

# Comparison of Different ICT Kits for HBsAg and Anti HCV Using Gold Standard ELISA

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## Abstract

**Objectives:** To compare rapid tests (ICT) with 4<sup>th</sup> generation ELISA (gold standard) for hepatitis B and C infection.

**Settings:** Biochemistry and Serology Laboratory of the Pakistan Medical Research Council, Research Centre, Jinnah Post Graduate Medical Centre, Karachi. Study was done over six months.

**Materials and Methods:** ELISA confirmed 200 samples for HBsAg and 200 for Anti-HCV were selected, making a total of 400 samples, 200 were positive and 200 negative on ELISA. These samples were further tested on three different brands of most frequently used rapid ICT Kits for HBsAg and Anti HCV. The sensitivity, specificity, negative predictive value, positive predictive value and cost effectiveness were compared using 4<sup>th</sup> generation ELISA as gold standard. The Rapid kits for HBsAg that were analysed included Acon (USA), Determine (Abbott) and Intec (China) and for Anti HCV they were Acon (USA), Membrane (Canada) and Nobis (Germany).

**Results:** Out of 100 positive and 100 negative tests for HBsAg confirmed on ELISA, all rapid kits showed comparable results with ELISA. The sensitivity and negative predictive value of Intec China (98%) and Determine Abbot (98%) were similar to each other however, these were higher when compared to Acon USA (95%). The rapid kit by Intec China was cheaper to the other two rapid kits and was therefore, the most cost effective rapid kit. The specificity and positive predictive value of all three HBsAg ICT kits was 100% and in agreement with ELISA. Out of 100 HCV positive and 100 HCV negative cases confirmed on ELISA, the rapid test by Acon USA showed maximum sensitivity. The sensitivity and negative predictive values of Acon USA were higher (93%) as compared to Membrane - Canada (89%) and Nobis-Germany (86%). The specificity and positive predictive values of Acon were comparatively lower (93%) but did not significantly vary when compared with Membrane Canada (97%) and Nobis German (96%).

**Conclusions:** The rapid ICT Kits for HbsAg and anti HCV were equally sensitive and specific when compared with ELISA. These rapid kits are cheaper and easy to perform and their use should be encouraged especially in rural setting.

**Policy statement:** ELISA confirmed rapid HBV, HCV kits being cheaper but sensitive and specific should be used for screening cases especially in rural setting.

**Key words:** HBsAg, HCV, ELISA, rapid tests.

## Introduction

Hepatitis B (HBV) and C (HCV) are serious global health problems. Approximately 500 million people i.e. one fifth of the world population are chronically infected with HBV and HCV<sup>1,2</sup>. About 1.5 million people die every year from HBV and HCV related chronic liver disease such as end stage cirrhosis and HCC (Hepatocellular carcinoma). Worldwide, over two billion people have been infected with HBV and more than 350 million have chronic life long infection<sup>3</sup>. In Pakistan the prevalence of HbsAg is 2.5% and that of HCV 5% showing almost 12 million population is exposed to these viruses<sup>4</sup>.

Different methods are used for the diagnosis of hepatitis including ICT, ELISA, EIA and PCR. ELISA, EIA and PCR methods are expensive and are used in well equipped labs and major tertiary care hospitals. Rapid diagnostic ICT kits are a good choice as they are less expensive and do not need high tech manpower or infrastructure<sup>5</sup>. Since 1990s, rapid tests are available for detection of HIV infection. They were intended for field survey diagnosis, emergency and home testing. In addition rapid test for Anti HIV, HBsAg and Anti HCV have been used for blood screening in many resource-poor areas to save resources and overcome lack of funding, equipment and electrical supply.

The rapid ICT kits are known to have less sensitivity and specificity than EIA but some have sensitivity and specificity comparable to EIA<sup>6,7</sup>. 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

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negative results. The present study was designed to check the sensitivity and specificity of at least three different rapid kits of HBsAg and Anti HCV which are frequently used in different labs and hospitals of Karachi and to compare with already confirmed cases on ELISA. The ultimate goal of this study was to recommend most reliable and cost-effective rapid kits for the diagnosis of HBV and HCV in areas where advance diagnostic facilities are not available.

### Subjects and Methods

Three most commonly used brands of rapid diagnostic kits for HBsAg and Anti HCV in different laboratories of Karachi were selected for the study. ELISA 4<sup>th</sup> generation was used as gold standard for comparative evaluation. Prior to the purchase of rapid ICT kits, a market survey was done in the major government and private hospitals of Karachi to find out which kits are being commonly used by these outlets. For HBsAg: Acon USA, Intec China, and Determine Abbot were selected and for Anti HCV: Acon USA, Membrane Canada, and Nobis German were selected.

