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Original Article **Open Access**

Assessment of the performance of six *in vitro* diagnostic kits for qualitative detection of hepatitis B virus surface antigen (HBsAg) in human serum or plasma in Lomé, Togo

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Abstract:

Background: Several *in vitro* diagnostic (IVD) test kits for hepatitis B surface antigen (HBsAg) are commercially available. The question is whether they are performing well for both screening and diagnosis or not? Thus, this study aimed to evaluate the performance of six commercially available HBsAg detection kits in Togo.
Methods: This study was conducted at the National Reference Center for HIV/STI testing in Lomé (CNR-VIH/IST), Togo. Reference sera used for the assessment were collected from blood donors and patients with history of hepatitis B viral (HBV) infection between 2008 and 2014, and includes 200 non-reactive HBsAg and 150 reactive HBsAg sera that were confirmed with a reference method which consisted of the combination of an ELISA, a RDT, and a molecular test. Four ELISA kits (EKOlab ELISA-HBsAg; HEPALISA ULTRA; HEPALISA; Murex AgHBs Version 3) and two RDTs kits (ACON AgHBs and OnSite HBsAg Rapid Test-Cassette) were then evaluated using these serum samples. The EPI-INFO software version 7.2 was used to determine the 95% confidence interval and performed statistical analysis.
Results: Reference serum samples were collected from the population with 65.0% under 40 years of age and 61.2% males. The sensitivity of the 4 ELISA tests compared to the reference method was 100%. Apart from the HEPALISA test with a specificity of 100.0%, the specificity of the other three ELISA tests (Murex HBsAg version 3, HEPALISA ULTRA and EKOlab ELISA-HBsAg) were 98.4%, 97.3% and 91.8% respectively. For the RDTs, the sensitivity of ACON HBsAg and OnSite HBsAg Rapid Test-Cassette was 70.0% and 95.6% respectively while the specificity was 100.0% for both.
Conclusion: The ELISA tests evaluated were more sensitive than the RDTs, and HEPALISA test was the most efficient. Of the two RDTs, the OnSite HBsAg Rapid Test-Cassette was more sensitive. Our findings highlight the need for onsite verification of *in vitro* diagnostic kits for qualitative detection of hepatitis B surface antigen before their routine use in Togo.

Keywords: HBV, HBsAg, Performance, IVD test

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Évaluation des performances de six kits de diagnostic *in vitro* pour la détection qualitative de l'antigène de surface du virus de l'hépatite B (HBsAg) dans le sérum ou le plasma humain à Lomé, Togo

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Résumé:

Contexte: Plusieurs kits de test de diagnostic *in vitro* (DIV) pour l'antigène de surface de l'hépatite B (HBsAg) sont disponibles dans le commerce. La question est de savoir s'ils fonctionnent bien pour le dépistage et le diagnostic ou non? Ainsi, cette étude visait à évaluer les performances de six kits de détection de HBsAg disponibles dans le commerce au Togo.
Méthodes: Cette étude a été réalisée au Centre national de référence pour le dépistage du VIH/IST à Lomé (CNR-VIH/IST), Togo. Les sérums de référence utilisés pour l'évaluation ont été collectés auprès de donneurs de sang et de patients ayant des antécédents d'infection virale par le virus de l'hépatite B (VHB) entre 2008 et 2014, et comprennent 200 sérums HBsAg non réactifs et 150 sérums réactifs HBsAg qui ont été confirmés avec une méthode de référence consistant en la combinaison d'un ELISA, d'un TDR et d'un test moléculaire. Quatre kits ELISA (EKOlab ELISA-HBsAg; HEPALISA ULTRA; HEPALISA; Murex AgHBs Version 3) et deux kits TDR (ACON AgHBs et OnSite HBsAg Rapid Test-Cassette) ont ensuite été évalués à l'aide de ces échantillons de sérum. Le logiciel EPI-INFO version 7.2 a été utilisé pour déterminer l'intervalle de confiance à 95% et a effectué une analyse statistique.
Résultats: Des échantillons de sérum de référence ont été prélevés dans la population dont 65,0% ont moins de 40 ans et 61,2% d'hommes. La sensibilité des 4 tests ELISA par rapport à la méthode de référence était de 100%. Hormis le test HEPALISA avec une spécificité de 100,0%, la spécificité des trois autres tests ELISA (Murex HBsAg version 3, HEPALISA ULTRA et EKOlab ELISA-HBsAg) était respectivement de 98,4%, 97,3% et 91,8%. Pour les TDR, la sensibilité de la cassette de test rapide ACON HBsAg et OnSite HBsAg était respectivement de 70,0% et 95,6% tandis que la spécificité était de 100,0% pour les deux.
Conclusion: Les tests ELISA évalués étaient plus sensibles que les TDR et le test HEPALISA était le plus efficace. Sur les deux TDR, la cassette de test rapide OnSite HBsAg était plus sensible. Nos résultats soulignent la nécessité d'une vérification sur site des kits de diagnostic *in vitro* pour la détection qualitative de l'antigène de surface de l'hépatite B avant leur utilisation en routine au Togo.

