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HBsAg variants: Diagnostic-escape and diagnostic dilemma

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ABSTRACT

A wide variety of commercial assays is available for the detection of hepatitis B surface antigen (HBsAg). Clearly, the sensitivity of an assay to detect a variant is dependent on the anti-HBs usage. Thus, it is not surprising that there are examples of variants that cannot be detected by all assays. Data from Europe, Asia and Africa about HBsAg variants which are not recognized by either monoclonal or polyclonal antibodies specific for wild-type group ‘a’ determinant, but positive by DNA polymerase chain reaction (PCR) in chronic patients and from vaccinated children are increasing. This would impose a challenge for public health issues of hepatitis B virus. In this review we tried to summarize the discrepancies between results of HBsAg assays and to explain some rationales for these inconsistencies.

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1. Introduction

The hepatitis B virus (HBV) envelope proteins can be translated from a single open reading frame: L (large), M (middle), and S (small) or hepatitis B surface antigen (HBsAg). Within the latter, the region between aa 100 and 160 is termed the major hydrophilic region (MHR). This comprises amino acids (aa) 99–160 that encompass the group-specific ‘a’ determinant (Fig. 1). Two major loops are proposed in the “a” determinant, defined by multiple potential disulfide bridges, between aa 139/147 (or 149), and 121/124 and conserved cysteines at positions 124, 137, 139 and 149 have been found to be essential for the antigenicity and presumably for the conformation of the protein (Fig. 1).[1–5] The anti-HBs (antibody to HBsAg) response comprises mainly antibodies that recognize this epitope cluster. The antigenic epitopes of the ‘a’ region have been analyzed by binding studies to synthetic peptides using anti-HBs monoclonal/polyclonal antibodies.[6–8] These studies use antigens with point-mutated positions within the second ‘a’ loop (aa 139–147) demonstrated significant alteration in antigenic properties in some cases.[9] These data document the impact of both conformational and physicochemical properties of aa at a given position on the antigenicity of the epitope cluster.

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2. HBsAg-escaped mutants

The escape mutants within immune epitopes of HBV constitute a significant role and isolated cases of infection with HBV variants bearing substitutions in these regions, are predicted to escape from the following situations:

2.1. Vaccine-escape variants

The success of the vaccine strategy has now been challenged by the discovery of mutant hepatitis B viruses showing amino acid exchanges in surface protein HBsAg, which might lead to reduction or even abolished binding of vaccine-induced neutralizing antibodies[10]. Some of these “escape mutants” have been described so far (Table 1), all showing changes in the “a” determinant of HBsAg, which is assumed to be the main target for neutralizing antibodies,[11–14] especially in children who acquired HBV infection despite the presence of surface antibody (anti-HBs).[10,15–17] The prevalence of the *a* determinant mutants has been reported to be significantly higher in children who received plasma-derived vaccines than in children who received recombinant vaccines.[18] Some of these variants, especially G145R coexisted with a minor wild-type virus, and subsequently were reverted back to predominance by the wild-type virus. This could be due to the waning of vaccine-induced anti-HBs, which initially targeted the variants and thus eventually was taken over by the wild-type virus.[19,20] The G145R mutation and other less prevalent mutations of HBsAg a determinant epitopes that allow for infection in successfully vaccinated persons have been reported with various mutational frequencies

