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A Comparative Assessment of Immunochromatography, Elisa and PCR for the Detection of Hepatitis B Surface Antigen in Human Plasma

Awadh Sahu[1], Naresh Kumar Dewangan[2], Sumit Gupta[3]

[1]PhD Scholar, Shree Shankaracharya Professional University Bhilai (C.G.) India
[2]PhD Guide, Assistant Professor Department of Life Science, Shree Shankaracharya Professional University Bhilai (C.G.) India
[3]PhD, Co- Guide, Assistant Professor Department of Microbiology SBDMS Govt. Medical College and Hospital Korba (C.G.) India

Abstract: ***Hepatitis B virus (HBV) is a common blood borne infectious agents causing high morbidity and mortality that constitute the major global health challenge These viruses are mainly spread through the translation of contaminated blood and blood products. HBV is a DNA virus belonging to the Hepadnaviridae family The primary laboratory screening test for the early detection of HBV infection is the immunoassay for hepatitis B surface antigen (HBsAg). Various advanced analytical techniques are currently employed for HBV diagnosis, including Immunochromatographic Test (ICT), Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescent Microparticle Immunoassay (CMIA), and Polymerase Chain Reaction (PCR). Findings from various studies indicate that rapid testing methods may not always ensure the accuracy of results, thereby increasing the risk of bloodborne infections during transfusion. In the early stages of infection or during the recovery phase, viral titers are often low, leading to reduced signal optical density (Low SOD) in test outcomes. These low-positive cases may go undetected by rapid screening methods such as ICT but can be identified through more sensitive assays like ELISA. and PCR However, it is essential to emphasize that standardization of diagnostic methods is critical before confirming an infection. A key consideration in the use of screening tests is that they must demonstrate high diagnostic performance across all stages of the disease to ensure reliable detection. In the present study, a comparison was done between ELISA, PCR, CLIA and ICTs for the screening of HBsAg. This study aims at comparing the analytical measures between ELISA, PCR, CLIA and rapid cards for better diagnosis of HBsAg in forthcoming years. From this study results, it was found that for HBsAg screening, ELISA, PCR, CLIA was more sensitive than the rapid card tests and equally specific to the rapid card tests. In this study, the sensitivity of ICT was 83.4%,specificity 100%, PPV 99.4%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%.(Prabha, P. et al.2022), the sensitivity of ICT was 97 %,specificity 100%, PPV 90.4 %, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%.(Rahaman, S. et al. 2023), the sensitivity of ICT was 80%,specificity 100%, PPV 97.8.%, NPV 100 % (Chameera, E.W.S. et al.2013), the sensitivity of ICT was 97.1%,specificity 100%, PPV 98.5.%, NPV 100 % and the sensitivity, specificity, PPV and NPV PCR was 100% (Hasan, K. N.et al. 2017), the sensitivity of ELISA was 78%,specificity 76 %, PPV 75.%, NPV 80% and the sensitivity, specificity, PPV and NPV PCR was 100% (Navvabi, N. et al. 2021) , the sensitivity of ELISA was 100%,specificity 100%, PPV 100%, NPV 100% and the sensitivity, specificity, PPV and NPV TRUE NAAT PCR was 100% (Samom, P., et al .2023) ,the sensitivity of ELISA was 92%,specificity 100%, PPV 100.%, NPV 97% and the sensitivity, specificity, PPV and NPV PCR was 100% (Aftab, H. T.,et al. 2023) ,the sensitivity of ICT was 92%,specificity 97%, PPV 93%, NPV 96 % and the ELISA was sensitivity95%, specificity99.82%, PPV95% and NPV 99% (Ali, I., et al. 2024) ,the sensitivity of ICT was 98%,specificity 100%, PPV 100.%, (Hayder, I., et al. 2012), the sensitivity of ICT was 82%, specificity 100%, PPV 91%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100% (Asaduzzaman, M., et al. 2012), the sensitivity of ICT was 100%, specificity 100%, PPV 100%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%. and sensitivity, specificity, PPV and NPV PCR was 100%. (Habibi, A., et al. 2016) ,the sensitivity of ICT was 99.7%,specificity 100%, PPV98% (Al-Matary, A. M., et al. 2016) , the sensitivity of ICT was92.98%,specificity 100%, PPV 99.4%, NPV 94.73 % and the sensitivity CLIA was 100%, specificity100%, PPV100% and NPV 97% and the sensitivity, specificity, PPV and NPV PCR was 100% (RENGARAJ, R., et al. 2024) ,the sensitivity of ICT was 99.27%,specificity 85.52%, PPV 95.58%, NPV 97.38 % and the ELISA was sensitivity 98.79%, specificity89.04%, PPV92.85% and NPV98.08% and sensitivity, specificity, PPV and NPV PCR was 100%. (Irfan, S., et al. 2018), the sensitivity of CLIA was 96.77%, specificity 96.07% PPV 93.75%, NPV 98% and the sensitivity, specificity, PPV and NPV ELISA was 100%., (Majumder, P., et al. 2017). This study does not have 100 % in the sensitivity and specification of the rapid test, but still some good brand rapid kits also have 100 % sensitive and specification, while the rapid test can also be done in rural areas and small labs, but after getting positive in the Rapid Test, it should be confermed with other mathods.***

