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Evaluation of Two Commonly Used Commercial Immunochromatographic and ELISA Screening Kits for the Detection of Anti-HCV Antibodies among Patients in North Central Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EIB, SAA, VJU and HII designed the study, wrote the protocol, and managed the analyses of the study. Author EIB performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Hepatitis C Virus infection presents a major public health threat globally. The advent of different immunoassays for the detection of specific markers for the diagnosis of the infection since the discovery of the virus is a positive development, but their varied degrees of sensitivity and specificity is a matter of public health concern.
Aim: To evaluate the efficiency of two commercial rapid test kits for the detection of anti-HCV antibodies against a third generation Enzyme Immunoassay (EIA) used as a gold standard.
Methodology: A total of 500 patient plasma samples screened by ELISA (Autobio Diagnostics, China) were subjected to further screening using two rapid test (immuno chromatographic) strips supplied by Global Diagnostics (USA) and Wondfo Biotech Diagnostic Products (China).
Results: Of the 500 samples, anti HCV was detected in 79(15.80%) by ELISA, 59(11.80%) by Wondfo strip, whereas only 45(9.00%) by Global strip method. This gave Wondfo Kit a sensitivity of 75.0%, specificity of 99.0%, overall accuracy of 95.2%, positive predictive value of 93.6%, negative predictive value of 95.4% positive likelihood

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ratio of 75.0, negative likelihood ratio of 0.25 and Kappa value of 0.803, while Global Kit had a sensitivity of 57.0%, specificity of 100.0%, overall accuracy of 93.2%, positive predictive value of 100%, negative predictive value of 92.5%, positive likelihood ratio of 0.57, negative likelihood ratio of 0.43 and Kappa value of 0.672.

Conclusion: The result pattern reveals a marked or significant variation in sensitivity of the test kits. It is therefore recommended that third generation ELISA should be used for blood donors screening, to reduce transmission of hepatitis C virus through blood transfusion. Where the use of ELISA is practically unavailable in health facilities like in remote rural areas or poorest developing countries, the used of rapid strips can be adopted provided their performance are validated before its adoption. We recommend the use of PCR for detection of HCV RNA as a supplement to ELISA in laboratories or blood banks that can afford it.

Keywords: Commercial kits evaluation; Anti-HCV; ELISA; rapid kits.

1. INTRODUCTION

Hepatitis C is found worldwide with some countries having chronic infection rates as high as 5% and above. According to the World Health Organization there are about 150 million people chronically infected with the hepatitis C virus (HCV), and more than 350,000 people die every year from hepatitis C-related liver disease [1]. Hepatitis C virus estimated prevalence by WHO Regions showed that Africa had the highest prevalence with 5.3%,Eastern Mediterranean 4.6%, Western pacific 3.9%, South-East Asia 2.15%, Americas 1.7% and Europe had the least with 1.03% [2]. HCV infection occurs frequently and is highly endemic in Nigeria. This high prevalence has been confirmed by various studies from different parts of Nigeria among selected groups. Alao et al. [3] reported 5.4% among blood donors in Makurdi, Olokoba et al. [4] reported 2.4% among blood donors in Yola; Isa et al. [5] reported 1.8% among blood donors in ABUTH Kaduna while Ogunro et al. [6] reported a prevalence of 9.2% among pregnant women in Osogbo; and Buseri et al. [7] reported a prevalence of 0.5% HCV antibodies among pregnant women in Benin City.

The hepatitis C virus (HCV) was described for the first time in 1989. However, it is still being transmitted today to persons of every age, gender and race in all regions of the world. The discovery of the Hepatitis C virus ended a period of intensive research aimed at finding the agent responsible for 80% of transfusion associated ("non-A, non-B") hepatitis cases [8,9].

HCV is a small-enveloped virus with one single-stranded positive-sense RNA molecule of approximately 9.6kb. It is a member of the *Flaviviridae* family. This viral family contains three genera, flavivirus, pestivirus, and hepacivirus. Based on phylogenetic analyses it has been proposed to introduce a fourth genus, named *Pegivirus*, containing GBV-A-like viruses, GBV-C and GBV-D [10]. To date, only three members of the *Hepacivirus* genus have been identified, HCV, GB virus B (GBV-B) and the recently detected canine *Hepacivirus* (CHV) [11].

