Diagnostic Methods of HBV and HDV infections

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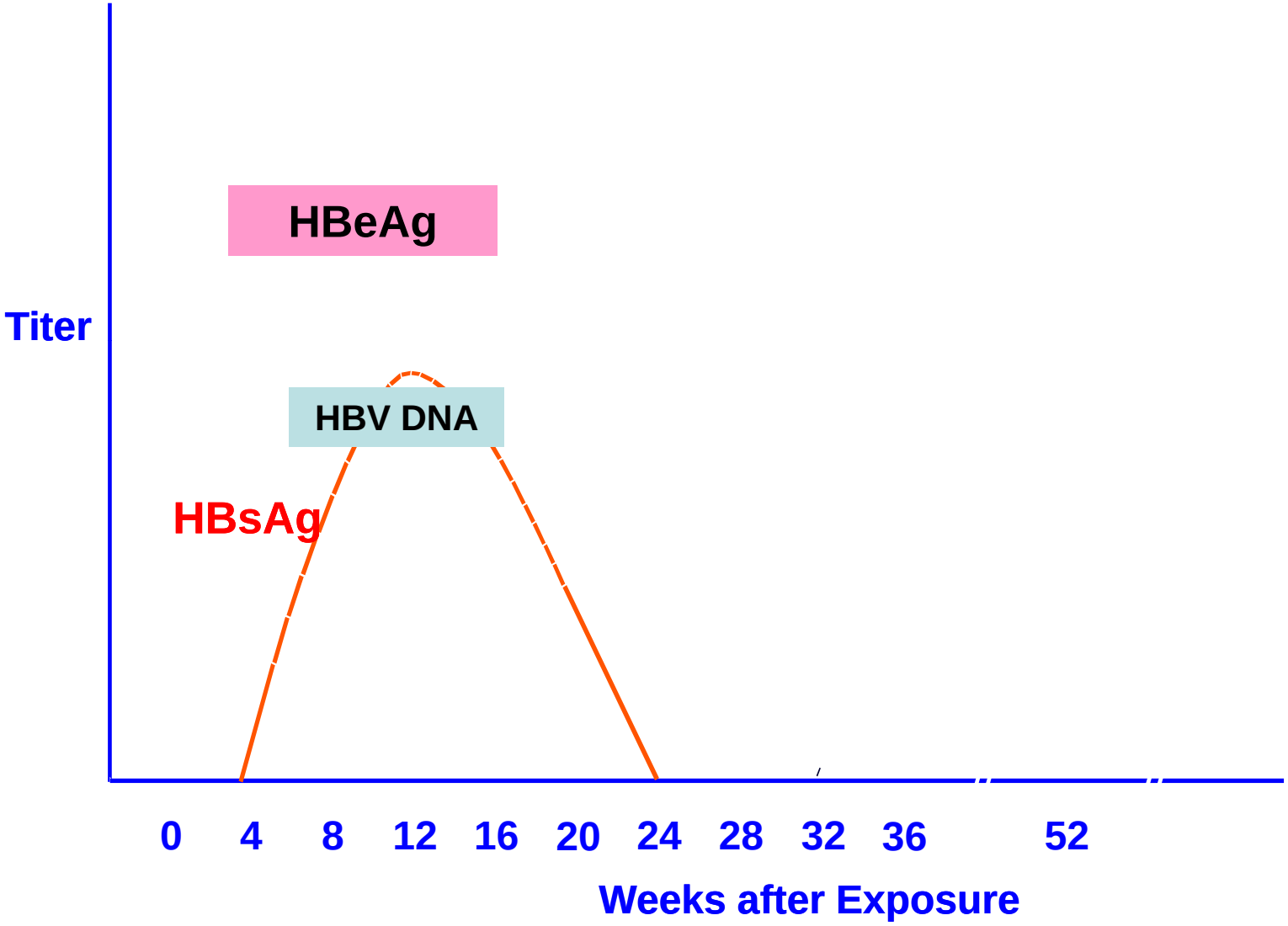
Hepatitis B-laboratory diagnosis