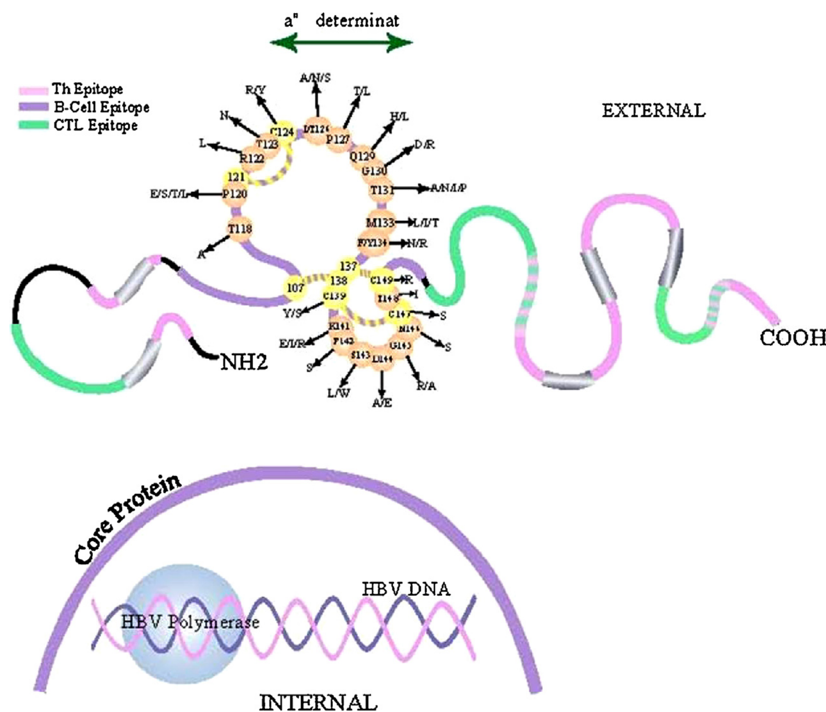

Fig. 1. Schematic of the HBsAg MHR (including "a" determinant). Cysteine residues are shown as white circles and disulphide bridges are indicated as S–S links. The changes selected by Antibodies (natural, used in assays and vaccine-induced) occur upstream and downstream of the a determinant are indicated. The carboxy-terminal end of the protein contains two alpha helices are shown as cylindrical structures.

from countries that implemented the Expanded Program on Immunization (EPI). Geographical differences, the time interval that has elapsed since launching the immunisation programme and particular vaccination strategies may play a role in discrepancies between results. For example, the prevalence of HBsAg "a" determinant variants was 39% in Singapore,[21] 22% in Taiwan,[22] 12% in the UK[20] and 0% in south Africa.[23] interestingly, "a" determinant mutations rarely have been reported from high HBV endemic area even from vaccinated individuals borne to HBsAg positive mothers.[19,24–27] Furthermore, the emergence of such variants has not raised concern about the efficacy of immunisation programme, as the breakthrough infections in the vaccinated cohorts have not totally attributed to the vaccine-induced mutants, and other factors such as: *in vitro* HBV infection of the fetus,[19] high maternal viral load,[28] host genetic factors,[29] improper administration of the HBV vaccine, timing of the vaccine particularly that of the birth dose[10], and a break in the vaccine cold chain[30] have been inculpated for the vaccine's failure. Collectively, data from different studies indicated that the presence of vaccine escape mutants can be considered rather negligible with respect to vaccination programmes worldwide.

2.2. HBIg-escape variants

Passive immunisation is now generally combined with active immunisation induced by vaccine, providing immediate protection and more durable immunity. However, HBsAg mutants have also been reported in association with escape from therapy with specific immunoglobulin in newborns from carrier women[10,19,31–33] and in patients who underwent liver transplantation (LT) (Table 1),[13,17,34–36] indicating that when it is used, the hepatitis B immunoglobulin (HBIg) part of a vaccine programme can be the major driving force for the selection of vaccine escape mutants.[24,37] Regarding HBIg prophylaxy against intrauterine HBV infection, there are no data to support the emergence of such variants after HBIg alone (without vaccine administration), and also,

Table 1
The most frequent medically selected amino acid changes in HBsAg. The causal selection pressures are: V, vaccine; L, lamivudine; mAb, monoclonal antibody; HBIG, hepatitis B immune globulin; Fam, famciclovir. *Stop codon mutation.

