Received: 4 October 2017 | Accepted: 25 March 2018

DOI: 10.1111/jvh.12915

ORIGINAL ARTICLE

WILEY JOURNAL OF VIRAL HEPATITIS JVH

Detection of in vivo hepatitis B virus surface antigen mutations—A comparison of four routine screening assays

M. Gencay[1] | A. Seffner[2] | S. Pabinger[3] | J. Gautier[4] | P. Gohl[5] | M. Weizenegger[2] | D. Neofytos[1] | R. Batrla[1] | A. Woeste[6] | H. S. Kim[7] | G. Westergaard[1] | C. Reinsch[8] | E. Brill[5] | P. T. T. Thuy[9] | B. H. Hoang[10] | M. Sonderup[11] | C. W. Spearman[11] | G. Brancaccio[12] | M. Fasano[13] | G. B. Gaeta[12] | T. Santantonio[13] | W. E. Kaminski[5]

[1]Roche Diagnostics International Ltd, Rotkreuz, Switzerland

[2]Department of Molecular Genetics and Microbiology, MVZ Labor Dr. Limbach & Kollegen GbR, Heidelberg, Germany

[3]Health and Environment Department, Molecular Diagnostics, Austrian Institute of Technology, Vienna, Austria

[4]Cerba Spécimen Services, Saint-Ouen l'Aumône, France

[5]Bioscientia, Institute for Medical Diagnostics GmbH, Ingelheim, Germany

[6]Roche Diagnostics GmbH, Penzberg, Germany

[7]Department of Laboratory Medicine, Yonsei University College of Medicine, Severance Hospital, Seoul, Korea

[8]Roche Diagnostics GmbH, Mannheim, Germany

[9]Hepatology Department, Medic Medical Center, Ho Chi Minh City, Vietnam

[10]Gastroenterology Department, Ho Chi Minh City University Medical Center, Ho Chi Minh City, Vietnam

[11]Division of Hepatology and Department of Medicine, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

[12]Infectious Diseases and Viral Hepatitis Unit, University of Campania Luigi Vanvitelli, Naples, Italy

[13]Infectious Diseases Unit, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Correspondence
Wolfgang E. Kaminski, Bioscientia Institut für Medizinische Diagnostik GmbH, Ingelheim, Germany.
Email: wolfgang.kaminski@bioscientia.de

Funding information
Roche Diagnostics International Ltd

Summary

An important requirement for a state-of-the-art hepatitis B surface antigen (HBsAg) screening assay is reliable detection of mutated HBsAg. Currently, there is a striking shortage of data regarding the detection rates of in vivo HBsAg mutations for these clinically important assays. Therefore, we compared the detection rates of four commercial HBsAg screening assays using a global cohort of 1553 patients from four continents with known HBV genotypes. These samples, which represent the broadest spectrum of known and novel HBsAg major hydrophilic region (MHR) mutations to date, were analyzed for the presence of HBsAg using the Roche Elecsys® HBsAg II Qualitative, Siemens ADVIA Centaur XP HBsAg II, Abbott Architect HBsAg Qualitative II and DiaSorin Liaison® HBsAg Qualitative assays, respectively. Of the 1553 samples, 1391 samples could be sequenced; of these, 1013 (72.8%) carried at least one of the 345 currently known amino acid substitutions (distinct HBsAg

Abbreviations: CI, confidence interval; COI, cut-off index; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MHR, major hydrophilic region.

mutation) in the HBsAg MHR. All 1553 patient samples were positive for HBsAg using the Elecsys® HBsAg II Qual assay, with a sensitivity (95% confidence interval) of 99.94% (99.64%-100%), followed by the Abbott Architect 99.81% (99.44%-99.96%), Siemens ADVIA 99.81% (99.44%-99.96%) and DiaSorin Liaison® 99.36% (98.82%-99.69%) assays, respectively. Our results indicate that the Elecsys® HBsAg II Qual assay exhibits the highest sensitivity among the commercial HBsAg screening assays, and demonstrate that its capacity to detect HBV infection is not compromised by HBsAg MHR mutants.