Mots-clés: VHB, AgHBs, Performance, diagnostic *in vitro*

Introduction:

Hepatitis B virus (HBV) is a DNA virus that causes acute and chronic hepatitis in humans. It is estimated that two billion people worldwide have contracted HBV and 240 million are chronic carriers of the virus. Every year, about 600,000 people die from late-onset HBV infection. HBV infection is hyper endemic in sub-Saharan Africa and Asia and is thought to be the major etiological factor in more than 75% of patients with chronic liver disease (1-3). It remains the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC), two major causes of mortality worldwide (4).

Most of HBV-infected persons remain asymptomatic for long periods but are at risk of progressive liver disease and can transmit the virus to other susceptible individuals. Early identification of infection is thus important. The primary marker for screening and laboratory diagnosis of HBV infection is hepatitis B surface antigen (HBsAg), a component of the virus envelope that is also found in the blood in large amount as non-infectious subviral particles. This small envelope protein is the first viral marker to appear after HBV infection, and can be found in the blood before the onset of symptoms or abnormalities of blood chemistry. Thus, HBsAg test has been at the forefront of blood screening for HBV infection (5).

Immunoassays for detection of HBsAg required the use of highly sensitive and specific test reagents. In recent years, there has been an increase in the frequency of HBV which HBsAg antigenic determinants have undergone one or more mutations as a result of natural or therapeutic pressure induced by therapy or vaccination. These mutations modify the structure of the epitope with possible consequence of non-recognition of the mutated antigens by the antibodies used in the HBsAg screening reagents (6,7). Therefore, the objective of this study is to conduct an on-site assessment of the performance of 6 diagnostic test kits manufactured for HBsAg detection in humans.

Materials and Method:

Study setting

The study was conducted at the National Reference Center for HIV/STI (CNR-VIH/IST) located at the Sylvanus Olympio University Teaching Hospital (CHU SO), Lomé, Togo. One of the activities of this laboratory required by the Ministry of Health, is the evaluation of all HIV, viral hepatitis and STIs diagnostic test kits that can be used in the country for diagnosis or screening of donor's blood for transfusion.

Subjects and sample collection

Five ml of venous blood was obtained in EDTA Vacutainer tubes from each donor received at the National Blood Transfusion Center and in dry Vacutainer tubes from patients with history of HBV infection at the CHU SO, Lomé, Togo. The blood sample was centrifugated at 3000rpm for 5 minutes, then 1-2ml serum or plasma obtained after centrifugation were stored at -20°C in CNR-VIH/IST.

Detection of HBsAg by the IVD test kits and reference method

On these samples, we first prepared our reference panel by using a combination of an ELISA test kit (Monolisa HBsAg ULTRA™, BIO-RAD, France) and RDT kit (Determine HBsAg, Abbott™, Japan) in line with the WHO recommendation (8), and confirmed negative samples by a molecular test. Thus, on all plasma negative with the two tests, HBV DNA viral load was carried out on 500µL of plasma with the m2000rt Abbott Real Time HBV DNA assay (Abbott, USA), according to the manufacturer's protocol. We considered as true positive (TP) any sample that gave a positive result on both tests, and true negative (TN) as any sample that tested negative in both tests and in the molecular test.

The HBsAg was then detected from the serum using four different enzyme linked immunosorbent assay (ELISA) kits and two rapid diagnostic test kits The four ELISA tests were EKOlab ELISA-HBs Ag (EKOlab, Russia), HEPALISA ULTRA (Laboratoire J. Mitra & Co., India), HEPALISA (Laboratoire J. Mitra & Co., India), and Murex AgHBs Version 3 (Murex Biotech Limited., UK), and the two RDTs were ACON AgHBs (ACON Laboratories, NC., USA) and OnSite HBsAg Rapid Test-Cassette (CTK Biotech, Inc., USA) (Table 1). The tests were performed in accordance with each manufacturer's protocol.

Statistical analysis

The EPI-INFO software version 7.2 was used to determine the 95% confidence interval (CI) of each value. The performance of each test was obtained by determining the sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV). The sensitivity was defined as the ability of the test kit to truly identify persons infected [Se=TP/(TP+FN) x 100], specificity as the ability of the test kit to truly identify persons not infected [Sp=TN/(TN+FP) x 100], PPV as the ability of the assay to correctly identify actual infected persons among all who tested positive with the particular kit used [PPV=TN/(TN+FP) x 100], while NPV was defined as the ability of the assay to correctly identify actual non-infected persons among those who tested negative with the kit used [NPV=TN/(TN+FN) x 100].