A total of 400 (ELISA Confirmed) samples both for HBsAg and Anti HCV were selected and tested on three different ICT kits. Two hundred HBsAg samples included 100 positive and 100 negative samples. Two hundred Anti HCV samples included 100 positive and 100 negative samples. The sample size was based on the prevalence of previous study conducted in Pakistan, where prevalence was 2.5% for HBsAg and 4.9-5.3% for Anti HCV<sup>4,8</sup> at 95% confidence interval and 3% absolute precision. For calculating the sample size we used maximum prevalence of Anti HCV i.e 5% for both HBsAg and Anti HCV. Using Computer program "OpenEpi Version 2.

At PMRC, JPMC serology lab, the ELISA confirmed stored positive and negative samples of HBsAg and Anti HCV were run on rapid tests which, were procured from the market. Dual infections with HBV and HCV and repeat samples of same patient were excluded. The sensitivity, specificity, negative predictive value and positive predictive value were calculated.

The data was analyzed using computer statistical package of social sciences (SPSS) Version 11.0. This included sensitivity, specificity, positive and negative predictive values for all rapid ICT kits<sup>9</sup> i.e Determine (Abbot), Intec (China) and Acon ( USA), for HBs Ag and Acon (USA), Membrane (Canada) and Nobis (Germany) for Anti HCV. These results were then compared with Gold standard ELISA.

Sensitivity is the ability of the screening test to give a positive finding when the person tested has the disease. it is expressed as percentage.

$$\frac{\text{Person with the disease detected by the screening test}}{\text{Total number of person tested with the disease}} \times 100$$

Specificity is the ability of the test to give a negative finding when the persons tested are free of the disease under study. it is also expressed as a percentage.

$$\frac{\text{Person without the disease detected negative by the screening test}}{\text{Total number of person tested without the disease}} \times 100$$

Positive Predictive value is the percentage of true positives among total positives.

Negative Predictive value is the percentage of true negatives among total negatives.

### Cost-effectiveness analysis

For cost effective analysis cost of ICT kits/device and their sensitivity was compared using ELISA as Gold standard and by that comparison the most sensitive and cost effective kit was identified.

### Results

The results of different ICT kits on the basis of sensitivity and specificity were compared for HBsAg and Anti HCV and are depicted in Table-1 & 2. The overall performance of rapid ICT kits was reasonably well. The specificity and positive predictive value of all HBsAg rapid kits were 100% while for Anti HCV it was 97% for Membrane 96% for Nobis and 93% for Acon. The sensitivity and negative predictive value for HBsAg ICT kit for Intec China and Determine Abbot was 98% and for Acon USA 95%. For Anti HCV it was 93% for Acon, 86% for Nobis Germany and 89% for Membrane Canada. ACON USA was the most sensitive rapid ICT kit in detecting Anti HCV while Intec China and Determine Abbot showed similar sensitivity for HBsAg detection.

On cost analysis for HBsAg Intec. China was found to be the most cost effective device when compared with other two. In case of anti HCV evaluation Acon USA showed higher sensitivity as compared to Nobis Germany and Membrane Canada (Table-3), although true negative rates from Acon were less as compared to the other devices. Some false positive and false negative results were also obtained specially with border line cases. It was observed that all ICT kits were able to pick HBsAg and Anti HCV antibodies negative samples reasonably well.

The sensitivity, specificity, negative and positive predictive values of all the devices are shown in Table-1 & 2. For HCV, Acon showed higher sensitivity and negative predictive value as compared to other strips/devices while specificity and positive predictive value of Membrane and Nobis were found higher when compared with the Acon but this kit was more cost effective in detecting chronic anti HCV (Table-3).

**Table 1: Evaluation of rapid HBsAg kits with ELISA.**

Kit for Hepatitis "B"		ELISA (Gold standard)			Results for screening test (kit)							
		Reactive	Non Reactive	Total	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		(n=100)	(n=100)									
ACON	Reactive	95	-	95	95	100	-	5	95	100	100	95
	Non Reactive	5	100	105								
INTEC	Reactive	98	-	98	98	100	-	2	98	100	100	98
	Non Reactive	2	100	102								
DETERMINE	Reactive	98	-	98	98	100	-	2	98	100	100	98
	Non Reactive	2	100	102								

TP; true positive, FP; false positive, PPV; positive predictive value, TN; true negative, FN; false negative, NPV; negative predictive value

**Table 2: Evaluation of rapid Anti HCV kits with ELISA.**

Kit for Hepatitis "C"		ELISA (Gold standard)			Results for screening test (kit)							
		Reactive	Non reactive	Total	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		(n=100)	(n=100)									
ACON	Reactive	93	7	100	93	93	7	7	93	93	93	93
	Non Reactive	7	93	100								
NOBIS	Reactive	86	4	90	86	96	4	14	86	96	95	87
	Non Reactive	14	96	110								
MEMBRANE	Reactive	89	3	92	89	97	3	11	89	97	97	90
	Non Reactive	11	97	108								

TP; true positive, FP; false positive, PPV; positive predictive value, TN; true negative, FN; false negative, NPV; negative predictive value

Results for HBsAg are depicted in Table-1 & 3. For HBsAg the specificity and positive predictive value of all three kits were similar to that seen with the Gold standard ELISA (100%). The sensitivity and negative predictive value of Intec China and Determine Abbott were higher when compared to Acon USA but between the two kits there was no difference in the sensitivity and negative predictive value. Intec however, was cheaper and thus cost effective kit for the screening of HBsAg (Table-3).