KEYWORDS
"a" determinant region, HBsAg mutations, HBV mutations, mutation spectrum

1 | INTRODUCTION

More than 200 million people worldwide carry the hepatitis B virus (HBV) infection,[1] which is associated with chronic liver illness in nearly half of the cases. Moreover, it poses a serious threat to blood transfusion safety, particularly in highly endemic countries.[2,3] In the clinical setting, detection of the hepatitis B surface antigen (HBsAg) by serological screening assays is key to establishing a diagnosis of HBV infection.

Increased mutation rates in the overlapping genes encoding the HBsAg and the HBV polymerase have been observed in patients with chronic HBV infection and may lead to escape of the virus from the host's defence mechanisms and the action of antiviral drugs.[4,5] The major hydrophilic region (MHR) of the HBV surface antigen gene spans amino acid residues 99-170, which is composed of several conformational epitopes.[6] Mutations in the region comprising residues 120-147 have been associated with diagnostic failure[6-12] as the majority of the antibodies used in commercial HBsAg immunoassays are also directed to this segment.[6,13] The immunodominant MHR "a" determinant region includes amino acid residues 124-147, which is divided into two subdomains—the first loop (aa 124-137) and the second loop (aa 139-147). Mutation-induced amino acid changes in this critical region may give rise to false-negative HBsAg immunoassay results; for example, the mutation G145R represents a frequently diagnosed and vaccine-escape mutation.[14] The major characteristic of the G145R mutation-containing virus is that it is stable and capable of replicating.[15] Other diagnostically relevant immune-escape mutations are located at—but not limited to—amino acid positions 120, 126, 129, 130, 131, 133, 142, 143 and 144.[7,8,11,16,17] Thus, the reliable detection of diagnostic and immune-escape-associated HBsAg mutations is a critical requirement for state-of-the-art HBsAg diagnostic screening assays.[6,18,19]

Previous studies have generally relied on laboratory-generated "recombinant" HBsAg mutants to evaluate the detection performance of commercial HBsAg assays.[6,19,20] However, a major shortcoming of the use of recombinant HBsAg mutants in this setting is that the resulting proteins do not necessarily reflect the complete and complex biostructure of the mutated HBsAg protein as it occurs in vivo in chronically HBV-infected patients. Moreover, synthetically produced HBsAg mutants by definition only represent a limited spectrum of the true diversity of HBsAg variants that exist worldwide. To address these difficulties, we assessed for the first time, the capacity of commercial HBsAg screening assays to detect HBsAg mutants in a four-continent cohort of patients with HBV infection. To date, this global cohort represents the largest pool of known HBsAg variants, as assessed by ultra-deep sequencing, worldwide (n = 345).

2 | MATERIALS AND METHODS

2.1 | Study population

This prospective observational study included samples from 1553 patients with HBV infection; blood samples were obtained between 17 January 2007 and 6 February 2015. Patients were defined as chronic HBV if they were diagnosed with HBV >6 months before enrolment (n = 562) and as "unselected random HBV" (n = 991) if samples were obtained from blood donation centres or vendors in Europe, South Africa and the USA. The study aimed to ensure that all HBV genotypes A-G were included. Additional inclusion criteria for randomly selected HBV-positive samples were HBV DNA (>100 IU/mL) or HBsAg positivity.

The samples of chronic HBV cases were collected in Korea and Vietnam with written informed consent obtained from each patient, and the study protocol was approved by the Ethics Committees of the participating hospitals in Korea (Severance Hospital, Seoul) and Vietnam (Medic Medical Center and Ho Chi Minh City University Medical Center, Ho Chi Minh City). In South Africa, random samples from HBV-positive blood donors were obtained with Ethics Committee approval (South African National Blood Services, Johannesburg). Institutional Review Board and Ethics Committee approvals were already available for the unselected random samples that were obtained from commercial vendors in the USA and Europe (SlieaGen, Austin, TX; Discovery Life Sciences, Los Osos, CA; and Boca Biolistics, Coconut Creek, FL). Deidentified random samples that were included in this study were provided by Bioscientia (Ingelheim, Germany). Ethics approval is not required for deidentified random samples in Germany.