Detection of HBV infection involves

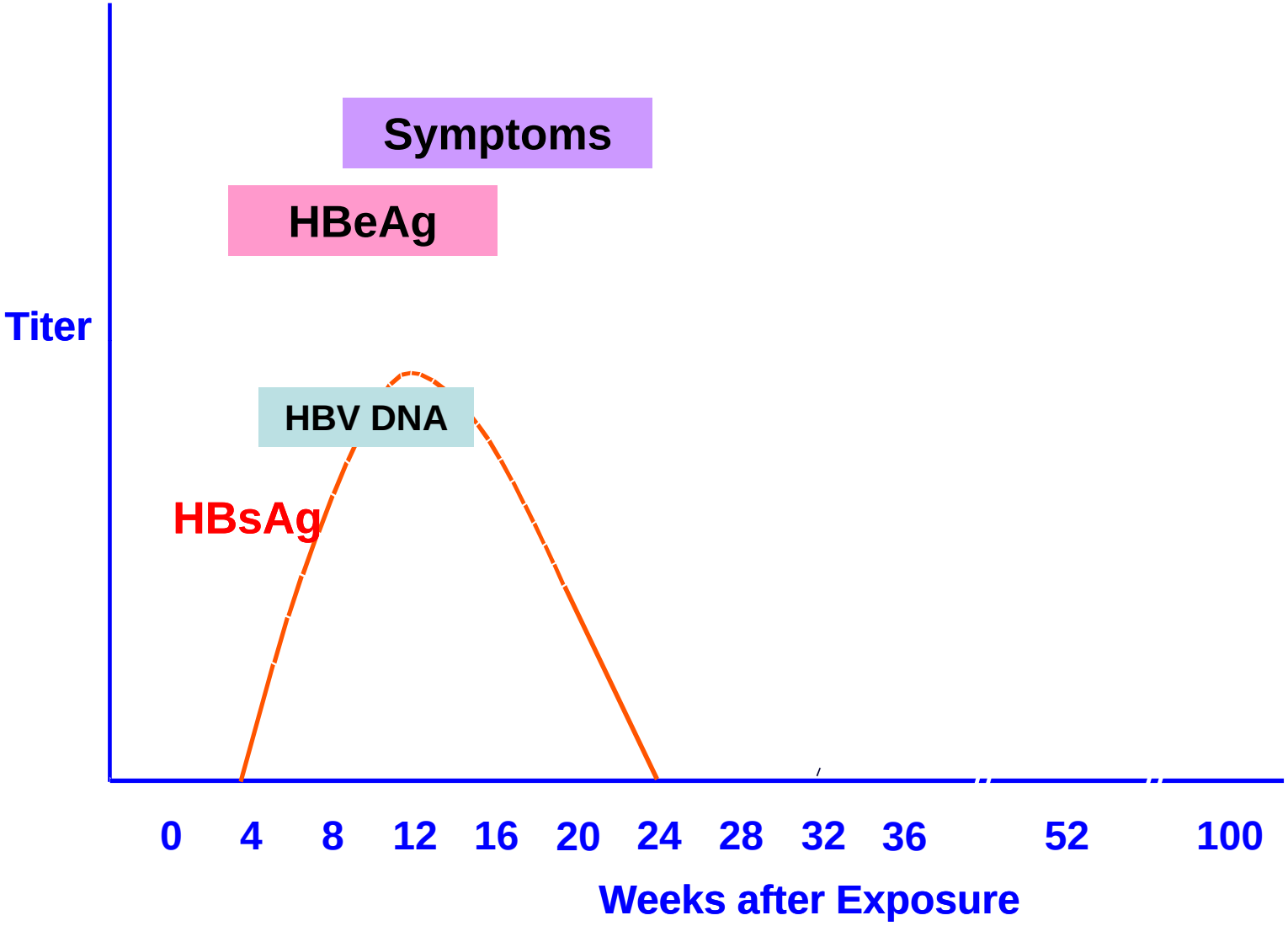
detecting the presence of:

– Viral genetic material
– Viral proteins (antigens)
– Antibody response to viral antigens

