**Supplemental Table 1: FDA-Approved HBV DNA Quantification Assays**

| Test | Standardized Against | Amplification and Detection Methods | LOQ and LOD | Dynamic Range of Quantification |
|---|---|---|---|---|
| Cobas AmpliPrep/Cobas TaqMan HBV test, version 2.0 | 2nd WHO standard | Real-time PCR<br>Florescent detection | LOD = 9.0 IU/ml in plasma and 19.0 IU/ml in serum<br><br>LLOQ of 20 IU/ml | 20 to 1.7 × $10^8$IU/ml (1.3 to 8.2 log IU/ml), |
| Abbott Real-Time HBV Assay | 1st WHO standard | Real-time PCR<br><br>Florescent detection | LOD =6.4 IU/ml in plasma and 3.8 IU/ml in serum<br><br>LLOQ = 10 IU/ml, | 10 to 1 × $10^9$ IU/ml (1.0 to 9.0 log IU/ml), |
| Aptima HBV Quant Assay | 3rd WHO standard | Transcription-mediated PCR<br><br>Florescent detection | LOD =5.6 IU/ml in plasma and 4.3 IU/ml in serum<br><br>LLOQ =10 IU/ml | 10 to 1 × $10^9$IU/ml (1.0 to 9.0 log IU/ml), |
| Procleix® Ultrio™ Assay | 1st WHO standard | Transcription-mediated PCR<br><br>Florescent detection | | |

**Supplemental Table 2: Role of HBV DNA Testing in Different Clinical Scenarios**

| **Diagnosis** | **Monitoring in patients off treatment** | **Patients on treatment** |
|---|---|---|
| Only marker of infection during window phase of acute infection | Useful to define phases of infection (HBV DNA plus ALT and HBeAg status) | Defines response to antiviral therapy: progressive decline in HBV DNA viral load expected<br>If failure to achieve ≥1 log decline by week 12 of treatment = nonresponse |
| Marker of HBV infection in persons with chronic HBV infection but HBsAg mutants resulting in falsely-negative HBsAg | Defines thresholds for HBV DNA for consideration of antiviral therapy<br>• If HBeAg-positive, HBV DNA >10,000 IU/mL needed<br>• If HBeAg-negative, HBV DNA >200 IU/mL needed<br>If cirrhosis, any level of HBV DNA | Diagnosis of virologic breakthrough: ≥1-log increase in HBV DNA from nadir or ≥100 IU/mL if previously undetectable |
| Identifies occult HBV infection (HBsAg negative, anti-HBc positive and HBV DNA positive) | Useful to differentiate HBV flare (increase in ALT and HBV DNA) from ALT elevation unrelated to HBV (increase in ALT without significant change in HBV DNA) | Identifies risk group for liver-related complications among those with decompensated cirrhosis (low versus undetectable HBV DNA on treatment) |

**Supplemental Figure 1: Diagnostic markers in acute hepatitis B infection**

Following exposure, an eclipse phase of ~8 days occurs, followed by the window phase when HBV DNA is detectable but HBsAg not yet present. HBsAg will be detectable ~35 post-exposure, followed by anti-HBc (first IgM, then IgG), with resolution of infection accompanied by decline in HBsAg and rise in anti-HBc. A second window period may occur between loss of HBsAg and detection of anti-HBs (varies with assays used) – and duration of this time, the only marker of infection is anti-HBc. Note, over time the levels of anti-HBs may decline and become undetectable, again leaving anti-HBc as the only marker of prior HBV exposure.

**Supplemental Figure 2: Hepatitis B Genotypes**

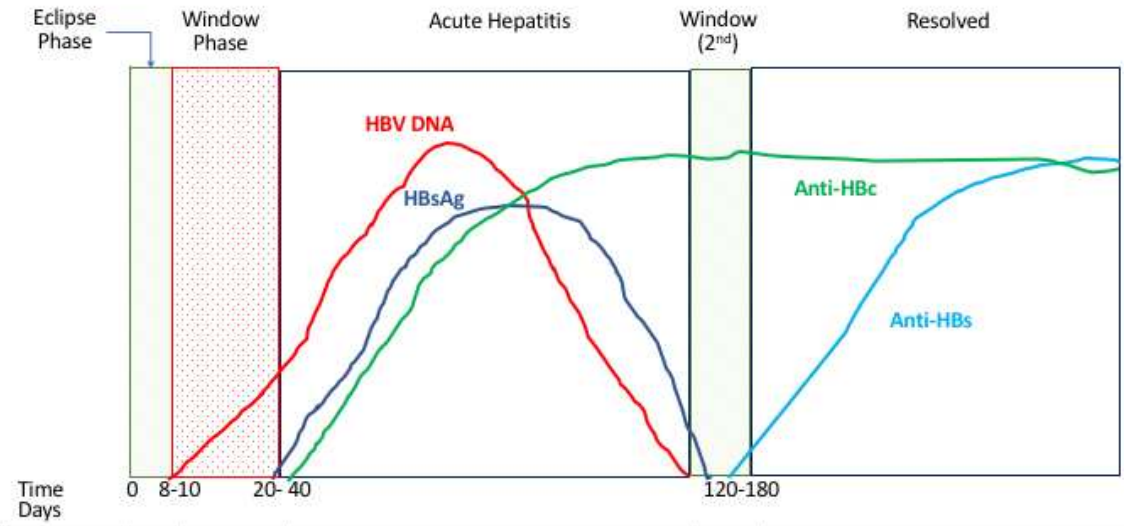
Of the most common genotypes, B and C are predominantly found throughout Asia, Genotype A and D found in parts of Europe, India and Africa and Genotype E is restricted to West Africa. In North America, the most common genotypes as described by the Hepatitis B Research Network are B (39%) and C (33%), followed by A (18%), D (8%), and E (3%), reflecting the largely foreign-born population of Asian and Africans {Congly, 2013 #23558} [87]. Less commonly found, genotype G is prevalent in parts of Europe and US, genotype H in Central and South America, genotype I in Laos and Vietnam, and the newest, genotype J in the Ryukyu Islands of Japan. Infections with mixed genotypes are also known to occur.