Amino acid position	Wild-type	Mutant	Cause
120	P	E-S-T	V-L-HBIG
123	T	N	HBIG
124	C	R-Y	mAb-HBIG
126	I/T	A/N/S	V-HBIG
129	Q	H/L	V
130	G	D/R	L-HBIG
133	M	L	V
134	F/Y	N/R	HBIG
141	K	E/I	V-mAb
142	P	S	V-mAb
144	D	A/E	V-HBIG
145	G	R	V-L-HBIG
146	N	S	V
148	T	I	V
149	C	R	V
157	A	D/R	V-L
158	F	Y	L
164	E	D	L-Fam
175	L	S	L
179	F	Y	L
181	Q	H	V
182	V	*	L
183	F	C	V
184	V	A	V
193	F	S	L
194	V	A	V
195	I	M	L
196	W	S	L
198	M	I	L
204	N	S	V
207	Q	R/S	L
208	I	T	V

injection of HBIg in carrier mothers did not increase the mutation rate of infants who received the HBV vaccine.[38] Some of these mutations arose after HBIg administration alone (aa: 123 and 134), whereas in a majority of studies combination of variations within the surface protein have been reported in the presence of either HBIg/HBV vaccine or HBIg/antivirals (in LT settings) (Table 1). In LT cases, it is not clear yet if the emergence of mutations would be directly related to HBIg dosage, however, most studies reported "a" determinant mutations after prophylaxis with high dose HBIg.[39–41] On the withdrawal of hyperimmune globulin, the mutants are often lost and the wild-type again becomes dominant.[2] Further, this pattern of mutation after passive HBIg prophylaxy was reported regardless of the presence or the absence of concomitant antiviral therapy, especially lamivudine.

2.3. *Natural immune-escape variants*

Besides vaccination-associated HBsAg mutants, mutations in the HBV surface gene have been reported in carriers who did not receive HBIg or vaccine[42,43] suggesting a 10.8–11.2% frequency of HBsAg variants among chronic HBV carriers Asian with dominant genotype D.[44–47] However, a lower frequency has been confirmed in healthy blood donors[48], and in a random population screening in Singapore, with 0.8% of HBsAg variants detected.[49] It seems that the consequence of selection pressure posed by anti-S antibodies would be the emergence of immune escape mutations in this protein which no longer could be recognized by the host immune system. The results would be the presence of virus (and sometimes with a high level of viral load) in a chronically infected patient. Interestingly, patients with both HBsAg and anti-HBs positive have been reported in 10–25% of chronic active, hepatitis B "e" antigen (HBeAg)-positive patients.[50] Thus, the escape phenomenon from the neutralising anti-HBs could be one of the mechanisms of persistence in these vaccine/therapy naïve patients.

2.4. *Therapy-escape*

Although the initial effect of nucleos(t)ide analogous (NA) in suppressing HBV replication and reducing alanine aminotransferase activity is promising, the emergence of drug-resistant variants reduces considerably the effect of the drugs, especially with the widespread use of lamivudine.[51–54] Drug resistance has been associated with the emergence of polymerase gene mutations that localized within the catalytic domain of the RT region. These compensatory mutations occur due to the overlapping nature of polymerase and surface proteins (Fig. 1), thus, the reciprocal influences within them are inevitable; some of which are associated with alterations in the 'a' determinant of the HBsAg protein[51,55], that may have the potential to become vaccine escape mutants[52]. The overlapped areas encompassed within the distal part of the A–B inter-domain as well as the B domain of RT (residues 80–236); and residues 72–228 of the RT and surface proteins, respectively. Common surface therapy-escape mutations have been reported to occur between aa residues 120 and 208 (Table 1),[51,52,56] of which the rtA181T/sW172 mutant has a dominant negative secretion effect leading to seroclearance of HBV surface antigen, where vaccine-escape-like mutants might be selected.[57] In this regard, in many *in vitro* studies, the replication efficiencies of polymerase/surface variants versus wild type have been compared, however, in an elegant study, Torresi et al. found that the vaccine-escape mutant, G145, and the lamivudine-selected mutant E164D/I195M showed minimal binding of anti-HBs antibody (collected from volunteers who had been vaccinated with a second-generation HBV vaccine) while the E164D, M198I, I195M and W196S lamivudine-selected mutants also showed reduced binding to anti-HBs antibodies, raising the possibility that lamivudine-selected HBsAg protein changes may have the potential to escape neutralization by vaccine induced anti-HBs antibody. There is lack of data on interaction between surface protein reactivity with other new NA antivirals: adefovir, entecavir, tenofovir, and telbivudine are lacking and the potential of these new nucleoside analogs to select HBV mutants which reduce the efficacy of the current HBV vaccine has not been established yet.