Acute Hepatitis B Virus Infection



Acute Hepatitis B Virus Infection

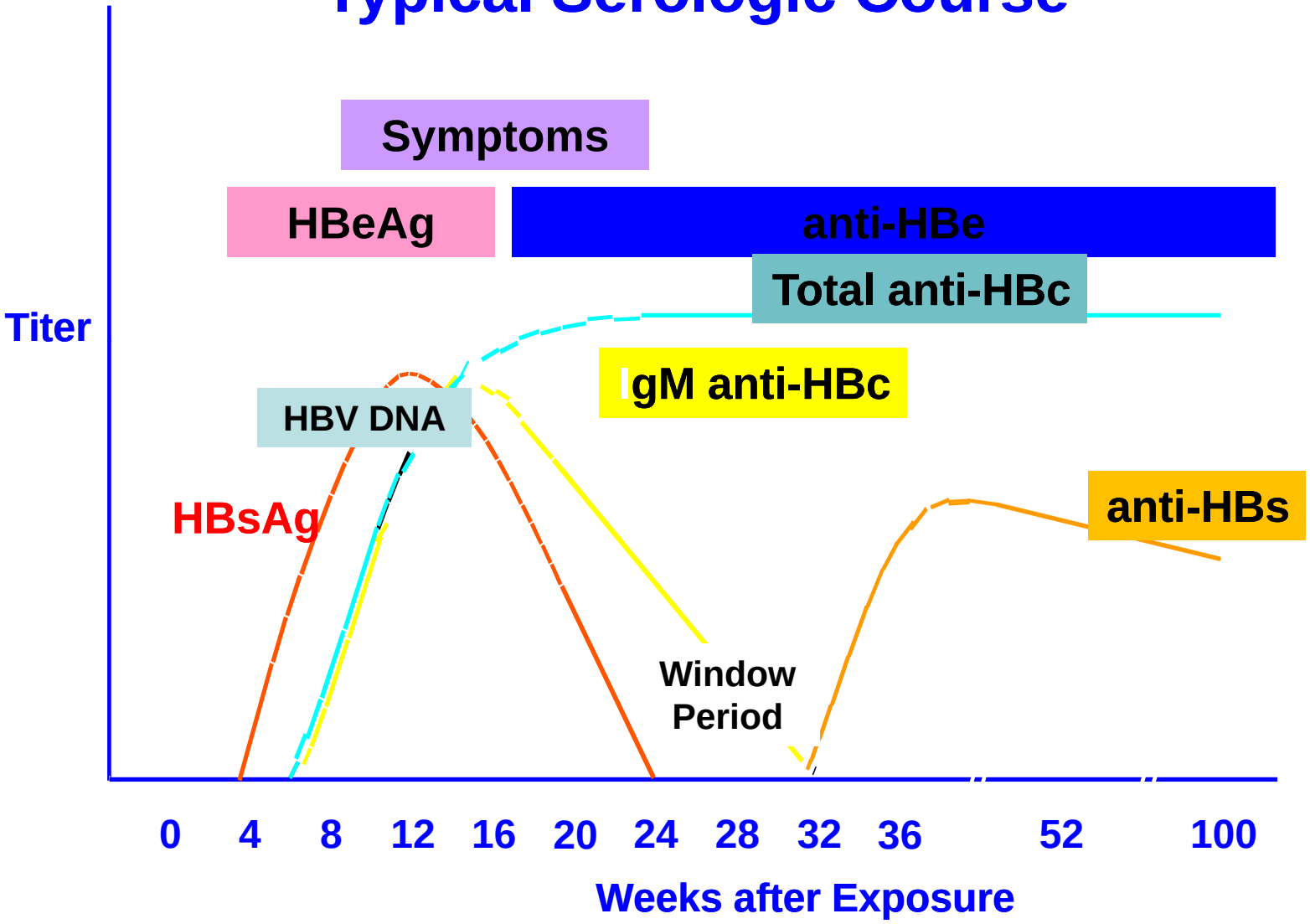


Diagnosis of acute hepatitis

Diagnosis of HBV infection has generally relied on interpretation of hepatitis B specific serology and biochemical markers of liver damage