Common symptoms of hepatitis C infection such as fatigue, muscle ache, loss of appetite or nausea are unspecific and, in many cases, mild or not present. Consequently, hepatitis C is often diagnosed accidentally and, unfortunately, remains heavily under-diagnosed. It is estimated that only 30-50% of individuals infected with HCV are aware of their disease and can take advantage of treatment options and avoid the risk of further transmission of the

virus [12]. Untreated hepatitis C advances to a chronic state in up to 80% of people, which leads to liver cirrhosis in 20-40% with an accompanying risk of hepatic decompensation, hepatocellular carcinoma and death [13].

Hepatitis C virus (HCV) is mainly transmitted through contact with blood and blood products. The majority of HCV-seropositive individuals will have persistent viraemia. More than half of all patients will develop chronic hepatitis, and in 20% infection will lead to cirrhosis with all the subsequent complications, such as ascites, encephalopathy, variceal bleeding and hepatocellular carcinoma. Chronic HCV infection often runs an asymptomatic course and only 25 to 30% of infected persons seek medical attention for symptoms attributable to HCV infection. Early detection is of key importance in order to prevent complications of HCV-related liver disease [14].

The risks factors for the transmission of HCV include: Intravenous drug use, dental care, previous abortion, dilation and curettage (D&C), previous surgery, unprotected sexual exposures with multiple sexual partners, transfusion of blood and blood products, hemodialysis, employment in the health care field, birth to an HCV-infected mother and tattooing [15-17].

Current treatment for HCV infection is not highly effective and at least 90% of the patients who need treatment are unable to afford it. Immunization for passive prophylaxis of the hepatitis infection is not readily available. Public health interventions therefore continue to be the only effective method of preventing HCV infection. These include screening blood and blood products before transfusion, effective use of universal precautions and contraceptive barrier methods, use of disposable sharps and promotion of health education on HCV infection and its prevention. However, any strategy to prevent HCV infection must be based on accurate data [9,18]. This study aimed at evaluating the performance of two commercial immuno-chromatographic rapid kits with a third generation ELISA kit.

2. MATERIALS AND METHODS

2.1 Study Area

The North Central Nigeria is made up of six states and Abuja the capital of Nigeria. This study covered three states which comprised of Plateau State, Nasarawa State and Benue State. The study population focused on blood donors and women attending antenatal clinics in the selected areas of the study area.

2.2 Sampling Method

Ethical approvals were obtained from the Research Ethics Institutional Review Boards of Jos University Teaching Hospital, Plateau State, Federal Medical Centre Keffi, Nasarawa State and Federal Medical Centre Makurdi, Benue State before the commencement of the work. Consent forms were administered randomly to subjects who gave their consent prior to sample collection.

2.3 Inclusion and Exclusion Criteria

All those that consented and were non HIV patients within the study population were included in the study. While all those who declined their consent and those infected with HIV were excluded from the study.

2.4 Sample Collection

Five (5)mls of blood was collected in an anti-coagulated tube. The plasma was separated and stored in a freezer at -20 until ready for use.

2.5 Assay Procedure

The samples were all screened for antibody to HCV (anti-HCV) using a third generation ELISA Kit manufactured by Autobio Diagnostics, China; a one step Hepatitis C virus Test Strip (Global Laboratory Products, USA) and a one step Hepatitis C virus Test Strip (Wondfo Biotech Diagnostic Products, China) in accordance to the manufacturer's instructions. According to the manufacturers, the ELISA kit has a sensitivity of 100.0% and specificity of 99.5%, Global strip has a sensitivity of 99.0%, and specificity of 98.6% while Wondfo Biotech strip has a sensitivity of 99.0% and specificity of 99.8%.

The sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for the strip method were calculated based on the third generation ELISA kit as the gold standard and using the following formula:

Percentage Sensitivity=a/(a+c)×100%
Percentage Specificity=d/(b+d)×100%
Efficiency=(a+d)/(a+b+c+d)×100%
Percentage Positive Predictive Value=a/(a+b)×100%
Percentage Negative Predictive Value=d/(c+d)×100%
Where:
a = number of true positives
b = number of false positives
c = number of false negatives
d = number of true negatives
Likelihood Ratio Positive (LR+)=Probability of positive test in those with disease/Probability of positive test in those without disease
LR+=Sensitivity/(1-Specificity)
Likelihood Ratio Negative (LR-)=Probability of negative test in those with disease/Probability of negative test in those without disease
LR-=(1-Sensitivity)/Specificity.

