=100)	HCV Non-reactive (n=100)
Age (year); median (range)		33, (5-84)	40, (5-75)
Sex	Male (N %)	80	63
	Female (N %)	20	37

Table 3: Evaluation of anti-HCV rapid card tests with ELISA.

		ELISA Test (Reference Test)			Results of Rapid Card Tests			
		Positive (n=100)	Negative (n=100)	Total	TP	TN	FP	FN
Meriscreen	Positive	97	0	97	97	100	0	3
	Negative	3	100	103				
Accurate	Positive	95	0	95	95	100	0	5
	Negative	5	100	105				
Oscar	Positive	93	0	93	93	100	0	7
	Negative	7	100	107				
BioLab	Positive	98	0	98	98	100	0	2
	Negative	2	100	102				

TP: True positive, TN: True negative, FP: False positive, FN: False negative

Table 4: Performance characteristics of rapid card tests evaluated in this study.

Characteristics	Meriscreen	Accurate	Oscar	BioLab
Sensitivity (95% CI)	97% (91.48% to 99.38%)	95% (88.72% to 98.36%)	93% (86.11% to 97.14%)	98% (92.96% to 99.76%)
Specificity (95% CI)	100% (96.38% to 100%)	100% (96.38% to 100%)	100% (96.38% to 100%)	100% (96.38% to 100%)
PPV	100%	100%	100%	100%
NPV (95% CI)	97.09% (91.62% to 99.03%)	95.24% (89.49% to 97.92%)	93.46% (87.49% to 96.69%)	98.04% (92.69% to 99.50%)
Accuracy (95% CI)	98.50% (95.68% to 99.69%)	97.50% (94.26% to 99.18%)	96.50% (92.92% to 98.58%)	99.00% (96.43% to 99.88%)

PPV: Positive predictive value, NPV: Negative predictive value.

DISCUSSION

Early detection of HCV infection is crucial to stop morbidity and mortality and rapid point-of-care card tests are playing an important role for the fast diagnosis of HCV infection; however, their compromised sensitivity and specificity may harm HCV control programs. In the present study, analytical performance of four different commercially available anti-HCV rapid card tests was investigated and compared with ELISA test (Reference standard) as the screening assay for the detection of anti-HCV antibody.

For the first time, present study evaluating analytical performance of Meriscreen, Accurate, Oscar and Biolab anti-HCV rapid card test. As per the guidelines from the

drug controller general of India for anti-HCV rapid immunodiagnostic kits, acceptance criteria for the sensitivity and specificity are >99% and ≥98%, respectively.[17] In the present study, only Biolab kit qualifies the acceptance criteria for both sensitivity and specificity. Other rapid cards kits had very good specificity, but demonstrated relatively low sensitivity and as a result do not meet the mandatory standards.

In India, HCV prevalence is between 0.5 and 1.5 per cent with approximately 12–18 million people living with HCV.[18,19] However, there is wide variation in the state-to-state prevalence as very few studies for the prevalence of HCV have been done. On the basis of published reports HCV prevalence in the Punjab state (5.2%) is the maximum and on the other hand Maharashtra state has the lowest (0.09%) HCV prevalence.[20,21] The Government of India has launched the National Viral Hepatitis Control Program on 28th July 2018 with the aim of ending viral hepatitis by 2030 and provides free diagnosis and treatment for the patients of viral hepatitis including hepatitis C.[22] To reduce the burden of HCV, there is an urgent need to scale up identification and treatment of HCV as both play fundamental roles to control the hepatitis disease burden.

Rapid card test are a simple and cost effective option and has the potential to diagnose clinical specimen quickly, subsequently timely initiation of the treatment further decreases chances of chronic condition and progress of complications. Rapid tests which show 100% sensitivity and 100% specificity are better choices over the ELISA test or other time consuming and laborious techniques. These rapid card tests can be used extensively in the resource limited settings and may reduce the challenges of sample collection and transportation to the high-end laboratories. Even though inconsistent performance of different rapid card tests estimated in the present study, some of those have the sensitivity and specificity acceptable for HCV detection particularly in patients requiring urgent treatment.

Limitations

The current study has certain drawbacks. The study's sample size was smaller than average because only 200 clinical specimens were included. Second, this study used of stored, frozen clinical samples. Third, other virology tests and biochemical parameters were not determined and fourth, Fourth, a comparative cost effectiveness of all kits was not included. All tests were carried out in the reference virology laboratory in the ideal environmental conditions by the trained lab staff therefore, the results of all tests may differ in peripheral lab settings where a variety of factors, including environmental conditions, specimen type (whole blood, plasma, or serum) and technical expertise may influence the outcome of the test.

CONCLUSION

The results of the current investigation show that BioLab's anti-HCV fast card tests had higher sensitivity and specificity than Meriscreen, Accurate, and Oscar RCTs, and they met the Drug Controller General of India's acceptance standards. In house validation of all rapid card tests are strongly recommended before their diagnostics use on clinical specimens.

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