A Comparative Evaluation for Detection of Hepatitis B Surface Antigen and Anti HCV Antibodies by Rapid Diagnostic Tests and ELISA Method at Govt. Medical College, Dungarpur, India

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ABSTRACT

Hepatitis B virus and hepatitis C virus share common modes of transmission i.e. by blood and blood products mainly and also noticed in drug addicts. Hepatitis B virus and Hepatitis C virus have been recognized as a major pathogen causing significant morbidity and mortality throughout the world including India. The most important markers for HBV and HCV infection are HBsAg and Anti HCV antibodies respectively. Detection of infection markers for these agents is a major challenge in a resource poor setting. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary. The aims of this study were to compare Rapid tests with ELISA for Hepatitis B and Hepatitis C virus infection. The ultimate goal of this study was to recommend most reliable and cost-effective rapid card test for the diagnosis of HBV and HCV in areas where advance diagnostic facilities are not available. The present study was conducted in the clinical microbiology laboratory of Govt, Medical College, Dungarpur from period of 1\textsuperscript{st} March, 2019 to 15\textsuperscript{th} May 2019 to comparative evaluate the detection of HBsAg and Anti HCV antibodies by rapid diagnostic tests and ELISA method. In our study, total no. of 1756 samples were tested for HBsAg and 188 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. On testing for 1756 samples of HBsAg, 78 samples were reactive by ELISA but only 70 were reactive in Rapid tests and Out of 188 samples for HCV, 7 were reactive for Anti HCV antibodies by ELISA but only 6 samples were reactive by Rapid tests. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary. The card test’s sensitivity and specificity is comparable with ELISA. These rapid kits are cheaper and easy to perform and their use should be encouraged at rural settings. ELISA is much more sensitive than rapid tests for screening of infections like HBsAg and HCV.

Keywords
HBV, HCV, HBsAg, Anti HCV antibodies, Rapid test, ELISA

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Introduction

The Viral Hepatitis caused by Hepatitis B Virus is termed as “Serum Hepatitis”. 1-5% infected people act as chronic carriers of HBV Virus. Major part of the chronic carriers secretes hepatitis B surface antigen (HBsAg) into blood and other secretions of the body like saliva and vaginal fluid. These chronic carriers are potentially infectious to other seronegative people. HBsAg has been accepted as a universal and the most reliable
seromarker in case of acute HBV infection due to its appearance almost 2-4 weeks. Within HBVs, antigenic diversity is recognized in the surface antigens. HBsAg particles contain common “a” antigen, linked to two sets of mutually exclusive determinants, “d” or “y” and “w” or “r” giving the four main types-adw, adr, ayw and ayr. (19, 20, 22) Hepatitis C Virus was identified in 1989 as the main aetiological agent of non-A, non-B hepatitis (NANBH) accounting for greater than 90% of post-transfusion hepatitis cases. It is considered to be the major cause of acute chronic hepatitis, liver cirrhosis and hepatocellular carcinoma throughout the world. It is therefore necessary to correctly diagnose Hepatitis C infection. The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding anti-HCV in serum/plasma. (3, 5, 16, 17) Conventional ELISA is regarded as the most commonly used screening technique for general population in Tertiary Care Hospitals of India, but due to limitations of ELISA tests like high costs, unavailability in many laboratory and testing sites, involvement of costly instruments, time taking nature and requirement of highly skilled personnel for interpretation, rapid tests that are user friendly are gaining more importance and warrants comparison of performance. (11) Rapid diagnostic kits are a good choice as they are less expensive and do not need high technical manpower or infrastructure. (14) Were intended for field survey diagnosis, emergency and home testing. The rapid card test is known to have less sensitivity and specificity than EIA but some have sensitivity and specificity comparable to EIA (2). The ultimate goal of this study was to recommend most reliable and cost-effective rapid card test for the diagnosis of HBV and HCV in areas where advance diagnostic facilities are not available. The present study was conducted to compare the efficacy of ELISA test kits and Rapid test kits for screening in rural area and general population of India.

The present study was conducted to compare the efficacy of ELISA test kits and Rapid test kits for screening Hepatitis B and Hepatitis C infection. The present study was designed to check the sensitivity and specificity of rapid card test of HBsAg which are frequently used in different laboratories and hospitals and to compare with already confirmed cases on ELISA. The ultimate goal of this study was to recommend most reliable and cost-effective rapid card test for the diagnosis of HBV and HCV in areas where advance diagnostic facilities are not available. This study was conducted to aid in early detection and treatment of HBV and HCV infections and its prevention in community.

