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# Performance of HBsAg point-of-care tests for detection of diagnostic escape-variants in clinical samples

Cédric Hirzel MD [1], Stefan Pfister PharmD [2], Meri Gorgievski-Hrisoho [2],Gilles Wandeler MD [1], Samuel Zuercher MD [2]

1 Department of Infectious Diseases, Bern University Hospital and University of Bern, Switzerland

2 Institute for Infectious Diseases, University of Bern, Bern, Switzerland

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**Corresponding author:** Cédric Hirzel

Universitätsklinik für Infektiologie und Reisemedizin

Inselspital

Poliklinikträkt 2

CH-3010 Bern

Phone: +041 (0)31 632 00 18

Fax: +041 (0)31 632 31 76

e-mail: cedric.hirzel@insel.ch

# Highlights

- HBsAg point-of-care tests have slightly lower sensitivities than standard methods.
- We assessed test characteristics of point-of-care tests using HBsAg mutated viruses.
- The point-of-care tests accurately diagnosed mutated Hepatitis B viruses.
- HBsAg mutations do not affect the sensitivity of the evaluated tests.

# Abstract

**Background:** Hepatitis B viruses (HBV) harboring mutations in the a-determinant of the Hepatitis B surface antigen (HBsAg) are associated with reduced reactivity of HBsAg assays.

**Objectives:** Evaluating the sensitivity and specificity of three HBsAg point-of-care tests for the detection of HBsAg of viruses harboring HBsAg mutations.

**Study design:** A selection of 50 clinical plasma samples containing HBV with HBsAg mutations was used to evaluate the test characteristics of three HBsAg point-of-care tests (Vikia®, bioMérieux, Marcy-L`Étoile, France. Alere Determine HBsAg™, Iverness Biomedical Innovations, Köln, Germany. Quick Profile™, LumiQuick Diagnostics, California, USA) and compared to the ARCHITECT HBsAg Qualitative® assay (Abbott Laboratories, Sligo, Ireland).

**Results:** The sensitivity of the point-of-care tests ranged from 98% to 100%. The only false-negative result occurred using the Quick Profile™ assay with a virus harboring a D144A mutation.

**Conclusions:** The evaluated point-of-care tests revealed an excellent sensitivity in detecting HBV samples harboring HBsAg mutations.



# Keywords

Hepatitis B virus, diagnostic-escape variants, HBsAg mutations, point-of-care test

# Background

The mainstay of Hepatitis B virus (HBV) infection diagnosis is the detection of the HBV surface antigen (HBsAg) [1]. In recent years an increasing number of HBsAg point-of-care (POC) tests have become available. POC tests, which use the principle of immunochromatography as well as enzyme immunoassays and chemiluminescence immunoassays, are based on the detection of the antigenic determinant ("a-determinant"). The a-determinant is located between amino acid position 99 and 160 of the HBsAg [2]. However, in a recent study from The Gambia, POC tests had a slightly lower sensitivity than the standard serological methods [3] . In diagnostic-escape variants, mutations in the a-determinant of the HBsAg are thought to influence the performance of HBsAg assays [2]. The diagnostic performance for mutant HBV has been shown to differ across commercial HBsAg assays, depending on which anti-HBsAg reagents are used [4]. Thus, the different capacity in detecting HBV diagnostic-escape variants between POC tests and standard HBsAg assays could be an explanation for the lower sensitivity of POC tests.

# Objectives

To determine the performance of three commercial HBsAg POC tests (Vikia®, bioMérieux, Marcy-L`Étoile, France. Alere Determine HBsAg™, Iverness Biomedical Innovations, Köln, Germany. Quick Profile™, LumiQuick Diagnostics, California, USA) in detecting HBV with HBsAg mutations of the antigenic determinant from clinical samples.

# Study Design

We retrospectively screened all samples for HBsAg mutations that were sent to our reference laboratory for HBV genotyping between January 2010 and December 2013. All samples with any mutation of the HBsAg with the exception of serotype- (amino acid positions 122, 127, 140, 159, 160) or genotype- ( T118A, T125M, A128V) specific HBsAg polymorphisms [5, 6] were considered for this analysis. Twenty randomly selected HBsAg negative samples were used as negative controls. The HBV viral load was measured using

COBAS AmpliPrep®/COBAS TaqMan® HBV test 2.0 (Roche Diagnostics, Indianapolis, USA).