TABLE 1 List of genotype-specific reference sequences used for phylogenetic analyses

Subtype	GenBank accession number
A1	U87734
A2	AY168427
B1	AB073855
B2	AY217359
B3	AB033554
B4	AB100695
C1	AB014368
C2	AF209393
C3	AF208876
C4	AB048704
D1	AY161157
D2	Z35716
D3	AF061523
D4	AB048701
E	AB091262
F1	AY264390
F2	AY264396
G	M74499
H	AF369536

2.2 | HBV genotype analysis and surface antigen mutant detection

HBV mutations of all patient samples were detected as recently published.[21] Briefly, PCR primers were designed to cover the S gene (defined as the region encoding amino acids 83-227) and HBV DNA was extracted from 200 μL serum or plasma samples using the MagNA Pure 96 instrument and the MagNA Pure 96 DNA kit (Roche Applied Science). Ultra-deep sequencing was performed following the instructions in the GS Junior+ Sequencing Method Manual (454/Roche Life Science, version April 2014). The evaluation of genotypes was carried out by phylogenetic analysis.

2.3 | Phylogenetic analysis

The raw sequencing output was processed by an integrated variant calling pipeline. First, the files were demultiplexed to assign the reads to the corresponding samples. Each sequencing read was then checked for sufficient length (250 bp), and primers and adapters were removed. Following this, reads were clustered using the UCHIME algorithm and the consensus sequence was assigned to the correct main type and subtype (Table 1). Genotypes with at least 100 reads and a Phred quality score >20 on both strands were subjected to variant calling in which we kept variants with an allele frequency >5%. Each identified variant was reported to the user in a summary table.

2.4 | HBsAg assays

All 1553 patient samples were tested as singletons using the fully automated Elecsys® HBsAg II Qual assay (Roche Diagnostics GmbH, Mannheim, Germany), the Abbott Architect HBsAg Qualitative II assay (Abbott Diagnostics, Wiesbaden, Germany), the ADVIA Centaur XP HBsAg II assay (Siemens Healthcare Diagnostics, Erlangen, Germany) and the DiaSorin Liaison® HBsAg Qualitative assay (DiaSorin S.p.A., Saluggia, Italy), respectively, according to the manufacturer's recommendations. Confirmatory testing was not performed given that all samples were originally obtained from HBV-infected patients or donors. Cut-off index (COI) values (Table 2) were used for the interpretation of the results of the four HBsAg qualitative assays.

2.5 | Statistics

Statistical analysis was independently performed at the AIT Austrian Institute of Technology (Vienna, Austria) in a multistep approach including data preprocessing, formatting, calculation and evaluation. For this, Python (version 2.7.6) scripts and the scientific computing module NumPy were used. Graphical visualizations were created using the matplotlib package.

3 | RESULTS

We collected plasma or serum samples from 1553 patients with HBV infection. The geographical distribution of the samples was as follows: Africa (n = 435; Cameroon, Dem. Republic of Congo, Guinea-Bissau, Ivory Coast, Senegal, South Africa and Sudan), Asia (n = 653; Philippines, South Korea, Thailand and Vietnam), Europe (n = 72; France, Germany and Spain), Latin America (n = 35; Argentina, Nicaragua, Peru and Venezuela), Middle East (n = 79; Saudi Arabia),

TABLE 2 Interpretation criteria of the commercial hepatitis B surface antigen (HBsAg) assays

Interpretation of results (COI)	Elecsys® HBsAg II Qual	Abbott Architect HBsAg Qual II	Siemens ADVIA Centaur XP HBsAg II	DiaSorin Liaison® HBsAg I
Negative	<0.9	<1.0	<1.0	<0.9
Positive	≥1.0	≥1.0	≥1.0	≥1.1
Greyzone/borderline	0.9 ≤ s/co <1.0	NA	NA	0.9 < × <1.1

COI, cut-off index.

TABLE 3 Sensitivity of the Elecsys® hepatitis B surface antigen (HBsAg) II Qual assay vs other HBsAg qualitative assays

	Elecsys® HBsAg II Qual	Abbott Architect HBsAg Qual II	Siemens ADVIA Centaur XP HBsAg II	DiaSorin Liaison® HBsAg I
Number of samples tested	1553	1553	1552[a]	1553
Sensitivity (95% CI)	99.94% (99.64%-100%)	99.81% (99.44%-99.96%)	99.81% (99.44%-99.96%)	99.36% (98.82%-99.69%)

CI, confidence interval.
[a]One sample was excluded from analysis because of sample volume restriction.

USA (n = 234) and unknown (n = 45). The mean age of chronic HBV patients and randomly collected donors was 39.7 (SD 13.14) and 39.9 (SD 12.50) years, respectively (age information for 395 deidentified or random samples was not available). HBV DNA positivity was confirmed in all patient samples.

