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Diagnostic Accuracy of Combo Immunochromatographic Assay Kit in Comparison with ELISA for Detection of HBsAg, anti-HCV Antibody, and anti-HIV Antibody

Raza Ullah Asif, Eijaz Ghani, Muhammad Ali Rathore, Saadiya Mushtaq, Adnan Shehzad, Hina Saeed

Department of Virology, Armed Forces Institute of Pathology, Rawalpindi/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To determine the diagnostic accuracy of combo Immunochromatographic (ICT) kit compared with Enzyme Linked Immunosorbent Assay (ELISA) to diagnose Hepatitis B, Hepatitis C, and HIV infection.

Place and Duration of Study: Department of Virology, Armed Forces Institute of Pathology Rawalpindi, Pakistan, from Jul to Dec 2023.

Study Design: Diagnostic accuracy study.

Methodology: A total of 1217 blood specimens were collected and tested for HBsAg, Anti-HCV Ab, and Anti-HIV Ab using both an ICT kit and ELISA. The results of positive and negative specimens on ICT were compared with ELISA, which was taken as gold standard. Sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy, and Kappa coefficient were calculated.

Results: Regarding HBsAg detection, the ICT kit showed 97.8% sensitivity, 99.8% specificity, a positive predictive value of 92.0%, and a negative predictive value of 99.9%. For anti-HCV antibodies, sensitivity was 98.8%, specificity 99.6%, a positive predictive value 93.5% and a negative predictive value 99.9%. For anti-HIV antibodies, sensitivity, specificity, positive predictive value, and negative predictive value were all 100.0%. Overall, the ICT kit demonstrated 99.0% diagnostic accuracy with a Kappa coefficient above 0.9, indicating excellent agreement with ELISA results.

Conclusion: The study highlighted the combo ICT kit as a cost-effective alternative to ELISA for detecting Hepatitis B, Hepatitis C, and HIV infections. With 99% diagnostic accuracy and a Kappa coefficient above 0.9, it demonstrated high sensitivity, specificity, PPV, and NPV.

Keywords: Enzyme-Linked Immunosorbent Assay, Hepatitis B Surface Antigens, Hepatitis C Antibodies, HIV, Immunoassay, Point-of-Care Testing.

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INTRODUCTION

Among immunoassays, Immuno-chromatographic Technique (ICT) has firmly occupied its position as the most demanded and popular rapid test method that is fully aligned to the modern concept of point-of-care testing in specialties like medicine, veterinary, ecology and zoology, and is widely used in the diagnosis of both infectious and non-infectious diseases.[1,2]

Hepatitis B, Hepatitis C and HIV are chronic viral diseases which cause significant mortality and morbidity. In Pakistan, prevalence of Hepatitis B is 1.98%, Hepatitis C is 6% and HIV infection is 0.2%.[3-5] HBsAg is the first serological marker which appears as early as 1-2 weeks post exposure.[6] Presence after six months indicates chronic carrier state.[7] Anti-HCV Ab appears 8-10 weeks post exposure.[8] A reactive result is consistent with recent or resolved infection. It may become undetectable in hemodialysis patients and those taking immunosuppressive therapy. Anti-HIV Ab appear 4-6 weeks post exposure and is present in both acute and chronic phase of infection.[9]

Different methods used for the diagnosis of these viral infections are ICT, ELISA, Chemiluminescence Immunoassay (CLIA) and Polymerase chain reaction (PCR). ELISA, CLIA and PCR are expensive methods and are used in well-equipped labs and major tertiary care hospitals.[10] ICT is a cost-effective rapid screening test for the qualitative detection of infection in whole blood, plasma, or serum specimen. The test utilizes recombinant antigens and antibodies to selectively detect elevated levels of viral antibodies and antigens respectively in different viral infections. 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.

Correspondence: Dr Raza Ullah Asif, Department of Virology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

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The aim of this study was to determine the diagnostic accuracy of combo ICT kit in comparison with ELISA for diagnosing Hepatitis B, Hepatitis C and HIV infections.

METHODOLOGY

This diagnostic accuracy study was conducted at the Department of Virology, Armed Forces Institute of Pathology Rawalpindi over a period of six months from July to December 2023. The study was approved by Institutional Ethical Review Board (vide IRB certificate No. FC-VIR22-25/READ-IRB/23/2609).

Inclusion Criteria: Patients of either gender with age ranging from 2 to 65 years, presenting with clinical suspicion of disease, were included.

Exclusion Criteria: Patients on immunosuppression, chemotherapy, dialysis and those with a history of immunological disorders were excluded.

The prevalence of Hepatitis C (6%) was relatively higher as compared to Hepatitis B and HIV Infection and was therefore applied for sample size calculation. 3-5 Buderer's formula was used for sample size calculation which came out to be 1217.[11] Non-probability consecutive sampling technique was used for sample collection.