Keywords: HBsAg diagnosis, ELISA vs ICT, hepatitis B screening, diagnostic test sensitivity, blood transfusion safety

1. Introduction

Hepatitis B virus (HBV) is a common blood borne infectious agents causing high morbidity and mortality that constitute the major global health challenge. About one fifth population of the world is consistently infected with HBV. estimated 1.5 million deaths are caused by HBV-related diseases. (Hayder *et al*. 2012). These viruses are mainly spread through the translation of contaminated blood and blood products. However, other important methods of infection, such as use of intravenous drug, close personal contact, and sharing needles, razors and other things. (Ahmed *et al*. 2009).

HBV is a DNA virus belonging to the Hepadnaviridae family. The envelope of the virus surrounds the nucleo-capsid which comprises the DNA genome and the viral matrix.HBV can survive for at least seven days outside the body which makes HBV more infectious than HIV. HBV infection is silent in those infected at an early stage of their

life. Acute HBV infection has an incubation period of 4-12 weeks.

Hepatitis B virus (HBV) infection is often symptomatic; However, chronic infection can lead to progressive liver fibrosis, which is usually clear after many years. Around 75% of acutely infected individuals develop chronic hepatitis B, which often turns into liver cirrhosis and hepatic malignancy (Ahmed *et al*. 2009).

Hepatitis B Surface Antigen (HBsAg) emerges during the incubation period and can usually be detected in most cases during the production of the disease and acute stages. In young adults, HBsAg is usually cleaned by the bloodstream within 5–6 months. However, its persistence for more than six months reflects a chronic career stage. (Risin et al., 2009).

The primary laboratory screening test for the early detection of HBV infection is the immunoassay for hepatitis B surface antigen (HBsAg). Various advanced analytical techniques are currently employed for HBV diagnosis, including Immunochromatographic Test (ICT), Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescent Microparticle Immunoassay (CMIA), and Polymerase Chain Reaction (PCR).

The ELISA, CMIA and PCR methods are found to be expensive and are used in advanced laboratories and major tertiary care hospitals. ICT, using by the most of the laboratories as a rapid diagnostic analytical method are now considered a good choice because of its low price, less involvement of skilled technician and infrastructure (Rahman *et al*. 2008). The rapid ICT kits are known to have less sensitivity and specificity than ELISA.

Enzyme-Linked Immunosorbent Assay (ELISA) is a wet-laboratory analytical technique that utilizes a solid-phase enzyme immunoassay to detect the presence of specific antigens or antibodies in a liquid-phase sample. This method integrates enzyme-labeled antigens or antibodies, immune complex formation, enzymatic catalysis, signal detection, and quantitative analysis. The combination of these processes enhances its specificity and sensitivity, making ELISA one of the most reliable immunoassays for detecting biological molecules.

Immunochromatographic Test (ICT) strips are utilized for the screening of hepatitis. Their mechanism of action is based on the fundamental principle of antigen-antibody interaction, where antibodies present in the test serum or plasma bind to protein-coated particles. This complex then migrates chromatographically along the membrane via capillary action, interacting with recombinant antigens immobilized on the membrane. This reaction results in the formation of a visible color line in the test region, indicating a positive result.

PCR is a technique used in molecular biology to create several copies of a certain DNA segment. PCR has made it possible to generate millions of copies of a small segment of DNA. The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase. (Bhadoria,A.S.*at al*.2022).

Findings from various studies indicate that rapid testing methods may not always ensure the accuracy of results, thereby increasing the risk of bloodborne infections during transfusion. In the early stages of infection or during the recovery phase, viral titers are often low, leading to reduced signal optical density (Low SOD) in test outcomes. These low-positive cases may go undetected by rapid screening methods such as ICT but can be identified through more sensitive assays like ELISA. and PCR However, it is essential to emphasize that standardization of diagnostic methods is critical before confirming an infection. A key consideration in the use of screening tests is that they must demonstrate high diagnostic performance across all stages of the disease to ensure reliable detection.