There is currently no role for molecular testing in the diagnosis of acute hepatitis B other than in the detection of asymptomatic patients during pre transfusion screening of blood products

Acute Hepatitis B Virus Infection with Recovery
Typical Serologic Course



Serological Tests for HBV

- **Antibody assays**
  - **anti-HBs**
  - **anti-HBe**
  - **anti-HBc**
    - **IgM**
    - **Total**

Antigen assays

- **HBsAg**
- **HBeAg**

Confirmatory Screening Assays
HBsAg neutralisation

Interpretation of common HBV serology patterns

HBsAg	HBeAg	IgM Anti-HBc	IgG Anti-HBc	Anti-HBe	Anti-HBs	HBV-DNA	Interpretation
-	-	-	-	-	-	-	Susceptible to HBV Early in HBV incubation period
-	-	-	-	-	-	+	**Early in acute HBV** **Possible HBV variant infection**
+	-	-	-	-	-	+	**Early in acute HBV**
+	+	+	-	-	-	+	**Early in acute HBV**
+	+	-	+	-	-	+	**Chronic HBV(high infectivity)**
-	-	+	-	-	-	+	**Window period** **acute HBV**
+	-	-	+	+	-	-	**low infectivity** **Non-replication**
+	-	-	-	+	-	+	**Core mutation**
-	-	-	+	+	+	-	**Recovery**
					+		**Immunization response**

False positive HBsAg results and neutralization assays

As with all infectious disease assays, nonspecific binding can occur. In a large retrospective study found that weakly positive results are often falsely positive and require confirmation

In the case of tests for HBsAg, manufacturers provide a neutralization test that can be used to confirm true positivity of the results.

samples that were initially reactive in HBsAg testing but failed neutralization; their interpretation should, therefore, have been HBsAg-negative

False-negative HBsAg results

Window period

It has been recognized for many years that there is a delay between HBV infection and appearance of HBsAg; during this “window” period, the individual may still be infectious.

clinical sensitivity

Diagnostic (clinical) sensitivity maybe related to the analytical performance of the assay used.

The Studies on evaluated 17 different diagnostic reagent sets available in Europe, results showed that detection limits varied by 5- to 10-fold between the most sensitive and least sensitive assays, depending on the serotype of HBsAg tested

Other forms of HBsAg-negative HBV infection

Occult HBV infection

Most adults with acute HBV infection lose HBsAg and develop both anti-HBc and anti-HBs, HBV DNA remains present in small amounts, both in the circulation and within the liver

At present, it is not clear whether assays with better analytical sensitivity would be able to detect such “occult” infections

variants of HBsAg

The infecting strains of HBV in such cases often have mutants that impair HBV replication, leading to low concentrations of HBV DNA in the circulation

HBsAg “escape” mutants

The presence of HBsAg mutants that are not detected by HBsAg assays and that can cause infection despite the presence of anti-HBs