2.5. *Diagnostic-escape variants*

There have also been cases of infection that have been missed because of failure of current serological assays to detect some variant forms of HBsAg. In these situations, the HBsAg mutants may arise in patients with HBV infection but diagnosed negative for HBsAg. Again, mutations affecting the HBsAg 'a' determinant region can potentially render HBsAg undetectable by some laboratory tests.[58–60] These variants may cause breakthrough infections in immunised persons.

3. HBsAg escape mutations

3.1. *Mutations of MHR region*

Multiple aa changes in surface- exposed regions of HBsAg, including mutational deletions have been found to abolish the reactivity of monoclonal and polyclonal anti-HBs diagnostic antibodies against the 'a' loop epitope cluster, and most anti-HBs in sera from vaccinees are targeted to an epitope between aa 139 and 147.[61]

Amino acid substitution within the MHR can allow replication of HBV in vaccinated persons, as much of the antibody induced by current vaccines does not recognise critical changes in this surface antigen domain.[62] These variants are summarized in Fig. 1 and Table 1. In many studies, mutations in vaccinated children were preferentially located in the 'a' determinant,[63,64] in contrast to the more randomly located mutations in unvaccinated children.[23,65]

In general, replacement in the presumed second 'a' determinant loop, including amino acid positions 144 and 145, are more often associated with anti-HBs immune pressure than mutations in other epitopic domains of the MHR (Fig. 1). Mutations at aa positions 120, 143, 144, and 145 can result in poor reactivity with *dly* subtyping monoclonal antibodies.[66,67] As this specificity is defined by the residue at aa 122, this indicates the discontinuous nature of many of the epitopes in this antigenic region. Subtype-related variation can also alter binding of antibodies to regions linearly distant.[66,67]

Changes located outside the 'a' determinant region were reported from immunized infants in Singapore born to HBV carrier mothers.[68,69] Interestingly, some of these mutant proteins had reduced binding to monoclonal antibodies against the 'a' determinant, and some were most likely transmitted vertically as they were isolated both from infant and maternal serum. Their role in vaccination failure is currently unknown. Some of those variants were within the major hydrophilic loop of HBsAg, but mutations at positions 183 and 184 were also found. As some of these mutations had decreased binding to an 'a'- specific monoclonal antibody, their functional analysis will contribute to the understanding of the antigenic structure of the HBV envelope.

The region between aa 118 and 123 was identified as a hot spot for insertions by investigators, illustrating its immunological importance.[7,70,71] Further, amino acid substitutions have been found in residues downstream (38–98) and upstream (between 164 and 215) of MHR region of native and or recombinant surface proteins with different binding capacities to antibodies.[60,72–75]

In an attempt to study the sensitivity of modern assays for the detection of HBsAg, investigators evaluated the performance of different assays for the detection of single, double and triple recombinant and/or native HBsAg mutants (Table 1).[72,74–78] Assays were

Table 2
Proposed antigenic epitopes within HBsAg.

Sequence	Cell subsets	HLA restriction	Ref
100–160	B	Not HLA restriction	130
19–28	Th	ClassΠ	79
28–51	CTL	Class I HLA-A2	85
80–98	Th	ClassΠ	79
171–179	CTL	ClassI	84
175–184	CTL	Class I HLA-A2	80
186–197	Th	ClassΠ	80
206–215	CTL	ClassI HLA-A2	80
215–223	Th	ClassΠ	79

challenged with native and/or recombinant mutants in positions upstream and downstream of "a" determinant (Fig. 1). Interestingly, the majority of HBsAg tests were able to detect the mutants (data not shown).