**Materials and Methods**

The present study was conducted in the clinical microbiology laboratory of Govt, Medical College, Dungarpur from period of 1\textsuperscript{st} March, 2019 to 15\textsuperscript{th} May 2019 to comparative evaluate the detection of HBsAg and Anti HCV antibodies by rapid diagnostic tests and ELISA method. A total of 1944 blood samples were collected from the outdoor and indoor patients of Govt, Medical College, Dungarpur and its allied hospitals. Out of these, total no. of 1756 samples were tested for HBsAg and 188 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. The results of the reactive sample by ELISA were compared with the rapid tests. The collected blood was allowed to clot & serum was separated. The sample were stored at 2-8\textdegree c & tested within 7 days of collection. Patients’ serum samples were subjected to following tests for detection of Anti-HCV antibodies.

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Rapid test (also called RDT or Rapid Diagnostic test, ICT or Immuno chromatographic Test)

LINE immunoassay for detection of HBsAg.

DOT immunoassay for detection of Anti-HCV antibodies.

ELISA test

For Detection of HBsAg for Detection of Anti-HCV antibodies.

For Detection of Anti-HCV antibodies.

Hepalisa Ultra Test

This is a 4th generation micro well ELISA test for the detection of hepatitis B surface antigen (HBsAg) in Human Serum / Plasma. HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection [12, 14]. This Kit is manufactures by J. Mitra& Co. Pvt. Ltd. New Delhi, India.

Principle: 4th Generation Hepalisa Ultra is a solid phase enzyme linked immunosorbent assay (ELISA) based on the “Direct Sandwich” principle. The microwells are coated with Monoclonal antibodies with high reactivity for HBsAg. The samples are added in the wells, followed by standard procedure. The intensity of developed blue colour is proportional to the concentration of HBsAg in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450 nm spectrophotometrically. Test procedure & results were interpreted as per the manufacture’s guidelines. Sample were interpreted as reactive for HBsAg (HBsAg positive) or non-reactive for HBsAg (HBsAg negative). Sample found to be reactive initially by HEPALISA ULTRA test were again tested by visual rapid test which is HEPACARD test.

Hepacard

The 3rd Generation HEPACARD is a rapid, visual, sensitive and qualitative in vitro diagnostic test for the detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma. The principal antigenic determinant is “a” which is common to all HBV serotypes. In addition, two pairs of sub specific determinants have been identified, d/y & w/r, which are apparently mutually exclusive. Four antigenic combinations are therefore possible: adw, adr, ayw and ayr. This Kit is manufactures by J. Mitra & Co. Pvt. Ltd. New Delhi, India. Principle: - HEPACARD is a one step immunoassay based on the antigen capture, or “sandwich” principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg and test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate. Interpretation: - Results are noted as per manufactures guidelines and results were interpreted accordingly. Appearance of pink coloured line, one each in test region “T” and control region “C” indicates that the sample is REACTIVE for HBsAg.

HCV Micro ELISA test

The 3rd generation HCV Microlisa is an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies against HCV (anti-HCVs) in human serum or plasma. This kit is manufactured by J. Mitra& Co. Pvt. Ltd. New Delhi, India. Principle: The 3rd generation HCV Microlisa is based on a highly sensitive
technique, Enzyme Linked Immunosorbent Assay which detects antibodies against HCV in human serum and plasma. The 3rd generation HCV Microlisa utilizes a combination of antigen with the sequence of both HCV structural and non-structural antigen i.e. CORE, E1, E2, NS3, NS4 and NS5. The results were read on Microplate spectrophotometer at 450 nm. Cut off value was calculated as per the manufacturer's guidance and the results were interpreted accordingly. Cut off value = 0.1xPCx + 0.1, PCx = Mean absorbance of positive control

Interpretation:- According to their absorbance values, samples were interpreted as either reactive for HCV antibody (HCV positive) or non-reactive for HCV antibody (HCV negative) if test specimens with absorbance value within 10% below the cutoff should be considered suspect for the presence of antibodies and should be retested in duplicate. Sample found to be reactive initially by HCV Microlisa test were again tested by visual rapid test which is HCV TRI-DOT test.

**HCV TRI-DOT**

The 4th Generation HCV TRI-DOT is a rapid, visual, sensitive and qualitative in vitro diagnostic test for the detection of antibodies to Hepatitis C Virus in human serum or plasma. They are for the putative core (structural), protease/helicase NS3 (non-structural) NS4 (non-structural) and replicase NS5 (non-structural), regions of the virus in the form of two test dots "T1" & "T2" to provide a highly sensitive and specific diagnostic test. This Kit is manufactures by J. Mitra & Co. Pvt. Ltd. New Delhi, India. Principle: 4th generation HCV TRI-DOT has been developed and designed using modified HCV antigens representing the immune dominant regions of HCV antigen. HCV antigens are immobilized on a porous immune filtration membrane. Interpretation: - Results are noted as per manufactures guidelines and results were interpreted accordingly. If test dots T1, & T2, either both dark and light in colour (pink), result should be considered reactive for antibody to HCV. If only control dot appear it indicates that the sample is non-reactive for anti-body to HCV. Sample found to be positive for HCV antibodies by both HCV Microlisa test & HCV TRI-DOT method would be further tested for hepatitis B Surface antigen by ELISA test.

