Performance of three commercial rapid diagnostic tests for the detection of hepatitis B surface antigen (HBs Ag) in Lao PDR

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Abstract

Objective: This study aimed to determine sensitivity, specificity, and accuracy of RDT used in 4 health care centres in Vientiane capital versus ELISA.

Methods: A study was then conducted among 1,729 patients who underwent three different RDTs for surface antigen of hepatitis virus (Boson, CTK and Coretest) in two public central hospitals and two private clinics in Vientiane Capital, to compare sensitivity, specificity, and accuracy of RDTs versus ELISA.

Results: The mean age (95%CI) of the patients was 28.7 years old and the sex ratio was balanced. 13.71% of the patients had positive HBsAg as detected by ELISA, while this was only 8.9% for RDTs. All three types of RDTs had a sensitivity of 54% and specificity of 97%. There was no difference in accuracy, sensitivity, specificity, positive and negative predictive values between RDTs.

Conclusions: This study revealed higher prevalence of HBsAg among young adults who were present in health care facilities in Vientiane than previously described in Laos. All three RDTs studied had low sensitivity but high specificity; therefore, they are likely to miss many cases of Hepatitis B infection and should be replaced or backed up by more accurate methods.

Keywords: Hepatitis B, ELISA, Laos, rapid diagnostic test

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Introduction

According to WHO, 240 million people are chronically infected with hepatitis B virus (HBV) worldwide WHO [1]. In Laos the prevalence of HBV (HBsAg positivity) varies from 2.9% to more than 8% [2-3]. In Vientiane capital, people visiting health centres and suspected to be infected with HBV are commonly tested with rapid immunochromatographic diagnostic tests (RDTs). Three commercial brands are commonly used in Vientiane: Boson Biotech HBsAg RDT, CTK Biotech "Onsite" HBsAg RDT and Coretest One step HBsAg RDT. None of them have official recommendations; they are bought by health center authorities according to their price. They have many advantages, as they do not require expensive specialized equipment, trained technicians, they can be stored at room temperature (at least under air conditioning atmosphere in tropical environments) and they allow individual use with fast processing. Nevertheless, to the best of our knowledge, no data are available on the performance of these RDTs and the manufacturers provide poor information on specificity and sensitivity of their kits. Moreover, according to our experience at the Centre d'Infectiologie Lao-Christophe Mérieux in Vientiane (CILM), about 20% of patients diagnosed HBsAg positive at hospitals and private clinics with rapid test diagnosis are false positives. In this context we decided to determine the performance of rapid tests of different brands in use in hospitals or private clinics of Vientiane Capital, versus ELISA, which is considered as a gold standard.

Methodology

Study design:

A prospective, cross-sectional study was carried out in Vientiane at the out- and in- patient Departments (OPD and IPD) of 2 public hospitals (Mahosot Hospital, Military hospital) and 2 private clinics (Mitthaphab and Lao-Viet). Patients were enrolled from September 2015 to June 2016. All patients who were prescribed HBV detection were proposed to participate. Exclusion criteria were: patients unwilling to participate already diagnosed by ELISA or rapid test non-interpretable. Socio-demographic characteristics of patients and their potential risk behavior were compiled in table 1.

Sample size, sampling method and procedure:

The sample size was determined according to Naing et al. [4], taking into account 95%CI, 10% desired precision and 9% prevalence HBV infection in Laos.

After participants were recruited as per eligibility inclusion criteria by clinicians, written informed consent, and answered a medical questionnaire.

For each patient, 3 mL of blood was collected and split in 2 samples, one for RDT (for immediate determination) and one for ELISA determination. ELISA tests were performed on micro plate 96 wells (Monolisa HbsAg ULTRA®, Bio Rad). RDTs were also performed according to manufacturer's instructions. Sensitivity, Specificity, Positive Predictive Value (PPV) Negative Predictive Value (NPV) and Accuracy of the RDTs were calculated and defined according to Penn State Science recommendations [5].

Ethical considerations:

The study was approved by the Laos ethical review committee of the National Institute of Public Health (Ref. No 046NIOPH/NECHR) and written informed consent was obtained from all included patients.

Statistical Analyses:

All information's participants and laboratory results were recorded in the file maker program, and then exported to Excel and used table 2x2 to calculate the sensitivity, specificity, PPV, NPV and data were analyzed by STATA version 10.

Results

1,740 samples were collected, 810 came from public health facilities (Mahosot hospital and Military hospital) and 930 from private clinics (Mitthaphab and from Lao-Viet). 10 were lost and one was not interpretable, therefore 1,729 samples were included in the study. For RDT tests, 190 were tested with Boson Biotech, 529 with CTK Biotech "OnSite" and 920 with Coretest® One, according to Naing calculation (fig.1).