Performance of kits for each lot was evaluated in terms of sensitivity, specificity, positive predictive value, negative predictive value and efficiency which can be defined as follows [19]:

Sensitivity = It is the ability of an assay kit to detect truly infected individuals and very small amounts of analyte.
Specificity = It is the ability of an assay kit to correctly identify all the uninfected individuals and there should be no false positives.

Positive Predictive Value (PPV) = It is the ability of a test to identify actually infected individuals among all persons giving a positive result with the kit being used.

Negative Predictive Value (NPV) = It is the ability of a test to identify correctly the real non infected individuals among all persons giving a negative result with the kit being used.

Efficiency = It is the overall ability of a test to correctly identify all positives as positive and all negatives as negative. This is also referred to as 'accuracy'.

2.6 Statistical Analysis

The data obtained from the study were analyzed by Kappa statistic using SPSS 15.0 software. The Kappa statistics is used to test interrater reliability. The importance of rater reliability lies in the fact that it represents the extent to which the data in the study are correct representations of the variables measured. Measurement of the extent to which data collections (raters) assign the same score to the same variable is called interrater reliability. Kappa usually range from 0 to +1, but like most correlation statistics, the Kappa can range from -1 to +1, with larger values indicating better reliability. Generally, a kappa >.70 is considered satisfactory.

3. RESULTS

A comparative study on the positivity and negativity of ELISA and the two rapid immunochromatographic Kits used for the detection of anti-HCV as showed in Table 1 revealed that of the 500 samples screened 79(15.8%) were positive for anti-HCV using ELISA, 59(11.8%) were positive using Wondfo rapid strip and 45(9.0%) were positive using Global rapid strip. Of the 79 ELISA positive samples, Wondfo kit detected 59 positives, giving 20 false negative, while, of the 421 negative samples screened, 417 were negative and 4 were positive. So also, of the 79 ELISA positive samples analysed by Global kit, 45 samples were positive and of the 421 ELISA negative samples screened, all of them were negative.

Table 1. Comparative positivity and negativity of ELISA and two rapid immuno-chromatographic kits for detection of anti-HCV

Test Kit	No. Screened	No. positive (%)	No. negative (%)	TP	FP	TN	FN
ELISA	500	79(15.8)	421(84.2)	-	-	-	-
WONDFO	500	59(11.8)	441(88.2)	59	4	417	20
GLOBAL	500	45(9.0)	445(91.0)	45	0	421	34

Key: TP=True Positive; TN=True Negative; FP=False Positive; FN=False Negative

The comparison of ELISA (Reference) technique with the two commercially available chromatographic rapid Kits for the detection of anti-HCV as shown in Table 2 revealed that out of 79 anti-HCV positive and 421 anti-HCV negative by ELISA, Wondfo Kit had a sensitivity of 75.0%, specificity of 99.0%, overall accuracy of 95.2%, positive predictive value of 93.6%, negative predictive value of 95.4%,positive likelihood ratio of 75,negative likelihood ratio of 0.25 and Kappa value of 0.803,while Global Kit had a sensitivity of 57.0%, specificity of 100.0%, overall accuracy of 93.2%, positive predictive value of 100%, negative

predictive value of 92.5%, positive likelihood ratio of 0.57, negative likelihood ratio of 0.43 and Kappa value of 0.672.

Table 2. Performance characteristics of HCV rapid kits used for comparative evaluation with reference to ELISA

Methods	S	SP	OA	PPV	NPV	LR+	LR-	Kappa
Wondfo	75.0%	99.0%	95.2%	93.6%	95.4%	75.0	0.25	0.803
Global	57.0%	100.0%	93.2%	100.0%	92.5%	0.57	0.43	0.672

Key: S=Sensitivity; SP=Specificity; OA=Overall Accuracy; PPV=Positive Predictive Value; NPV=Negative Predictive Value; LR+=Positive Likelihood Ratio; LR-=Negative Likelihood Ratio