3.2. 2.2- Mutations outside of the MHR region

Being a structural protein, HBsAg is an immune target. The distribution of the mutations within known surface protein immune epitopes reflects the virus-host interaction with a prolonged infection period. HBV surface proteins contain both B and T cell antigenic epitopes. Mutations in T cell epitopes may theoretically influence the anti-HBs antibody profile through the interaction between CD4$^+$ helper T cells and B cells.[2] Appropriate reactivity of T cells is a prerequisite for adequate anti-HBs production after infection with HBV, as well as after hepatitis B vaccination. Thus, the T-cell epitopes of HBsAg being targets for recognition by T cells should also be affected.

The humoral response to HBsAg is T-cell-dependent. At least four regions within HBsAg present epitopes for major histocompatibility complex (MHC) class II restricted CD4$^+$ T cells (Table 2).[79,80] The effect of HBsAg T-cell epitope variants on the cellular immune response has been studies by Bauer et al. in individuals vaccinated against HBV[81] Six of 23 different variants in two HBsAg Th epitopes were shown to be responsible for inadequate T-cell reactivity. Similarly, the MHC class I-restricted CDB+CTL response plays a key role in suppressing HBV infection. Schirmbeck et al.[82] showed that in H-2b mice even small changes in amino acid residues within two different CTL epitopes that mimic natural variants of adw2, ayr and adr, completely eliminated the immunogenicity of each epitope. A majority of mutations up- and downstream of MHR in different studies were found to be potentially responsible for vaccine breakthrough, HBsAg undetectability[7,8,33,70,83] which unpredictably were found to be located within the known either Th or CTL (or both) epitopes of surface protein (Table 2).[79,80,84,85]

Nonetheless, accumulating evidence indicates that the genomic heterogeneity of the virus does not account for HBsAg negative status in some cases.[33,86,87]

3.3. 2.3- Mutation in other HBV proteins

In other studies, however, the failure of HBsAg detection was not fully explained by S gene mutations at all.[88,89] Furthermore, multiple mutations outside the surface protein could be found within the core,[90] pre-S[90,91] and polymerase[92] with known functional and/or immune epitope reactivity. In our study on 21 children who were born from HBsAg positive mothers and were non-reactive to two separate HBsAg tests, of 14 available HBV surface proteins, 10 harbored G145R. Three isolates did not contain any mutation within the surface protein at all, however, they had single and/or double mutations and deletions in pre-S2, X and polymerase proteins.[33]

Multiple alterations in the genome possibly have a synergistic effect in downregulation of HBsAg production, and an isolated cause for mutation in a particular gene or regulatory region could not be established. Thus, it appears that the nondetectability of HBsAg in serum may arise because of several mechanisms, caused by alterations in the structural, functional, and regulatory regions of the HBV genome making it below the sensitivity of standard enzyme linked immunosorbent assay tests.[87,90,93]

Mutation outside the surface protein may also influence HBV replication capacity. According to earlier studies, these mutants have also been reported to be less "replication fit" compared to wild-type virus *in vitro*, providing a plausible explanation for the low HBV DNA levels.[94,95]

4. HBsAg diagnostic dilemma

HBsAg detection is the main stay for the diagnosis of infection and the routine screening of blood donors and pregnant women. Several studies have addressed the impact of escape mutants on the sensitivity of HBsAg assays. The assays were challenged with native mutants and with recombinant mutant samples obtained by site directed mutagenesis or by cloning and expression of amplified sequences from escape mutant strains. These recombinant antigens were constructed to reflect mutations described in the literature occurring throughout the S gene. Due to difficulties to collect clinical samples containing natural HBsAg mutants, most of the recent data regarding the capability of commercial HBsAg immunoassays to detect HBsAg mutants have been drawn from studies involving artificial materialsobtained by those recombinant procedures.

4.1. HBsAg mutants in vitro reactivity, inconsistency in results

A large number of HBsAg mutants are thought to be able to influence the performance of commercial assays (Table 3). Most companies have either assessed their current diagnostic assays or produced new versions in order to better detect a range of variants, both natural and clinically selected. The introduction of sensitive techniques for detection of HBV DNA has highlighted this issue, as a significant proportion of persons can be DNA-positive in the absence of HBsAg, so called occult hepatitis B (OBH).