75% of the patients were from Vientiane Capital, while 25% from outside of the capital city. Sex ratio was 1, mean age was 28.7 years old and 55% of patients were married. The largest occupational group was the group of government staff (41%). 40% completed secondary school. About 5% of all participants had been vaccinated and nearly 77% of them had never been tested for HBV before (Table 1).

Among the 1,729 patients enrolled in this study, 154 (8.9%) were positive for HBsAg by RDTs, while 237 (13.71%) were positive with ELISA (fig.2).

There was no statistical difference (P<0.001) between the three RDTs for Sensitivity, Specificity, PPV, NPV and accuracy (Table 2).

Poor or no indication on accuracy was given by the manufacturer and to the best of our knowledge, only one article referred to CTK.

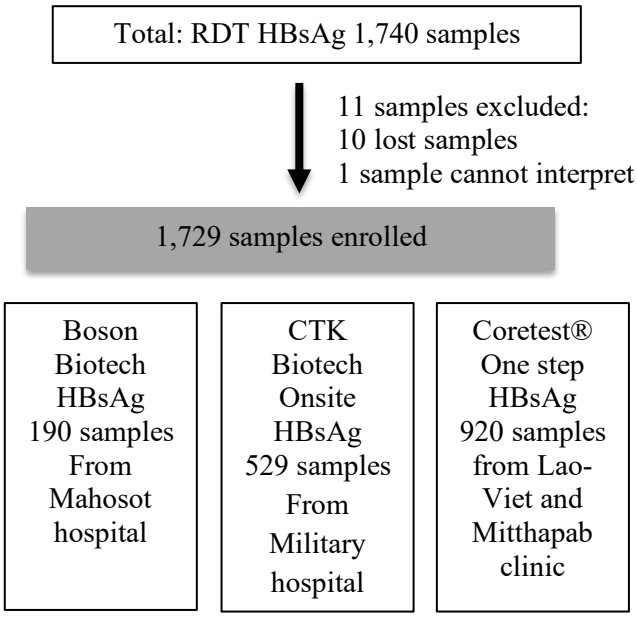

Figure 1: Structure of the studied cohort

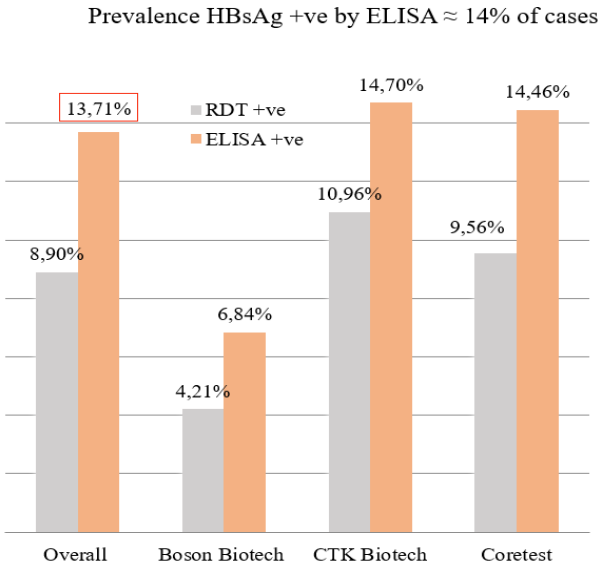

Figure 2: Prevalence of HBs Ag+ tested by ELISA vs RDTs

Discussion

HBV is a highly infectious virus that can cause a long-term silent infection leading in some cases to liver cancer. It is then necessary to be able to detect the presence of the virus to control transmission and the dynamic of the infection. In many developing countries, rapid diagnostic tests are widely used to detect HBsAg for both diagnosis and screening of acute and chronic HBV infections, although ideally, screening should be done using more advanced and accurate methods such as ELISA.

As results obtained are crucial to take therapeutic decision, it is important for doctors to know the limit of the test that has been done for the diagnostic of this pathology.