3.1 | Mutations in the MHR "a" determinant region

Details of mutations identified in the MHR "a" determinant region and their distribution by geographical region and HBV genotype have been reported previously for the 1553 HBV-positive patient samples.[21] Briefly, 1391 of the 1553 samples (89.5%) were successfully genotyped and analyzed for mutations in the "a" determinant region prior to HBsAg measurement (the genotype or "a" determinant region mutations could not be identified for technical reasons in the remaining 162 samples). All HBV genotypes (A-G) were represented across the 1391 sequenced samples, of which 1013 (72.8%) samples contained a total of 2780 mutations. More specifically, and further to previously reported, 547 samples contained 1569 mutations located outside the immunodominant "a" determinant region and 844 samples contained 1211 mutations located within the immunodominant "a" determinant region. These 1211 mutations in the immunodominant "a" determinant region of 844 samples were subgrouped under "first loop" (83.8%, 1015/1211) and "second loop" (16.2%, 196/1211) mutations. A summary of the specific mutations identified was also reported previously.[21] A total of 98 (7.1%) sequenced samples revealed drug resistance-relevant polymerase region mutations. The overlapping mutation rate between the polymerase region and HBsAg gene was observed in 78 of 98 (79.5%) samples, and this rate was higher in genotype B and C samples. The majority of overlapping mutations occurred in the first loop of the "a" determinant region. The average coverage per mutation site that passed the cut-off criteria was 914-fold.

3.2 | Performance of commercial HBsAg assays

In this global cohort (n = 1553 samples), sensitivity of the Elecsys® HBsAg II Qual assay was 99.94% (95% CI: 99.64%-100%); followed by the Abbott Architect assay, 99.81% (95% CI: 99.44%-99.96%); the Siemens ADVIA assay, 99.81% (95% CI: 99.44%-99.96%); and the DiaSorin Liaison® assay, 99.36% (95% CI: 98.82%-99.69%) (Table 3). Of note, only one of the 1553 HBV-positive samples yielded a false-negative result utilizing the Elecsys® HBsAg II Qual assay. This sample was also negative using the Abbott Architect, Siemens ADVIA and DiaSorin Liaison® assays (Table 4). In additional serological analysis, this sample was found to be negative for anti-HBc immunoglobulin (Ig) M and anti-HBc (total) antibodies, but HBV DNA positive. Detailed sequence analysis revealed that this sample did not carry an HBsAg mutation. It is thus most likely that this sample represents a very early phase of HBV infection. Altogether, 10 samples generated discrepant results (detectable by one assay but not another) in all four commercial HBsAg assays. Whereas four of these carried mutations in the "a" determinant region, six did not exhibit any mutations in the MHR (Table 4). Unlike the three other assays, the DiaSorin Liaison® HBsAg assay failed to detect the HBsAg in all four of these mutation-bearing samples. Of note, one highly complex sample, which carried five substitutions (K122R, Q129H, G130N, M133T and D144A, respectively) and showed a mutation rate of 10%-73% depending on amino acid location, was successfully detected by the Elecsys® HBsAg II Qual assay but none of the three other assays. In a reciprocal setting, one nonmutated HBV sample was detected solely by the Elecsys® HBsAg II Qual assay and was missed by the three other HBsAg qualitative assays. The number of samples that could not be detected by each assay was as follows: Elecsys® HBsAg II Qual assay, n = 1; Abbott Architect HBsAg Qual II, n = 3; Siemens ADVIA Centaur XP HBsAg II, n = 3; and DiaSorin Liaison HBsAg I, n = 10.

A total of 52 different mutations have previously been associated with diagnostic and immune-escape problems (including P120T, G145R, G145A, M133L, Q129H, G130N, S143L, T126S, D144A, T131I and P142S).[22-35] All of these HBsAg variants were successfully detected by the Elecsys® HBsAg II Qual assay, and there was no difference in detection of these mutations between the commercial assays included in the study. The Elecsys® HBsAg II Qual assay and other commercial assays included in the study successfully identified all 78 samples with HBsAg gene mutations and overlapping POL mutations, as well as all samples with G145R and K122R mutations.