A number of explanations for the persistence of HBV DNA in HBs-negative samples have been proposed, including HBV DNA in low copy numbers,[96,97] altered host immune response,[98] genetic variations of the S gene,[58] viral DNA integration in the host genome,[99] infection of peripheral blood mononuclear cells [100], immune complexes in which HBsAg is hidden[101,102], and interference of other viruses such as hepatitis C virus (HCV)[103–106] and human immunodeficiency virus (HIV)[107–110]. OHB in apparently healthy HBV chronic carriers is essentially found in four types of clinical conditions: (1) recovery from infection defined by the presence of anti-HBs (spontaneous HBsAgseroclearance in a carrier) [111]; (2) chronic hepatitis, where the infection is related to escape mutants that are not (or are only poorly) recognized by either natural polyclonal or monoclonal antibodies in the assays [10]; (3) low-replicative phase of chronicity at the healthy carriage stage marked by the presence of hepatitis B core antibody (anti-HBc), with or without detecTable anti-HBe[112] and (4) chronic hepatitis or healthy carriage without any marker of HBV infection other than HBV DNA [113,114]. This illustrates the fact that some patients may have had serological markers of HBV infection, but subsequently lost them while still continuing to have a low-grade HBV infection.[114]

Although currently available HBsAg assays are superior to their predecessors, there is a need for further development with ongoing assessment of assay ability to detect a wide range of HBsAg variants. Several studies compare the sensitivity of modem assays for the detection of HBsAg variants in different panels.[60,115–118] Those current assays are much improved with manufacturers now using

Table 3
Variation in sensitivity of HBs antigen detection by various assays. Samples obtained by natural and/or recombinant constructs.

AA position	Reactivity/(%)	No. of HBsAg assays	Reference
T118A	19(100%)	19	70,115
P120G/S/L/T	17(68%)	25	75,76,115,126,131
C121S	1(14.2%)	7	126
K/E/R122I	21(67.7%)	31	72,122,126
T123N	23(63.8%)	36	60,72,122,124,126,132
T123A	3	3	133
C124R/Y	6(60%)	10	72,78,122
T125M	3(100%)	3	76
T126S/N	35(100%)	35	115,123–125,131,132
P127T/L	4(80%)	5	76,127
A128T	Weak	3	76
G/Q129H	39(97.5%)	40	115,123–125,132,133
G130D/R/N	6(60%)	10	86,131
T131A/N/I/P	28(84.8%)	33	70,72,78,86,115
M133I/L/T	49(92.4%)	53	60,73,115,123–125,127,131,132
Y134S/L/N	9(100%)	9	72,131
P135S	7(77.7%)	9	115
C137W	3(75%)	4	72
C138R	1(50%)	2	78
C139Y	3 75(%)	4	72
T/L/K140I/Q/E	11(73.3%)	15	72,78,115
P142L/S	19(76%)	25	72,115,127
143L	2 (%)/1 weak	3	131
D144E/A/G	49(80%)/2 weak	61	34,60,70,72,115,122–125,132
G145R	37(54.4%)/10 weak	68	60,78,86,115,123–126,131–135
G145A	9(75%)	12	115,127
G145K	6(75%)	8	72,122
C147S	3(75%)	4	72
T123N/T143S	3	5	124
T126S+G145R	1(20%)/4 weak	5	75
T131N G145R	0	4	86
G130D, G145R	0	4	86
P142L/S-G145R	48(76%)/8 weak	63	72,115,122–125,133
N/DE144A-G145R	9(42.8%)/3 weak	21	115,124,125
T126S/G145R	12(66.6%)	18	115,123,124
Y100C/P120T	1(14.2%)/3 weak	7	60
I195M	2(100%)	2	136
W196 Stop	1(50%)	2	136

a mixed monoclonal/polyclonal combination to minimize unexpected missing of HBsAg through epitope loss.

