

Evaluation of the Performance of Two Rapid Immunochromatographic Tests for Detection of Hepatitis B Surface Antigen and Anti HCV Antibodies Using Elisa Tested Samples

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Study Design: An experimental study conducted at the King Edward Medical University, Lahore during the period between November 2009 and January 2010. Four rapid immunochromatographic assays – ONE STEP HBsAg *One Check*, One step HBsAg test *Accurate*; Rapid anti HCV test *One Check* and Hepatitis C virus one step device *Accurate* were evaluated for detection of hepatitis B surface antigen and anti HCV. A collection of ELISA tested samples (57 tested for Hepatitis B by ELISA, 68 tested for Anti HCV antibodies by ELISA) were stored at -20°C and subsequently tested with rapid ICT tests (devices) (*Accurate* and *One Check* brands). Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of these tests were calculated using ELISA as the gold standard.

Aims: The study was aimed to evaluate the performance of two commercially available rapid immuno-chromatographic testing (ICT) kits for HBsAg and anti HCV antibodies.

Materials and Methods: Elisa tested samples for HBV (n = 57) and HCV (n = 68) were allotted random numbers to the positive and negative specimens. The medical officers allotting the numbers and the one performing the tests were double blind.

Results: Among the 38 ELISA HBV positive sera, 20 were positive by Hepatitis B surface antigen one check and 19 were positive by accurate. Among the 53 ELISA Anti HCV positive sera 24 were positive by one check, 32 were positive by accurate and 4 were weakly positive by accurate. The sensitivity for rapid Hepatitis B surface antigen was found to be 53% and 50 % for one check and accurate respectively. The sensitivity of rapid HCV devices was found to be 66% and 45% for *Accurate* and *One Check* kits respectively. The negative predictive value for rapid HBsAg devices was 51% and 49% for *One Check* and *Accurate* kits respectively. The positive predictive values for HCV were 96% and 97% with *One Check* and *Accurate* respectively; for HBV, these were 100% and 95% with *Accurate* and *One Check* respectively.

Conclusion: These results suggest that the rapid immuno-chromatographic kits for HBsAg and HCV have only limited efficacy and should be backed by superior methods like ELISA and PCR where possible.

Key words: HCV, hepatitis, ELISA, immunochromatographic test, screening.

Introduction

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis are caused by Hepatitis A virus, Hepatitis B virus and Hepatitis C virus. The presence of HBs Ag in serum or plasma is an indication of active Hepatitis B infection either acute or chronic. There are currently about 350 million people worldwide who are chronically infected with hepatitis B virus (HBV) (3, 8), 15 to 40% of whom will develop serious sequelae during their lifetime.⁴

In Pakistan, prevalence of HCV is 4-9% in general population and 1.8-7.5% in healthy blood donors.⁶ Hepatitis B virus sero prevalence is 5-8% in general population and 3.5-5% in blood donors.⁷

Ideally, these chronic carriers should be identified and medical interventions implemented to reduce the risk of premature death. The considerable morbidity and mortality associated with serum hepatitis initiated vigorous efforts to identify the causative agent(s). Screening of blood donors

for hepatitis B virus (HBV) became available in industrialized countries around 1970. However, the agent causing parenterally transmitted hepatitis non-A, non-B remained hidden for almost 20 years until the discovery of hepatitis C virus (HCV) in 1989. Once identified, simple antibody tests for screening and diagnostic purposes became available in 1990-91.¹

Past exposure to hepatitis C virus (HCV) is mostly determined by testing for specific antibodies using an approved enzyme immunoassay (EIA). The presence of antibody shows that the patient has been infected with the virus but does not indicate whether the infection is acute, chronic or resolved.⁹ The absence of antibody usually shows that the patient has not been infected. The recommendations for the screening of Hepatitis B also call for testing the serum or plasma specimens by ELISA tests.¹⁰ Elisa tests are generally costly so far the instruments and chemicals are concerned. Cheaper immunochromatographic tests developed by different pharmaceutical and diagnostic firms are advocated and

recommended with claims of high sensitivity and specificity. These tests do not have the requirement for using any instruments. The two most commonly available immunochromatographic kits for screening of the patients for Hepatitis B and C are Accurate and one Check. The claims for specificity and sensitivity for Hepatitis B and Hepatitis C immunochromatographic devices by Accurate fall in the range of 95-99% (both for specificity and sensitivity).¹¹ For One check for these values have been claimed to be ranging from 97-100%. These claims are usually made on the basis of studies carried by workers observing ideal conditions of manufacture, transport and storage. It is common knowledge that these conditions may not be observed for especially in underdeveloped countries where cost is a great consideration. Under such conditions it becomes pertinent to test the claims of the companies under the prevalent conditions of manufacture, storage and transport before the purchase.^{12,13}

Materials and Methods

Elisa tested samples for HBV (n = 57) and HCV (n = 68) were stored at -20°C. Random numbers were allotted to the positive and negative specimens. The medical officers allotting the numbers and the one performing the tests were double blind. All the conditions for the storage of the kits were

strictly followed. All the tests were done in one go. The sera were thawed on the day of doing the tests. The tests were performed as per manufacturer’s instructions. The results were read and recorded by two independents medical technologists and supervised by the consultant.