Informed voluntary consent was taken from all the participants. A predesigned proforma was used to collect demographic data and clinical features. Whole blood specimens from participants were collected in clot activator tubes under aseptic measures. After centrifugation at 4000 rpm for 10 minutes, the serum was separated and tested for HBsAg, Anti-HCV Ab, and Anti-HIV Ab using both ICT kit (LDS R-test Rapid, Pak) and ELISA (EVOLIS Twin plus BIORAD, USA).

The results of positive and negative specimens on ICT were compared with ELISA, which was taken as a gold standard.

Data was analyzed on Statistical package for Social Sciences (SPSS) version 27. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, and Kappa co-efficient were calculated. Kappa co-efficient was used to determine the extent of agreement between the result values of two different diagnostic methods.

RESULTS

A total of 1217 patients were enrolled in the study among which 757(62%) were males and 460(38%) were females with a mean age of 45 years.

Amidst the 1217 specimens tested for HBsAg, 46 were true positive, 1168 were true negative, 2 were false positive, and 1 was detected as false negative by combo ICT kit. The sensitivity of the ICT kit for HBsAg was 97.8%, with a specificity of 99.8%. PPV was 92%, and NPV was 99.9% as mentioned in Table-I.

Table-I: Comparison of Combo Immunochromatographic (ICT) kit with Enzyme Linked Immunosorbent Assay (ELISA) for Detection of HBsAg (n=1217)

ICT	ELISA	
	Test Positive (ELISA)	Test Negative (ELISA)
Test Positive (ICT)	46(3.8%)	02(0.2%)
Test Negative (ICT)	01(0.1%)	1168(95.9%)
Sensitivity= True Positive/(True Positive +False Negative) = 97.8%		
Specificity= True Negative / (True Negative +False Positive) = 99.8%		
Positive Predictive Value= True Positive/ (True Positive+ False Positive) = 92.0%		
Negative Predictive Value= True Negative/ (True Negative +False Negative) = 99.9%		
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 99.8%		

Of all the specimens tested for anti-HCV antibody, 80 were true positive, 1131 were true negative, 5 were false positive, and 1 was detected as false negative by combo ICT kit. The sensitivity of the ICT kit for anti-HCV antibody detection was 98.8%, with a specificity of 99.6%. The PPV was 93.5%, and NPV was 99.9% as mentioned in Table-II.

Table-II: Comparison of Combo Immunochromatographic (ICT) Kit with Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HCV (n=1217)

ICT	ELISA	
	Test Positive (ELISA)	Test Negative (ELISA)
Test Positive (ICT)	80(6.6%)	05(0.4%)
Test Negative (ICT)	01(0.1%)	1131(92.9%)
Sensitivity= True Positive/(True Positive +False Negative) = 98.8%		
Specificity= True Negative / (True Negative +False Positive) = 99.6%		
Positive Predictive Value= True Positive/ (True Positive+ False Positive) = 93.5%		
Negative Predictive Value= True Negative/ (True Negative +False Negative) = 99.9%		
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 99.5%		

Among the specimens tested for anti-HIV antibody, 22 were true positive, and 1195 were true negative. No specimen was detected as false positive or false negative. The sensitivity, specificity, PPV, and NPV for anti-HIV antibody detection were all 100% as mentioned in Table-III.

Table-III: Comparison of Combo Immunochromatographic (ICT) Kit with Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HIV (n=1217)

ICT	ELISA	
	Test Positive (ELISA)	Test Negative (ELISA)
Test Positive (ICT)	22 (1.8%)	00 (0.0%)
Test Negative (ICT)	00 (0.0%)	1195 (98.2%)
Sensitivity= True Positive/(True Positive +False Negative) = 100.0%		
Specificity= True Negative / (True Negative +False Positive) = 100.0%		
Positive Predictive Value= True Positive/ (True Positive+ False Positive) = 100.0%		
Negative Predictive Value= True Negative/ (True Negative +False Negative) = 100.0%		
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 100.0%		

Overall, the diagnostic accuracy of combo ICT kit was found to be more than 99%, with a Kappa coefficient exceeding 0.9, indicating excellent agreement between ICT and ELISA results for the detection of HBsAg, anti-HCV antibody, and anti-HIV antibody.

DISCUSSION

In this study, combo ICT kit was compared with ELISA for screening of HBsAg, anti-HCV and anti-HIV antibodies. Sensitivity of combo ICT kit for HBsAg, anti-HCV and anti-HIV antibodies was 97.8%, 99.8% and 100% respectively and specificity was 99.8%, 99.6% and 100% respectively, exhibiting its reliability as an effective alternative to ELISA for screening of viral diseases in resource-limited settings.

ICT kits are not only sensitive and specific but are rapid and cost-effective also. ELISA, CLIA, Immunoblot, PCR and other advanced methods are laboratory based, time-consuming and require trained staff. Whereas ICT enables early detection where laboratory facilities or trained manpower are not available or there is issue of accessibility. It also helps in mitigating the risk of "loss to follow up" when results are not immediately available. It is also a method of choice when elevated costs in laboratory expenses associated with other methods cause reluctance in screening of general population.

