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Field performance of HBsAg rapid diagnostic tests in rural Ethiopia

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ABSTRACT

Point-of-care rapid diagnostic tests (POC-RDTs) are widely used to screen and diagnose hepatitis B virus (HBV) infection and are often the only available diagnostic tools in resource-limited settings. The aim of this study was to evaluate the validity of three hepatitis B surface antigen (HBsAg) POC-RDTs (Healgen®, Advanced Quality™ and Determine™) in an area with high prevalence of HBV in eastern Ethiopia. Results were compared with a commercial enzyme linked immunosorbent assay (ELISA) as gold standard. Quantification of HBsAg was performed in false negative samples.

A total of 511 subjects were screened, of whom 81 (15.9 %) were HBsAg-positive with the gold standard. All three POC-RDTs were positive in 65 of the 81 positive samples, yielding a sensitivity (95 % confidence interval) of 80.2 % (70.3–87.5) and a specificity of 99.8 % (98.7–100 for Healgen® and Determine™; 98.6–100 for Advanced Quality™). False negatives were observed in 16 patients associated with low levels of HBsAg (median 1.5 IU/mL). All three POC-RDTs had reasonably high sensitivity and excellent specificity, but false negative results were observed in patients with low titres of HBsAg. Thus, these POC-RDTs might be useful to identify patients in need of HBV treatment, but cannot be recommended as blood donor screening tests.

1. Introduction

Hepatitis B virus (HBV) infection is the most common cause of liver disease worldwide. Approximately 2 billion have serological evidence of past or present infection with the highest prevalence found in east Asia and sub-Saharan Africa (World Health Organization, 2017a). Around 257 million people are living with chronic hepatitis B and an estimated 887,000 deaths annually are attributable to HBV, mostly due to decompensated cirrhosis and hepatocellular carcinoma.

Timely diagnosis is critical for reducing the burden of HBV-related mortality and morbidity. Effective screening is also crucial to reduce transmission and increase public awareness. In sub-Saharan Africa, 5–10 % of the adult population is living with chronic HBV infection (World Health Organization, 2016). In Ethiopia, the estimated overall pooled seroprevalence of hepatitis B surface antigen (HBsAg) is 6.0 % (Schweitzer et al., 2015; Yazie and Tebeje, 2019). However, access to laboratory testing is limited and the majority of infected individuals are unaware their serological status.

HBsAg is a key serological marker for HBV infection. Enzyme immunoassays (EIA) and chemiluminescence immunoassays (CIA) are considered the most precise tools for detection of circulating HBsAg, but both assays require expensive equipment and high-quality laboratory

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infrastructure with well-trained technicians. Hence, point-of-care rapid diagnostic tests (POC-RDTs) detecting HBsAg have been developed to facilitate testing in resource-limited settings, and these are recommended by the World Health Organization (WHO) due to their relatively low cost, simplicity and high accessibility (World Health Organization, 2017b). Several systematic reviews of POC-RDTs detecting HBsAg have confirmed the diagnostic accuracy of many commercially available POC-RDTs (Shivkumar et al., 2012; Scheiblauer et al., 2010; Amini et al., 2017).

Although POC-RDTs are widely used throughout sub-Saharan Africa, studies on their accuracy in real-life are scarce. We therefore aimed to evaluate the reliability of three POC-RDTs widely used in Ethiopia (Determine™, Advanced Quality™ and Healgen®) using an enzyme linked immunosorbent assay (ELISA) as gold standard.

2. Material and methods

2.1. Study setting and participants

A case-control study was undertaken at Hiwot Fana Specialized University Hospital and Jugal Hospital in Harar, Ethiopia, between April 2015 and April 2016, as described previously (Orlien et al., 2018a,b). Participants in the present POC-RDT validation study were 18 years of age or older and were included either due to clinical and/or biochemical signs of chronic liver disease (n = 211) or they were healthy controls with normal alanine aminotransferase (ALT) and absence of clinical signs of liver disease (n = 300).