DNA was extracted using NucliSENS easyMAG® (bioMérieux, Paris, France). A fragment of the HBsAg was amplified in a primary PCR (pPCR) using the primers HBV_1F and HBV_4R [7]. If needed, a nested PCR (nPCR) was performed using the primers HBV P1F_f and HBV S6_r [8]. All PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN GMBH, Hilden, Germany). The purified amplicons were subjected to bidirectional Sanger sequencing using the primers HBV_1F [5] and HBV S6_r [8] for pPCR products and HBV P1F_f and HBV S6_r [7, 8] for nPCR products. Cycle sequencing was performed according to Platt et al [9]. After purification of the cycle sequencing products by the QIAGEN DyeEx® 2.0 Spin Kit (QIAGEN GMBH, Hilden, Germany) The electropherograms were acquired on a Applied Biosystems® 3130 genetic analyzer (Life Technologies Europe BV, Nieuwerkerk, Netherlands) and then processed using SeqMan® (DNASTAR Inc., Madison, WI, USA). For in silico sequence analysis and detection of HBsAg mutations the open access interpretation tool geno2pheno was used [10].

The performance of three HBsAg POC tests (Vikia®, Alere Determine HBsAg™, Quick Profile™) previously validated in a French cohort [11] was compared with that of the ARCHITECT HBsAg Qualitative® assay, which has an excellent sensitivity in detecting HBsAg mutants [12]. False-negative and borderline POC test results were repeated twice. The ARCHITECT HBsAg Quantitative® assay was additionally performed in samples with false-negative POC tests and in samples harboring the same mutations as the false-negative ones. This allowed determining if false-negative results were caused by lower HBsAg levels. All tests were performed according to the manufacturer`s instruction.

# Results

Of 153 samples sequenced between 2010 and 2013, 50 contained HBsAg mutations. Forty-one different single or combined mutations were detected (Table 1). With the exception of six

samples containing the mutations T118S, T126A, T126N, H129L, Y134R or W196L, all mutant variants had been previously associated with reduced sensitivity for HBsAg detection [2], occurrence of occult HBV infection [13-15], reduced binding of anti-HBsAg antibodies [16] or reduced HBsAg secretion [17]. The median HBV viral load was 14`937 IU/ml (IQR 1`139- 329`750 IU/ml). Genotype D was the most prevalent (52.0%, 13/50) followed by A (26.0%, 13/50), B (10.0%, 5/50), C (6.0%, 3/50), E (4.0%, 2/50) and F (2.0%, 1/50).

The sensitivity and specificity of the HBsAg POC tests were excellent (Table 2). The only false-negative test occurred using the Quick Profile™ assay with a HBV diagnostic escape variant harboring the single mutation D144A (HBV viral load 432 IU/ml; quantitative HBsAg 140.4 IU/ml). Of note, the Quick Profile™ assay produced a borderline positive result using another sample harboring the mutation F134A/D144A (HBV viral load 603 IU/ml; quantitative HBsAg 14.8 IU/ml) but was clearly positive for a sample with a D144A/G145A (HBV viral load 41`850`456 IU/ml, quantitative HBsAg 998.7 IU/ml) and a Y100C/Y134H/D144A (HBV viral load 22`815 IU/ml, quantitative HBsAg 1146.5 IU/ml) mutation. The electropherograms of the four samples containing a D144A mutation showed single peaks at the amino acid position 144. Therefore the correct identification of viruses harboring the D144A mutation could not be explained by the presence of non-mutated HBV sub-populations.

# Discussion

This is the first study to assess the performance of HBsAg POC tests in diagnosing HBV harboring HBsAg mutations from clinical samples. We showed that the sensitivity and specificity of the assays were excellent. One false-negative and one borderline positive test occurred, both using the Quick Profile™ assay.

Bottero et al tested the performance of the identical HBsAg POC tests using whole blood samples in a large cohort in France [11]. They found high sensitivities (Vikia® 96.5%, Alere Determine HBsAg™ 93.6%, Quick Profile™ 90.5%) and specificities (Vikia® 99.9%, Alere Determine HBsAg™ 100%, Quick Profile® 99.7%). Because of the low HBV viral loads in the samples with false-negative POC test results, they were not able to investigate whether

false-negatives were caused by HBsAg mutations or by other factors. The sensitivity of POC tests was even higher in our study, despite analyzing HBV samples harboring HBsAg mutations. However, we did not have samples with low viral loads, as we only included those which were successfully sequenced and therefore the sensitivities of the POC test may be overestimated. We used plasma, which, according to the manufacturer`s information, leads to a slightly higher sensitivity than whole blood with the Vikia® assay. However, this is not true for the Alere Determine™ - and unknown for the Quick Profile™ assay.

