



HAL
open science

Development and clinical validation of loop-mediated isothermal amplification (LAMP) assay to diagnose high HBV DNA levels in resource-limited settings

Jessica Vanhomwegen, Aurélia Kwasiborski, Abou Diop, Laure Boizeau, Damien Hoinard, Muriel Vray, Raymond Bercion, Babacar Ndiaye, Amélie Dublineau, Satoru Michiyuki, et al.

► To cite this version:

Jessica Vanhomwegen, Aurélia Kwasiborski, Abou Diop, Laure Boizeau, Damien Hoinard, et al.. Development and clinical validation of loop-mediated isothermal amplification (LAMP) assay to diagnose high HBV DNA levels in resource-limited settings. *Clinical Microbiology and Infection*, 2021, S1198-743X (21), pp.00149-X. 10.1016/j.cmi.2021.03.014 . inserm-03237803

HAL Id: inserm-03237803

<https://inserm.hal.science/inserm-03237803v1>

Submitted on 5 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

TITLE

Development and clinical validation of loop-mediated isothermal amplification (LAMP) assay to diagnose high HBV DNA levels in resource-limited settings

RUNNING TITLE

LAMP to diagnose high HBV DNA levels

AUTHORS

Jessica Vanhomwegen,^a Aurélia Kwasiborski,^a Abou Diop,^c Laure Boizeau,^b Damien Hoinard,^a Muriel Vray,^{d,e} Raymond Bercion,^c Babacar Ndiaye,^c Amélie Dublineau,^d Satoru Michiyuki,^f Jean-Claude Manuguerra,^a Virginie Sauvage,^b Daniel Candotti,^b Abdoulaye Seck,^{c,g} Syria Laperche,^b Yusuke Shimakawa,^{d,#}

AFFILIATIONS

^a Unité de recherche et d'expertise Environnement et risques infectieux, Institut Pasteur, Paris, France

^b Département des Agents Transmissibles par le Sang, Centre National de Référence Risques Infectieux Transfusionnels, Institut National de Transfusion Sanguine (INTS), Paris, France

^c Laboratoire de Biologie Médicale, Institut Pasteur de Dakar, Dakar, Sénégal

^d Unité d'Epidémiologie des Maladies Émergentes, Institut Pasteur, Paris, France

^e Unité d'Epidémiologie des Maladies Infectieuses, Institut Pasteur de Dakar, Dakar, Sénégal

^f Eiken Chemical Co.,Ltd, Fundamental Research Laboratory, 1381-3 Shimoishigami Ohtawara, Tochigi, Japan

^g Faculté de Médecine, Pharmacie et d'Odontologie, Université Cheikh Anta Diop de Dakar, Dakar, Sénégal

CORRESPONDING AUTHOR

Yusuke Shimakawa

Unité d'Épidémiologie des Maladies Émergentes, Institut Pasteur, 25-28 rue du Dr Roux, 75015 Paris, France

Phone: +33 (0) 1 40 61 38 87

Email: yusuke.shimakawa@pasteur.fr

WORD COUNT

Abstract 250 words

Main text 2499 words

ABSTRACT

Objectives

A massive scale-up of testing and treatment is indicated to globally eliminate hepatitis B virus (HBV) infection. However, access to a polymerase chain reaction (PCR), a key test to quantify HBV DNA levels and determine treatment eligibility, is limited in resource-limited countries. We developed and evaluated loop-mediated isothermal amplification (LAMP) assay to diagnose clinically important HBV DNA thresholds defined by WHO ($\geq 20,000$ and $\geq 200,000$ IU/mL).

Methods

Pan-genotypic primer sets were designed on conserved HBV gene regions. Accuracy of LAMP to identify highly viraemic patients was evaluated in 400 and 550 HBV-infected people in France and Senegal, respectively.

Results

Our primers successfully detected eight major HBV genotypes/sub-genotypes ($A_{1/2/3}/B/C/D/E/F$) with a detection limit ranging between 40-400 IU/mL. In France, the area under the receiver operating characteristic curve (AUROC), sensitivity and specificity of bead-based extraction and real-time turbidimetric LAMP were 0.95 (95% CI: 0.93-0.97), 91.1% and 86.0%, respectively, to diagnose HBV DNA $\geq 20,000$ IU/mL; and 0.98 (0.97-0.99), 98.0% and 94.6% for $\geq 200,000$ IU/mL. The performance did not vary by viral genotypes. In Senegal, using a field-adapted method (reagent-free boil-and-

spin extraction and inexpensive end-point fluorescence detection), the AUROC, sensitivity and specificity were 0.95 (0.93-0.97), 98.7% and 91.5%, respectively, to diagnose HBV DNA $\geq 200,000$ IU/mL. The assay was not adapted to discriminate low-level viraemia.

Conclusions

We developed a simple, rapid (60 minutes), and inexpensive (US\$8/assay) alternative to PCR to diagnose high viremia $\geq 200,000$ IU/mL. HBV-LAMP may contribute to eliminating HBV mother-to-child transmission by identifying high-risk pregnant women eligible for antiviral prophylaxis in resource-limited countries.

KEYWORDS

LAMP; hepatitis B; diagnosis; sensitivity and specificity; Africa

INTRODUCTION

Worldwide, 257 million people are chronically infected with hepatitis B virus (HBV) and 887,000 annually die from cirrhosis or liver cancer [1]. In 2016, WHO adopted a global strategy to eliminate HBV as a public health threat by 2030, and increasing treatment uptake from 8% to 80% in HBV-infected people eligible for antiviral therapy has become a key intervention [2]. Since >95% of HBV-infected people live in low-income and middle-income countries (LMICs) and only 12-25% of infected people are eligible for anti-HBV therapy [3,4], it is crucial to expand diagnostic services to assess treatment eligibility in these countries.

After testing positive for HBV surface antigen (HBsAg), a nucleic acid test (NAT) to quantify serum HBV DNA is required to determine the treatment eligibility. WHO recommends to initiate nucleos(t)ide analogues in HBV-infected patients with high viral load ($\geq 20,000$ IU/mL) who have persistently abnormal alanine transaminase (ALT) levels [5]. More recently, to prevent mother-to-child transmission (MTCT), tenofovir is recommended for HBsAg-positive pregnant women with very high viraemia ($\geq 200,000$ IU/mL) [6]. However, access to real-time polymerase chain reaction (PCR), a standard NAT to quantify HBV DNA, is severely limited in LMICs [7,8]. PCR is expensive (US\$20-130/assay), requires well-equipped laboratories and trained personnel [5,9]. WHO therefore underlines a critical unmet need for simple, low-cost point-of-care tests to evaluate HBV replications [1].

