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Evaluation of screening effectiveness of hepatitis B surface antigen and anti-HCV rapid test kits in Pakistan

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Abstract:

OBJECTIVE: The current study was conducted to evaluate the performance and screening effectiveness of commercially available rapid screening kits in comparison with chemiluminescence immunoassay (CLIA) and polymerase chain reaction (PCR).

MATERIALS AND METHODS: This single-center, cross-sectional study was conducted at the Department of Pathology and Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, PIMS, Islamabad, from January to April 2019. A total of 10 commercially available immunochromatographic test (ICT) devices and one CLIA kit (LIAISON XL) were tested for their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy using 100 positive and 100 negative samples each for HBV and HCV, respectively.

RESULTS: The sensitivities and specificities of ICT kits for hepatitis B surface antigen were 65% and 70% (Hightop), 67% and 85% (RightSign), 62% and 73% (Wondfo), 70% and 80% (Accu-Chek), 68% and 77% (Fastep), 73% and 85% (Abon), 77% and 83% (ImmuMed), 80% and 90% (Insta-Answer), 67% and 81% (BioCheck), and 72% and 83% CTK Biotech, respectively. Similarly, the sensitivities and specificities of different ICT kits for HCV were 69% and 80% (Hightop), 76% and 83% (RightSign), 69% and 81% (Wondfo), 78% and 79% (Accu-Check), 68% and 68% (Fastep), 63% and 73% (Abon), 71% and 70% (ImmuMed), 79% and 68% (Insta-Answer), 62% and 66% (BioChek), and 69% and 78% CTK Biotech, respectively. The sensitivity and specificity of Diasorin Liaison Murex assay for both HBV and HCV were found to be 100% when compared with PCR. The PPV, NPV and Accuracy were determined accordingly.

CONCLUSION: Rapid testing ICT devices for both HBV and HCV available in Pakistan were found to have a variable degree of sensitivity and specificity when compared with CLIA and PCR. Comparatively expensive but quality methods are more reliable as compared to rapid devices.

Key words:

Blood banks, HBV, HCV, Pakistan, Screening

Introduction

Hepatitis B and C are a serious public health issue across the globe. Screening for these infectious agents is a major challenge for blood banks in developing countries where resources are limited. Pakistan is a developing country with a population of 200 million. The blood transfusion services in the country are fragmented and majority of the blood banks are functioning without following appropriate standards and guidelines.[1] The annual blood collection is estimated to be 3.0 million with predominant reliance on replacement donations (~85%) and about 10%–15% donations are voluntary, nonremunerated.[2] Screening for

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transfusion-transmitted infections (TTIs) is particularly very critical in a country such as Pakistan where the prevalence of HBV and HCV in the general population is 2.5% and 6.2%, respectively.[3,4] Due to the poorly organized system with limited regulatory control, the standard of serological screening leaves much to be desired in majority of the blood centers.

The National Blood Policy and Strategic Framework (2014–2020) is the national policy for the safe blood transfusion. The section "*Cluster: 3, Core Business*" underlines the importance of TTI screening and ensures 100% screening of TTIs on donated blood as well as ensures proper resource allocation for adequate and sustainable supply of validated screening assay and required accessories.[5]

With no national regulatory control over the TTI screening kits, many poor quality, substandard, and cheap options are available. Therefore, in the majority of the blood centers, poor quality rapid kits are utilized which have never been accurately evaluated and validated at a national level. In addition, there is no national strategy for a testing technique and algorithm, the screening equipment is not properly maintained and calibrated, and human resource technical capacity issues also exist. As a result, the risks of acquiring infections following transfusions remain very significant.[6] The seroprevalence of HBV and HCV in blood donors is reported at 2.35% and 3.26%, respectively.[7] In addition, the large community of thalassemia patients with multiple transfusions per year accumulates higher percentages of HBV (8.4%) and HCV (56.8%) infections.[8] The detection of HBV and HCV is very critical considering their prevalence and the degree of pathogenicity. The cost of each type of screening test varies so does the quality. The most common method used for screening TTIs is rapid immunochromatographic tests (ICTs) which are prone to false negativity and positivity.[9] The false-negative results are seldom verified which leads to residual risk of transmission through blood transfusion.[10] Hence, the current study was designed to evaluate the screening performance of rapid kits in comparison with chemiluminescence immunoassay (CLIA) and polymerase chain reaction (PCR). This will assist national and provincial health authorities to make informed decisions about the selection of assays to be used and therefore assist in formulating a screening strategy for the country.