**Bio-Safety**

All standard precautions, bio-safety measures & biomedical waste managements in our study according to Biological waste management’s Rules 1998 were observed.

**Results and Discussion**

In our study, A total of 1944 blood samples were collected from the outdoor and indoor patients of Govt, Medical College, Dungarpur and its allied hospitals. Out of these, total no. of 1756 samples were tested for HBsAg and 188 samples for HCV by ELISA method (gold standard) as a confirmatory method and samples found reactive were again tested by rapid test kits. On testing for 1756 samples of HBsAg, 78 samples were reactive by ELISA but only 70 were reactive in Rapid test

Out of 188 samples for HCV, 7 were reactive for HCV by ELISA but only 6 samples were reactive by Rapid test.

Table shows that using ELISA as a gold standard confirmatory method, sensitivity of rapid card test for HBsAg was 89.74%, specificity was 100%, PPV(positive predictive value) was 100%, NPV(negative predictive value) was 99.52%, Diagnostic accuracy was 99.54%, Youden’s index was 0.897 and F1 Score was 0.945 whereas sensitivity of rapid card test for Anti HCV Antibodies was 85.71%, specificity was 100%, positive predictive value was 100%, negative
predictive value was 99.45%, Diagnostic accuracy was 99.47%, Youden’s index was 0.857 and F1 Score was 0.923.

In the present study ELISA was compared with the rapid kits for the screening of HBsAg and Anti HCV Antibodies. For HBsAg and Anti HCV Antibodies screening, rapid tests are equally sensitive to ELISA and yet they are cheaper and quicker. Within the rapid tests, the sensitivity and specificity was same but there were variations in the cost. (14) ELISA and other advanced methods are laboratory based, time consuming and require trained personnel. Rapid test enables early detection at sites where laboratory facilities or trained manpower are not available or there is issue of accessibility. (18, 19) The rapid tests reduce the potential for loss of follow up of a case when results are not given straight away. The high laboratory cost is another factor that reduces the willingness to screen the general population. Ideally rapid devices should have a high degree of sensitivity and a reasonable specificity so as to minimize false positive and false negative results (14) (Fig. 1 and Table 1–6).

**Table.1** Comparative Evaluation of *Rapid Test Kits with ELISA* for HBsAg and Anti HCV Antibodies Tests

| TESTS           | Reactive by ELISA | Percentage Reactivity | Reactive by RAPID TESTS | Percentage Reactivity | False Negative by RAPID TESTS |
|-----------------|-------------------|-----------------------|-------------------------|-----------------------|-------------------------------|
| HBsAg           | 78                | 4.44%                 | 70                      | 3.98%                 | 0.46%                         |
| Anti HCV Antibodies | 7            | 3.72%                 | 6                       | 3.19%                 | 0.53%                         |

**Table.2** Comparative Evaluation of HBsAg CARD Rapid Test Kit with HBsAg ELISA Test

| Rapid Card Test     | HBsAg    | **ELISA Reactive** | **ELISA Non Reactive** |
|---------------------|----------|--------------------|------------------------|
| Rapid Reactive      | 70       | 0                  |
| Rapid Non reactive  | 08       | 1678               |
| Total cases         | 78       | 1678               |

P value <0.001

**Table.3** Comparative Evaluation of HCV DOT Rapid Test Kit with Anti HCV Antibodies ELISA Test

| Rapid Card Test     | Anti HCV Antibodies | **ELISA Reactive** | **ELISA Non Reactive** |
|---------------------|---------------------|--------------------|------------------------|
| Rapid Reactive      | 06                  | 0                  |
| Rapid Non reactive  | 01                  | 181                |
| Total cases         | 07                  | 181                |

P value <0.001
Table 4 Comparative evaluation of rapid test kits with ELISA as a gold standard confirmatory method

| Tests                          | Sensitivity | Specificity | PPV  | NPV  | Diagnostic effectiveness (accuracy) | Youden’s index | F1SCORE |
|-------------------------------|-------------|-------------|------|------|------------------------------------|----------------|---------|
| HBsAg by Rapid card test      | 89.74%      | 100%        | 100% | 99.52% | 99.54%                             | 0.897          | 0.945   |
| Anti HCV Antibodies by Rapid card test | 85.71%      | 100%        | 100% | 99.45% | 99.47%                             | 0.857          | 0.923   |