A total of 614 individuals were evaluated for eligibility, of whom 511 were screened for HBsAg and included in the analysis. Among the 103 study participants who were excluded, 89 were ineligible as cases in the case-control study and excluded prior to blood sampling; 11 individuals refused blood sampling and withdrew their consent; and, 3 patients deteriorated rapidly and were withdrawn from the study for compassionate reasons.

2.2. Laboratory tests

Blood was collected by venous puncture for immediate processing; serum was separated for immediate testing and further storage in aliquots at -20 °C. Standard biochemical tests were analysed locally, within 24 h, using a semi-automatic biochemistry analyser DR-7000D (DIRUI, Changchun, China) and HumaLyzer 3000 (HUMAN, Wiesbaden, Germany).

2.3. POC-RDTs

The Determine™ (Alere, Waltham, MA, USA), Advanced Quality™ One Step HBsAg test (InTec PRODUCTS, Xiamen, China) and Healgen® HBsAg Rapid Test Cassette (Zhejiang Orient Gene Biotech, Zhejiang, China) POC-RDTs were evaluated for their accuracy to detect HBsAg locally using separated serum samples according to the manufacturers' instructions. All three tests are qualitative tests based on lateral flow immune chromatographic techniques for lateral association of monoclonal and polyclonal antibodies specific for HBsAg. Each rapid test cassette had a control indicator showing if the test was performed correctly. Gold standard testing of serum HBsAg was undertaken at Aklilu Lemma Institute of Pathobiology in Addis Ababa using a commercial ELISA (Elisys Uno, HUMAN, Wiesbaden, Germany; or Architect, Abbott Diagnostics, IL, USA), and the laboratory technicians were blinded to the POC-RDT results.

2.4. HBsAg quantification

Samples that were false negative with the POC-RDTs, and an equal number of randomly selected true positive samples, were analysed at Oslo University Hospital in Norway using a chemiluminescent microparticle immunoassay for quantification of HBsAg (Abbott Diagnostics, Weibaden, Germany). The analysis was performed on an automated laboratory analytic platform (Abbott Architect).

2.5. Statistical methods

The statistical analyses were performed in SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were summarized as frequencies, whereas continuous variables were presented as median and interquartile range (IQR). Comparisons between the groups were performed using the Pearson Chi-Square test for categorical variables and Mann-Whitney U for continuous variables. A p-value <0.05 was considered significant throughout the study. Area under the receiver operating characteristics (ROC) curve, sensitivity, specificity, positive and negative predictive values were used to estimate test performances and calculated using SPSS and an online open source statistic module (OpenEpi Diagnostic or Screening Test Evaluation 1.0, 2020). The *Strengthening the Reporting of Observational studies in Epidemiology* (STROBE) statement guidelines were followed (von Elm et al., 2007).

2.6. Ethics

The study was approved by the National Research Ethics Review Committee (NRERC, Ref. No.: 3.10/829/07) in Ethiopia and by the Regional Committees for Medical and Health Research Ethics (REK Sør-Øst Ref. No.: 2014/1146) in Norway. The study was conducted in accordance with the Declaration of Helsinki (World Medical Association (2013)). Written informed consent was obtained from all study subjects. All HBsAg positive study subjects were referred to a treatment program for hepatitis B at Hiwot Fana Specialized University Hospital.

3. Results

3.1. Study population

Overall, there were more men (64.2 %) than women and the median age was 30 (IQR 24–50) years. The HBsAg-positive individuals were significantly more likely than the HBsAg-negatives to be male (81.5 *vs.* 60.9 %; $p < 0.001$), ethnic Oromo (86.4 *vs.*72.1 %; $p = 0.024$), Muslim (88.9 *vs.* 71.6 %; $p < 0.001$) and farmers (63.0 *vs.* 40.7 %; $p < 0.001$).