Materials and Methods

This single-center, cross-sectional study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, PIMS, Islamabad, from January to April 2019. The study subjects selected were representatives of the donor population who came to donate blood at the study site. Universally accepted inclusion criteria for blood donors were followed, i.e., aged between 18 and 60 years, weighing more than 50 kg, and having a blood hemoglobin level of more than 12 g/dl. A thorough medical history was taken to ensure that donors meet the inclusion criteria. The exclusion criteria included a history of jaundice, malaria, drug addiction, anemia, repeated transfusions, and any evidence of cardiac, renal, or pulmonary disease.

Blood samples from prospective blood donors were collected in ethylenediamine tetraacetic acid tubes. Plasma was separated from blood by centrifugation at 5000 rpm for 10 min at 4°C. Screening for HBV and HCV infections was carried out by CLIA on the DiaSorin LIAISON® XL Murex. (S.p.A., Saluggia, Italy) as per the standard protocol provided by the manufacturer. Specimens with concentration values ≥0.05 IU/ml were considered reactive. Samples tested positive for hepatitis B surface antigen (HBsAg) or anti-HCV during routine screening were further confirmed by PCR using ONE-STEP RT-PCR PreMix kit. The samples tested negative for HBV (HBsAg) and HCV (anti-HCV) were also confirmed through PCR. A total of 100 PCR-positive samples and 100 PCR-negative samples for each HBV and HCV were used for the quality assessment of ten commercially available rapid testing ICT devices for HBV and HCV, respectively. All ICT assays were carried out following the manufacturers' instructions. The testing was performed by qualified trained staff under the supervision of researchers with extensive experience in the field of clinical diagnostics. Sensitivity and specificity of each ICT kit were determined by calculating number of true-positive, false-positive, true–negative, and false-negative results in comparison to PCR results. Sensitivities and specificities were compared with fully automated LIAISON® XL Murex HBsAg and anti-HCV antibodies Quant assays (DiaSorin S. p. A., Saluggia, Italy), a CLIA.

The study protocol was approved by the Ethical Committee of the SZAB Medical University, Islamabad, Pakistan. The participants were informed regarding the purpose of sample collection and study objectives, and informed consent was obtained.

For each HBsAg and anti-HCV kit, sensitivity and specificity with 95% confidence intervals (CI) were calculated after determining true-positive, false-positive, true–negative, and false-negative results of each kit compared to PCR. Positive predictive values (PPV) and negative predictive values (NPV) (with 95% CI) were also calculated. The data were entered and analyzed using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA).

Results

Sensitivities and specificities of different ICT kits for HCV, namely Hightop, RightSign, Wondfo, Accu-Check, Fastep, Abon, ImmuMed, Insta-Answer, BioChek, and CTK Biotech are given in Table 1. Similarly, sensitivities and specificities for each of the kits for the detection of HBsAg are given below in Table 2.

Among various rapid ICT devices, the highest accuracy for the detection of HBsAg was shown by Insta-Answer (85%), while Hightop and Wondof equally had the lowest accuracy index (67.50%). The RightSign® showed the highest accuracy index for HCV detection (79%) while BioChek® had 64% accuracy index.

The Liason Murex® assay showed 100% sensitivity and specificity for both HBV and HCV, respectively, when compared with PCR results.

Discussion

The universally used diagnostic and blood screening marker for HBV is HBsAg. An individual positive for HBsAg is considered to be infected with HBV and is therefore potentially infectious.[11] The performance evaluation results of 10 rapid kits showed a great degree of variability. The performance of Liason XL Murex assay was also compared with the PCR detection method which showed equal consistency, reliability, and accurate results for all samples including the controls. In several countries, similar studies have already been conducted. The data showed variation in the detection capacity of rapid devices related to their specificity and sensitivity.[12-15]

In France and Ghana, Vikia®test and Determine® were validated in two studies. The observed sensitivities were 70.7% and 69.3%, respectively, while sensitivities were 90.0% and 88.3%, respectively.[16,17] A comparative study conducted in Srilanka using CORTEZ® and Onsite® HBsAg rapid kits described less efficiency of rapid kits with variable results as compared to the ELISA method.[18] In a study in India, the Hepacard® assay was found to have a sensitivity of 79% and specificity of 98.9%.[13] A similar study performed by Maity *et al*., to evaluate the rapid HBsAg devices manufactured by J. Mitra Co., SPAN Diagnostic Pvt. Ltd., and Standard Diagnostic Co., found variable specificities and sensitivities for the diagnosis of HBV.[19] A Pakistani study showed 100% sensitivity of latex agglutination and ICT method with a specificity of 91.7% and 99.2% for HBsAg.[20] Another Indian study reported 97.47% specificity and 78.94% sensitivity when compared to the results of Meriscreen® HBsAg with that of ELISA.[21] In Iran, Ansari *et al*. evaluated six rapid detection methods taking quantitative PCR results as a gold standard method. The rapid tests with strips or devices showed the sensitivity between 97.5% and 99.2% and specificity of 97.5 and 99.2% and concluded that negative results generated by the rapid method must not be excluded unless tested with gold standard.[22]