Loop-mediated isothermal amplification (LAMP) assay is a NAT using DNA polymerase with high auto-cycling strand displacement activity and six specially designed primers

[10]. In contrast with PCR requiring a thermal cycler, LAMP can be performed under constant temperature (60-67 °C) using a simple heat block or water bath. Moreover, LAMP has the following characteristics allowing its use as a rapid, reliable and inexpensive point-of-care test in LMICs: high amplification efficiency enabling rapid detection of nucleic acids; generation of a large amount of amplified products allowing the use of a simplified low-cost detector; and high tolerance to well-known PCR inhibitors facilitating the process of DNA extraction [11]. A commercial kit to detect tuberculosis or malaria costs only US\$6-8/reaction [12]. LAMP has therefore been put on the WHO List of Essential In Vitro Diagnostics, and recommended to be used in primary care setting to diagnose tuberculosis [13].

We therefore developed a pan-genotypic HBV-LAMP to diagnose high viral loads defined by the WHO guidelines ($\geq 20,000$ and $\geq 200,000$ IU/mL) [5,6,14] using two amplification protocols: a semi-quantitative real-time LAMP and a qualitative end-point LAMP. We then conducted clinical validation in France and Senegal.

METHODS

Primer design

Conserved HBV gene regions were selected by multiple sequence alignments of all genome sequences available in GenBank and National Reference Center of Infectious Risks in Blood Transfusion (CNR-RIT) database for eight genotypes (A-H). A set of six degenerated, pan-genotypic primers were designed using LAMP explorer software

(Premier Biosoft). Primer specificity and genotypic coverage were verified *in silico* by BLAST analysis. Twenty-six newly designed candidate primer sets and three from previous publications [15–17] were evaluated.

Real-time LAMP

Analytical validation was performed using real-time turbidimetric LAMP (Loopamp™ LA-500, Eiken Chemical, Japan). This was carried out in a 25 µL reaction volume with 5 µL of the nucleic acid sample, 40 pmol of forward and backward inner primers, 5 pmol of forward and backward outer primers, 20 pmol of loop forward and backward primers, sterile deionized water, a commercial freeze-dried reagent (Loopamp™ DNA Amplification Reagent D, LMP207, Eiken), 30mM KCl and 8 units of Bst 2.0 polymerase (New England Biolabs, USA). Reaction mixture was incubated at a constant temperature (65°C) for 60 minutes. Amplified DNA products were detected in real-time by measuring turbidity generated with a release of magnesium-pyrophosphate.

End-point LAMP

In Senegal, we evaluated end-point HBV-LAMP specifically designed to identify high-risk pregnant women ($\geq 200,000$ IU/ml) to prevent MTCT [6,18,19]. The optimal time-to-result threshold obtained from the real-time LAMP to diagnose $\geq 200,000$ IU/ml (*i.e.*, 40 minutes) was applied to stop a reaction using end-point LAMP incubator (HumaLoop M, HUMAN, Germany). The incubator accommodates 16 tubes/run; each run consisted of 40 minutes of isothermal amplification at 65°C, followed by 5 minutes of inactivation of

the enzyme at 80°C. Reaction tubes were read by naked eyes under ultraviolet light in the fluorescence visualization unit of HumaLoop.

Analytical validation

Analytical sensitivity and genotypic coverage were assessed using five replicates of 10-fold serial dilutions of the quantified synthetic DNAs. From GeneCust and NIBSC, we obtained plasmids containing synthetic DNA of the complete HBV genomes representing eight major genotypes/sub-genotypes (A1/A2/A3/B/C/D/E/F) with the following GenBank ID: AY233277/AY738140/AM180624/AB073843/AB111946/AB104712/AB194948/AY090456. For each genotype, an additional 15 replicates were tested at the lowest HBV DNA concentration detected in all five replicates. The limit of detection (LoD) was defined as the lowest concentration that was detected in $\geq 95\%$ of 20 replicates. Reproducibility (intra-assay and inter-assay variability) was evaluated by testing eight replicates per sample and per experiment by three independent operators using a clinical sample (genotype A3, HBV DNA: 1,200 IU/mL) and reported as a coefficient of variation of time-to-result values. Analytical specificity was evaluated using a panel of genomic extracts of HCV/HIV/cytomegalovirus/parvovirus.

Field-adapted extraction methods

To optimize HBV DNA extraction methods for LMICs, we evaluated efficiency of two simplified low-cost protocols: a magnetic bead-based extraction (Dynabeads SILANE Viral NA, Thermo Fisher Scientific, US) and a reaction-free “boil-and-spin”. The boil-

and-spin, originally described by Nyan [17], was optimized as below; plasma samples were diluted twofold in nuclease-free water, heated at 95°C for 5 minutes and centrifuged at 12000 g for 10 minutes. The supernatant (10 µL) was subsequently used to amplify HBV DNA. Using a serially diluted plasma sample infected with HBV genotype A1, viral DNA recovery following an in-house real-time PCR [20] was compared between three extraction methods: bead-based, boil-and-spin, and a reference spin-column extraction kit (QIAamp Viral DNA Mini Kit, Qiagen, USA).

Clinical validation in France

The real-time HBV-LAMP was retrospectively validated at the CNR-RIT, Paris, using well-characterized plasma samples from deferred blood donors found positive for HIV, HBV, or HCV across France. HBV DNA, HCV RNA, and HIV-1 RNA were detected/quantified using TaqMan-based COBAS system (Roche Diagnostics, France, LoD for HBV DNA: 4.8 IU/mL). HBV DNA-positive samples were genotyped by sequencing a 445-bp region in the S gene [21]. Of those collected in 2004-2018, 400 HBV mono-infected cases and 50 HBV DNA-negative controls (30 HIV mono-infected and 20 negative for HBV and HIV and HCV) were randomly selected. An investigator blinded to the PCR results performed the bead-based extraction and real-time HBV-LAMP.