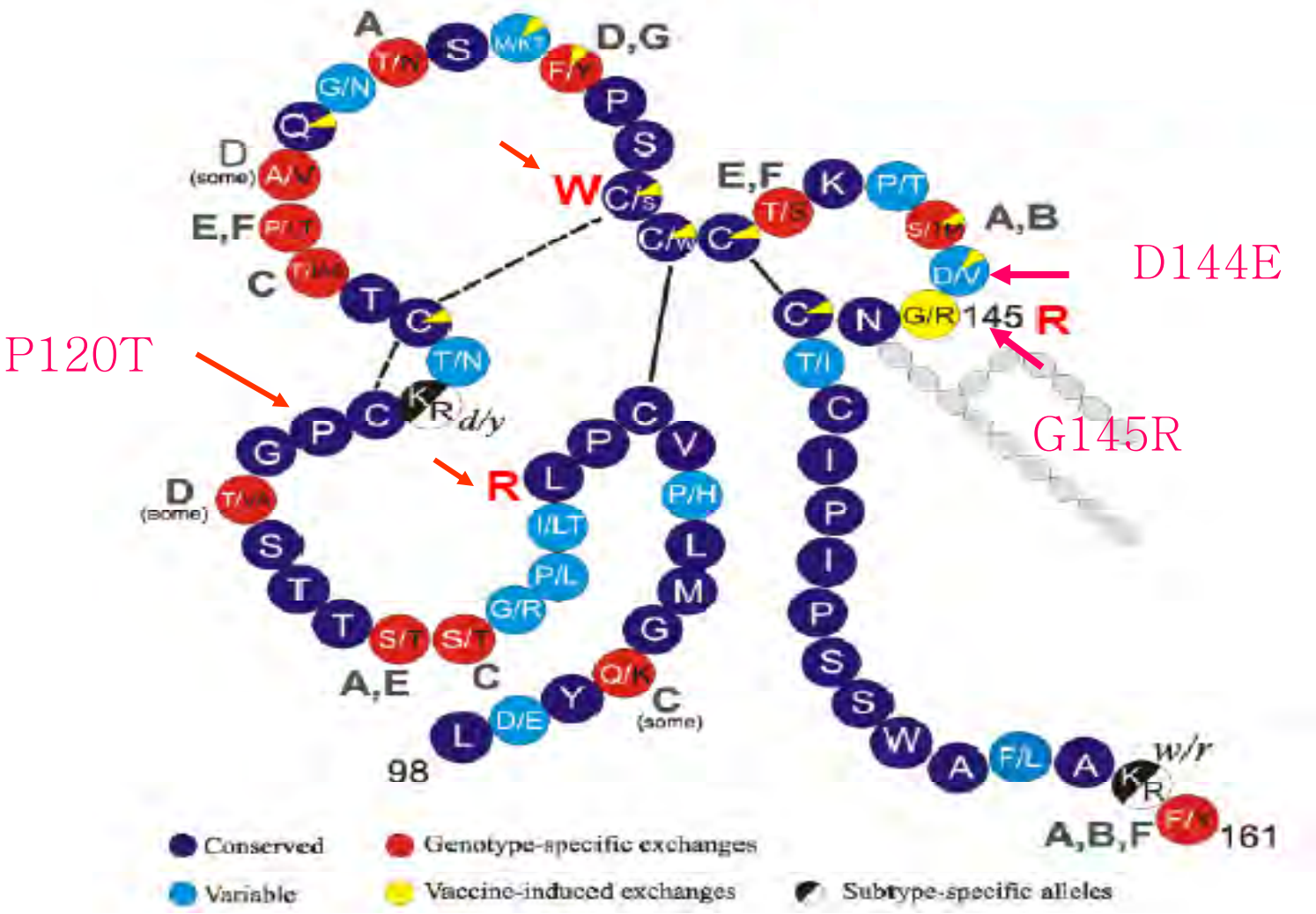
Individuals exposed to strains with mutations in critical positions in the HBsAg “a” determinant may become infected despite having what are thought to be protective titers of anti-HBs; additionally, such mutants may escape detection by HBsAg assays

Vaccine / HBIG escape mutants

Immune escape mutants

Changes in the amino acids within the *a determinant, particularly* between 137-147, disable surface antigen domain recognition by neutralizing antibodies.

Genotype- and vaccine-escape induced specific exchanges in the a determinant of SHBs