Most of the specimens in virology lab are analyzed for simultaneous screening of HBsAg, anti-HCV and anti-HIV antibodies. Compared to ELISA, which requires three to four hours for test completion, ICT test results are available within 20 minutes. Secondly, ELISA cannot be performed for a single or small number of samples, since it would be quite uneconomical.

In resource-limited settings, combo ICT kit is more cost-effective as the price is five times less than ELISA kit. Moreover, it will minimize pre-analytical errors as specimen will be used only once for testing three different parameters and will encourage HIV screening that is otherwise missed during routine workup. The disadvantage of ICT is that kits are quite susceptible to unfavorable storage conditions. So, it is essential to do periodic quality control checks to avoid false positive or false negative results.

A study was conducted by Tiwari *et al*. at Jhalawar Medical College, Rajasthan, India, in which ICT kit was evaluated for HBsAg detection. Sensitivity of this ICT kit was 95.12%, specificity was 99.82%, PPV was 95.12%, NPV was 99.82%, diagnostic accuracy was 99.65%, and Kappa statistic value was 0.949 that is quite similar to our test results.[10] Another study also reported similar findings.[11]

Another study was conducted by Sadeghi *et al*. at Tabriz University of Medical Sciences, Iran in 2021 in which ICT kits were evaluated for detection of HBsAg, anti HCV and anti-HIV antibodies. For Anti-HIV antibodies, sensitivity and specificity of ICT kit was 96.6% and 98.7% respectively whereas for both HBsAg and anti-HCV antibodies, sensitivity and specificity were 90% and 100% respectively.[12]

Results of another study conducted by Das *et al*. in 2018 showed sensitivity of combo ICT kit for HBsAg, anti-HCV and anti-HIV to be 80%, 66.7% and 100% respectively whereas specificity was 100%, 98.9% and 100% respectively.[13]

A similar study was conducted by Ayodeji *et al*. for Hepatitis B screening by ICT and ELISA method. In this study, sensitivity of rapid ICT kit for HBsAg was 81.8%and specificity were 100%.[14]

Another study was conducted by Al-Matary *et al*. at Jibla University Hospital Yemen in 2022, in which four different ICT kits were compared for detection of HBV, HCV, and HIV infections. Sensitivity results of these kits for HBsAg were 25% to 75%, for anti-HCV 60% to 80% and for anti-HIV, all the kits exhibited 100% results. Whereas specificity results of these ICT kits for HBsAg were 95.9% to 99.7%, for anti-HCV 98.2% to 99.2%, and for anti-HIV 91.2% to 99.2%.[15]

One study by Sanou *et al*., which was carried out in Burkina Faso, evaluated different ICT kits for HBsAg detection. All ICT kits exhibited specificity ≥99.0% and sensitivity ranged from 90.8 % to 92.8%. PPV ranged from 95.6% to 98.9% and NPV ranged from 96.2% to 97.4% whereas Kappa coefficient was more than 0.916.

Muñoz-Chimeno *et al*., at Carlos-III Institute of Health Madrid, Spain, studied three ICT kits from Abbott Diagnostics, USA, which were evaluated for detection of HBsAg, anti HCV and anti-HIV antibodies separately taking ELISA as a reference method. These ICT kits showed sensitivity for HBsAg, anti-HCV and anti-HIV to be 66.7%, 98.8% and 100% respectively and specificity to be 100% for all three parameters. Whereas diagnostic accuracy was 99.6%, 97.7% and 100% respectively.[17]

Ince *et al*. conducted research at Çanakkale Onsekiz Mart University, Turkey in 2022 in which

different ICT kits were evaluated for detecting HBsAg, anti-HCV and anti-HIV antibodies in serum. These kits exhibited sensitivity of more than 99.1% for HBsAg and 100% for both anti-HCV and anti-HIV antibodies whereas specificity was 98.7%, 99.2% and 100% respectively.[18]

All these studies revealed overall high sensitivity and specificity of ICT kits compared to laboratory-based immunoassays for routine use in resource limited settings and as a "point of care test" in specialized settings.

LIMITATIONS OF STUDY

The main limitation of this study is its single-center setting, which may restrict the generalizability of the findings to other healthcare facilities or rural populations. Additionally, ELISA was used as the reference standard rather than more definitive molecular methods like Polymerase Chain Reaction (PCR), which could have provided a more accurate baseline. Furthermore, the study did not evaluate how the combo kit performs across varying viral loads or different stages of infection.

CONCLUSION

This study showed that combo ICT kit had a good diagnostic accuracy in comparison with ELISA for cost-effective and rapid detection of HBsAg, anti-HCV and anti-HIV antibodies. With overall diagnostic accuracy of more than 99% and Kappa coefficient surpassing 0.9, the combo ICT kit exhibited high sensitivity, specificity, PPV, and NPV across all tested parameters, reaffirming its reliability and comparability to established testing methods.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

RUA & EG: Data acquisition, data analysis, critical review, approval of the final version to be published.

MAR & SM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AS & HS: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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