Clinical validation in Africa

Field-adapted HBV-LAMP was retrospectively validated using 550 sera from HBsAg-positive patients stored at -80 °C at the Institut Pasteur de Dakar, Senegal. Viral loads

were quantified using a real-time PCR (AMPLIX, Biosynex, France, LoD: 26 IU/mL). To ensure good representation of the target conditions (HBV DNA $\geq 20,000$ and $\geq 200,000$ IU/mL), a total of 550 HBsAg-positive sera were randomly selected from four different strata defined by viral loads: undetectable (< 26 IU/mL, n=100); low (26-19,999 IU/mL, n=150); high (20,000-199,999 IU/mL, n=150); and very high ($\geq 200,000$ IU/ml, n=150). Viral genome extracted by the boil-and-spin method was mixed with the primer set and a commercial lyophilized reagent with calcein (LoopampTM RNA/DNA Amplification Reagent D, LMP247, Eiken) for both real-time and end-point HBV-LAMP. The study was approved by the Clinical Research Committee at the Institut Pasteur and the National Ethics Committee in Senegal, and reported in accordance with the STARD (Supplementary Table 1) [22].

Data analysis

Receiver operating characteristic (ROC) curve was used to select optimal cutoffs for the real-time LAMP (time-to-result) that maximize the sum of sensitivity and specificity to diagnose the target viral loads. Correlation between LAMP index (60 minutes/time-to-result (minutes)) and HBV DNA level (log IU/ml) was assessed using Pearson's correlation coefficient.

RESULTS

Analytical validation

Reaction conditions of HBV-LAMP were optimized to attain the best amplification efficiency and test reproducibility (data not shown). Of the 26 primer sets designed, the one with the best amplification efficiency was selected (Supplementary Table 2); this set performed better than those previously reported [15–17].

Eight major genotypes/sub-genotypes were all successfully detected by the turbidimetric HBV-LAMP with a LoD ranging from 40 to 400 IU/mL (Table 1). The intra-assay and inter-assay coefficient of variation was 8.51% and 7,94%, respectively. Genomic DNA extracts from HCV, HIV, cytomegalovirus, and parvovirus were not detected by HBV-LAMP. DNA extraction efficiency was comparable between the bead-based and the reference spin-column extraction, but inferior for the boil-and-spin (Supplementary Table 3).

Clinical evaluation in France

Supplementary Table 4 presents characteristics of 400 case samples. Median viral load was 3.3 log IU/mL (IQR: 2.7-4.0). There was broad distribution of HBV genotypes thanks to wide variation in their country of origin: genotype A (n=87), B (66), C (72), D (84), E (76), and F (15). Using the bead-based extraction and the real-time turbidimetric LAMP, a total of 282/400 (71%) HBV DNA-positive samples were detected, with a median time-to-result of 24.5 minutes (IQR: 20.1-30.4). All the samples undetected by the LAMP (n=118) had viral loads below our target condition of 20,000 IU/mL (median: 299 IU/mL, range: 11-18,296). In the samples detected by HBV-LAMP, a correlation

coefficient was $r=0.77$ ($p<0.0001$) between HBV-LAMP index and HBV DNA levels (Figure 1A). The correlation coefficient was $r=0.88$ and $r=0.19$ in samples with viral loads $\geq 20,000$ IU/mL and $<20,000$ IU/mL, respectively, demonstrating the linearity of the assay in samples with high viral loads. In each genotype, the linearity was similarly observed for the samples with viral loads $\geq 20,000$ IU/mL (Figure 1B).

Table 2 presents the performance of HBV-LAMP to discriminate between low and high viraemia. The AUROC was 0.95 (95% CI: 0.93-0.97) for $\geq 20,000$ IU/mL and 0.98 (0.97-0.99) for $\geq 200,000$ IU/mL. The optimal cutoff, sensitivity, and specificity were 23.3 minutes, 91.1%, and 86.0% for $\geq 20,000$ IU/mL, and 20.1 minutes, 98.0%, and 94.6% for $\geq 200,000$ IU/mL, respectively. The AUROC was high across the genotypes (Figure 2): A (0.92), B (0.98), C (0.96), D (0.93), E (0.95) and F (0.88) for $\geq 20,000$ IU/mL; and A (0.99), B (0.99), C (0.97), D (0.97), E (0.98) and F (0.96) for $\geq 200,000$ IU/mL. The performance was intermediate to discriminate viral load threshold of $\geq 2,000$ IU/mL (Table 2). None of the 50 samples negative for HBV DNA reacted with HBV-LAMP.

Clinical evaluation in Senegal

In 550 HBsAg-positive samples, median viral load was 4.4 log IU/mL (IQR: 2.3-5.6). The proportion detected by the real-time LAMP was 0% (0/100), 0% (0/150), 40.7% (61/150), and 98.7% (148/150), in undetectable (<26 IU/mL), low viraemia (26-19,999 IU/mL), high viraemia (20,000-199,999 IU/mL), and very high viraemia ($\geq 200,000$ IU/mL), respectively. In those detected by the LAMP ($n=209$), the median time-to-result

was 29.8 minutes (IQR: 25.9-37.2). The correlation coefficient between the HBV-LAMP index and viral loads was $r=0.80$ ($p<0.0001$) (Figure 1C). The AUROC of the real-time LAMP was 0.85 (95% CI: 0.82-0.87) for $\geq 20,000$ IU/mL and 0.98 (0.97-0.99) for $\geq 200,000$ IU/mL (Table 2). The optimal cutoff, sensitivity, and specificity were 60.0 minutes, 69.7%, and 100% for $\geq 20,000$ IU/mL, and 40.0 minutes, 94.0%, and 93.5% for $\geq 200,000$ IU/mL, respectively. HBV-LAMP was not adapted to diagnose viral loads $\geq 2,000$ IU/mL (Table 2).

Finally, the end-point LAMP was evaluated to diagnose very high viraemia ($\geq 200,000$ IU/mL). The proportion detected by the end-point LAMP, stopped at 40 minutes, was 0% (0/100), 0% (0/150), 22.7% (34/150), and 98.7% (148/150), in undetectable (<26 IU/mL), low (26-19,999 IU/mL), high (20,000-199,999 IU/mL), and very high viraemia group ($\geq 200,000$ IU/mL), respectively. The sensitivity and specificity of the end-point LAMP were 98.7% and 91.5% to diagnose $\geq 200,000$ IU/mL (Table 2).