*Sensitivity (also called the true positive rate, the recall, power, hit rate or probability of detection)-refers to a test's ability to designate an individual with disease as positive. Specificity (also called the true negative rate or selectivity)-refers to a test's ability to designate an individual who does not have a disease as negative. Positive predictive value (PPV also called the precision)-is the ability of an assay to identify actual infected individuals among all persons. Negative predictive value (NPV)-is the ability of an assay to identify correctly the real non-infected individuals among persons. Diagnostic accuracy (DA also called the Diagnostic effectiveness)-The accuracy of a test is its ability to differentiate the patient and healthy cases correctly. DA is affected by disease prevalence. Youden’s index (also called Yuoden J satstices)-It is a single statistic that captures the performance of a dichotomous diagnostic test. DA is affected by spectrum of disease, not by disease prevalence. F1Score-(also called theaka F-Score/F-Measure)-It is the harmonic mean (average) of the PPV and Sensitivity. It can be used as a single measure of performance of the test for the positive class.

Table 5 Comparison of Rapid Test and ELISA for HBsAg in other studies

| Author                  | Place          | HBsAg Positive by Rapid test | HBsAg Positive by ELISA |
|-------------------------|----------------|-------------------------------|-------------------------|
| Ijaj H et al., 2012 (6) | Pakistan       | 95                            | 100                     |
| Adeyemi AA et al., 2013 (1) | Nigeria     | 38                            | 71                      |
| EWS Chameera et al., 2015 (4) | Srilanka     | 04                            | 05                      |
| Mohammad K et al., 2016 (12) | India (Haryana) | 22                            | 30                      |
| Parth R et al., 2016 (13) | India (Guajrat) | 70                            | 80                      |
| Present study (2019)    | India(Dungarpur) | 70                            | 78                      |
Table.6 Comparison of rapid test and ELISA for Anti HCV antibodies in other studies

| Author               | Place              | Anti HCV Antibodies Positive by Rapid test | Positive by ELISA |
|----------------------|--------------------|-------------------------------------------|------------------|
| Ijaj H et al., 2012 (6) | Pakistan          | 07                                        | 12               |
| Adeyemi AA et al., 2013 (1) | Nigeria          | 00                                        | 04               |
| Mohammad K et al., 2016 (12) | India (Haryana)  | 93                                        | 100              |
| Present study (2019) | India (Dungarpur) | 6                                         | 7                |

Fig.1 Comparative evaluation of Rapid Test Kits with ELISA for HBsAg and Anti HCV antibodies test

This study agrees with previous studies in other countries, which have stated that rapid test kits are not sensitive enough to be used solely for the detection of HBsAg and HCV. Some studies suggest that the diagnostic performance of Rapid Test is comparable to ELISA. However in our study we found ELISA to be much more sensitive than Rapid Test. The our results were correlated with the Previous studies by Ijaj H et al., 2012 (Pakistan)\(^6\), Adeyemi AA et al., 2013 (Nigeria)\(^1\), EWS Chameera et al., 2015 (Srilanka)\(^4\), Mohammad K et al., 2016 (India)\(^12\) and Parth R et al., 2016 (India)\(^13\). The results were also correlated with the other studies by Kaur et al., (2000)\(^5\), Raj et al., (2001)\(^15\), Zahoorullah et al., (2001)\(^21\), Lin et al., (2008)\(^10\) and Khan et al., (2010)\(^9\). Comparison of studies conducted by other researchers showed slight variations in results. Results were varies with geographical distribution and social characteristic of population groups. Our study is a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population. We conclude that HBV and HCV directly affects epidemiology, morbidity, mortality, socioeconomic and preventive aspects, So particularly in developing countries like India, the present study and other similar studies by early detection of viral prevalence for in assessment of disease burden in community and in controlling the complications of HBV and HCV viral infections.

The ultimate goal of this study was to recommend most reliable and cost-effective
rapid kits for the diagnosis of HBV in areas where advance diagnostic facilities are not available. We reported that rapid test is less efficient than ELISA compared with conventional ELISA which needs long time. Rapid card test results are available within minutes. This will be very helpful in initiating immediate treatment and minimizing the serious complications and mortality. Conventional ELISA cannot be performed for single or small number of samples, since it would be quite uneconomical. Hence, they are used for testing of large samples. Rapid card test are quite susceptible to unfavourable storage conditions, so this is essential to do periodic quality control checks to avoid false positive or false negative results. These rapid card tests should be recommended only in resource limited poor settings, remote areas and peripheral health facilities for screening purpose. HBV and HCV are highly dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays such as ELISA. A major concern in utilizing rapid screening tests is that these tests should have a high degree of sensitivity and a reasonable level of specificity to minimize false positive and false negative results. HBV and HCV are highly dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays such as ELISA. Therefore, it is recommended that, rapid test kit should be used in conjunction with other immunoassay particularly ELISA technique.

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