Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Performance of rapid tests for detection of HBsAg and anti-HBsAb in a large cohort, France

Julie Bottero[1,2,3,*], Anders Boyd[1,3,†], Joel Gozlan[4,†], Maud Lemoine[5], Fabrice Carrat[1,2,6], Anne Collignon[7], Nicolas Boo[8], Philippe Dhotte[9], Brigitte Varsat[10], Gérard Muller[11], Olivier Cha[12], Odile Picard[3], Jean Nau[13], Pauline Campa[3], Benjamin Silbermann[14], Marc Bary[15], Pierre-Marie Girard[1,2,3], Karine Lacombe[1,2,3]

[1]*Inserm UMR-S707, Paris, France;* [2]*Université Pierre et Marie Curie, Paris, France;* [3]*AP-HP, Hôpital St Antoine, Service de Maladies Infectieuses, Paris, France;* [4]*AP-HP, Hôpital St Antoine, Laboratoire de Virologie, Paris, France;* [5]*AP-HP, Hôpital St Antoine, Service d'Hépatologie, Paris, France;* [6]*AP-HP, Hôpital St Antoine, Unité de Santé Publique, Paris, France;* [7]*Laboratoire de biologie médicale St Marcel, Paris, France;* [8]*Direction de l'Action Sociale, de l'Enfance et de la Santé (DASES), Paris, France;* [9]*Centre de dépistage anonyme et gratuit (CDAG) du Figuier, Paris, France;* [10]*Département des Examens Périodiques de Santé (DEPS), CPAM de Paris, France;* [11]*Centre de dépistage anonyme et gratuit (CDAG) de Belleville, Paris, France;* [12]*AP-HP, Hôpital St Antoine, Policlinique Baudelaire, Paris, France;* [13]*Centre d'accueil, de soins et d'orientation, Médecin du Monde, Paris, France;* [14]*Unité de consultation et de soins ambulatoires (UCSA), Maison d'arrêt de la santé, Paris, France;* [15]*Centre Croix Rouge du Moulin Joly, Paris, France*

Background & Aims: The systematic use of rapid tests performed at points-of-care may facilitate hepatitis B virus (HBV) screening and substantially increase HBV infection awareness. The aim of this study was to evaluate the effectiveness of such tests for HBsAg and anti-HBsAb detection among individuals visiting a variety of healthcare centers located in a low HBV-prevalent area.
Methods: Three rapid tests for hepatitis B surface antigen (HBsAg) detection (VIKIA®, Determine™ and Quick Profile™) and one test for anti-hepatitis B surface antibody (anti-HBsAb) detection (Quick Profile™) were evaluated in comparison to ELISA serology. Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV, respectively) and area under the ROC curve were used to estimate test performance. Non-inferiority criteria of the joint Se, Sp were set at 0.80, 0.95.
Results: Among the 3956 subjects screened, 85 (2.1%) were HBsAg-positive and 2225 (56.5%) had a protective anti-HBsAb titer. Test Se and Sp (lower bound of 97.5% CI) were as follows: 96.5% (89.0%), 99.9% (99.8%) for Vikia®; 93.6% (80.7%), 100.0% (99.8%) for Determine™; and 90.5% (80.8%), 99.7% (99.5%) for Quick Profile™; with all three tests achieving minimal non-inferiority criteria. False negatives were typically observed in inactive HBsAg carriers. The anti-HBsAb Quick Profile™ test had excellent specificity (97.8%) and PPV (97.8%) albeit low sensitivity (58.3%), thus failing to establish non-inferiority.
Conclusions: All three HBsAg rapid tests could be considered ideal for HBV screening in low HBV-prevalent countries, given the ease of use, rapidity, and high classification probabilities. The anti-HBsAb Quick Profile™ could be considered reliable only for positive tests.

Keywords: Hepatitis B; Rapid tests; Screening.
Received 27 September 2012; received in revised form 31 October 2012; accepted 6 November 2012; available online 23 November 2012
* Corresponding author. Address: Service de Maladies Infectieuses, Hôpital St Antoine, 184, rue du faubourg St Antoine, 75012 Paris, France. Tel.: +33 6 24 28 68 73; fax: +33 01 49 28 21 49.
E-mail address: julie.bottero@sat.aphp.fr (J. Bottero).
† These authors contributed equally to this work.
Abbreviations: CHB, chronic hepatitis B; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; Anti-HBsAb, anti-HBs antibody; Anti-HBsAc, anti-hepatitis B core antibody; ELISA, enzyme-linked immuno-assay; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; AUROCs, area under the receiving operator curve; FPF, false positive fraction; TPF, true positive fraction.