The panels included different HBV genotypes and HBsAg subtypes, as well as mutants displaying amino acid changes in most of the residues that have been found to impair the performance of HBsAg tests, either alone or in combination with other substitutions. Significant numbers of samples containing either natural or recombinant HBsAg mutants have been used in different studies to assess the performance of commercial assays. Most samples displayed multiple mutants and the results obtained confirmed that the performances of most assays were impaired significantly by amino acid changes in residues 120–124, 130–133, 142 and 144–145 (Table 3). G145R has also been shown to exhibit various degrees of altered binding of HBsAg in different commercial assays.[70,119,120] Accordingly, constructing HBsAg variants, particularly G145R, in yeast led to markedly reduced binding by monoclonal and polyclonal antibodies,[14,121] indicating that this change alone may be important in the cases of vaccine escape described to date. However, naturally occurring R145 variants are often detectable with monoclonal antibody-based assays, albeit at reduced sensitivity. Whether these cases also had very high levels of serum HBsAg or whether there are antigenic subtleties after expression in yeast is unclear.

However, others also showed that the presence of a particular mutation (i.e., 144A, 145A, and 145R, either solely or in combination) in a clinical sample had not always the same effect on the response of the assay (Table 3).[72,115,122–125] T he different relative proportion of wild-type strain and other viral variants accompanying the mutant in the sample [see below]. Other potentially relevant substitutions involving residues 118, 125–127, 134–139 had no significant effects on the results.[70,72,74,75,115,122–124,126,127]

4.2. HBsAgassays results;reasons for discrepancies

Despite the fact that all assays were not equally able to detect expressed HBsAg variants. The differences in the results might be followed by several reasons. The main reason for discrepancies in the assay results may be due to the use of anti-HBs antibodies with variable specificities and sensitivities against different HBsAg epitopes. It is obvious that the ability of an assay to detect a variant depends critically on the choice of anti-HBs usage. In general, these samples were detected by assays that used polyclonal antibodies in the capture and/or detection phases. Assays that contained mono antibodies for both phases of the assay appeared to perform less efficiently in detecting this set of variants.

Second, in any analysis of this nature, standardization of the number of HBsAg particles is required; having a similar, if nonidentical amount of 'standard' antigen to compare, the variant is crucial for the interpretation of results. The fundamental difficulty in the *in vitro* characterization of all these variations is that it is difficult to quantify the expressed HBsAg in a way that does not depend on its antigenicity. Expressed HBsAg is not absolutely ideal for characterizing variation as measured reactivity depends on both on antigenicity and total amount of protein. Because of the transient nature of expression and relatively small number of cells, the lower assays reactivity would be expected. Counting of particles by electron microscopy is too labor-intensive and variable (Carman, unpublished data).

Third, in the case of natural variants, HBsAg mutants are present as major or minor population pool as a quasispecies, direct sequencing of PCR products cannot detect a variant present in the sample as a minor population, and the true proportion of patients carrying HBsAg variants should, therefore, be higher than found in studies. Subsequently, some cases of mutants may be overlooked or interpreted as false positives, for example, when a reactive HBsAg is found concurrently with an anti-HBs positive result and a second assay is negative for HBsAg.

Finally, data regarding frequency of the different HBsAg mutants among the carriers from a given population are, scarce, and the selection of a panel of recombinant mutants for testing assay performance is unlikely to fit with the reality of that population.[43,123,124] Due to different HBV genotypes-studied, it appears that variants can have different antigenic expression depending on their genotype/subtype background. However, Mizouchi found that all 10 diagnostic kits evaluated in the study were able to detect HBsAg irrespective of all three genotypes A–C.[128]

Regardless, if the level of antigenemia is low, as is generally the case in healthy blood donors with inactive hepatitis B, low levels of circulating HBsAg mutants may not be detected, even if the immunoassay is capable of detecting the recombinant form of the corresponding mutant.[76,129]

5. Concluding remarks

At present, it is very important to find out new escape mutants and further investigate their distribution. Testing of a wide panel of natural HBsAg mutants with the current and with the modified assay have confirmed that amino acid changes in residues 143–145 are likely the main source of failures in detection of HBV and changes in the design of the HBsAg detection assays may improve significantly their ability to detect particular HBsAg mutants.

Conflict of interest

None declared.

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