Rapid tests may yield false test outcomes due to the prozone effect, especially during the initial phase after infection when the viral load is high and there are high antigen concentrations [6] and due to genotype variations, that may influence test sensitivity [7]. Serological tests, such as enzyme immunoassays (EIA), have high accuracy in detection of serological markers, such as HBsAg, anti-HBc and anti- HCV. However, the tests are expensive, require complex instrumentation, and are not feasible in rural remote district hospitals in low-income countries. Advances in diagnostic technology have resulted in rapid tests for identification of serological markers. However, the accuracy of these rapid tests as claimed by the manufacturers is normally based on sero-conversion test panels, which do not necessarily reflect the antibody or antigen spectrum in the population studied. It is possible that test accurate on pre-arranged panels may yield falsely high-performance indicators. Results of this study indicate that the three RDTs commonly used in Vientiane are almost similar between us for Sensitivity, Specificity, PPV, NPV and accuracy. They have high specificity, NPV and accuracy for the detection of HBsAg, which is similar with report from Sri Lanka and Pakistan [8]. Boson RDT has 95,6% sensitivity and 96% specificity (According to the fabricant), 80% sensitivity and 100% specificity for CTK (Khan et al. 2010) and 60% sensitivity and 100% specificity [7] for Core. Nevertheless, we showed herein that ELISA that able to detect 1.5 more positive patients than RDT. Moreover, RDTs were shown to be less predictive compared to other RDT recommended by WHO [9]. In our study we should take into account cross-reactivity with other infectious diseases as Cruzetal [10] showed that some pathologies could yield confusing results and inconclusive clinical interpretations

As previously described by Paboriboune et al. (2017) there were more men positive for HBV than women (P-value=0.001). We also showed herein that tattoo/Piercing, IV Drugs use and low rate of being previously vaccinated against HBV infection are risk factors. The latter correlates with the fact that participants were born before the introduction HBV vaccine into the national program of vaccination for children and that the adult vaccination is not available for free in Lao.

Conclusion

The national health care system should consider HBV as a priority and facilitate access to health care services and vaccinate people for free.
ELISA HBsAg detection should be available in health care centres or at least RDTs recommended by WHO should be preferred over the one distributed nowadays in Vientiane.

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Competing interest

The authors declared no conflict of interest with any organization

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Table 1: Socio-demographic characteristics of participants and their HBV vaccination status

Variables		Frequency (n=1,729)	Percentage (%)	Median	IQR
Study sites	Lao-Viet/Mitthapab	920	53.15%		
	Military hospital	529	35.65%		
	Mahosot hospital	190	11%		
Gender	Male	873	50.49%		
	Female	856	49.51%		
Age (years old)				28.7	23.4-40.8
Material status	Married	961	55.58%		
	Single	734	42.45%		
	Widowed	29	1.68%		
	Divorced/Separated	5	0.29%		
Occupation	Government official	709	41.01%		
	Student	406	23.48%		
	Trader	225	13.01%		
	Wife house	186	10.76%		
	Worker	63	3.64%		
	Unemployed	22	1.27%		
	Retired	20	1.16%		
	Business	18	1.04%		
	Other	80	4.62%		
Level of Education	Secondary	699	40.43%		
	Technical	442	25.56%		
	University	401	23.19%		
	Elementary	153	8.85%		
	Illiterate	31	1.19%		
	Child	3	0.17%		
Consultation	OPD	1,633	94.45%		
	Hospitalized	96	5.55%		
HBV vaccination	Yes	81	4.7%		
	No	1,648	95.3%		

The results were not statistically different of regardless of the RDTs used. The trend for the most accurate was Boson Biotech HBsAg Sen. 53.85 %, Spec. 99.44% (PPV 87.5%, NPV 96.7%) and accuracy 96.31%. CTK HBsAg was used in 35.86% (619/1729) of cases with Sen. 54.95 %, Spec. 98.48 % (PPV 86.2%, NPV 92.69%) and 92.08% accuracy. Coretest's HBsAg was used in 53.15% (920/1729) of cases with 53.38% of sensitivity, specificity 97.84% (PPV 80.68%, NPV 92.69%) in 91.41% accuracy.

Table 2: Sensitivity, Specificity, PPV, NPV and accuracy of each individual HBsAg RDT (N=1,729)

Assay of HBsAg	%Sensitivity (95%CI)	% Specificity (95% CI)	% Positive predictive Value	% Negative predictive Value	% Accuracy (95% CI)
Boson Biotech HBsAg	53.85 (25.13-80.78)	99.44 (96.89-99.99)	87.5 (48.19-98.14)	96.7 (94.22-98.14)	96.31%
CTK Biotech "OnSite" HbsAg	54.95 (44.16-65.4)	98.48 (97.04-99.34)	86.21 (75.4-92.72)	92.69 (91-94.09)	92.08%
Coretest® One Step HbsAg	53.38 (44.54-62.08)	97.84 (96.56-98.74)	80.68 (71.77-87.28)	92.55 (91.19-93.71)	91.41%

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