DISCUSSION

This is the first study that developed a field-adapted pan-genotypic HBV-LAMP assay, aiming to assist clinicians to diagnose high viral loads indicating anti-HBV treatment eligibility. Irrespective of HBV genotypes, we found a sufficient accuracy of the real-time HBV-LAMP to diagnose clinically important viraemia of $\geq 20,000$ and $\geq 200,000$ IU/mL. Moreover, using the reagent-free boil-and-spin extraction and the low-cost robust

LAMP incubator, we observed high accuracy (sensitivity 98.7%, specificity 91.5%) of the end-point HBV-LAMP to diagnose very high viraemia $\geq 200,000$ IU/mL in Senegal.

Although LAMP has been evaluated to diagnose HBV, its primary focus has been to detect small amounts of HBV DNA rather than to identify highly viraemic patients who need antiviral therapy [15–17,23,24]. Two studies from China assessed LAMP to quantify HBV DNA [15,16]. However, both focused on local viral genotypes (B/C), and we confirmed a lack of reaction of these primers with other genotypes.

In order to optimize the assay for LMICs, we examined two distinct methods for viral genome extraction, and for amplification/detection. Table 3 compares their operational characteristics by referring to WHO-ASSURED criteria for ideal point-of-care tests in LMICs [25]. The bead-based extraction and real-time turbidimetry provides semi-quantitative results allowing to diagnose both WHO's viral load thresholds: $\geq 20,000$ to treat HBV-infected adults and $\geq 200,000$ IU/mL to prevent MTCT [5,6]. As a drawback, the turbidimeter costs US\$18,000 and extraction reagents requires a cold chain. In contrast, the boil-and-spin extraction and end-point fluorescence detection only provides qualitative results just to diagnose $\geq 200,000$ IU/mL, however, this is likely to be readily applicable to LMICs as a simple, rapid (60 minutes), and affordable (US\$8/reaction) point-of-care test to identify high-risk pregnant women for MTCT. To date, over a hundred end-point LAMP incubators (HumaLoop) have been deployed in LMICs for tuberculosis or malaria program; this only requires a two-day training for local laboratory technicians, no need for regular maintenance, and high throughput (70/day) with 16 modules [26,27]. The risk of contamination is minimal as results can be determined

without opening reaction tubes [28]. In Africa, a prospective study is ongoing to evaluate the feasibility, acceptability, and cost-effectiveness of HBV-LAMP in rural health centers. Moreover, a mobile solar panel for LAMP incubator has been commercialized [12], a potential solution to decentralize the assays in areas with limited electronic supply.

The study has limitations. First, HBV genotypes were not available in samples from Senegal, where the major genotypes are E and A [29,30]. Second, our assay was not adapted to detect low-level viraemia. Our objective was to develop a tool to assist clinicians to evaluate the WHO's treatment eligibility in LMICs, and not to monitor the response to treatment, to diagnose occult HBV infection or to define treatment eligibility in resource-rich countries. Third, HBV-LAMP was evaluated using different extraction methods according to the country. In France, we used bead-based extraction which provided better amplification efficiency (ie, faster time-to-result) than boil-and-spin; the latter does not completely remove inhibitors leading to a slower LAMP reaction. In contrast, boil-and-spin was used in Senegal because this method does not require reagent, resulting in a lower cost and no need for refrigerator.

To achieve the WHO's global elimination goals, it is critical to have a simple, reliable and affordable diagnostic test to evaluate active HBV replication. HBV-LAMP, an attractive alternative to PCR, deserves further field evaluation.

ACKNOWLEDGEMENTS

This work was supported by the Institut Carnot Pasteur Maladies Infectieuses [ValoExpress: ANR11-CARN017-01]; and the Alliance for Accelerating Excellence in Science in Africa (AESA) through Grand Challenges Africa [GCA/ISG/Round1/045]. We thank Cassandre von Platen, Sandrine Fernandes-Pellerin, Magali Herrant, Mai Ban, Yasuyoshi Mori, and Tsuyoshi Shindome.

CONFLICT OF INTEREST

MS is employee of Eiken Chemical. The others declare no conflict of interest.

REFERENCES

- [1] WHO. Global hepatitis report, 2017. Geneva, Switzerland: 2017.
- [2] WHO. Global Health Sector Strategy on Viral Hepatitis 2016-2021. Geneva, Switzerland: 2016.
- [3] Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018;3:383–403. [https://doi.org/10.1016/S2468-1253\(18\)30056-6](https://doi.org/10.1016/S2468-1253(18)30056-6).
- [4] Tan M, Bhadoria AS, Cui F, Tan A, Van Holten J, Easterbrook P, et al. Estimating the proportion of people with chronic hepatitis B virus infection eligible for hepatitis B antiviral treatment worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2021;6:106–19. [https://doi.org/10.1016/S2468-1253\(20\)30307-1](https://doi.org/10.1016/S2468-1253(20)30307-1).
- [5] WHO. Guidelines on hepatitis B and C testing. Geneva, Switzerland: 2017.
- [6] WHO. Prevention of mother-to-child transmission of hepatitis B virus: Guidelines on antiviral prophylaxis in pregnancy. Geneva, Switzerland: 2020.
- [7] Andriamandimby SF, Olive MM, Shimakawa Y, Rakotomanana F, Razanajatovo IM, Andrianinarivomanana TM, et al. Prevalence of chronic hepatitis B virus infection and infrastructure for its diagnosis in Madagascar: Implication for the WHO's elimination strategy. *BMC Public Health* 2017;17:636. <https://doi.org/10.1186/s12889-017-4630-z>.
- [8] Ishizaki A, Bouscaillou J, Luhmann N, Liu S, Chua R, Walsh N, et al. Survey of programmatic experiences and challenges in delivery of hepatitis B and C testing