4 | DISCUSSION

This is the first report to assess the detection rates of in vivo HBsAg mutations in four major clinically relevant screening assays using a

TABLE 4 Details of the 10 samples that displayed discrepant results in the four hepatitis B surface antigen (HBsAg) tests

HBV genotype	MHR mutation	HBV DNA load (IU/mL)	Cut-off index			
			Elecsys® HBsAg II Qual	Abbott Architect HBsAg Qual II	Siemens ADVIA Centaur XP HBsAg II	DiaSorin Liaison® HBsAg I
A	None	2130	**0.690**	**0.247**	**0.100**	**0.010**
A	None	1620	3482	**0.506**	**0.715**	**0.200**
A	Multiple mutations (F134I, M133T, K122R, Q129H and D144A)	364	1.184	**0.716**	**0.901**	**0.800**
E	None	774	3897	3163	1000	**0.010**
E	G130E	697	1999	2055	1000	**0.010**
E	Multiple mutations (A128V, T118V and T123S)	286	7314	1830	1000	**0.010**
E	T115A	549	5891	3704	1000	**0.010**
E	None	267	3729	3654	1000	**0.200**
C	None	1720	4852	2954	1000	**0.010**
C	None	663	4383	3847	1000	**0.100**

COI, cut-off index; MHR, major hydrophilic region. A value <1 is negative (highlighted in bold).

global cohort of 1553 patients with characterized HBV genotypes. This cohort, from four continents, represents the most comprehensive spectrum of systematically characterized HBsAg MHR mutations to date. All assays performed well; however, the Elecsys® HBsAg II Qual assay exhibited the highest sensitivity, and its capacity to detect HBV infection was least compromised by HBsAg MHR mutants, compared with the other three commercial assays evaluated.

In our previous study, we noted a considerably higher mutation frequency (72%) in the MHR than previously reported.[21] This high mutation rate in HBV-infected patients not only underlines the potential threat to the performance of HBsAg assays but also the complexity of in vivo HBsAg mutations. Mutations in the immunodominant "a" determinant region, between amino acid locations 124 and 147, can potentially reduce the binding affinity of the HBsAg capture antibody of the assay and have a clinical and diagnostic impact in the HBV-infected patient population.[6,18] Consequently, this specific region harbours all currently known diagnostic and vaccine-escape mutations of the HBV.[8,11,16,17,36,37] In the present study, all assays performed well, despite the presence of 2780 mutations in 1391 well-characterized samples, including mutations associated with diagnostic and vaccine-escape problems. In this study, we focused on variants in the MHR region localized between amino acid residues 99-170. The Elecsys® HBsAg II Qual assay was the only assay to detect all positive samples, providing a sensitivity of 99.94% for the diagnosis of HBV infection. Interestingly, one of the HBV DNA-positive nonchronic donor samples was found to be HBsAg negative by all commercial HBsAg assays. Surprisingly, it did not have any detectable hepatitis B core antibody (anti-HBc) IgG and IgM antibodies. It is very likely that this sample represents the HBsAg negative early window of infection. However, these samples are likely rare and of minimal concern; in a recent study, for example, such cases were identified in 23 of 4.4 million blood donations in The Netherlands.[38]

Our findings agree with those from previous investigations with the Elecsys® HBsAg II Qual assay using native clinical samples from HBV-infected patients.[39,40] For example, Mühlbacher and colleagues reported that the Elecsys® HBsAg II Qual assay achieved a sensitivity of 100% with 156 HBV samples with different genotypes and 696 preselected HBsAg-positive samples.[40] Similarly, Louisirirotchanakul et al[39] reported equivalent high sensitivities and specificities for the Elecsys® HBsAg II and Architect HBsAg assays; however, the Elecsys® HBsAg II assay recognized a HBV genotype A native mutant sample with multiple amino acid substitutions (F134I, M133T, K122R, Q129H and D144A) that the other three commercial assays failed to detect. Importantly, the Elecsys® HBsAg II Qual assay uses one polyclonal and three monoclonal antibodies to detect HBsAg, which may be an advantage as all three monoclonal antibodies are able to bind to the surface antigen simultaneously and recognize different epitopes located on the surface antigen without competing for target epitopes. In contrast, the other commercial assays use monoclonal capture and tracer antibodies alone.[39,41] Taken together, our findings provide confidence regarding the detection of the broad

spectrum of in vivo HBsAg gene mutations that were found in HBV-infected patient samples.