Our study endorsed the previous studies that more cautions should be adopted while screening blood

Table 1: Statistical evaluation of the results obtained from kits used for anti-hepatitis C virus detection

	Anti-HCV assays										
Statistical parameters	Rapid kits										Lison-XL
	Hightop	RightSign	Wondfo	Accu-Check	Fastep	Abon	Immu-Med	Insta answer	BioChek	CTK Biotech	
Sensitivity (%)	69	76	69	78	68	63	71	79	62	69	100
Specificity (%)	80	83	81	79	68	73	70	68	66	78	100
PPV	0.77	0.81	0.78	0.78	0.68	0.70	0.70	0.71	0.64	0.76	1.0
NPV	0.72	0.77	0.72	0.78	0.68	0.66	0.70	0.76	0.63	0.71	1
Accuracy index (%)	75.50	79.00	71.00	78.50	68.00	68.00	70.50	73.00	64.50	73.50	100

PPV=Positive predictive value, NPV=Negative predictive value, HCV=Hepatitis C virus

Table 2: Statistical evaluation of results obtained from kits used for hepatitis B surface antigen detection

	HBsAg assays										
Statistical parameters	Rapid kits										Liason-XL
	Hightop	RightSign	Wondfo	Accu-Check	Fastep	Abon	Immu-Med	Insta Answer	BioChek	CTK Biotech	
Sensitivity (%)	65	67	62	70	68	73	77	80	67	72	100
Specificity (%)	70	85	73	80	77	85	83	90	81	83	100
PPV	0.68	0.81	0.70	0.77	0.75	0.82	0.82	0.89	0.78	0.81	1.0
NPV	0.66	0.72	0.66	0.72	0.70	0.75	0.78	0.82	0.71	0.74	1
Accuracy index (%)	67.50	76.00	67.50	75.00	72.50	79.00	80.00	85.00	74.00	77.50	100

HBsAg=Hepatitis B surface antigen, PPV=Positive predictive value, NPV=Negative predictive value

donations with rapid devices. As blood donations are further processed in three components, one infected blood donation, if remain undetected due to poor quality of detection methods, will tragically affect at least three individuals at once. There is a great need to implement the national blood policy and strategic framework[5] under strict regulations by concerned blood regulatory authorities[23] regarding the screening of blood donations in Pakistan. Currently, different types of HBV and HCV testing kits are being used both in the government and private/NGO sector blood banks. Quality-assured kits must be used across the country under a national policy for the detection of HBV and HCV, which are not accurately detected by methods used currently. The current study has highlighted the gaps in the effectiveness of rapid testing devices for screening. It also revealed that the chemiluminescence assays and PCR are more reliable and accurate methods for the detection of HBV and HCV. However, the cost could be prohibitive. The selection, procurement, and the use of rapid screening kits, as well as quality control require urgent attention from the concerned authorities.

Conclusion

The current study reveals that most of the currently used rapid devices in Pakistan have low sensitivities and specificities for HBV and HCV screening of blood donations. The study provides valuable information to experts, administrators, and policy-makers to take informed decisions during selection and procurements of blood screening technologies to ensure the safety of blood transfusion and contribute to the prevention and control of transfusion-transmitted hepatitis infections in Pakistan.

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Conflicts of interest
There are no conflicts of interest.