- in low- and middle-income countries. *BMC Infect Dis* 2017;17:696.
<https://doi.org/10.1186/s12879-017-2767-0>.
- [9] Tordrup D, Hutin Y, Stenberg K, Lauer JA, Hutton DW, Toy M, et al. Additional resource needs for viral hepatitis elimination through universal health coverage: projections in 67 low-income and middle-income countries, 2016–30. *Lancet Glob Heal* 2019;7:e1180–8. [https://doi.org/10.1016/S2214-109X\(19\)30272-4](https://doi.org/10.1016/S2214-109X(19)30272-4).
- [10] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 2000;28:E63. <https://doi.org/10.1093/nar/28.12.e63>.
- [11] Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J Infect Chemother* 2009;15:62–9. <https://doi.org/10.1007/s10156-009-0669-9>.
- [12] FIND. Negotiated Prices LOOPAMP™ 2019. <https://www.finddx.org/pricing/loopamp/> (accessed January 7, 2020).
- [13] WHO. First WHO Model List of Essential In Vitro Diagnostics. Geneva, Switzerland: 2019.
- [14] WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva, Switzerland: 2015.
- [15] Cai T, Lou G, Yang J, Xu D, Meng Z. Development and evaluation of real-time loop-mediated isothermal amplification for hepatitis B virus DNA quantification: A new tool for HBV management. *J Clin Virol* 2008;41:270–6. <https://doi.org/10.1016/j.jcv.2007.11.025>.

- [16] Cai Z, Lou G, Cai T, Yang J, Wu N. Development of a novel genotype-specific loop-mediated isothermal amplification technique for Hepatitis B virus genotypes B and C genotyping and quantification. *J Clin Virol* 2011;52:288–94. <https://doi.org/10.1016/j.jcv.2011.08.013>.
- [17] Nyan D-C, Ulitzky LE, Cehan N, Williamson P, Winkelman V, Rios M, et al. Rapid Detection of Hepatitis B Virus in Blood Plasma by a Specific and Sensitive Loop-Mediated Isothermal Amplification Assay. *Clin Infect Dis* 2014;59:16–23. <https://doi.org/10.1093/cid/ciu210>.
- [18] Terrault NA, Lok ASF, McMahon BJ, Chang K-M, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018;67:1560–99. <https://doi.org/10.1002/hep.29800>.
- [19] European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67:370–98. <https://doi.org/10.1016/j.jhep.2017.03.021>.
- [20] Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, et al. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood* 2003;101:2419–25. <https://doi.org/10.1182/blood-2002-04-1084>.
- [21] Servant-Delmas A, Mercier M, El Ghouzzi MH, Girault A, Bouchardeau F, Pillonel J, et al. National survey of hepatitis B virus (HBV) polymorphism in asymptomatic HBV blood donors from 1999 to 2007 in France. *Transfusion* 2010;50:2607–18. <https://doi.org/10.1111/j.1537-2995.2010.02725.x>.

- [22] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Paul P, Irwig L, et al. RESEARCH METHODS & REPORTING STARD 2015 : an updated list of essential items for. *Radiology* 2015;277:826–32. <https://doi.org/10.1136/bmj.h5527>.
- [23] Akram A, Islam SMR, Munshi SU, Tabassum S. Detection of Hepatitis B Virus DNA among Chronic and potential Occult HBV patients in resource-limited settings by Loop-Mediated Isothermal Amplification assay. *J Viral Hepat* 2018;25:1306–11. <https://doi.org/10.1111/jvh.12931>.
- [24] Chen CM, Ouyang S, Lin LY, Wu LJ, Xie TA, Chen JJ, et al. Diagnostic accuracy of LAMP assay for HBV infection. *J Clin Lab Anal* 2020;34:1–9. <https://doi.org/10.1002/jcla.23281>.
- [25] Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. *Nat Rev Microbiol* 2004;2:231–40. <https://doi.org/10.1038/nrmicro841>.
- [26] Kaku T, Minamoto F, D’Meza R, Morose W, Boncy J, Bijou J, et al. Accuracy of LAMP-TB method for diagnosing tuberculosis in Haiti. *Jpn J Infect Dis* 2016;69:488–92. <https://doi.org/10.7883/yoken.JJID.2015.519>.
- [27] Global Laboratory Initiative. GLI Practical Guide to TB Laboratory Strengthening. Geneva, Switzerland: 2017.
- [28] Tomita N, Mori Y, Kanda H, Notomi T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat Protoc* 2008;3:877–82. <https://doi.org/10.1038/nprot.2008.57>.

- [29] Vray M, Debonne J, Sire J-M, Tran N, Chevalier B, Plantier J-C, et al. Molecular epidemiology of hepatitis B virus in Dakar, Sénégal. *J Med Virol* 2006;78:329–34. <https://doi.org/10.1002/jmv.20544>.
- [30] Jaquet A, Nouaman M, Tine J, Tanon A, Anoma C, Inwoley A, et al. Hepatitis B treatment eligibility in West Africa: Uncertainties and need for prospective cohort studies. *Liver Int* 2017;37:1116–21. <https://doi.org/10.1111/liv.13484>.

TABLES

Table 1. Detection limit of HBV-LAMP for eight reference HBV genotypes

HBV genotype	Real-time turbidimetric LAMP				
	Limit of detection (IU/mL)*	Mean time-to-result (minutes)	Number of replicates	Number detected	% detected
A1	400	27.54	20	19	95
A2	400	26.32	20	19	95
A3	400	24.41	20	20	100
B	400	24.61	20	19	95
C	40	33.54	20	19	95
D	400	27.41	20	20	100
E	400	32.04	20	19	95
F	400	29.13	20	20	100

* The limit of detection was defined as the lowest HBV DNA concentration that was detected in $\geq 95\%$ of 20 replicates.