Introduction

According to recent estimations, France has a low prevalence of chronic hepatitis B virus infection (CHB) as roughly 0.65% of those cases with health insurance are estimated to be infected [1,2]. Although the social security system provides a wide range of services targeted towards prevention and effective care, more than 280,000 people continue to live with chronic hepatitis B virus infection, of whom over 55% are unaware of their infection-status [1]. CHB diagnosis is therefore severely delayed in this group and often occurs when severe clinical repercussions, such as advanced stages of cirrhosis and/or hepatocellular carcinoma, are already present. As a result, it is estimated that over 1300 deaths per year are directly attributable to hepatitis B virus (HBV) in France [3].

Unawareness of HBV infection status could be explained by both the lack of knowledge among those at risk (i.e., subjects born in geographic regions with hepatitis B surface antigen (HBsAg) prevalence >2%, household contacts, sexual partners of subjects with CHB or intravenous drug users [4]) and the lack of recognition concerning the seriousness of its public health impact among general practitioners. Furthermore, the absence of national guidelines related to screening practices leads to further confusion, with highly variable screening protocols between healthcare

Research Article

centers. In order to remedy this inadequacy, the "National Hepatitis Plan, 2009–2012" [5] recommended increasing HBV screening and improving consistent reporting. One public health tool that could potentially drive such an increase is the use of rapid tests, which may facilitate access to screening services.

Until recently (2012), no HBV rapid test has been approved for use by European or North American regulatory agencies. Moreover, there have been very few studies validating their use in low HBV-prevalent countries, apart from those given by the tests' manufacturers, in which their performance has been mainly evaluated on serum samples rather than on whole blood specimens. We then aimed at conducting a multicenter, cross-sectional, single-arm evaluation of several rapid tests that could be used to identify the presence of serological markers typically used in screening for CHB infection.

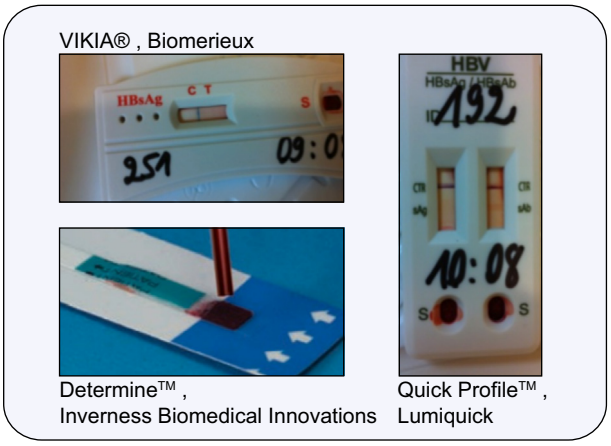

Fig. 1. Picture of the 3 rapid tests. (This figure appears in color on the web.)

Patients and methods

Study participants

From September 2010 to August 2011, 4000 subjects were recruited from ten, Paris-based healthcare centers whose aims involved screening, prevention and/or vaccination of diverse populations. Inclusion criteria for the present study were as follows: agreement to be screened for HBV, 18 years of age or older, and availability for a subsequent follow-up questionnaire via telephone. Participants without health coverage were also included [5]. All participants provided written informed consent and the protocol was approved by the Hôtel-Dieu Hospital Ethics Committee (Paris, France) in accordance with the Helsinki Declaration.