**Table 3: Sensitivity and cost effectiveness of diagnostic kits.**

ICT kits	Test	Sensitivity (%)	Cost (Rs)
Membrane Canada	HBs Ag	89	60
Nobis Germany	HBs Ag	86	60
Acon USA	HBs Ag	93	40
Acon USA	Anti HCV	95	40
Intec China	Anti HCV	98	35
Determine Abbott	Anti HCV	98	70

### Discussion

In the present study ELISA was compared with the rapid kits for the screening of chronic HBV and HCV infections. For both infections, rapid tests were equally sensitive to ELISA and yet they were cheaper and quicker.

Within the rapid tests, the sensitivity and specificity was same but there were variations in the cost. Globally quantitative immunoassay (EIA, ELISA and PCR etc.) is the most sensitive test which, is widely used at well equipped reference centres or central blood banks<sup>10,11</sup>. Rapid test are intended for qualitative detection of HBsAg in serum, plasma or whole blood where EIA methods are beyond access or cost<sup>12</sup>. ELISA, EIA, PCR 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. Most rapid tests are based on immunochromatographic principles<sup>13</sup>. The rapid tests reduce the potential for loss of follow up of a case when results are not given straight away<sup>14,15</sup>. The high laboratory cost is another factor that reduces the willingness to screen the general population<sup>15,16</sup>.

Due to their easy use and cheaper cost, the rapid tests are being used practically at all primary and most secondary health care facilities in Pakistan. Their use is maximum in the private sector which caters for 70-80% of the population and where cost is a major concern and for which sometimes quality is compromised. In Pakistan many cheaper rapid tests are available which are creating confusion among the end users for their reliability. Ideally

rapid devices should have a high degree of sensitivity and a reasonable specificity so as to minimize false positive and false negative results.

The present results are similar to the Iranian study where 6 rapid strips/devices were compared with gold standard and the results of rapid kits were comparable with the Gold standard<sup>17</sup>. In a study from India the rapid kits of HBsAg were found to be 100% specific and 93.4% sensitive<sup>18</sup>. In another study from Seoul, using rapid technique showed a 97% sensitivity and 100% specificity for detecting HBsAg<sup>19</sup>.

A Pakistani study showed 100% sensitivity of latex agglutination and ICT method with a specificity of 91.7% and 99.2% for HBsAg<sup>20</sup>. In another study ICT and ELISA were compared for detection of HBsAg in healthy individuals from Karachi and showed comparable sensitivity and specificity of ICT kits with ELISA technique<sup>21</sup>.

In the present study false positive results were less than false negative results. For HBsAg no false positive results were found while for Anti HCV 3%, 4% and 7% false positive results were found with Membrane, Nobis and Acon respectively which is much less than that reported from Hazara Pakistan<sup>22</sup>. In case of Nobis, the false negative results were higher. Although in many instances false positive results are preferable to false negative results when screening large groups, as positive serology triggers repeat testing with alternative method for case confirmations but false negative results may jeopardize blood safety. In our study false negative results were more comparable to false positive results, but overall the specificity results for both HBsAg and Anti HCV ICT kits were high i.e. 95-100%. These results are different to the study from Lahore Pakistan<sup>23</sup> where the specificity obtained with HBsAg and Anti HCV ICT Kits were 93 to 100% but the sensitivity was 50% for both HBsAg and Anti HCV. In the present study sensitivity was higher 86-93% for Anti HCV and 95-98% for HBsAg.

Present study showed that Acon had higher sensitivity (true positive rates) while, lower specificity or high number of false positive samples as compared to Membrane and Nobis in detecting Anti HCV but the difference was not significant. Some weak positive results were also found in Anti HCV which were seen in Acon with low reactivity or low titer positivity on ELISA, so it is necessary to read these rapid strips/devices carefully while reporting results.

The present study concludes that the overall performance of these rapid tests was not only compatible with currently established and advanced diagnostic methods but also cheaper. It can be recommended that ELISA comparable rapid devices may be allowed to be used for initial screening of hepatitis B and C especially, in remote areas or where cost is an issue.

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