Table 2. Performance of HBV-LAMP to diagnose high HBV DNA levels using different extraction/detection methods

	European study (n=400)	African study (n=550)	
	Method 1	Method 2	Method 3
Extraction method	Bead-based	Boil-and-spin	Boil-and-spin
Detection method	Real-time turbidimetry	Real-time turbidimetry	End-point fluorescence
To diagnose HBV DNA level $\geq 2\ 000$ IU/mL			
AUROC (95% CI)	0.86 (0.82-0.89)	0.80 (0.77-0.82)	N/A
Optimal cutoff	25.3 minutes	60.0 minutes	
Sensitivity, % (95% CI)	65.5 (58.5-72.1)	59.4 (54.0-64.5)	
Specificity, % (95% CI)	89.5 (84.4-93.4)	100 (98.2-100)	
To diagnose HBV DNA level $\geq 20\ 000$ IU/mL			
AUROC (95% CI)	0.95 (0.93-0.97)	0.85 (0.82-0.87)	N/A
Optimal cutoff	23.3 minutes	60.0 minutes	
Sensitivity, % (95% CI)	91.1 (82.6-96.4)	69.7 (64.1-74.8)	
Specificity, % (95% CI)	86.0 (81.7-89.6)	100 (98.5-100)	
To diagnose HBV DNA level $\geq 200\ 000$ IU/mL			
AUROC (95% CI)	0.98 (0.97-0.99)	0.98 (0.97-0.99)	0.95 (0.93-0.97)
Optimal cutoff	20.1 minutes	40.0 minutes	40.0 minutes
Sensitivity, % (95% CI)	98.0 (89.6-100)	94.0 (88.9-97.2)	98.7 (95.3-99.8)
Specificity, % (95% CI)	94.6 (91.6-96.7)	93.5 (90.6-95.7)	91.5 (88.3-94.0)

N/A, not applicable

Table 3. Operational characteristics of two HBV-LAMP methods according to the WHO ASSURED criteria for a point-of-care test in a resource-limited settings [25]

	Bead-based extraction & real-time turbidimetry	Boil-and-spin extraction & end-point fluorescence
<u>A</u>ffordable by those at risk of infection	(+) Reagent cost: US\$ 14 Instrument cost: US\$ 18,000	(+++) Reagent cost: US\$ 8 Instrument cost: US\$ 2,750
<u>S</u>ensitive (few false-negatives)	(+++) High clinical sensitivity to diagnose the two viral load thresholds defined by the WHO ($\geq 20,000$ IU/mL to treat chronic hepatitis B & $\geq 200,000$ IU/mL to prevent mother-to-child transmission)	(+) High clinical sensitivity to diagnose only one viral load threshold ($\geq 200,000$ IU/mL to prevent mother-to-child transmission)
<u>S</u>pecific (few false-positives)	(+++)	(+++)
<u>U</u>ser-friendly (simple to perform and requiring minimal training)	Field study ongoing	Field study ongoing
<u>R</u>apid (to enable treatment at first visit) and <u>R</u>obust (does not require refrigerated storage)	(+) 95 min (30 min for extraction & 60 min for amplification & 5 min for detection) (-) Reagent for bead-based extraction requires refrigerated storage	(+++) 60 min (15 min for extraction & 40 min for amplification & 5 min for detection) (+++) No cold chain requirement
<u>E</u>quipment-free	(-) Water bath Realtime turbidimetry	(-) Water bath Centrifuge Incubator with UV light
<u>D</u>elivered to those who need it	Field study ongoing	Field study ongoing

FIGURE LEGENDS

Figure 1. Correlation between HBV-LAMP index (60 min / time-to-result min) and HBV DNA levels quantified by PCR

1A. Using bead-based extraction in France (n = 282)*†

1B. Using bead-based extraction in France, by viral genotype (n = 282)*

1C. Using boil-and-spin extraction in Senegal (n = 209)*

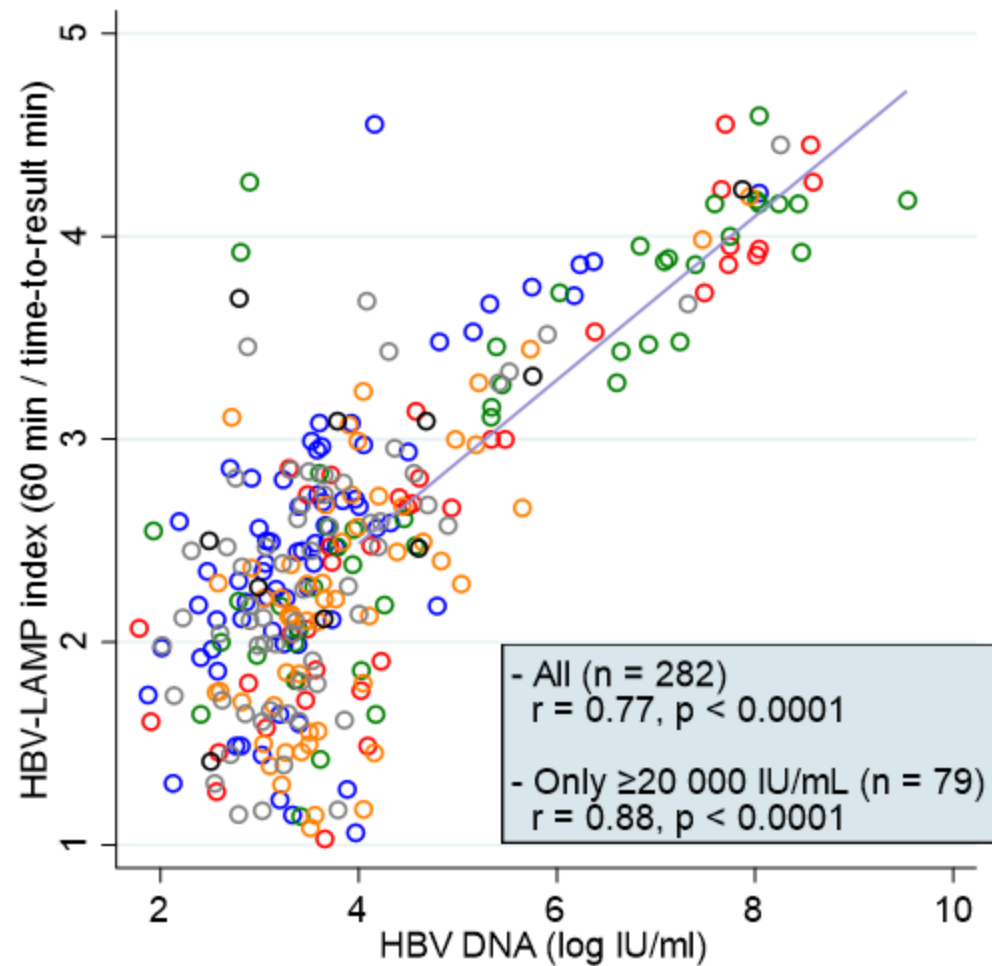
Figure 2. Receiver operating characteristic (ROC) curves to indicate elevated serum HBV DNA levels by different HBV genotypes (n=400)

2A. To diagnose HBV DNA level $\geq 20,000$ IU/ml

2B. To diagnose HBV DNA level $\geq 200,000$ IU/ml

Figure 1. Correlation between HBV-LAMP index (60 min / time-to-result min) and HBV DNA levels quantified by PCR

1A. Using bead-based extraction in France (n = 282)*†



* These analyses included only the samples detected using HBV-LAMP. The regression line was drawn in a range of HBV DNA levels ≥20,000 IU/mL.