In line with findings from Muhlbacher et al, we showed that a specific mutation did not always have the same effect on the result of the assay [18]. In our study the sample with a single D144A mutation was not detected by one of the tests, whereas for viruses harboring additional mutations, the result was either borderline positive or clearly positive. This phenomenon was not explained by lower quantities of HBsAg in the false-negative sample.

This was the first study to evaluate the sensitivity of HBsAg POC tests for diagnostic escape mutants using clinical samples with a wide variety of mutations and HBV genotypes. We recognize that in clinical settings, HBsAg POC tests are generally performed using whole blood and not serum or plasma. However, in light of recently published evidence, we did not expect the use of plasma to affect our results significantly [19].

In conclusion we demonstrated that the three HBsAg POC tests accurately diagnosed HBsAg diagnostic escape variants in plasma samples. Besides a potentially slightly reduced performance of the Quick Profile™ assay in detecting D144A mutants, our results indicate that HBsAg mutants do not relevantly affect the sensitivity of the evaluated POC tests.

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## Conflict of Interest

**Funding:** None

**Competing interests:** None

**Ethics approval:** Not required

# References


[1] Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-662.

[2] Alavian SM, Carman WF, Jazayeri SM. HBsAg variants: diagnostic-escape and diagnostic dilemma. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2013;57:201-208.

[3] Njai HF, Shimakawa Y, Sanneh B, Ferguson L, Ndow G, Mendy M, et al. Validation of Rapid Point-of-Care (POC) Tests for Detection of Hepatitis B Surface Antigen in Field and Laboratory Settings in the Gambia, Western Africa. Journal of clinical microbiology 2015;53:1156-1163.

[4] Ly TD, Servant-Delmas A, Bagot S, Gonzalo S, Ferey MP, Ebel A, et al. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. Journal of clinical microbiology 2006;44:2321-2326.

[5] Purdy MA, Talekar G, Swenson P, Araujo A, Fields H. A new algorithm for deduction of hepatitis B surface antigen subtype determinants from the amino acid sequence. Intervirology 2007;50:45-51.

[6] Tallo T, Tefanova V, Priimagi L, Schmidt J, Katargina O, Michailov M, et al. D2: major subgenotype of hepatitis B virus in Russia and the Baltic region. The Journal of general virology 2008;89:1829-1839.

[7] 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. Journal of virological methods 2011;177:31-37.

[8] Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, et al. Variant of hepatitis B virus with primary resistance to adefovir. The New England journal of medicine 2006;354:1807-1812.

[9] Platt AR, Woodhall RW, George AL, Jr. Improved DNA sequencing quality and efficiency using an optimized fast cycle sequencing protocol. BioTechniques 2007;43:58, 60, 62.

[10] Beggel B, Neumann-Fraune M, Doring M, Lawyer G, Kaiser R, Verheyen J, et al. Genotyping hepatitis B virus dual infections using population-based sequence data. The Journal of general virology 2012;93:1899-1907.

[11] Bottero J, Boyd A, Gozlan J, Lemoine M, Carrat F, Collignon A, et al. Performance of rapid tests for detection of HBsAg and anti-HBsAb in a large cohort, France. Journal of hepatology 2013;58:473-478.

[12] Lou SC, Pearce SK, Lukaszewska TX, Taylor RE, Williams GT, Leary TP. An improved Abbott ARCHITECT assay for the detection of hepatitis B virus surface antigen (HBsAg). Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011;51:59-63.

[13] Chamni N, Louisirirotchanakul S, Oota S, Sakuldamrongpanish T, Saldanha J, Chongkolwatana V, et al. Genetic characterization and genotyping of hepatitis B virus (HBV) isolates from donors with an occult HBV infection. Vox sanguinis 2014;107:324-332.

[14] Pei R, Grund S, Verheyen J, Esser S, Chen X, Lu M. Spontaneous reactivation of hepatitis B virus replication in an HIV coinfected patient with isolated anti-Hepatitis B core antibodies. Virology journal 2014;11:9.

[15] Motta JS, Mello FC, Lago BV, Perez RM, Gomes SA, Figueiredo FF. Occult hepatitis B virus infection and lamivudine-resistant mutations in isolates from renal patients undergoing hemodialysis. Journal of gastroenterology and hepatology 2010;25:101-106.