Comprehensive Review of Methods

A cross-sectional study was conducted from July 2019-August 2019 in a tertiary care hospital Tamilnadu India. A total of 200 blood samples were tested for HBsAg using both rapid Immuno Chromatographic Card (ICT) method (HEPAVIEW - one step test for HBsAg,Viola Diagnostic System) and Enzyme Linked Immuno Sorbent Assays (ELISA) (Merilisa HBsAg- Meril Diagnostics Pvt., Ltd.) method. the total 200 blood samples tested by HBsAg rapid card, 5 samples were positive and the remaining 195 were negative.

Table 1: Evaluation of RAPID and ELISA test

Test	Sensitivity	Specificity	PPV	NPV
ICT	83.4%	100%	99.4%	100%
ELISA	100%	100%	100%	100%

The overall performance of the rapid ICT for HBsAg was less sensitive to ELISA. So, only ELISA can be encouraged in all setups irrespective of their developmental and economical status not only to prevent the complications of Hepatitis B infection but also for early diagnosis and better treatment of patients **(Prabha, P. et al.2022)**

This cross sectional study was conducted at the Immunology laboratory, BIHS General Hospital, Dhaka Bangladesh. A total 240 sample of HBsAg test results of the patients were collected from Laboratory register book. All tests were carried out by ELISA & ICT methods and compare between them. Majority (76.3%) of the donors was within the age group of 19-30 years. Among the donors, male donors were predominant (93.75%) than female (6.25%). A total number of 240 HBsAg tests were done in ICT method and found 6 Positive (2.5%) of them. Then we done correspondence test to confirm these by ELISA method and found 100% negative results.

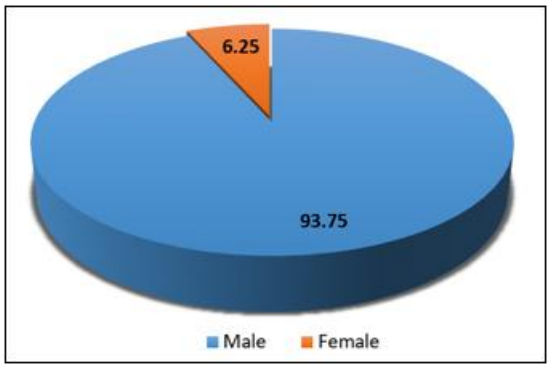


Figure 1: Gender distribution of the patients

Table 2: Distribution of seromarkers status analyzed by Immunochromatography (ICT) (n= 240)

Seromarkers	ICT				P value
	Positive		Negative		
	Number	%	Number	%	-
HBsAg	6	2.5	234	97.5	

Table 3: Distribution of seromarkers status by ELISA (n=240)

Seromarkers	ELISA				P value
	Number	%	Number	%	-
HBsAg	0	0	240	100	

This study shows ICT method were able to determine HBsAg negative samples reasonably well that was detected negative by ELISA. ELISA method is more specific and sensitive than that of ICT **(Rahaman,S. et al. 2023)**

This study was conducted at the department of Microbiology University of Peradeniya Shri Lanka. Two rapid tests, CORTEZ'S HBsAg one step and CTK Biotech's Onsite HBsAg, for the detection of HBsAg in the serum were compared using the SURASE B-96 (TMB) enzyme immunoassay (EIA) as the comparator. (n=50) were tested using the two rapid tests and the EIA. Based on the EIA, 5 sera were positive for HBsAg and 45 sera were negative for HBsAg. However, 4 sera were positive while 46 sera were negative for HBsAg when tested by Biotech's Onsite HBsAg. Three (n=3) sera were positive while 47 sera were negative for HBsAg when tested by CORTEZ'S HBsAg one step detection kit.

Table 4: Accuracy indices of the rapid assays

Accuracy indices	Sensitivity	Specificity	PPV	NPV
Onsite HBsAg rapid assay	80%	100%	100%	97.8%
CORTEZ'S HBsAg one step assay	60%	100%	100%	95.7%

Both rapid tests showed less sensitivity and NPV than the EIA. Sensitivity and NPV were higher with Onsite HBsAg than the CORTEZ'S HBsAg making the former slightly better than the latter for detecting serum HBsAg. According to our findings, the accuracy of rapid tests for HBsAg detection varied compared to EIA, suggesting the importance of validating rapid tests routinely when used in Diagnostic Laboratories **(Chameera, E.W.S. et al.2013)**

This cross sectional study was conducted at the Department of Biochemistry & Microbiology, School of Health and Life Sciences, North South University, Bashundhara, Dhaka Bangladesh The study comprised 203 samples, of which 103 had detectable and 100 had no detectable HBV DNA by real time PCR. these samples were tested with ICT kits from two companies: Onsite HBsAg rapid test (CTK biotech, Inc., USA) and HBsAg test Device (EXCEL®, USA). sensitivity of CTK and EXCEL were found to be 97.1% and 95.1% respectively. Negative predictive value (NPV) of CTK and EXCEL were 97.1% and 95.2% respectively. Both kits showed 100% specificity and 100% positive predictive value (PPV). HBV-DNA status strongly correlated with CTK (r = 0.971, p & lt; 0.001) and with EXCEL (r = 0.952, p < 0.001). Three and five moderate to high HBV load containing samples were undetected by CTK and EXCEL respectively.

Table 5: Comparison of Rapid HBsAg kit with Real-time PCR

Real time PCR (gold standard)/RIA				
Rapid HBsAg devices		Reactive	Non- reactive	
		N=103	N=100	Total
CTK	Reactive	100	0	100
	Non -reactive	03	100	103
EXCEL	Reactive	98	0	98
	Non- reactive	05	100	105

Table 6: True Positive and True Negative values for CTK and EXCEL compared to Real-time PCR

Parameter	True positive	True negative	Total sample
CTK	100(97.1%)	100(100%)	203
EXCEL	98(95.1%)	100(100%)	203
PCR	103(100%)	100(100%)	203

the study demonstrates that ICT based rapid test Devices might be considered suitable for screening of HBV infection as a point of care test. However, Nucleic Acid testing for Hepatitis B virus (NAT-HBV) should be implemented for donor screening for HBsAg. **(Hasan, K. N.et al. 2017)**

This study was conducted at the Medical University hospital Uremia Iran.The aim of the present study was to analyze the specificity of the ICT and ELISA diagnostic tests for Hepatitis B surface antigen and compare them with PCR testing. The study sample was made up of 140 men and 60 women referred to the Urmia Medical University hospital to undergo PCR testing for HBV diagnosis. The ELISA test was performed using the Pioneer Medicine Company kit (Tehran, Iran). The results of the HBsAg rapid test and the ELISA test were compared with the PCR test.

Table 7: Sensitivity, specificity, PPV, and NPV values of the immune chromatography method used in the experiments, compared with PCR testing.

Parameters	Sensitivity (%)	PPV	Specificity (%)	NPV
Immune chromatography	97	91	92	85
ELISA	78	76	75	80
PCR	100	100	100	100

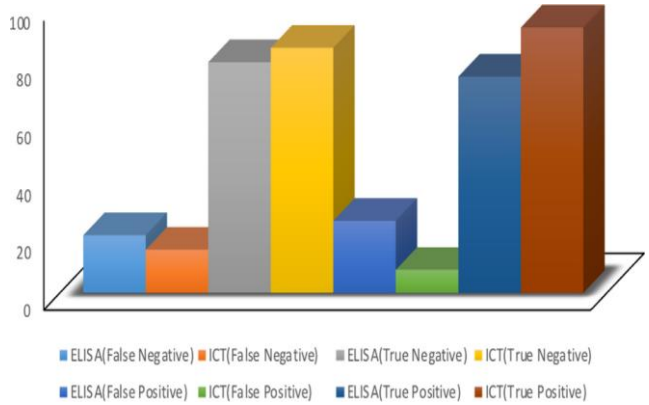


Figure 2: The accuracy relevance of the results of the immune chromatography method and the ELISA test.

According to our results, the Immunochromatographic test was accurate for diagnosing HBsAg in blood and the ELISA test had acceptable sensitivity and specificity, compared with PCR testing. **(Navvabi, N. et al. 2021)**

This study was a Hospital-based crosssectional study conducted at the Department of Microbiology, JNIMS, Imphal, Manipur, India, to compare ELISA and closed system PCR TrueNat for the detection of HBV and HCV among antinatal women attending a tertiary care medical institute. December 2020 to January 2022. Serum samples were tested using ELISA as well as on the TrueNat PCR. Out of 60 samples, using ELISA (QUALISA),

Table 8: Comparison of ELISA with TrueNat PCR for HBV detection.

Test		TrueNat		Total	p-value
		Positive	Negative		
ELISA	Positive	8	16	24	0.03*
	Negative	4	32	36	
Total		12	48	60	

Table 9: Comparison of ELISA with TrueNAT PCR for HCV detection.