3.2. POC-RDT results

All POC-RDTs yielded valid results on the first attempt, and there were no discrepancies between the three rapid tests. During the study period, there was shortage of supplies of the Advanced Quality™ test, and thus 17 samples (all HBsAg negative) were not tested with this particular POC-RDT.

3.3. Diagnostic accuracy

The diagnostic accuracy did not vary significantly between the POC-RDTs (Table 1). For all tests the sensitivity was 80.2 % (95 % confidence interval [CI], 70.3−87.5). For Healgen® and Determine™ the specificity was 99.8 % (95 % CI, 98.7−100), whereas for Advanced Quality™ 99.8 % (95 % CI, 98.6−100).

3.4. Characteristics of participants with false negative results

Of the 81 study participants who were HBsAg positive by ELISA, 16 (19.8 %) had false negative results with all three POC-RDTs. There were no significant differences in sex distribution (18.7 *vs.* 18.5 % women; $p = 0.979$) or age (median 35 *vs.* 30 years; $p = 0.152$) between participants with false negative results compared to subjects with true positive results.

Spare serum samples were available in 14 out of the 16 participants

Table 1
Diagnostic accuracy of selected rapid HBsAg tests compared to ELISA.

Rapid HBsAg test	HBsAg serology ELISA		AUROC (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+(95 % CI)	LR-(95 % CI)
	Positive (n = 81)	Negative (n = 430)							
Determine™ (Alere)									
Positive	65	1	0.90	80.2	99.8	98.5	96.4	345.1	0.198
Negative	16	429	(0.85–0.95)	(70.3–87.5)	(98.7–100)	(91.9–99.7)	(94.2–97.8)	(48.3–2468)	(0.175–0.224)
Advanced Quality™ (InTec)[1]									
Positive	65	1	0.90	80.2	99.8	98.5	96.3	331.4	0.198
Negative	16	412	(0.85–0.95)	(70.3–87.5)	(98.6–100)	(91.9–99.7)	(94.0–97.7)	(46.3–2370)	(0.175–0.224)
Healgen® (Orient Gene Biotech)									
Positive	65	1	0.90	80.2	99.8	98.5	96.4	345.1	0.198
Negative	16	429	(0.85–0.95)	(70.3–87.5)	(98.7–100)	(91.9–99.7)	(94.2–97.8)	(48.3–2468)	(0.175–0.224)

Abbreviations: HBsAg, hepatitis B surface antigen; **ELISA**, enzyme-linked immunosorbent assay; **AUROC**, area under the receiver operating characteristics curves; **CI**, confidence interval; **PPV**, positive predictive value; **NPV**, negative predictive value; **LR+**, positive likelihood ratio; **LR-**, negative likelihood ratio.
[1] The test cassette was not obtainable in 17 samples.

with false negative POC-RDT results, and these were quantified for HBsAg. For comparison, 16 random samples with true positive POC-RDT results were also tested. The false negative samples had significantly lower HBsAg levels (median 1.5 *vs.* 6271 IU/mL; $p < 0.001$) and lower liver transaminase activities (median ALT 24 *vs.* 35 IU/L; $p = 0.008$) than true positives. The lowest serum HBsAg level that showed POC-RDT reactivity was 20 IU/mL. With the exception of two outliers, false negatives had HBsAg levels below 41 IU/mL, suggesting a HBsAg cut-off level for all three POC-RDTs of around 20–40 IU/mL (1.3–1.6 $\log_{10}$ IU/mL) (Fig. 1).

4. Discussion

This study is, to the best of our knowledge, the first field evaluation of HBsAg POC-RDTs commonly used in Ethiopia. All three POC-RDTs were easy to use and yielded valid test results on the first attempt, underscoring their applicability in a rural resource-limited setting. The specificity was excellent for all POC-RDTs, but the sensitivity was only moderate at around 80 %. Interestingly, the more expensive WHO approved POC-RDT (Determine™) did not perform better than the low-cost POC-RDTs (Advanced Quality™ and Healgen®).