**Supplemental Figure 3A/B: Precore and Basal Core Promoter Mutations and HBeAg**

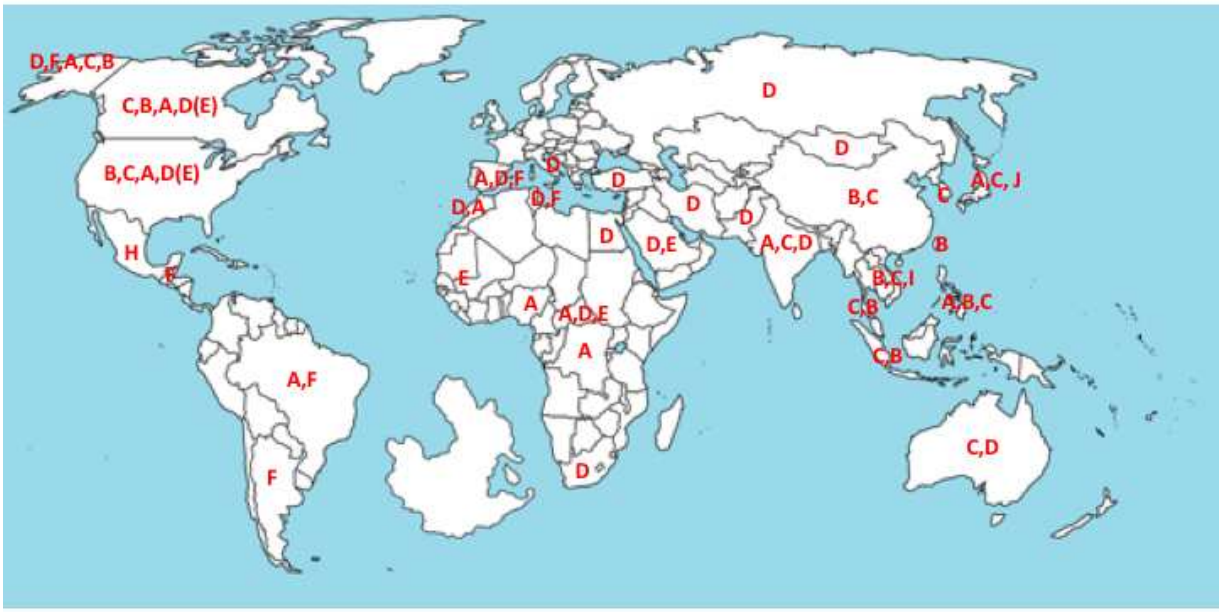
The precore mutation (G1896A, codon 28) inhibits translation of the precore protein due to frameshift mutations or premature stop codons (Figure 3A). The BCP (basal core promoter) mutations (nt1742-1849) suppress the production of precore mRNA at the transcriptional level resulting in defective synthesis of HBeAg (Figure 3B).

**Supplemental Figure 4: Precore Variants and HBV Genotype Associations**

The frequency of the precore variant varies by HBV genotype. The G->A1896 mutant cannot occur in HBV strains containing C at nucleotide 1858 because the change would disrupt the pre-exiting C-G Watson Crick base pairing and destabilizes the stem loop, which is critical in the replication cycle of the virus.




| | | | | | |
|---|---|---|---|---|---|
| Useful Diagnostic Tests | N O N E | HBV DNA | HBsAg<br>Anti-HBc (IgM)<br>HBV DNA | Anti-HBc IgG | Anti-HBc IgG/Total<br>Anti-HBs |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F
D
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D
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D,F
D
D
D
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E
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A
D
D
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C,B
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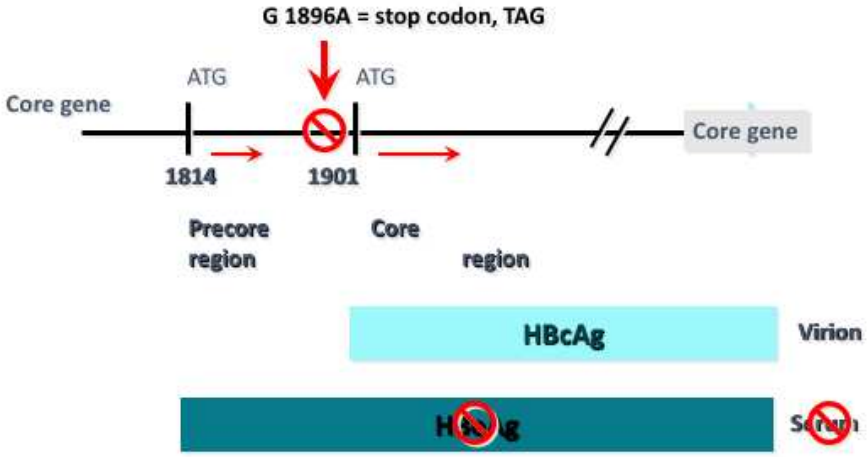
C,B
C,D

# Precore Variant



# Double BCP Variants