Test		TrueNat		Total	p-value
		Positive	Negative		
ELISA	Positive	3	11	14	0.04*
	Negative	2	44	46	
Total		5	55	60	

24 were found to be positive for HBV surface antigen (HBsAg) and 14 were found to be positive for HCV antibodies (HCV Ab). On further testing using 12 TrueNat chip-based closed system Real Time-PCR (RT-PCR) Test, 12 were found to be positive for HBV-DNA and 5 were found to be positive for HCV-RNA. TrueNat PCR could eliminate the problem of false positivity in the detection of HBV and HCV. It is useful to make clinical decisions on starting antiviral therapy and also in documenting the efficacy of the antiviral therapy. **(Samom, P., et al .2023)**

This study was to evaluate the effectiveness of ICT in comparison with ELISA for the detection of HBsAg and anti-HCV in a semi-urban, low-resource setting. In this cross-sectional study, 951 blood samples were collected from patients at a tertiary care hospital on the outskirts of Lahore.

Table 10: Gender Distribution

Gender	Frequency	Percent
Male	434	45.6%
Female	517	54.4%
Total	951	100.0%

Table 11: Age Group Distribution

Age	Frequency	Percentage
1-20	134	14.1 %
20-40	496	52.2%
41-60	297	31.2%
61-80	24	2.5%
total	951	100 %

Table 12: Hepatitis B and C detection by ICT

Status	frequency	Percentage
Cumulative positive	71	7.4%
Hep C positive	60	6.3%
Negative	880	92.6%
Total	951	100%

Table 13: Hepatitis B detection by ELISA

Status	frequency	Percentage
Positive	11	1.2%
Negative	940	98.8%

Table 14: Hepatitis C detection by ELISA

Status	Frequency	Percentage
Positive	60	6.3 %
Negative	891	93.7 %
total	851	100%

Samples were tested for HBsAg and anti-HCV using the rapid ICT method (Abbott Bioline HBsAg WB and Abbott Bioline HCV). Positive results were confirmed using ELISA (ANTEC Diagnostic Products, UK). focusing on the comparison of sensitivity, specificity, and overall concordance between the two testing methods. Out of 951 individuals tested, the ICT method identified 71 positive cases (7.4%), with 11 (1.2%) for HBsAg and 60 (6.3%) for anti-HCV. ELISA confirmed all 71 positive cases identified by ICT, indicating high concordance between the two methods. The gender distribution was 434 males (45.6%) and 517 females (54.4%), with the majority in the 21-40 year age group (52.2%). ICT is a reliable and cost-effective screening tool for HBsAg and anti-HCV in low-resource settings, with results highly comparable to those obtained from ELISA. **(Aftab, H. T., et al. 2023)**

This study aimed to compare the analytical sensitivity of an ELISA approach with a fast kit in identifying HBV infection in blood donors. This study used a cross-sectional design. Using the Rapid screening test kit, the ELISA approach was used to re-test 250 blood donor samples that had tested negative. 250 of 250 blood donor samples were analysed that were negative by fast test (ICT) but positive by ELISA method. Each blood sample was tested for HBsAg using both ELISA and ICT techniques. The blood was drawn, processed, and tested according to standard procedures, with serum separated via centrifugation and analyzed using the ICT kit initially, followed by confirmation through ELISA, employing the CMIA principle on the ARCHITECT i1000SR immunoassay analyzer. The target variables hepatitis B surface

antigen (HBsAg) were determined by ELISA. Out of 250 samples, 250 were negative by the ICT method whereas positive by the ELISA method., Table 15: Comparing the Performance Metrics of ELISA and ICT

Sensitivity	95.12	92
Specificity	99.82	97
Positive Predictive Value	94	93
Negative Predictive Value	99	96
Overall Accuracy	97.6	94.6

respectively, while the negative predictive value (NPV) is 99% for ELISA and 96% for ICT. Overall accuracy is higher for ELISA at 97.6% compared to ICT's 94.6%. ELISA is reliable for detecting HBsAg in blood donors and is better than fast kits in determining hepatitis B virus infection**. (Ali, I., et al. 2024)**

To compare rapid tests (ICT) with 4th generation ELISA (gold standard) for hepatitis B and C infection. Biochemistry and Serology Laboratory of the Pakistan Medical Research Council, Research Centre, Jinnah Post Graduate Medical Centre, Karachi. Study was done over six months. ELISA confirmed 200 samples for HBsAg and 200 for Anti-HCV were selected, making a total of 400 samples. Out of 400 samples, 200 were positive and 200 negatives 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 4th 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). Out of 100 positive and 100 negative tests for HBsAg confirmed on ELISA, all rapid kits showed comparable results with ELISA. 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.

Table 16: Evaluation of rapid HBsAg kits with ELISA.