[16] Torresi J, Earnest-Silveira L, Deliyannis G, Edgtton K, Zhuang H, Locarnini SA, et al. Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. Virology 2002;293:305-313.

[17] Huang CH, Yuan Q, Chen PJ, Zhang YL, Chen CR, Zheng QB, et al. Influence of mutations in hepatitis B virus surface protein on viral antigenicity and phenotype in occult HBV strains from blood donors. Journal of hepatology 2012;57:720-729.

[18] Muhlbacher A, Weber B, Burgisser P, Eiras A, Cabrera J, Louisirirotchanakul S, et al. Multicenter study of a new fully automated HBsAg screening assay with enhanced sensitivity for the detection of HBV mutants. Medical microbiology and immunology 2008;197:55-64.

[19] Freeya Njai H, Shimakawa Y, Sanneh B, Ferguson L, Ndow G, Mendy M, et al. Validation of rapid point-of-care (POC) tests for the detection of hepatitis B surface antigen (HBsAg) in field and laboratory settings in The Gambia, West Africa. Journal of clinical microbiology 2015.

## Table 1

HBsAg variants used for test evaluation

| HBsAg mutation (Genotype) | n | HBsAg mutation (Genotype) | n |
|---|---|---|---|
| Y100C[1] (A1) | 1 | G130N[2]/T131N[2] (D3) | 1 |
| Y100C[1]/P120T[2] (A1) | 1 | T131I[2] (D4) | 1 |
| Y100C[1]/T118R/P120A /Y134L/D144E[2] (D1) | 1 | T131N[2] (B2) | 1 |
| Y100C[1]/Y134H/D144A[2] (D3) | 1 | T131P[2] (D1) | 1 |
| T118K/P120T[2] (C2) | 1 | T131N[2]/I195M[4] (A1) | 1 |
| T118S (D3) | 1 | M133I[2] (A2) | 1 |
| P120L[2] (D3) | 1 | M133L[2] (B2, B2) | 2 |
| P120S[2] (B4, D2, D3) | 3 | M133T[2] (A1, D4) | 2 |
| P120S[2]/G145R[2] (D3) | 1 | M133L[2]/G145A[2] (B2) | 1 |
| C124Y[2]/P135S[2] (D1) | 1 | M133T[2]/I195M[4] (C1) | 1 |
| T126A (A2) | 1 | Y134R (E) | 1 |
| T126I[1] (C2) | 1 | F134A/D144A[2] (D3) | 1 |
| T126N (D3) | 1 | P135S[2] (D4) | 1 |
| T126N/Q129R[5] (D1) | 1 | C139Y[2] (D1) | 1 |
| T126N/Q129R[5]/G145A[2] (D1) | 1 | S143L[2] (F2) | 1 |
| H129L (A1) | 1 | D144A[2] (D3) | 1 |
| Q129A/G130R[2]/T131N[2]/M133T[2]/F134V[3] (D3) | 1 | D144A[2]/G145A[2] (D3) | 1 |
| Q129H[2]/G130R[2]/T131N[2]/M133T[2]/F134V[3] (D3) | 1 | I195M[4] (A1, A2, D1, E) | 4 |
| G130N[2] (A2) | 1 | W196L (A1, A2, D3) | 3 |
| G130R[2] (D2) | 1 | W196S[4] (A2) | 1 |
| G130R[2]/T131N[2] (D3) | 1 | | |

1) Associated with occult HBV [13, 15]
2) Associated with reduced sensitivity of HBsAg assays [2]
3) Associated with occult HBV in combination with additional mutations [14]
4) Associated with reduced binding to anti-HBs antibodies [16]
5) Asociated with reduced HBsAg secretion [17]

**Table 2**

Test characteristics of HBsAg point-of-care tests compared to CMIA (ARCHITECT HBsAg Quantitative assay; Abbott Laboratories, Sligo, Ireland)

| | HBsAg serology CMIA | | Sensitivity | Specificity |
|---|---|---|---|---|
| | positive | negative | | |
| VIKIA® | (n=50) | (n=20) | 100% | 100% |
| positive | 50 | 0 | | |
| negative | 0 | 20 | | |
| DETERMINE™ | (n=50) | (n=20) | 100% | 100% |
| positive | 50 | 0 | | |
| negative | 0 | 20 | | |
| QUICK PROFILE™ | (n=50) | (n=20) | 98% | 100% |
| positive | 49 | 0 | | |
| negative | 1 | 20 | | |