Table 1: Characteristics of hepatitis B surface antigen *in vitro* diagnostic test kits

S/N	Test	Class	Manufacturer	Storage (°C)	Sample	Test Volume (microliter)
1	EKOlab ELISA-HBs Ag	ELISA	EKOlab, I Budennogo Str., Elektrogorsk, Russia,145530	2 – 8	Serum/Plasma	100
2	HEPALISA ULTRA	ELISA	Laboratoire J. Mitra & Co. Pvt. Ltd. A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 O20-INDIA	2 – 8	Serum/Plasma	100
3	HEPALISA	ELISA	Laboratoire J. Mitra & Co. Pvt. Ltd. A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 O20-INDIA	2 – 8	Serum/Plasma	100
4	Murex AgHBs Version 3	ELISA	Murex Biotech Limited. Central Road, Temple Hill, Dartford DA1 5LR UK	2 – 8	Serum/Plasma	75
5	ACON AgHBs	RDT	ACON Laboratories, Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121, USA	2 – 30	Serum/Plasma	50
6	OnSite HBsAg Rapid Test-Cassette	RDT	CTK Biotech, Inc. 10110 Mesa Rim Road, San Diego, CA92121, USA	2 – 30	Serum/Plasma/ Whole blood	45-55 40-50

Results:

The panel of reference samples used to evaluate these HBsAg test kits was gradually established from 2008 to 2014. It consisted of 350 samples, including 200 non-reactive HBsAg samples and 150 reactive HBsAg samples. All these samples were collected from the population with 65% under 40 years of age and 61.2% males. The sample size assessed ranged from 100 to 274 depending on the number of tests available per kit (Table 2).

The sensitivity of the 4 ELISA test kits was 100% as there were no false negative reactions with any of them. Apart from the HEPALISA test which gave no false positive results, the others 3 ELISA tests gave false positive results with 15 of 182 negative sera for the EKOlab ELISA-HBsAg test, 3 of 183 negative sera for HEPALISA ULTRA and 3 of 150 negative sera for Murex AgHBs Version 3 testing positive, giving specificity of 100.0%, 98.4%, 97.3% and 91.8% respectively. The PPV of these ELISA test kits ranged from 85.5% to 100.0% and the NPV was 100.0% for all of them.

In comparison, the sensitivity of the RDT kits were 70.0% for ACON HBsAg and 95.6% for OnSite HBsAg Rapid Test-Cassette, while both kits gave a specificity value of 100.0%. The PPV for the 2 kits was 100.0% while the NPV were 76.9% and 95.7% respectively for ACON HBsAg and OnSite HBsAg Rapid Test-Cassette (Table 2).

Discussion:

We conducted a field evaluation to assess the performance of 6 IVD test kits for hepatitis B virus infection screening. These tests detect the surface antigen (HBsAg) of the hepatitis B virus. The screening for viral hepatitis B is a dire need in our environment. While a lot have been done to provide quality and reliable screening for HIV infection with a well-defined algorithm, this is not yet the case for viral hepatitis, including hepatitis B.

Data from an unpublished study showed that about 10 RDTs without WHO prequalification are available in Togo and used at different levels of healthcare services. One of CNR-VIH/IST missions is to ensure the quality and performance of test kits used for the diagnosis of HIV, hepatitis B and C, and syphilis in Togo. This involves evaluating the on-site performance of the proposed test kits.

Table 2: Performance of hepatitis B surface antigen *in vitro* diagnostic test kits

	Test	Year of evaluation	Number of samples	True positive	False positive	False negative	True negative	Sensitivity (%) (CI)	Specificity (%) (CI)	PPV (%) (CI)	NPV (%) (CI)
ELISA	HEPALISA ULTRA	2014	184	71	3	0	110	100 (93.6 - 100)	97.3 (91.9 - 99.3)	95.9 (87.8 - 98.9)	100 (95.8 - 100)
	HEPALISA	2014	182	68	0	0	114	100 (93.3 - 100)	100 (95.9 - 100)	100 (93.3 - 100)	100 (95.9 - 100)
	EKOlab ELISA-HBs Ag	2015	273	91	15	0	167	100 (95 - 100)	91.8 (86.5 - 95.2)	85.8 (77.4 - 91.6)	100 (97.2 - 100)
	Murex AgHBs Version 3	2015	274	124	03	0	147	100 (96.3 - 100)	98 (93.8 - 99.5)	97.6 (92.7- 99.4)	100 (96.8 - 100)
RDT	OnSite HBsAg Rapid Test-Cassette	2015	181	87	0	4	90	95.6 (88.5 - 98.6)	100 (94.9 - 100)	100 (94.7 - 100)	95.7 (88.8 - 98.6)
	ACON AgHBs	2008	100	35	0	15	50	70 (55.2-81.7)	100 (91.1 - 100)	100 (87.7 - 100)	76.9 (64.5 - 86.1)