HBV		ELISA			ICT							
		R (n=100)	NR (N=100)	Total	TP	TN	FP	FN	SEN%	SPE%	PPV%	NPV%
ACON	R	95	-	95	95	100	-	5	95	100	100	95
	NR	5	100	105								
INTEC	R	98	-	98	98	100	-	2	98	100	100	98
	NR	2	100	102								
Determine	R	98	-	98	98	100	-	2	98	100	100	98
	NR	2	100	102								

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

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 ELISA confirmed rapid HBV, HCV kits being cheaper but sensitive and specific should be used for screening cases especially in rural setting. **(Hayder, I., et al. 2012)**

This study was performed at the health screening centre of Dhaka Crown Medical Center, Uttar Badda, Dhaka North City Corporation, Bangladesh. It aimed to determine the seroprevalence of this disease by screening the apparently healthy outbound staff who were intending to go abroad for labor work. It also aimed to compare between ICT and ELISA test, ELISA was used as gold standard for comparative evaluation. 5,500 healthy subjects tested on ELISA and ICT kits using separate panelsera

Table 17: Showing RAPID and ELISA test

Test	ELISA positive	ELISA negative	PPV%	NPV%	ICT positive	ICT Negative	PPV%	NPV%
	50	0	100	100	45	5	100	97

The seroprevalence of HBV was 0.82% (45) by the ICT device and 0.91% (50) by ELISA test. Samples with OD values less than 1.000 in ELISA test could not detect HBsAg by using ICT method. Rapid assays must be used with caution and it is also important to validate these rapid assays by testing them in a given population to assess the effectiveness of these assays in detecting HBsAg in diagnostic laboratories. They should be recommended only in poor settings or remote areas to assess prevalence of HBV infection. **(Asaduzzaman, M., et al. 2012)**

This study we aimed to compare the efficacy of ELISA and rapid ICT kits with gold standard real time PCR for the detection of HBV in high risk patients. In a cross-sectional study, 100 patients consisting of hemodialysis, thalassemia and hemophilia were assessed by real time PCR as the gold standard for detection of HBV DNA, fourth-generation ELISA kits including Dia-pro®, Pishtazteb®, Pasteur® and ICT Ecotest® were applied for detection of HBSAg. Compared with real time PCR,

Table 18: Evaluation of RAPID, ELISA and PCR test

Test	Sensitivity	Specificity	PPV	NPV
ICT	100%	100%	100%	100%
ElISA	100%	100%	100%	100%
PCR	100%	100%	100%	100%

Recent improvements in the monoclonal antibody production against mutant hepatitis B virus and their application in fourth-generation ELISA and rapid ICT kits resulted in significant increase in sensitivity and specificity of these methods. Thus, in emergency cases or lack of real-time PCR instruments, rapid ICT and ELISA kits could be suggest for detection of HBsAg. **(Habibi, A., et al. 2016)**

This study aimed to compare the analytical sensitivity between rapid tests and the ELISA method for detecting HBV, HCV, and HIV infection among blood donors. Four hundred (400) blood donor samples were tested using the Rapid Test Kits (INTEC, SD, ABON, and CLUN), and the ELISA method was used as a confirmatory test. Out of 400 blood samples tested for viral infection, HBV, HCV, and HIV were detected in 8, 10, and 2 samples, respectively, using the ELISA technique. This study observed that the rate of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), in addition to determining the diagnostic accuracy rate and error rate for all rapid diagnostic kits in detecting HBV, HCV and HIV are less accurate and associated with more false negatives compared to the ELISA technique.

Table 19: Evaluation of rapid test kit for hbv compared with the elisa techniques

ELISA n=400		R						8				
		NR						392				
RESULT FOR SCREENING TEST HBV n=400												
ICT		TOTAL	TP	TN	FP	FN	P%	SEN%	SPE%	PPV	NPV	DA
INTEC	R	14	6	384	8	2	3.2	75	98	42.9	99.5	97.5
	NR	386										
SD	R	8	2	386	6	6	2	25	98.5	25	98.5	97
	NR	392										
ABON	R	6	5	391	1	3	1.5	62.5	99.7	83.3	99.2	99
	NR	394										
CLUN	R	22	6	376	16	2	5.5	75	95.9	27.3	95.5	95.5
	NR	378										

r, reactive nr, non- reactive, tp true positive, tn true negative, fp false positive fn false negative p prevalence sen sensitivity spe specificity ppv positive predictive valvue npv negative predictive valvue da diagnostic accuracy

This study showed a significant difference in sensitivity between ELISA and rapid diagnostic immunochromatographic technique (ICT) groups; therefore, rapid diagnosis is not suitable for testing the quality of infectious markers for blood donors. **(Al-Matary, A. M., et al. 2016)**