The present study has several major strengths: (i) inclusion of a large number of samples from HBV-infected patients across different geographical locations, (ii) coverage of HBV genotypes A-G in a large number of samples, (iii) inclusion of well-characterized HBsAg gene mutated samples and (iv) use of the highly sensitive next-generation sequencing method for the sequencing and mutation characterization of the samples.[21] As a result, we were able to evaluate the mutated HBsAg detection performance of commercial HBsAg assays based on the *native* form of surface antigen mutated samples, which more realistically reflects the clinical situation and associated diagnostic challenges. In previous studies, "recombinant" HBsAg mutants were routinely used to validate the mutant detection performance of HBsAg assays.[6,25] However, there are notable limitations with the use of recombinant mutants, including challenges associated with their preparation and stabilization, aggregation, solubility, degradation during storage, impact of freeze-thaw cycles and lack of standardization in preparation and reproducibility by other laboratories.[42-44] Additionally, as demonstrated in our previous study, the "a" determinant region mutations in native samples are complex, with sample-to-sample mutation rates varying between 5% and 100%.[21] Some of the comparator assays were assumed to be effective for mutant detection based on the use of recombinant proteins; however, our study relying on native HBV samples suggests that such studies do not necessarily reflect the *bona fide* in vivo situation.[25,45]

In summary, the results of this large-scale global cohort study indicate that the Elecsys® HBsAg II Qual assay exhibits the highest sensitivity among the commercial HBsAg screening assays, which all performed well for the detection of HBV infection. Based on the results from a large number of sequenced native samples, including 345 different types of HBsAg mutation, we conclude that the capacity of the Elecsys® HBsAg II Qual assay to detect HBV infection is not compromised by HBsAg MHR mutants.

ACKNOWLEDGEMENTS

The authors would like to thank Albert Kriegner for his support on HBV mutation analysis using AIT/Platomics software. Special thanks go to Professor Markus Nauck (Bioscientia, Institute for Medical Diagnostics GmbH, Ingelheim, Germany) for his contribution to the study and to Bill Johnson (Roche Diagnostics, Indianapolis, USA), Gengqin Su and Michael Walter (Roche Diagnostics GmbH, Penzberg, Germany) for their excellent data management and Medrio database support. Editorial support under the guidance of the authors was provided by Kim Brown of Roche Diagnostics International Ltd, Rotkreuz, Switzerland, and by Lucy Carrier of Gardiner-Caldwell Communications, Macclesfield, UK (supported by funding from Roche Diagnostics International Ltd).

DISCLOSURES

The study was sponsored by Roche Diagnostics, who were involved in the study design, and data collection, interpretation and analysis. M.G., D.N., R.B. and G.W. are employees of Roche Diagnostics International Ltd, Rotkreuz, Switzerland. A.W. and C.R. are employees of Roche Diagnostics GmbH, Penzberg, Germany. A.S. and M.W. are employees of Labor Limbach, which received research funding from Roche Diagnostics. S.P. is an employee of AIT, which has received funds from Roche Diagnostics for carrying out statistical analysis. W.K., M.N., P.G. and E.B. are employees of Bioscientia GmbH, which received research funding from Roche Diagnostics. P.T.T. is an employee of Medic Medical Center, Ho Chi Minh City, Vietnam, which received research funding from Roche Diagnostics. B.H.H. is an employee of Ho Chi Minh City University Medical Center, Ho Chi Minh City, Vietnam, which received research funding from Roche Diagnostics. M.S. and C.W.S. are employees of UCT and Groote Schuur Hospital, Cape Town, South Africa, which received research funding from Roche Diagnostics. C.W.S. has received speaker fees and a travel grant from Gilead and is an advisory board member for AbbVie. G.B. and G.B.B. are employees of Infectious Diseases and Viral Hepatitis Unit, Second University of Naples, Italy, which received research funding from Roche Diagnostics. J.G. is an employee of Cerba Spécimen Services.