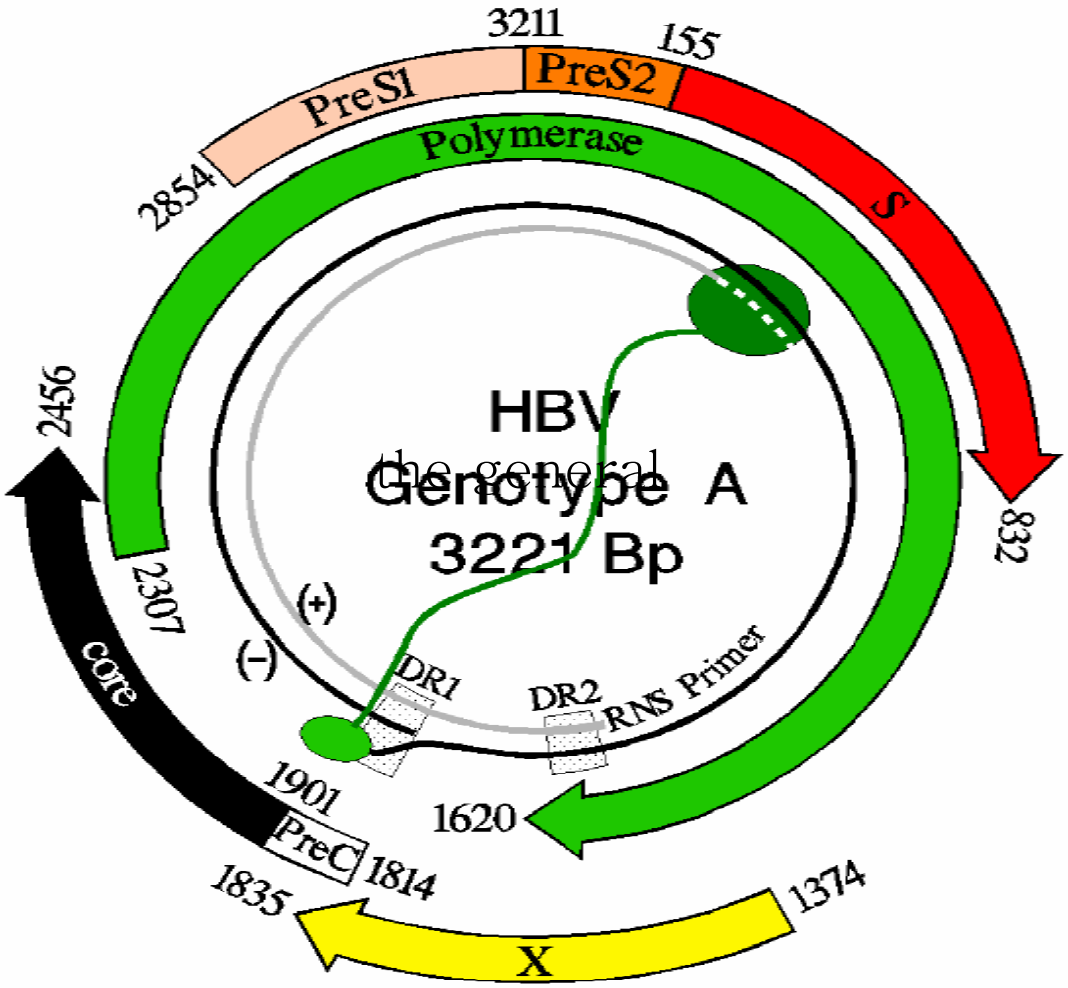


Overlap and mutation in the P gene

The nucleotide at rt204 in the *P gene is associated with resistance to LAM,* and entecavir (ETV), and the rtM204V/I mutation typically results in a sI195M, sW196S, sW196L or a terminal codon in the overlapping *S gene*

In previous studies, LAM selected HBsAg mutants with reduced anti-HBs binding capacity, and secretion of HBsAg was prevented with a mutant strain due to the stop codon

HEPATITIS B VIRUS GENOME



Analytical performance

At the least, laboratories should know the analytical performance of their assays near the cutoff concentration, and use neutralization assays in samples with weakly positive HBsAg results.

Because mutants can occur at many positions in the “a” determinant, the expert panel consensus statement suggested that assays will need to include more than one monoclonal antibody to detect all mutant strains

Discordant results

Samples with discordant results

- (e.g., those positive for both HBsAg and anti-HBs,
- or samples with positive HBeAg or HBV DNA but negative HBsAg)

should be evaluated for the presence of mutant strains;

this implies that panels of tests should be performed whenever HBsAg results are suspect.

- Laboratories should communicate with clinicians on the status of patients with questionable HBsAg results; important parts of the clinical history include
- immune status of the individual,
- and results of any previous tests that may have been performed using another assay.

