Immune Escape Mutant Detection Using Commercially Available Methods for Hepatitis B Surface Antigen Serological Testing

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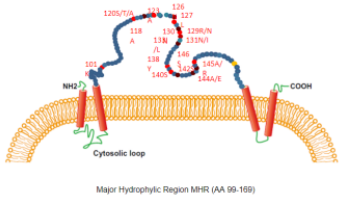
Introduction

While overall infection rates of Hepatitis B virus (HBV) in the United States (US) remain stable, as many as 2.2 million persons are still chronically infected with HBV[1]. Persons who inject drugs (PWID) are at a higher risk of HBV infection and since 2009 three states (KY, TN, WV) have reported up to a 114% increase in cases of acute HBV infection due to higher infection rates among a non-Hispanic white populations (30–39 years), and PWID[2]. Hepatitis B vaccination is recommended as primary prevention for adults who are at increased risk for HBV infection, including PWID. However, data from the National Health Interview Survey indicate that Hepatitis B vaccination coverage is low among adults in the general population[3], and it is likely to be lower among PWID.

Hepatitis B Surface Antigen (HBsAg) is the first serological marker to appear after HBV exposure and infection; this marker is included in the recommended panel for acute hepatitis diagnosis, and accurate detection is necessary for early and accurate diagnosis. Serological testing challenges exist for HBsAg due to the high degree of genetic variability which can further be exacerbated by endogenous and exogenous pressures (such as HBV approved therapies). The immunodominant major hydrophilic region (MHR, Figure 1) may have one or more mutations described as *"immune escape mutations"* which can decrease or abrogate HBsAg binding to antibodies used in immunoassays. Although the prevalence of these mutations is not well documented in the United States, international studies have shown that up to 18% of all HBV patients[4] and 79% of HBV-reactivated patients (vs 3.1% of control patients; $p < 0.001$) carry HBsAg mutations localized in immune-active HBsAg regions[5].

Because immunoassay manufacturers target the MHR, mutations in this region may lead to missed infections. A panel of 10 recombinant samples with one or more mutation/s was tested by five FDA approved HBsAg immunoassays to show not all samples with mutations are detected by all immunoassays.

Figure 1: HBsAg MHR[6]



Materials and Methods

Mutant Panel: In order to obtain a reliable in-house panel, the surface antigen gene sequences containing defined mutations were cloned in the pcDNA3.1(+) vector designed for expression in mammalian cells. Inserted genes were verified by sequencing and the expression vector used to transiently transfect human HeLa cells.

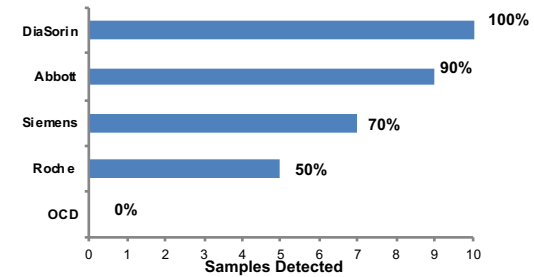
Sample	Mutation
MUTANT-01	T123N
MUTANT-02	T123N-T124S
MUTANT-03	P142L-F/Y143H-D144E-G145-R
MUTANT-04	I110R-SS117I-G119R-T123N
MUTANT-05	122+DT
MUTANT-06	122+DT-G145R
MUTANT-07	G145R
MUTANT-08	D114A
MUTANT-09	P142L-G145R
MUTANT-10	P142S-G145R

Table 1: Description of mutations in recHBsAg mutant panel.

Serological Methods: Serological testing was performed by two commercial laboratories using the following automated immunoassay methods: Roche cobas e 411 (Roche), Siemens ADVIA Centaur (Siemens), Ortho Clinical Diagnostics VITROS® (OCD), and Abbott ARCHITECT (Abbott). Testing was also performed on the LIAISON® XL (DiaSorin) at DiaSorin SpA.

Results

Figure 2: Detection of recHBsAg mutants



Results

The recently FDA approved DiaSorin LIAISON® XL method for HBsAg qualitative detection was the only method that was able to detect all rec HBsAg samples with immune escape mutations.

Additional immunoassay manufacturer results:

- Abbott: detected 9 of the 10 samples, falsely calling only Mutant-03 nonreactive.
- Siemens: detected 7 mutant samples, also falsely calling Mutant-03 as well as Mutant-04 and Mutant-06 negative.
- Roche: accurately detected half of the mutant samples, but in addition to those false negatives by Siemens also was unable to detect Mutant-02 and Mutant-05.
- OCD: showed the poorest overall performance without detecting any of the samples with mutations in the immunodominant region, including Mutant-07, which is the most commonly noted mutation in HBsAg.

Conclusions

Overall the performance of FDA approved immunoassays at immune escape mutant detection is variable by vendor and it may be important to further investigate suspected false negative HBsAg results. Due to new therapies and the increase in persons who are taking immunomodulators, antivirals or immunosuppressive medications, incidence of mutations will continue to rise in the United States. The DiaSorin LIAISON® XL method for qualitative HBsAg detection exhibited the best detection of recHBsAg mutants most likely due to the assay design which targets not just the immunodominant MHR region, but additionally targets the cytosolic loop and transmembrane sequences. This unique assay design allows for accurate detection of HBsAg even in the presence of immune escape mutations.

Footnotes/References

[1]Kowdley KV. et al. Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. Hepatology 2012;56:422–33.

[2]Vellozzi SS. et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2018;67(No. RR-1):1–31.

[3]Hung M. et al Vaccination Coverage among Adults in the United States, National Health Interview Survey, 2017

[4]Gautam Ray. Current Scenario of Hepatitis B and its Treatment in India. J Clinical and Translational Hepatology 2017; 5(3).

[5]Salpini R. et al. Additional N-Glycosylation sites in HBV surface antigen characterizes immunosuppression-driven HBV reactivation and alter HBsAg recognition in vitro ESCV 2018. Poster.

[6]Adapted from: Huang CH. et al. Influence of mutation in hepatitis B virus surface protein on viral antigenicity and phenotype in occult HBV strains from blood donors. J. Hepatology 2012; 57 (4); 720-9.

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