ORCID

W. E. Kaminski http://orcid.org/0000-0003-0745-6855

REFERENCES

1. WHO. Hepatitis B factsheet. 2017. http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed 4 October 2017.
2. Song Y, Bian Y, Petzold M, Ung CO. Prevalence and trend of major transfusion-transmissible infections among blood donors in Western China, 2005 through 2010. *PLoS ONE*. 2014;9:e94528.
3. Apata IW, Averhoff F, Pitman J, et al. Progress toward prevention of transfusion-transmitted hepatitis B and hepatitis C infection–sub-Saharan Africa, 2000-2011. *Morb Mortal Wkly Rep*. 2014;63:613-619.
4. Wang L, Liu H, Ning X, Gao F. Sequence analysis of the S gene region in HBV DNA from patients positive for both HBsAg and HBsAb tests. *Hepatol Res*. 2010;40:1212-1218.
5. Khedive A, Norouzi M, Ramezani F, et al. Hepatitis B virus surface protein mutations clustered mainly in CTL immune epitopes in chronic carriers: results of an Iranian nationwide study. *J Viral Hepat*. 2013;20:494-501.
6. Coleman PF. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis*. 2006;12:198-203.
7. Avellon A, Echevarria JM. Frequency of hepatitis B virus 'a' determinant variants in unselected Spanish chronic carriers. *J Med Virol*. 2006;78:24-36.
8. Ma Q, Wang Y. Comprehensive analysis of the prevalence of hepatitis B virus escape mutations in the major hydrophilic region of surface antigen. *J Med Virol*. 2012;84:198-206.
9. Mallory MA, Page SR, Hillyard DR. Development and validation of a hepatitis B virus DNA sequencing assay for assessment of antiviral