4. DISCUSSION

A comparative study on the positivity and negativity of ELISA and the two rapid Immuno chromatographic Kits used for the detection of anti-HCV in this study revealed that the ELISA Kit showed superiority over the two rapid immunochromatographic techniques. This is in consonance with the findings of Khan et al. Hussain et al. Muhibi et al. [20-22] who reported that ELISA proved to be more sensitive than the rapid immunochromatographic techniques. Although, the rapid screening methods are the commonly used techniques adopted by diagnostic laboratories in the study area, simply because they are cheaper and less expensive than ELISA or other techniques, and considering the superiority of ELISA over the rapid diagnostic techniques, there is need to adopt ELISA technique for screening anti-HCV especially for transfusion purposes to minimize chances of transfusing the virus to uninfected individuals.

The comparison of ELISA technique which served as the goal standard in this study with the two commercially available immunochromatographic rapid Kits for the detection of anti-HCV revealed that Wondfo Kit had a sensitivity of 75.0%, specificity of 99.0%, positive predictive value of 93.6% and negative predictive value of 95.4%. This result showed a satisfactory reliability of Wondfo when compared with ELISA method than with Global kit. Generally, the lower predictive values obtained in this study is similar to most screening techniques and this is the main draw back in adopting screening techniques for diagnostic purposes.

Global Kit had a sensitivity of 57.0%, specificity of 100.0%, positive predictive value of 100% and negative predictive value of 92.5%.This finding is comparable with a similar work carried out by Muhibi et al. [22] who reported that Global strip gave 68.8% sensitivity, 100% specificity, while the positive and negative predictive values were 100% and 97.42% respectively. Global kit is excellent in performance when considering specificity, but its major setback is, its poor sensitivity.

Based on the data obtained in this study Wondfo proved to be more efficient than Global kit. This was supported by both the positive and negative likelihood ratios and the Kappa statistic test obtained in this study. Kappa has a range from 0-1.00, with larger values signifying better reliability. Generally, a Kappa of >0.70 is considered satisfactory. The efficiency of rapid immunochromatographic kits was compared with the ELISA kit to assess their reliability with the use of Cohen's Kappa test [23]. A level of agreement above 0.75 was considered to be excellent [23,24]; in this study Kappa agreement value of 0.803 was recorded for Wondfo kits, which falls within the acceptable range, while Global kit was 0.672 which was below the acceptable range [25].

The range of sensitivity in this study was lower than those stated in the manufacturer's manuals. The sensitivity differences noted could accrue to different immuno chromatographic flow characteristics, antigen composition, concentration, or deposition or strength of colorimetric indication due to antibody binding. Conformational epitopes have been demonstrated to be more immune reactive than linear recombinant proteins [26]. A higher sensitivity and specificity claims of the products by the manufacturers may just be a market strategy to convince their customers to patronize their products as against other competitors.

The study suggested that apart from likely defects on the products from the manufacturers of the kits, poor handling or storage from the manufacturer to the users of the kits might have resulted in the significant decrease in the sensitivity for the rapid immunochromatographic kits compared to the ELISA. Possible explanations for heat-related decrease include impact on protein antigens, instability of reagents, and/or damage to the lateral flow matrix resulting in inhibition of lateral flow chromatography [27]. The specificity in this study was not significantly affected and this agreed with an earlier report [27].

5. CONCLUSION

The efficiency of the two rapid immunochromatographic kits when compared with ELISA kit which was used as the gold standard, proved the superiority of ELISA over the two kits. The study showed that of the two rapid kits Wondfo kit proved to be more sensitive than the Global kits. Considering the consequences of transfusing an infected blood to an uninfected patient, the use of ELISA technique for screening blood before transfusion and where it cannot be affordable, the use of two to three rapid kits with high sensitivity and specificity can be adopted to minimize cases of transfusing positive samples.

CONSENT

All participants who are all adults comprising of blood donors and pregnant women gave written informed consent. The consent form were filled and signed by all who participated in the study.

ETHICAL APPROVAL

Ethical approvals were obtained from the Research Ethics Institutional Review Board of Jos University Teaching Hospital, Plateau State (JUTH/DCS/ADM/127/XIX/5103), Federal Medical Centre Keffi, Nasarawa State and Federal Medical Centre Makurdi, Benue State (FMH/FMC/MED.108/VOL.1/112) before the commencement of the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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