Sensitivities of Four Commercial Hepatitis B Virus Surface Antigen (HBsAg) Assays in Detection of HBsAg Mutant Forms

Mutations in hepatitis B virus surface antigen (HBsAg) involving amino acid substitution may affect the performance of commercial HBsAg assays.

(i) analytical sensitivity performance with a national reference HBsAg panel

(ii) the detection of HBsAg mutants by studying a panel of 35 HBsAg mutants (

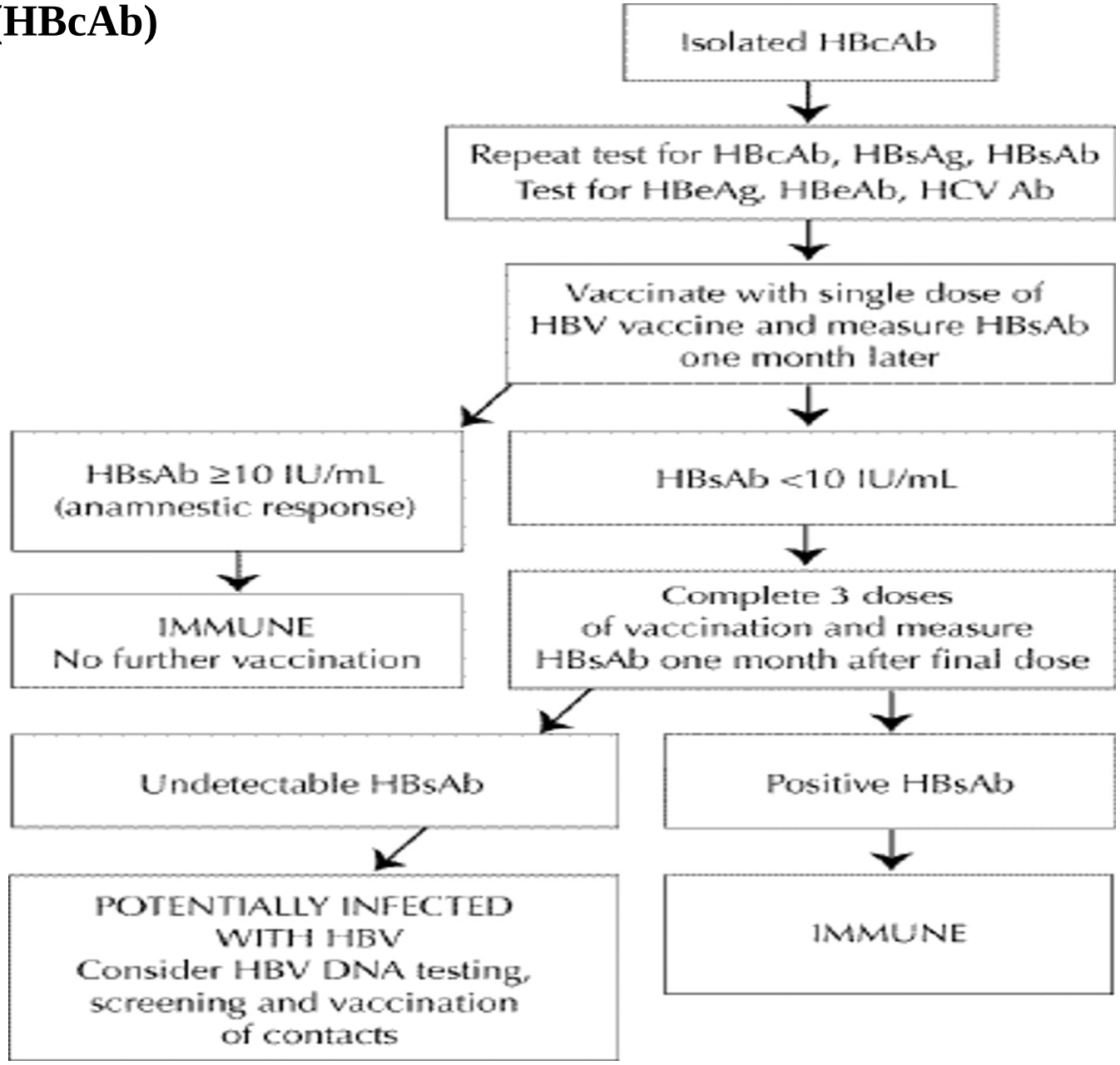
The limits of detection of these assays were from 0.089 to 0.121 ng/ml

The sensitivity performances for mutant virus detection varied, ranging from 37.1% to 91.4%.