References

1. Zaheer HA, Waheed U. National baseline survey on monitoring and evaluation of blood screening systems in Pakistan. J Blood Dis Transfus 2015;6:265.
2. Zaheer HA, Waheed U. Blood safety system reforms in Pakistan. Blood Transfus 2014;12:452-7.
3. Qureshi H, Bile KM, Jooma R, Alam SE, Afridi HU. Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. East Mediterr Health J 2010;16 Suppl: S15-23.
4. Al Kanaani Z, Mahmud S, Kouyoumjian SP, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Pakistan: Systematic review and meta-analyses. R Soc Open Sci 2018;5:180257.
5. National Blood Policy and Strategic Framework; 2014-20. Available from: http://sbtp.gov.pk/wp-content/uploads/2019/06/nbp-nsf-2014-20-final-2-1.pdf. [Last accessed on 2019 Jun 20].
6. Zaheer HA, Waheed U, Tahir S, Nasir K . National Blood Banks Data Collection Report; 2018. Available from: https://www.sbtp.gov.pk/wp-content/uploads/2019/10/National-Data-Collection-Report-2018.pdf. [Last accessed on 2019 Jul 27].
7. Zaheer HA, Saeed U, Waheed Y, Karimi S, Waheed U. Prevalence and trends of Hepatitis B, Hepatitis C and human immunodeficiency viruses among blood donors in Islamabad, Pakistan 2005-2013. J Blood Dis Transf 2014;5:217.
8. Farooq A, Waheed U, Zaheer HA, Rauf A, Arshad A, Muhammad Arshad M. Incidence of Hepatitis B and C viruses in Thalassaemia major patients. Pakistan J Zool 2018;50:1191-4.
9. D'Angelo RG, Klepser M, Woodfield R, Patel H. Hepatitis C virus screening: A review of the Oraquick Hepatitis C virus rapid antibody test. J Pharm Technol 2014;31:13-9.
10. World Health Organization. Screening Donated Blood for Transfusion-Transmissible Infections: Recommendations: Blood Screening, Quarantine and Release. Geneva: World Health Organization; 2009. p. 5. Available from: https://www.ncbi.nlm.nih.gov/books/NBK142991/. [Last accessed on 2019 Jun 10].
11. Ngui SL, Hallet R, Teo CG. Natural and iatrogenic variation in hepatitis B virus. Rev Med Virol 1999;9:183-209.
12. Bjoerkvoll B, Viet L, Ol HS, Lan NT, Sothy S, Hoel H, *et al*. Screening test accuracy among potential blood donors of HBsAg, anti-HBc and anti-HCV to detect hepatitis B and C virus infection in rural Cambodia and Vietnam. Southeast Asian J Trop Med Public Health 2010;41:1127-35.
13. Raj AA, Subramaniam T, Raghuraman S, Abraham P. Evaluation of an indigenously manufactured rapid immunochromatographic test for detection of HBsAg. Indian J Pathol Microbiol 2001;44:413-4.
14. Kim MH, Kang SY, Lee WI. Evaluation of a new rapid test kit to detect hepatitis C virus infection. J Virol Methods 2013;193:379-82.
15. Khan JK, Lone DS, Hameed A, Munim MR, Bhatti M, Khattak AA. Evaluation of the performance of two rapid immunochromatographic tests for detection of HBsAg and anti HCV antibody using ELISA tested samples. Ann King Edward Med Univ 2010;16:84-7.
16. Geretti AM, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, *et al*. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. J Clin Microbiol 2010;48:3223-30.
17. Bottero J, Boyd A, Gozlan J, Lemoine M, Carrat F, Collignon A, *et al*. Performance of rapid tests for detection of HBsAg and anti-HBsAb in a large cohort, France. J Hepatol 2013;58:473-8.
18. Chameera EW, Noordeen F, Pandithasundara H, Abeykoon AM. Diagnostic efficacy of rapid assays used for the detection of hepatitis B virus surface antigen Sri Lankan. J Infect Dis 2013;3:21-7.
19. Maity S, Nandi S, Biswas S, Sadhukhan SK, Saha MK. Performance and diagnostic usefulness of commercially available enzyme linked immunosorbent assay and rapid kits for detection of HIV, HBV and HCV in India. Virol J 2012;9:290.
20. Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. Indian J Med Sci 2000;54:432-4.
21. Mohammad KF. Comparison between rapid immuno-chromatographic device test and ELISA in detection of HBsAg and anti-HCV antibodies. Int J Recent Sci Res 2016;7:9129-2132.
22. Ansari MH, Mir-Davood O, Vahid M. Comparative evaluationof Immunochromatographic rapid diagnostic tests (Strip and Device) and PCR methods for detection of human hepatitis B surface antigens. Hepat Mon 2007;7:87-91.
23. The Gazette of Pakistan. Islamabad Transfusion of Safe Blood Ordinance. Ministry of Law, Justice, Human Rights and Parliamentary Affairs, Law, Justice and Human Rights Division, Government of Pakistan; 2002. Available from: http://www.sbtp.gov.pk/media/1344/ict-bt-ordinance-2002.pdf. [Last accessed on 2019 Jul 20].