CI = Confidence Interval; ELISA = Enzyme Linked Immuno-Sorbent Assay; RDT = Rapid Diagnostic Test; PPV = Positive Predictive Value; NPV = Negative Predictive Value

The major challenge is to choose the right reference test i. e. the gold standard. For our purpose, we first defined our reference panel based on combination of an ELISA test (Monolisa HBsAg ULTRA™, BIO-RAD, France) and RDT (Determine HBsAg, Abbott™, Japan) in line with WHO recommendation (8). In addition, we confirmed negative samples by molecular tools. Our assessment showed all the four ELISA tests to be more sensitive (100.0% sensitivity) than the two RDTs, and amongst these four, HEPALISA kit along with the two RDTs had specificity of 100.0%. These findings indicate clearly that one of these ELISA test kits can be chosen for HBV screening of blood donors in Togo. In terms of performance, our findings demonstrated the efficiency of the ELISA kits in the order HEPALISA>Murex AgHBs Version 3>HEPALISA ULTRA>EKOlab ELISA-HBs-Ag, and of the RDTs in the order OnSite HBsAg Rapid Test-Cassette>ACON AgHBs.

Although the ELISA tests appeared to be superior, they are expensive and time consuming to perform. Despite the significant burden of disease due to HBV and HIV co-infections, and the advances and opportunities for treatment, the majority of people infected with HBV remain undiagnosed and unaware of their infection (9). It is estimated that less than 5% of people with chronic hepatitis B or C viral infection know their status (8,9), and in low-and-middle-income settings, this is even lower (<1%). This is also particularly poor in key populations such as people who inject drugs, prisoners, sex workers and men who have sex with men, for whom access to care and treatment are already challenging (10).

Our goal is to increase accessibility to hepatitis B testing by implementing HBV screening in community, primary care and district hospital in Togo. If we want to increase access to HBV testing, we need accurate, practical and affordable assays that may be used at service delivery of hepatitis B testing or near to point of care (11). Although, many brands of RDTs claim to possess these abilities, the ACON AgHBs we evaluated here showed lack of sensitivity. The consequence will be misidentification of HBV infection cases. Although the RDT assays had low sensitivity, their high specificity indicates that they may be useful for screening in regions endemic for HBV such as Togo (12). Moreover, OnSite HBsAg Rapid Test-Cassette can be used on whole blood and be stored at laboratory temperature like the ACON AgHBs. Some studies conducted in lower-middle-income countries such as Cameroon (13) and Nigeria (14), identified another type of RDT kit to be more sensitive.

For all the six test kits, the PPV was greater than 85.0% and 100.0% for the 2 RDTs, which somewhat further support their use in endemic region where people who test negative could complete their screening by either an EIA or PCR technique when these tools are available, to increase accuracy of screening. In Togo, previous studies have reported HBV prevalence ranging approximately between 9% to 19% (15-17). Thus, in Togo HBV RDT could be used in key populations at care services and in high risk population such as those in the age range 20-39 years old in Lomé (17) and on students (18) during screening campaigns. However, for blood transfusion, only ELISA assays or RDT kits with high performance as regard sensitivity or specificity should be used for HBsAg screening for blood transfusion (19-22).

One limitation to this study is the fact that these evaluations were not done at the same period, and sample sizes for the different kits were also not the same, situations that could have effect on comparisons of performance between the test kits we assessed.

Conclusion:

From this study, the ELISA test kits showed better performance than the RDTs. A good assay for an infectious agent such as HBV from a diagnostic point of view is one with a high positive predictive value and high specificity (23). The most effective test kit in this study is HEPALISA and the most effective RDT is OnSite HBsAg Rapid Test-Cassette. Our findings highlight the need of onsite assessment and verification of HBV diagnosis test kits before their routine use in the country.

We recommend that assays including rapid test kits used for HBsAg screening should be validated by the CNR/VIH before use for routine screening in Togo. It is crucial to expand hepatitis testing services, especially in community-based settings, where there are challenges with cost, transport and venipuncture requirements. The RDTs with best accuracy in this study can be used as an alternative screening tool for HBV infection at the community or primary healthcare level, more so that they are simple to perform by minimally trained community workers.

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