Operational Definition:

Evaluation: This will be done in terms of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of the tests using ELISA as the gold standard.

Results

Table 1: *Specimens Collected For Testing Anti-HCV.*

Elisa positive	53
Elisa negative	15

Table 2: *Specimens Collected for Testing Hepatitis B.*

Elisa positive	38
Elisa negative	19

Table 2: *Summary of Results for Anti HCV.*

1. One Check vs ELISA		ELISA (n = 68)	
		Positive (n = 53)	Negative (n = 15)
One Check (n = 68)	Positive (n = 25)	24	1
	Negative (n = 43)	29	14

2. Accurate vs ELISA		ELISA (n = 68)	
		Positive (n = 53)	Negative (n = 15)
Accurate (n = 68)	Positive (n = 36)	35	1
	Negative (n = 32)	18	14

(Note: 4 of the positive results by accurate were weak positive)

Table 3: *Summary of Results for Hepatitis B.*

1. One Check vs ELISA		ELISA (n = 57)	
		Positive (n = 38)	Negative (n = 19)
One Check (n = 57)	Positive (n = 20)	20	0
	Negative (n = 37)	18	19

2. Accurate vs ELISA		ELISA (n=57)	
		Positive (n = 38)	Negative (n = 19)
Accurate (n = 57)	Positive (n = 20)	19	1
	Negative (n=37)	19	18

Discussion

Confirmation of diagnosis in hepatitis B and C viral infection and assessment of prognosis is based on wide array of advanced immunological, molecular and histological assays. The immunological techniques include 2nd generation ELISA, 3rd generation EIA and RIBA. While molecular/genetic testing includes qualitative, quantitative and signal enhancement detection of viral genomic fragments through PCR, RT-PCR, TMA or bDNA, whereas, invasive assessment includes examination of liver biopsy. But these techniques are costly and less frequently available in economically deprived countries. On the other hand a major concern in using rapid ICT screening kits is their variable degree of sensitivity and specificity. An ideal rapid test would have a high degree of positive predictive value (PPV) and low degree false negative results.

Results of the present study showed that the sensitivity and negative predictive values of rapid ICT kits used for HBsAg

and anti-HCV antibodies screening were significantly low. On the other hand specificity and positive predictive values of the rapid testing kits was fairly high, both for HBsAg and anti-HCV antibodies.

Since additional positive samples were detected by *ELISA* for both viruses, this could be due to short incubation period of the ICT employed in the study. Characteristically short incubation tests do not detect low affinity or low concentration of antibodies as compared to the classic type of immunoassays which employ longer incubation times allowing reaction to proceed to completion. It should be noted that the ICT may identify HBsAg and anti-HCV antibodies negative samples reasonably well, but, because of their short incubation times the assays do not always identify low affinity/low concentration of antibodies.

There is no second opinion about the superiority of advanced immunological and molecular techniques upon the rapid testing devices. But alarmingly the results obtained in this study depict that sensitivity claims of companies are mere boast. There was around 50% less sensitivity with rapid devices as compared to the *ELISA* in our study. In positive cases this difference was more marked while in negative *ELISA* sera, the difference was not so appreciable. Hence, ICT fall short of being the ideal screening tests for the detection of anti-HCV and HBsAg as there are a large number of false negative results resulting in low sensitivity and negative predictive values. Our results are nearer to the studies done in India where the *ELISA* method has been proven to be more sensitive and specific as compared with rapid test methods.^{22,23} But still the studies relating to this comparison are much scarce as the trend is more towards the superior assays. Further in-depth studies, using large sample size at different hospitals and health centers and employing advanced confirmatory techniques (e.g. RIBA, PCR) are needed to explore more information about these two clinically important human diseases.

Conclusion

The rapid test technique is though cheap but it is not helpful in the diagnosis and treatment of HBV/HCV positive patients because almost half the patients are left in doubt about their diagnosis. It is thus strongly recommended that where possible only the Elisa/PCR assays should be attempted to be 100% sure about the identification of HBV/HCV viruses.

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Table 4: *Quality Evaluation Parameters of Designed Diagnostic Assay.*

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic Accuracy
Anti HCV by One check	45%	93%	96%	35%	56%
Anti HCV by Accurate	66%	93%	97%	43%	72%
Hepatitis B by One check	53%	100%	100%	51%	68%
Hepatitis B by Accurate	50%	95%	95%	49%	65%

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