A cross-sectional study was done with serum samples collected from patients visiting the hospital over a period of six months. Serum samples were obtained from 57 patients suspected of HBV infection. The results of ICT, ECLIA, and HBV DNA viral load (by Polymerase Chain Reaction (PCR)) were cross-tabulated and assessed for differences in diagnostic sensitivity. The positivity and correlation of the ICT and ECLIA with PCR were estimated. Out of 57 samples, 53 (92.98%) tested positive in the ICT card test, and 54 (94.74%) were positive in the ECLIA method. McNemar's test showed that the sensitivity of ICT and ECLIA differed significantly compared to HBV DNA PCR. There was a significant positive correlation between ECLIA and HBV-DNA PCR (Spearman correlation, r- value=0.28, p-value=0.035).

Table 20: Evaluation of RAPID and ELISA test

Test	Total Sample	Sensitivity	Specificity	PPV	NPV
ICT	53	92.98		(92.98%)	
ECLIA	54	94.73		(94.74%)	
PCR	57	100%	100%	100%	100%

The findings suggest that in settings where accurate diagnosis is critical, particularly for screening and monitoring treatment efficacy, molecular assays remain the preferred choice despite their higher cost and complexity. However, in resource-limited settings, ECLIAs can still play a valuable role in HBV screening programs. **(RENGARAJ, R., et al. 2024)**

The current study was done to evaluate the efficacy of the chemiluminescence, ELISA and immune chromatographic (ICT) test to Real Time-PCR (RT_PCR) for establishing the diagnosis of hepatitis B virus (HBV) in indoor and outdoor patients of Department of Medicine, Rajendra Institute of Medical Sciences Ranchi, Jharkhand This descriptive, cross-sectional study was carried out in 475 patients. The demographic data and patient's serum samples of the subjects was collected. The samples were analysed by chemiluminescence, ELISA, ICT & real RT-PCR for HBV. In the present study, of total 70 by chemiluminescence, 64 by ELISA, 61 by ICT and 63 by RT PCR were found positive for HBV. By both RT PCR and chemiluminescence 63 samples were positive. Total 2 patients were positive for HBV by RT PCR and negative by chemiluminescence while 7 patients were negative for RT-PCR and positive by chemiluminescence. Total 8 patients were positive for HBV by RTPCR and negative by ELISA while 5 patients were negative for RT- PCR and positive for ELISA. Total 11 patients were positive for HBV by RT PCR and negative by ICT while 3 patients were negative for RT- PCR and positive for ICT. On the basis of above findings chemiluminescence, ELISA and ICT specificity was 98.32%, 98.79%, 99.27%, sensitivity was 97.01%, 89.04%, 85.52%, positive predictive value was 90.27%, 92.85%, 95.58% and negative predictive value was 99.51%, 98.08%, 97.38%. The HBsAg negative patients should also be screened with HBc total antibody for suspecting the exposure which could be later confirmed by RT PCR. RT-PCR method is accredited as a specific and reliable method suitable for screening of HBV and should be recommended

for establishing exact and final diagnosis of these patients. **(Irfan, S., et al. 2018)**

Table 21: Various technique performance.

Technique	Total sample tested	Positive	Negative	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
PCR	475	65	410	100	`100	100	100
Chemiluminescence		70	405	98.32	97.01	90.27	99.51
ELISA		64	411	98.79	89.04	92.85	98.08
Immunochromatography		61	414	99.27	85.52	95.58	97.38

The HBsAg negative patients should also be screened with HBc total antibody for suspecting the exposure which could be later confirmed by RT PCR. RT-PCR method is accredited as a specific and reliable method suitable for screening of HBV and should be recommended for establishing exact and final diagnosis of these patients**. (Irfan, S., et al. 2018)**

This study was to compare ELISA and chemiluminescence immunoassay (CLIA) for the detection of anti-HCV. A total of 91 samples were tested for the detection of anti-HCV by CLIA and ELISA)

Table 22: Sensitivity and Specificity of CLIA while ELISA using as Gold Standard

Sensitivity	96.77%
Specificity	96.07%

Table 23: Positive predictive value and negative predictive value of CLIA while using ELISA as Gold standard

Positive Predictive Value (PPV)	93.75%
Negative Predictive Value (NPV)	98%

Out of 91 samples, 30 (33%) were non-reactive by both CLIA and ELISA. 32(35.2%) samples were reactive by CLIA, 2(6.25%) CLIA reactive samples were found to be non-reactive by ELISA. 29 (31.87%) samples were interpreted as borderline on CLIA. By using ELISA technique as gold standard for Anti-HCV detection our results showed 96.07% specificity and 96.66% sensitivity for CLIA technique. Detection of anti-HCV by CLIA is comparable with ELISA. CLIA can help in detecting early infection compared to ELISA and is suitable in large sample volume laboratories. **(Majumder, P., et al. 2017)**