Rapid test comparisons and gold standard

Approximately 10 ml of whole blood was collected into a tube without any additive from each participant. Before the blood had yet to coagulate, a few drops were immediately removed from the sample and used for each rapid test according to manufacturers' instructions. Anticoagulant was not added to the sample because only serum was required for subsequent study procedures. Three tests for HBsAg detection (VIKIA®, Biomerieux, Marcy-l'Étoile, France; Determine™, Inverness Biomedical Innovations, Köln, Germany; Quick Profile™, Lumiquick, Santa Clara, CA, USA) and one test for anti-HBs antibody (anti-HBsAb) detection (Quick Profile™, Lumiquick) were evaluated (Fig 1). These qualitative tests are based on the principle of immunochromatography, in which membrane chromatography is used to determine the presence of polyclonal antibodies specific for HBsAg or anti-HBs antibody within a test region. In order to determine participants' "true" HBV status, serum was processed from whole blood and tested using a commercially-available enzyme-linked immuno-assay (ELISA) (MONOLISA AgHBS Ultra, anti-HBs plus, anti-hepatitis B core antibody-anti-HBc-plus, BIO-RAD, Hercules, USA). Only results of this testing were relayed to participants and their general practitioner. All participants found to have active HBV infection were asked if they would like to schedule a medical visit, during which a complete evaluation would be performed at a specialized clinic and therapy options would be discussed, if necessary. Additionally, all HBsAg-positive specimens had HBsAg quantification done using the ARCHITECT HBsAg enzyme-linked immunoassay (Abbott Laboratories, Rungis, France), and HBV DNA quantification, using the commercial quantitative polymerase chain reaction assay COBAS Taqman 48 HBV (Roche Diagnostic Systems, Meylan, France). For one specimen, HBV sequencing was performed on the *pol*/*S* region, as previously described [6]. The sequence was analyzed with the "HBV tool" accessible online at http://www.hiv-grade.de/cms/grade/hbv-tool.html.

Quality control of rapid tests

Rapid tests were performed immediately after the participant's sample was taken and in the same room as where blood collection occurred. Staff noted the date and time at which all tests were performed. Each rapid test had a control indicating whether the sample sufficiently migrated along the membrane (i.e., the test was performed correctly). In the event of an invalid test, two other attempts were made at most in order to achieve a valid result. Valid test results were then read within 30 min by two independent, previously-trained staff members (for a total number of 5 clinic research associates). Only results that the two readers agreed upon were included. However, if one reading was indeterminate while the other was definitive, the definitive reading was taken as the final result.

Statistical analysis

Rapid tests were compared to ELISA, which served as the gold standard. Sensitivity (Se), specificity (Sp), positive and negative predictive value (PPV and NPV, respectively), positive and negative likelihood ratio (LR+ and LR−, respectively) were estimated. Area under the ROC curves (AUROCs) were also calculated and compared between rapid tests using a test of equality of ROC areas. Inter-rater agreement was determined using the Kappa statistic, without taking into account indeterminate results.

Using a previously described method [7], we powered the study in order to test desirable levels of the pair [false positive fraction (FPF), true positive fraction (TPF)] at (0.02, 0.95). Non-inferiority criteria were then selected with minimally acceptable (FPF, TPF) at (0.05, 0.80), reflecting the importance of decreasing the number of false positives while increasing the number of cases identified [8]. We aimed at testing a one-sided, null hypothesis assuming a joint power of 0.90 and type I error (α) of 0.05. After accounting for an estimated prevalence of 2.0% from previous population-based studies within Paris [1] and correcting calculations on a 90% probability that the sample obtained will be at least as large as required, the minimum number of participants needed was 3384 and 489 (for enough diseased and non-diseased subjects, respectively). As both FPF and TPF are considered, the joint 95% confidence region is given from the 97.5% ($\sqrt{1-\alpha} = \sqrt{95\%}$) univariate intervals. For ease in clinical interpretation, we report the sensitivity (TPF) and specificity (1-FPF). Statistical analyses were performed using STATA (v11.2, College Station, TX, USA) statistical software.

Results

Study participants

At the end of the study, a total of 3956 subjects had at least one HBV rapid test with ELISA results and were hence included in the analysis. As discordant inter-rater results were excluded and the HBsAg Determine™ test was not available at the beginning of the study, but rather six months later, the number of participants varied among rapid tests (VIKIA®, N = 3928; Quick Profile™ HBsAg test, N = 3922, anti-HBsAb test, N = 3739; Determine™, N = 2472).

HBsAg rapid tests

Operator success and indeterminate results

Successful results were obtained on first attempt for the majority of rapid tests (Vikia®: 99.8%; Determine™: 100%; Quick Profile™: