ORIGINAL ARTICLE

COMPARATIVE EVALUATION AND ACCURACY OF ICT, CLIA AND NAT FOR THE DETECTION OF HEPATITIS B, C AND HIV IN THE BLOOD DONORS

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Background: A sensitive and specific donor screening strategy is essential for the prevention of transfusion-transmitted infections (TTI). The study was conducted to ascertain the comparative efficacy of ICT, CLIA and NAT methods. **Methods:** This cross-sectional analytical study was conducted in Regional Blood Center Abbottabad, Pakistan from 1st April to 25 August 2022. 6233 donors were screened for Hep B, C, and HIV by testing simultaneously with ICT, CLIA and NAT. **Results:** Active Hep B, C and HIV Infection was present in 0.51%, 0.28% and 0.00048% donors respectively. The sensitivity was found to be higher for HBV and HIV with CLIA as compared to ICT but was equal for HCV with both. whereas specificity was the same with both CLIA and ICT for all three viruses. PPV was higher with ICT for HBV and HCV, but for HIV it was found higher by CLIA. NPV was higher for all three viruses by CLIA as compared to ICT. **Conclusion:** In case rapid testing devices are used for the initial screening of blood in countries with limited resources, positive cases must be confirmed by CLIA and if possible, then by NAT because of missing cases in the window period and false positive cases.

Keywords: Blood Banking/Transfusion Medicine; Clinical Pathology; Hematology; Microbiology; Hematopathology; CLIA; NAT; ICT

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INTRODUCTION

Hepatitis B, Hepatitis C and HIV are caused by Hepatitis B (HBV), Hepatitis C (HCV) and HIV viruses. HBV and HCV cause liver inflammation and produce disease symptoms resulting in either acute or chronic hepatitis, severe liver damage and liver cirrhosis. Hepatocellular carcinoma may be the end point of these infections.[1–3]

The World Health Organization (WHO) reported that more than 350 million people are affected by HBV,170 million by HCV and 38.4 million People by HIV in 2021. In Pakistan, 2.5%, 6.2% and 1 % of the general population is affected by HBV, HCV and HIV respectively.[3,4]

The survival time of HBV outside the body is seven days and during this period, the virus can infect people if exposed to body fluids or infected blood. HCV infection, also called non-A and non-B Hepatitis also spread by the reuse of contaminated syringes and other medical apparatuses without proper sterilization, infected blood and its products and unsterilized instruments for piercing, shaving etc. Both viruses can spread from mother to foetus as well. Various preventive strategies are being used around the world to prevent the spread of these infections. Health care setups around the world are executing several strategies to prevent the spread of these Transfusion Transmitted Infections (TTI) such as blood and blood product screening before transfusion use of disposable syringes, prevention of reuse of syringes and disposal of used needles and syringes.[5,6]

Several diagnostic tools exist for the diagnosis and screening of these infections in blood donors as well as in the common population. Most of the blood centres, having inadequate laboratory setup, use rapid devices at the time of initial screening of the blood donors. Immunochromatographic tests (ICT) are simple test devices giving quick results, intended to detect the presence or absence of target antigen or antibody in the sample with the advantage that it does not need any specialized and costly equipment. The coated antigen produces a reaction colour giving positive results but, in some cases, very weak stripes cause incorrect result readings and hence need a skilled and experienced observer to avoid any false result.[7]

The Chemiluminescent Immunoassay (CLIA) is a biochemical test utilizing the immunoassay method which measures the concentration of a substance in a fluid such as serum,

water or blood by looking at the reaction of antibodies against the antigen. This method is employed for detecting HIV, syphilis HBsAg, and HCV, in the blood and is used in the blood centers for donor screening.[8] The CLIA uses a derivative of luminol with peroxidase and H_2O_2 (or another enzymatic system which produces H_2O_2 as oxidase glucose or uricase) plus the addition of (a derivative of phenol, such as P-iodophenyl) which increases light emission up to 2800 times. Thus, CLIA can detect the presence of antibodies at extremely low concentrations (limit of detection=zeptomole 10^{-21}mol).[9]

The Nucleic Acid Amplification Test (NAT) is a screening test technology that can significantly narrow the infectious window period by detecting the presence of viral DNA/RNA with the shorter window period, resulting in to increase in the safety of the blood transfusions.[10] It has proved to be very sensitive in analyzing even the DNA-RNA part of the blood. Using NAT, the viruses can be detected even before the antibodies are formed.[11–13]

To ensure the provision of safe blood and to control TTIs, the government of Pakistan has established regional blood centres (RBC) at divisional levels with the funding of the German government. RBC-Abbottabad was established and made a function in 2021 and provides blood products to the major hospitals in the Hazara division in the north of Pakistan.

MATERIAL AND METHODS

This cross-sectional analytical study was conducted in RBC Abbottabad, Pakistan. A total of 6233 blood donors (both voluntary and exchange) from 1st April to 25th August 2022 were included in the study. All the donors were screened for HIV, HBV, HCV, Malaria and Syphilis HBV, HCV and HIV were screened by ICT, CLIA and NAT. NAT is the gold standard for the detection of these viruses as per the guidelines of WHO.

Statistical analysis: the data was analyzed using SPSS version 21. The Sensitively, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated with NAT as the gold standard. The Youden's J index was also estimated for comparative analysis of different tests.

RESULTS

6233 blood donors, screened for TTIs, were included in the study.

HBV Infection was the most prevalent infection among the donors with a frequency of 0.51% (32) HCV infection was present in 0.28% (18) donors and HIV infections in 0.00048% (03) of donors. Figure-1 shows the positive cases detected by the three different methods (all positive cases are not found reactive by all the three methods)

The ICT showed a sensitivity of 73.1% for HBV, 81.8% for HCV and 50% for HIV while the Sensitivity of CLIA was 84.6%, 81.8% and 100% respectively for HBV, HCV and HIV.

The specificity of ICT and CLIA for all three infections is the same, i.e., 99.9%

Positive predictive value (PPV) for HBV was 82.6% with ICT and 78.6% with CLIA. For HCV it was 60% with ICT and 56.25% with CLIA, and for HIV it was 50% and 66.6% with ICT and CLIA respectively.

The negative predictive value (NPV) for HBV by ICT was 99.8% and 99.9% by CLIA. For HCV it was 99.9% with both ICT and CLIA. For HIV it was 99.9% by ICT and 100% by CLIA.

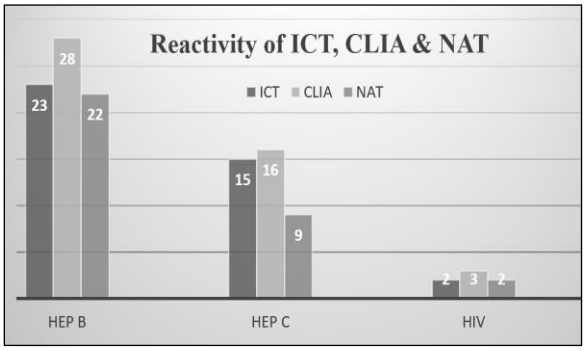

Figure-1: Reactivity of ICT, CLIA and NAT

Table-1: Performance analysis of different test with NAT as gold standard.

Test	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Youden's J Index
HBsAG					
ICT	82.6% (67.1-98.1%)	99.8% (99.8-99.9%)	73.1% (56.0-90.1%)	99.9% (99.8-99.9%)	0.73
CLIA	78.6% (63.3%-93.7%)	99.9% (99.8%-99.9%)	84.6% (70.7%-98.4%)	99.9% (99.8%-99.9%)	0.84
HCV					
ICT	60% (35.2-84.7%)	99.9% (99.9-100.0%)	81.8% (59.0-104.6%)	99.9% (99.8-99.9%)	0.81
CLIA	56.2% (31.9%-50.5%)	99.9% (99.9%-100.0%)	81.8% (59.0%-104.6%)	99.8 (99.8%-99.9%)	0.81
HIV					
ICT	50% (-19.2-119.2%)	99.9% (99.9-100.0%)	50% (-19.2-119.2%)	99.9% (99.9-100.0%)	0.49
CLIA	66.6% 13.3-120.0%)	100% (100-100.0%)	100% (100-100.0%)	99.9% (99.9-100.0%)	0.99

DISCUSSION

TTIs continue to be a risk factor in transfusion-related therapies and contribute a major portion to the disease-dependent socioeconomic burden in Pakistan. The recommended protocols for screening the blood and its products are not being followed mostly owing to the paucity of adequate screening services in public sector health institutions. HBV is the most common infection encountered in donors while the incidence of HCV, syphilis and malaria follows it.

WHO has recommended a set of guidelines for the screening of blood and blood products to prevent TTIs. The screening protocols for these viruses include screening of the blood initially by rapid diagnostic tests in health care setups which have limited resources and cannot afford to screen all the donors with CLIA and NAT, followed by confirmation by CLIA and further confirmation by NAT where needed. The NAT is the gold standard for the diagnosis of these viruses.

Our study, conducted in RBC Abbottabad, from 1st April 22 to 25th August 22, on 6233 donors found sensitivity of CLIA for HBV as 84.6% and specificity of 99.9% with 78.6% PPV and 99.9% NPV. Results of ICT show 73.1% sensitivity and 99.9% specificity with 82.6 %PPV and 99.8% NPV for HBV. Other studies also show almost the same results claiming NAT to be a superior test than CLIA or ICT. M Hassan shows 100% sensitivity and 70% specificity of CLIA for the detection of HBV and their PPV was 71% and NPV was 100%.[14]

Hayder, I show a sensitivity of 95-98% for HBV on ICT and a specificity of 100%.[15] Ly *et al* reported that some results of HBV are negative on CLIA (because these patients are HBsAg negative virus carriers, with immune silent infection.) but are HBV-DNA positive, which means they were positive when tested with NAT. It was concluded that some mutations and natural variations induced HBsAg conformational changes. Since many HBsAg immunoassays use monoclonal antibodies with epitopes directed against the major hydrophilic region, in particular against the "a" determinant aminoad substitution in that region, therefore they cause negative results in immunoassay.[16,17]

Chen and Kaplan in their study suggest that laboratories need to be attentive to the performance of their HBsAg assay. Laboratories should be aware of the analytical performance of their assays near the cutoff concentrations and should use neutralization assays with weakly positive HBsAg results. [18] In these low-index cases, NAT can easily detect the DNA of the virus.

As shown by the results of these studies, in the majority of HBV cases, the diagnostic CLIA method is acceptable except in low-indexed positive cases where further investigation with NAT is required.[19]

For HCV our study showed 81.8% sensitivity and 99.8% specificity with 56.2% PPV and 99.9% NPV on CLIA and 81.8% sensitivity and 99.9% specificity with 60%PPV and 99.9% NPV on ICT.

Arshi Naz used several ICT devices and reported a sensitivity of 90-98% and specificity of 59-72% for HCV. Their PPV was 69-79% and NPV was 87-93%.[4] Whereas another study showed 86-93% sensitivity and 93-97% specificity for HCV on ICT method by using different ICT devices.[15]

In the results for HIV testing in our study, sensitivity was 100% with CLIA and 50% with ICT. Specificity was 99.9% with CLIA and 99.9% with ICT. PPV was 66.6% with CLIA and 50% with ICT. NPV was 100% with CLIA and 99.9% with ICT.

Le Chang reported 100% sensitivity and 99.1% specificity for HIV by CLIA method in Chinese patients.[20]

The delay between the virus infection and the appearance of their antigen or antibodies or symptoms, called the window period is a well-recognized concept [21]During this window period, a person may still be infectious. ICT or CLIA methods cannot detect the infection during this period but with the use of NAT, these infections can be detected during this time, thus minimizing the risk of transmitting these diseases from carriers during this time, and reducing the disease burden.

In our study, we found 6 false positive HBV cases, 7 HCV and 1 HIV false positive case (positive on CLIA and negative on NAT). Studies show that heterophilic antibodies have been known to cause both false positive (e.g., HIV) and falsely elevated (e.g. prostate-specific antigen) immunoassay results.[22] These naturally acting human antibodies bind to a wide variety of chemical structures, including the animal antibodies often used in immunochemistry assay [23] and they can be neutralized by a reagent composed of specific inactivating binders. Active or resolved infection would each have resulted in the presence of another serological HBV marker.[24] Transient HBsAg have been observed in patients for up to 2 weeks after HBV vaccination.[25] To overcome this chemiluscent method limitation, and to be more accurate NAT method is preferable to CLIA or ICT. However, in resource-constrained countries, these methods can be used and are completely acceptable

CONCLUSION

Both ICT and CLIA performed well in the detection of TTI in blood donors, however, these methods are significantly less sensitive.

Although CLIA did not miss any blood sample positive by NAT, it misses cases in the window period

and also gives false positive results due to other heterophilic antibodies, or when a person is vaccinated or in some cases even when the infection is resolved, whereas ICT failed to detect small proportion of samples (11 sample) with active viremia.

Rapid testing devices are recommended by WHO in countries with limited resources only for initial screening but positive cases must be confirmed by CLIA and if possible, then by NAT where affordable these methods should be preferred over ICT as they are more sensitive, reliable and recommended to be used in routine screening.