† The color of circle indicates HBV genotypes.

Genotype 1: blue

Genotype 2: red

Genotype 3: green

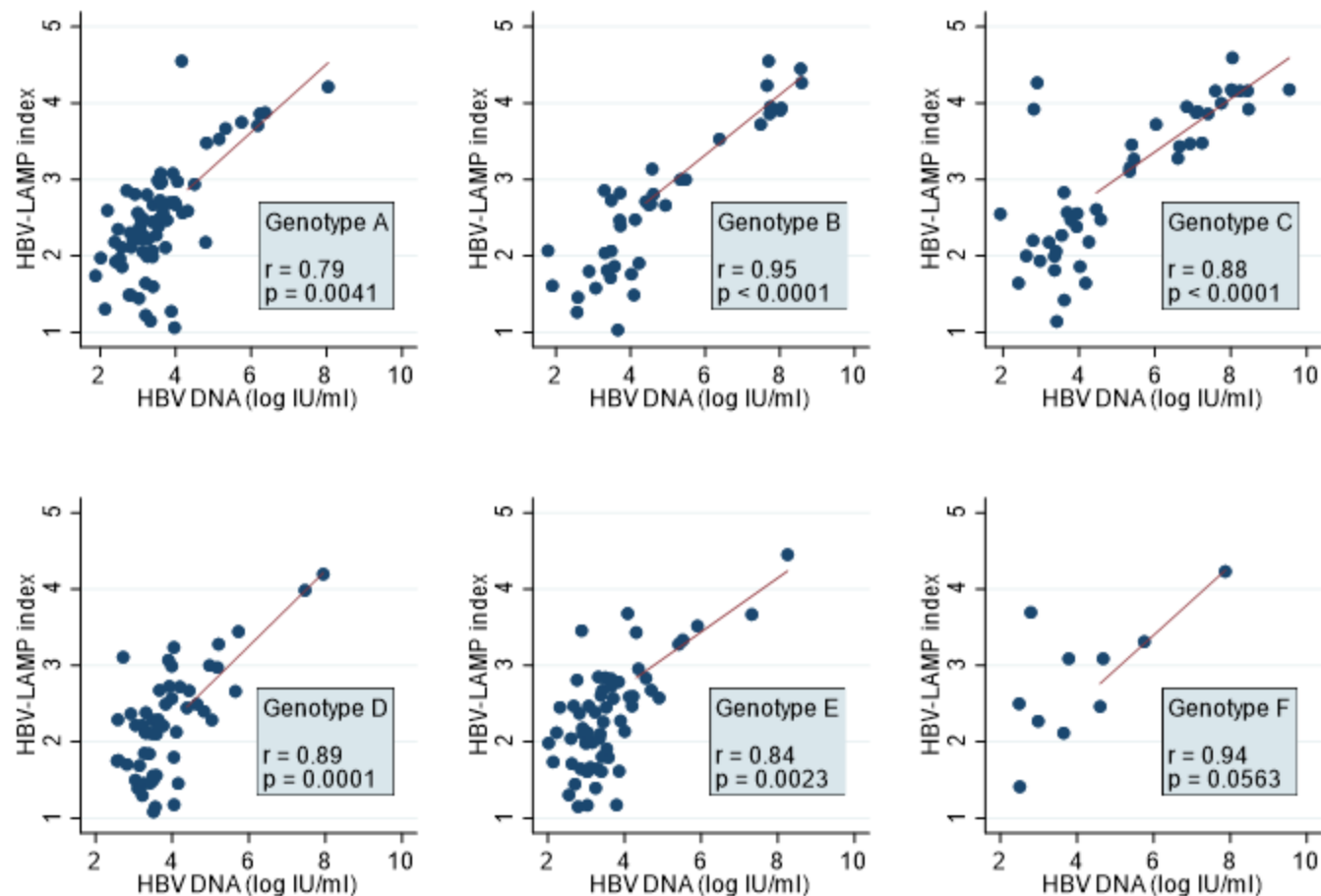
Genotype 4: orange

Genotype 5: gray

Genotype 6: black

Figure 1. Correlation between HBV-LAMP index (60 min / time-to-result min) and HBV DNA levels quantified by PCR

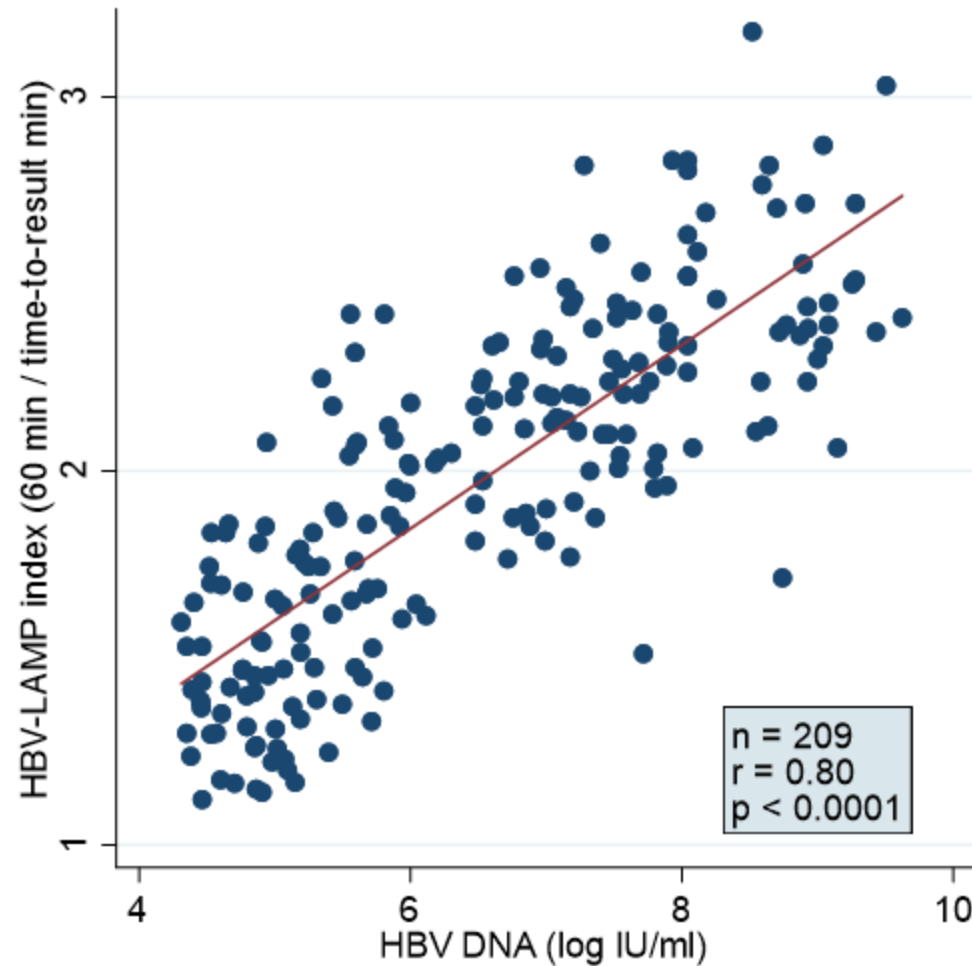
1B. Using bead-based extraction in France, by viral genotype (n = 282)*