resistance, viral genotype and surface antigen mutation status. *J Virol Methods*. 2011;177:31-37.
10. Pollicino T, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol*. 2014;61:408-417.
11. Seddigh-Tonekaboni S, Waters JA, Jeffers S, et al. Effect of variation in the common "a" determinant on the antigenicity of hepatitis B surface antigen. *J Med Virol*. 2000;60:113-121.
12. Yong-Lin Y, Qiang F, Ming-Shun Z, et al. Hepatitis B surface antigen variants in voluntary blood donors in Nanjing. *China Virol J*. 2012;9:82.
13. Servant-Delmas A, Mercier-Darty M, Ly TD, et al. Variable capacity of 13 hepatitis B virus surface antigen assays for the detection of HBsAg mutants in blood samples. *J Clin Virol*. 2012;53:338-345.
14. Roque-Afonso AM, Ferey MP, Belkhiri D, Dussaix E. HBs antigen mutants: prevalence, clinical and diagnostic implications. *Pathol Biol (Paris)*. 2005;53:563-568.
15. Zuckerman JN, Zuckerman AJ. Mutations of the surface protein of hepatitis B virus. *Antiviral Res*. 2003;60:75-78.
16. Lada O, Benhamou Y, Poynard T, Thibault V. Coexistence of hepatitis B surface antigen (HBs Ag) and anti-HBs antibodies in chronic hepatitis B virus carriers: influence of "a" determinant variants. *J Virol*. 2006;80:2968-2975.
17. Roque-Afonso AM, Ferey MP, Ly TD, et al. Viral and clinical factors associated with surface gene variants among hepatitis B virus carriers. *Antivir Ther*. 2007;12:1255-1263.
18. Cao GW. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol*. 2009;15:5761-5769.
19. Coleman PF. Surveillance for hepatitis B surface antigen mutants. *J Med Virol*. 2006;78(suppl 1):S56-S58.
20. La'ulu SL, Roberts WL. The analytic sensitivity and mutant detection capability of six hepatitis B surface antigen assays. *Am J Clin Pathol*. 2006;125:748-751.
21. Gencay M, Huebner K, Gohl P, et al. Ultra-deep sequencing reveals high prevalence and broad structural diversity of hepatitis B surface antigen mutations in a global population. *PLoS ONE*. 2017;12:e0172101.
22. Sheldon J, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother*. 2008;61:766-768.
23. Carman WF, Van Deursen FJ, Mimms LT, et al. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa, and Sardinia. *Hepatology*. 1997;26:1658-1666.
24. Carman WF. The clinical significance of surface antigen variants of hepatitis B virus. *J Viral Hepat*. 1997;4(suppl 1):11-20.
25. Coleman PF, Chen YC, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. *J Med Virol*. 1999;59:19-24.
26. Hou J, Wang Z, Cheng J, et al. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. *Hepatology*. 2001;34:1027-1034.
27. Ho M, Mau Y, Lu C, et al. Patterns of circulating hepatitis B surface antigen variants among vaccinated children born to hepatitis B surface antigen carrier and non-carrier mothers. A population-based comparative study. *J Biomed Sci*. 1998;5:355-362.
28. Ngui SL, O'Connell S, Eglin RP, Heptonstall J, Teo CG. Low detection rate and maternal provenance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxis in England and Wales. *J Infect Dis*. 1997;176:1360-1365.
29. Oon CJ, Chen WN, Goh KT, et al. Molecular characterization of hepatitis B virus surface antigen mutants in Singapore patients with hepatocellular carcinoma and hepatitis B virus carriers negative for HBsAg but positive for anti-HBs and anti-HBc. *J Gastroenterol Hepatol*. 2002;17(suppl):S491-S496.
30. Oon CJ, Lim GK, Ye Z, et al. Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. *Vaccine*. 1995;13:699-702.
31. Oon CJ, Tan KL, Harrison T, Zuckerman A. Natural history of hepatitis B surface antigen mutants in children. *Lancet*. 1996;348:1524.
32. Roznovsky L, Harrison TJ, Fang ZL, et al. Unusual hepatitis B surface antigen variation in a child immunised against hepatitis B. *J Med Virol*. 2000;61:11-14.
33. Chakravarty R, Neogi M, Roychowdhury S, Panda CK. Presence of hepatitis B surface antigen mutant G145R DNA in the peripheral blood leukocytes of the family members of an asymptomatic carrier and evidence of its horizontal transmission. *Virus Res*. 2002;90:133-141.
34. Ireland JH, O'Donnell B, Basuni AA, et al. Reactivity of 13 in vitro expressed hepatitis B surface antigen variants in 7 commercial diagnostic assays. *Hepatology*. 2000;31:1176-1182.
35. Moerman B, Moons V, Sommer H, Schmitt Y, Stetter M. Evaluation of sensitivity for wild type and mutant forms of hepatitis B surface antigen by four commercial HBsAg assays. *Clin Lab*. 2004;50:159-162.
36. Bian T, Yan H, Shen L, et al. Change in hepatitis B virus large surface antigen variant prevalence 13 years after implementation of a universal vaccination program in China. *J Virol*. 2013;87:12196-12206.
37. Torresi J. Hepatitis B antiviral resistance and vaccine escape: two sides of the same coin. *Antivir Ther*. 2008;13:337-340.
38. Lieshout-Krikke RW, Molenaar-de Backer MW, van Swieten P, Zaaijer HL. Surface antigen-negative hepatitis B virus infection in Dutch blood donors. *Eur J Clin Microbiol Infect Dis*. 2014;33:69-77.
39. Louisirirotchanakul S, Khupulsup K, Akraekthalin S, et al. Comparison of the technical and clinical performance of the Elecsys HBsAg II assay with the Architect, AxSym, and Advia Centaur HBsAg screening assays. *J Med Virol*. 2010;82:755-762.
40. Muhlbacher A, Weber B, Burgisser P, et al. Multicenter study of a new fully automated HBsAg screening assay with enhanced sensitivity for the detection of HBV mutants. *Med Microbiol Immunol*. 2008;197:55-64.
41. Louisirirotchanakul S, Kanoksinsombat C, Theamboonlert A, Puthavatana P, Wasi C, Poovorawan Y. Mutation of the "a" determinant of HBsAg with discordant HBsAg diagnostic kits. *Viral Immunol*. 2004;17:440-444.
42. Palomares LA, Estrada-Mondaca S, Ramirez OT. Production of recombinant proteins: challenges and solutions. *Methods Mol Biol*. 2004;267:15-52.
43. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. *Pharm Res*. 2010;27:544-575.
44. Manning MC, Patel K, Borchardt RT. Stability of protein pharmaceuticals. *Pharm Res*. 1989;6:903-918.
45. Zhang R, Wang L, Li J. Hepatitis B virus transfusion risk in China: proficiency testing for the detection of hepatitis B surface antigen. *Transfus Med*. 2010;20:322-328.

How to cite this article: Gencay M, Seffner A, Pabinger S, et al. Detection of in vivo hepatitis B virus surface antigen mutations—A comparison of four routine screening assays. *J Viral Hepat*. 2018;25:1132–1138. https://doi.org/10.1111/jvh.12915