2. Discussion

In the present study, a comparison was done between ELISA, PCR, CLIA and ICTs for the screening of HBsAg. This study aims at comparing the analytical measures between ELISA, PCR, CLIA and rapid cards for better diagnosis of HBsAg in forthcoming years. From this study results, it was found that for HBsAg screening, ELISA, PCR, CLIA was more sensitive than the rapid card tests and equally specific to the rapid card tests. In this study, the sensitivity of ICT was 83.4%,specificity 100%, PPV 99.4%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%.**(Prabha, P. *et al.*2022)**, the sensitivity of ICT was 97 %,specificity 100%, PPV 90.4 %, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%.(**Rahaman, S. *et al.* 2023)**, the sensitivity of ICT was 80%,specificity 100%, PPV 97.8.%, NPV 100 % **(Chameera, E.W.S. *et al.*2013)**, the sensitivity of ICT was 97.1%,specificity 100%, PPV 98.5.%, NPV 100 % and the sensitivity, specificity, PPV and NPV PCR was 100% **(Hasan, K. N.et al. 2017) ,**the sensitivity of ELISA was 78%,specificity 76 %, PPV 75.%, NPV 80% and the sensitivity, specificity, PPV and NPV PCR was 100% **(Navvabi, N. et al. 2021)** , the sensitivity of ELISA was 100%,specificity 100%, PPV 100%, NPV 100% and the sensitivity, specificity, PPV and NPV TRUE NAAT PCR was 100% **(Samom, P., et al .2023) ,**the sensitivity of ELISA was 92%,specificity 100%, PPV 100.%, NPV 97% and the sensitivity, specificity, PPV and NPV PCR was 100% **(Aftab, H. T.,et al. 2023)** ,the sensitivity of ICT was 92%,specificity 97%, PPV 93%, NPV 96 % and the ELISA was sensitivity95%, specificity99.82%, PPV95% and NPV 99% **(Ali, I., et al. 2024),** the sensitivity of ICT was 98%,specificity 100%, PPV 100.%, **(Hayder, I., et al. 2012)**, the sensitivity of ICT was 82%, specificity 100%, PPV 91%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100% **(Asaduzzaman, M., et al. 2012),** the sensitivity of ICT was 100%, specificity 100%, PPV 100%, NPV 100 % and the sensitivity, specificity, PPV and NPV ELISA was 100%. and sensitivity, specificity, PPV and NPV PCR was 100%. **(Habibi, A., et al. 2016)** ,the sensitivity of ICT was 99.7%,specificity 100%, PPV98% **(Al-Matary, A. M., et al. 2016)** , the sensitivity of ICT was92.98%,specificity 100%, PPV 99.4%, NPV 94.73 % and the sensitivity CLIA was 100%, specificity100%, PPV100% and NPV 97% and the sensitivity, specificity, PPV and NPV PCR was 100% **(RENGARAJ, R., et al. 2024)** ,the sensitivity of ICT was 99.27%,specificity 85.52%, PPV 95.58%, NPV 97.38 % and the ELISA was sensitivity98.79%, specificity89.04%, PPV92.85% and NPV98.08% and sensitivity, specificity, PPV and NPV PCR was 100%. **(Irfan, S., et al. 2018),** the sensitivity of CLIA was 96.77%, specificity 96.07% PPV 93.75%, NPV 98% and the sensitivity, specificity, PPV and NPV ELISA was 100%., (**Majumder, P., et al. 2017)**

This study does not have 100 % in the sensitivity and specification of the rapid test, but still some good brand rapid kits also have 100 % sensitive and specification, while the rapid test can also be done in rural areas and small labs, but after getting positive in the Rapid Test, it should be confermed with other mathods.

3. Conclusion

This study demonstrates that in detecting HBsAg, ELISA and PCR was more sensitive than ICT. These rapid test devices can be used as a screening test for HBsAg only when the resources are limited, remote regions and peripheral health centres for screening purposes. Hepatitis B virus can pose a serious threat of silent transmission and

spread among people and also create an urge for more sensitive assays as ELISA and PCR . The ultimate goal of this study was to recommend ELISA and PCR kits for the diagnosis of HBV irrespective of developmental and economical status of the area which detects positive cases correctly. ICT low resource places have a reliable and cost-effective screening tool for HBsAg the results of which are similar to the results obtained from ELISA. Its application can be significantly helpful in early detection and management of hepatitis in areas where access to more sophisticated diagnosis features is limited.

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