In previous reports, false-negative HBsAg POC-RDT results were associated with low levels of quantified HBsAg, low viral load, HBsAg mutants and certain viral genotypes (Coffin et al., 2019). The false negatives in our study were associated with low levels of quantified HBsAg and normal liver transaminases, suggesting that these were patients with a very low likelihood of progressive liver disease (Invernizzi et al., 2016). Thus, the reduced sensitivity of these POC-RDTs might be of less concern if the tests are used as a screen-and-treat diagnostic tool.

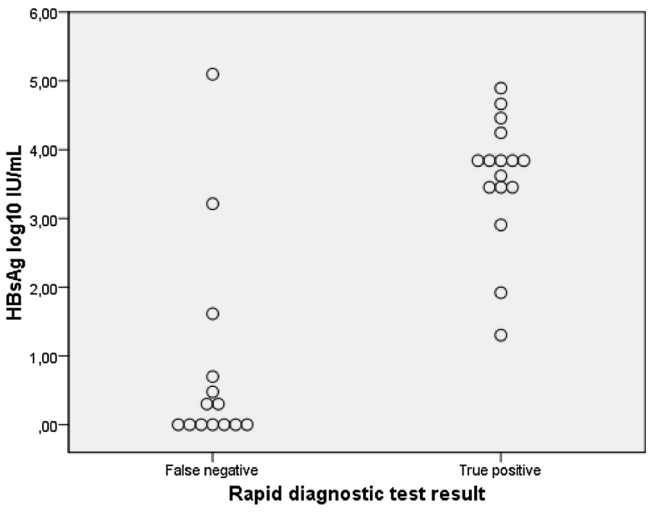

Fig. 1. Quantitative HBsAg levels in samples that were false negative *vs.* true positive with rapid diagnostic tests.

For the purpose of blood safety screening in blood banks, however, the sensitivity of these POC-RDTs is unacceptably low. HBV can be transmitted *via* blood transfusion from individuals with low, or even undetectable, levels of HBsAg in peripheral blood, since low-level production of virus might still occur in hepatocytes (Yuen et al., 2011). A recent review of sero-surveys among blood donors in Ethiopia observed a pooled estimated HBsAg seroprevalence of 4.9 % (Fite et al., 2020). If we apply our HBsAg POC-RDT performance results to real life, then one out of five individuals with chronic HBV infection will not be detected in blood banks if POC-RDTs are used for blood safety screening. Consequently, up to 1 out of 100 blood donations in Ethiopia might inadvertently transmit hepatitis B virus.

Our study had certain limitations. First, the sample size included a relatively small number of HBsAg positive cases and thus gives a relatively wide confidence interval of the sensitivity. Second, HBV sequencing was not performed because of lack of serum, and thus we were not able to relate a specific viral genotype or HBsAg mutant to the false-negative results. Third, a neutralization test or anti HBc serology in order to consolidate a real presence of HBsAg was not obtainable in the diagnostic work up in this study. Finally, the laboratory technician at the point of care was not blinded to the results of the other POC-RDTs, hence we cannot exclude that this might have biased the interpretation of the POC-RDTs.

In conclusion, the present study demonstrated the usefulness of selected POC-RDTs as a screen-and-treat diagnostic tool for HBV in a resource-limited setting. The low-cost POC-RDTs were in perfect agreement with the more expensive WHO approved POC-RDT. However, none of the POC-RDTs evaluated could be recommended for screening blood donations due to the risk of transfusion-transmitted HBV-infection.

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CRediT authorship contribution statement

Stian Magnus Staurung Orlien: Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Tekabe Abdosh Ahmed:** Resources, Writing - review & editing. **Nejib Yusuf Ismael:** Resources, Writing - review & editing. **Nega Berhe Belay:** Conceptualization, Methodology, Writing - review & editing. **Anne-Marte Bakken Kran:** Resources, Formal analysis, Writing - review & editing. **Svein Gunnar Gundersen:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Asgeir Johannessen:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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