AUTHORS' CONTRIBUTION

RI: Data acquisition, analysis, drafting, write-up. UF: Data analysis, writeup, proofreading. IU: Critical revision, Final Approval. AF: Drafting work, data entry. TS: Data Acquisition. AG: Data Acquisition. AB: Data Acquisition

REFERENCES

1. Lavanchy D. The global burden of hepatitis C. Liver Int 2009;29(Suppl 1):74–81.
2. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World J Gastroenterol 2016;22(4):1684–1700.
3. Alam MM, Zaidi SZ, Malik SA. Serology based disease status of population infected with Hepatitis B virus. BMC Infect Dis 2007;7:64.
4. Arshi N, Samina M, Imran N, Tahir S. Evaluation of efficacy of serological methods for detection of HCV infection in blood donors: a single centre experience. Pak J Med Sci 2018;34(5):1204–8.
5. Haim ST, Kazmi SU, Bagasra O. Seroprevelence of hepatitis B and C genotype among young apparently healthy females of Karachi-Pakistan. Libyan J Med 2008;3(2):66–70.
6. Nafees M, Farooq M, Jaffer G. Frequency of hepatitis B and C infection in general population of Lahore-pakistan. Biomedica 2009;25:106–11.
7. Kosack CS, Nick S. Evaluation of two rapid screening assays for detecting hepatitis C antibodies in resource-constrained settings. Trop Med Int Health 2016;21(5):603–9.
8. Kosack CS, Nick S, Shanks L. Diagnostic accuracy evaluation of the ImmunoFlow HCV rapid immunochromatographic test for the detection of hepatitis C antibodies. J Virol Methods 2014;204:6–10.
9. Murniasih A. Perbedaan Kadar HBsAg Sample Serum dan Plasma Metode CLIA Pada Pendonor (Doctoral dissertation, Universitas Muhammadiyah Semarang). [Internet]. 2018. [cited 2022 Oct 17]. Available from: http://repository.unimus.ac.id/2711/
10. ShrivaSTava M, MiShra S. Nucleic Acid Amplification testing (NAT): An innovative Diagnostic approach for enhancing blood safety. J Lab Med 2017;6(2):IRO1–6.
11. Sukanya B, Lokesh P. Seven years' experience in NAT testing of blood donors in a tertiary care centre. Int J Contemp Med Res 2019;6(7):g1–4.
12. Mathur A, Dontula S, Jagannathan L. A study of centralized individual donor nucleic acid testing for transfusion transmitted infections to improve blood safety in Karnataka, India. Glob J Transfus Med 2017;2(1):24–8.
13. Puspita R. Overview of Hepatitis B Virus Exposure on NAT and CLIA Blood Examination Methods at PMI Semarang City. Int J Seocol 2021;15:1–6.
14. Hassan M, Dawood M. Advanced biomedical research report. [Internet]. 2014. [cited 2022 Oct 17]. Available from: http/www.ncbi.nlm
15. Hayder I, Ahmed W, Alam SE. Comparison of Different ICT Kits for HBsAg and Anti HCV Using Gold Standard ELISA. Pak J Med Res 2012;51(3):72–6.
16. Ly TD, Servent Delmas A, Bagot S, Gonzalo S, Ferey MP, Ebel A, *et al*. Sensitivities of four new commercial hepatitis B surface antigen assay in detection of mutant forms. J Clin Microbiol 2006:44(7):2321–6.
17. Kim S, Kim JH, Yoon S, Park YH, Kim HS. Clinical performance evaluation of four automated chemiluminescence immunoassays for hepatitis C virus antibody detection. J Clin Microbiol 2008;46(12):3919–23.
18. Chen D, Kaplan LF. A new generation chemiluscent assay for hepatitis B surface antigen. Clin Chem 2006;52(8):1512–8.
19. Khadem-Ansari MH, Omrani MD, Rasmi Y, Ghavam A. Diagnostic validity of the chemiluminescent method compared to a polymerase chain reaction for hepatitis B virus detection in the routine clinical diagnostic laboratory. Adv Biomed Res 2014;3:116.
20. Chang L, Zhao J, Guo F, Ji H, Zhang L, Jiang X, *et al*. Comparative evaluation and measure of accuracy of ELISAs, CLIAs, and ECLIAs for the detection of HIV infection among blood donors in China. Can J Infect Dis Med Microbiol 2020;2020:2164685.
21. Dufour DR. Hepatitis B surface antigen assays: are they good enough for their current uses? Clin Chem 2006:52(8):1457–9.
22. Preissner M, Dodge LA, Kane DJ, Singh RJ, Grebe SK. Prevalence of heterophilic antibodies interference in 8 automated tumour marker immunoassays. Clin Chem 2005;51(1):208–21.
23. Tate T, Ward G. Interference in immunoassay. Clin Biochem Rev 2004;25:105–20.
24. Terrault NA, Bzowej NH, Chang KM. AASLD guidelines for the treatment of chronic hepatitis B. Hepatology 2016;63(1):261–83.

    Rysgaard CD, Morris CS, Drees D, Bebber T, Davis SR, Kulhavy J, *et al*. Positive hepatitis b surface antigen test due to recent vaccination: a persistent problem. BMC Clin Pathol 2012;12:15.

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