* These analyses included only the samples detected using HBV-LAMP. The regression line was drawn in a range of HBV DNA levels $\geq 20,000$ IU/mL.

Figure 1. Correlation between HBV-LAMP index (60 min / time-to-result min) and HBV DNA levels quantified by PCR

1C. Using boil-and-spin extraction in Senegal (n = 209)*



* These analyses included only the samples detected using HBV-LAMP. The regression line was drawn in a range of HBV DNA levels $\geq 20,000$ IU/mL.

Figure 2. Receiver operating characteristic (ROC) curves to indicate elevated serum HBV DNA levels by different HBV genotypes (n=400)

2A. To diagnose HBV DNA level $\geq 20,000$ IU/ml

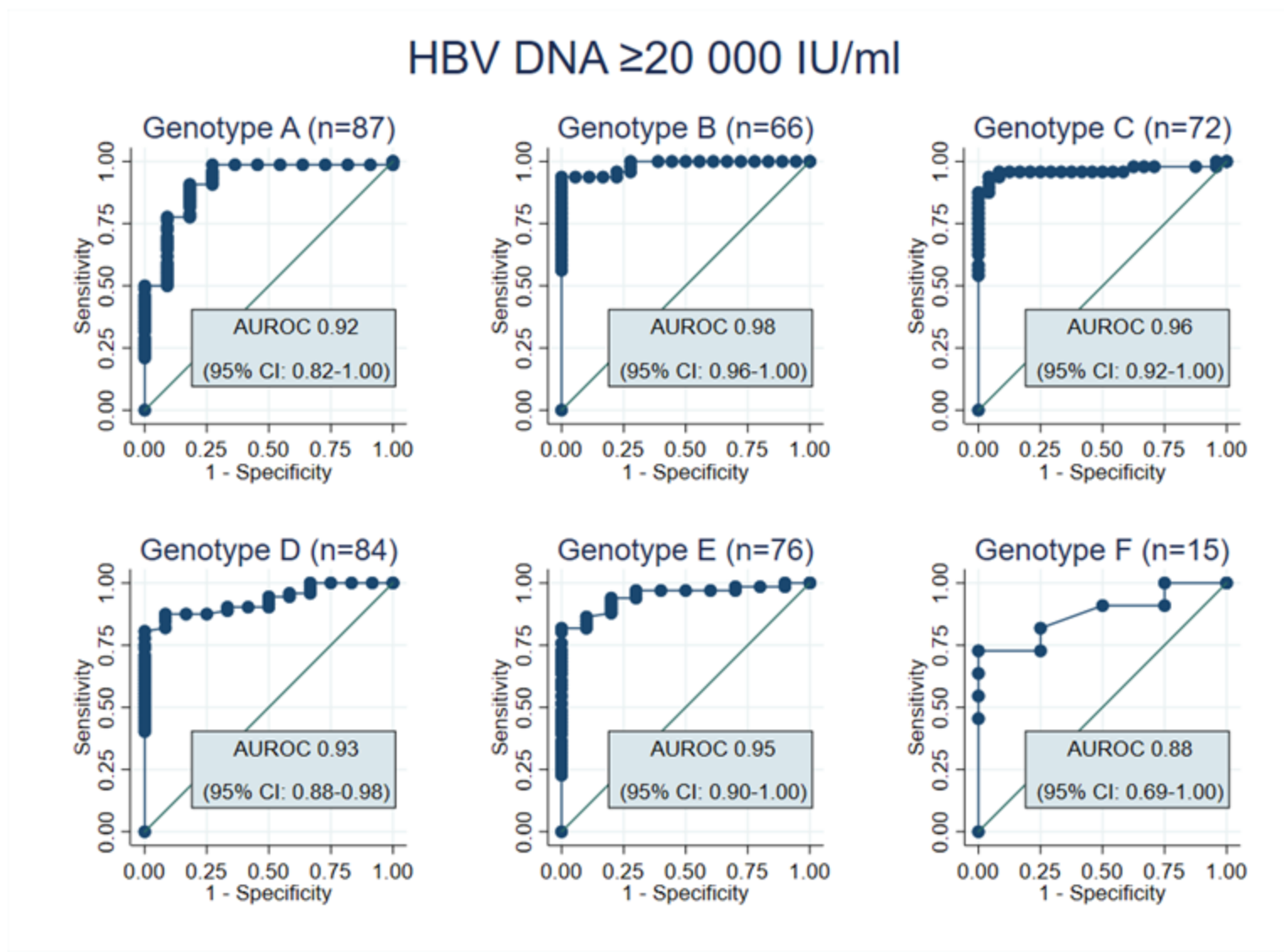


Figure 2. Receiver operating characteristic (ROC) curves to indicate elevated serum HBV DNA levels by different HBV genotypes (n=400)

2B. To diagnose HBV DNA level $\geq 200,000$ IU/ml