Core Alone

- 4 possible interpretation
- False positive anti-HBc result
- Window phase of Resolving acute infection
- Late immunity Stage
- Unresolved infection in late
- Repeated anti-HBc/ anti-HBs after 1-3 Months

Suggested algorithm for evaluation of patients with isolated hepatitis B core antibody (HBcAb)



Molecular assays

Four types of molecular assays are available for the diagnosis and management of HBV infection:

qualitative & quantitative (viral load)tests

genotyping assays

drug resistance mutation tests

core promoter/pre core mutation assays

Molecular assays

Molecular assays have begun to play increasingly significant roles in chronic hepatitis B(CHB)

TABLE 2. Useful markers for distinguishing chronic hepatitis B phases

Phase	/	3sAg	HBeAg	HBeAg antibody	HBV DNA (IU/ml)*	Liver histology
Immune tolerance	Usually normal	Present	Present	Absent	≥20,000	Usually normal; can have mild inflammation
Immune clearance	Elevated; can be episodic	Present	Present	Absent	≥20,000	Active inflammation
Inactive HBsAg carrier	Usually normal; can have flares	Present	Absent	Present	<20,000*	Degree of abnormality dependent on disease severity during clearance phase (mild inflammation to inactive cirrhosis)
HBeAg⁻ CHB	Periodic flares	Present	Absent	Present	>20,000 or <20,000	Active inflammation
Occult hepatitis B	Can be elevated	Absent	Absent	Present in recovered HBV infection	<20,000†	Ranges from normal to cirrhosis and HCC

QUANTITATIVE HBsAg ASSAYS
Correlation with serum HBV DNA

Although measuring serum HBV DNA is the gold standard for monitoring viral load, the technique for detecting qHBsAg is fairly easy and inexpensive, and the primary aim of initial clinical studies was to determine the relationship between qHBsAg and serum HBV DNA

qHBsAg might be particularly helpful in patients with undetectable HBV DNA, even with a highly sensitive polymerase chain reaction assay

The quantitative HBsAg assays

The quantitative HBsAg assays are used

to predict sustained virologic response (SVR) after the end of antiviral treatment.

The level of HBsAg in the serum provides the transcriptional activity of HBV cccDNA and is used as biomarker in chronically

Correlation with covalently closed circular DNA

An important qHBsAg issue is its association with covalently closed circular DNA (cccDNA)

However, to examine cccDNA, an invasive procedure is required, and qHBsAg has been suggested as a surrogate marker for cccDNA

cccDNA was significantly correlated with qHBsAg, suggesting that serial monitoring of qHBsAg might act as an additional marker to evaluate treatment response during antiviral therapy

Precore promoter/ precore mutations

The most important precore promoter mutations are A to T at nucleotide 1762 (A 1762 T) and G to A at nucleotide 1764(G 1764 A / T) that decreased HBeAg expression

The most important precore mutation is G to A at nucleotide 1896 (G1896A) that stopped HBeAg expression

viral load testing is useful

- Before therapy

baseline to predict the response to antiviral and the emergence of antiviral resistance, particularly to lamivudine

- During therapy (to predict flares, to change therapy)

viral load can be used as a primary end point to assess the response to nucleoside antiviral therapy

- In HBeAg- CHB, HBV DNA is the only virologic marker that can guide the decision to end treatment

The different types of nucleic acid molecular techniques

- Direct probe testing

First-generation assays for HBV DNA quantification in peripheral blood were based on solution Hybridization technology that they are better for identification than for detection because it is not as sensitive as amplification methods

- Amplification methods

used to improve the sensitivity of the nucleic acid testing technique that led to the development of second-generation assays with enhanced sensitivity (as low as 200 copies/ml)

- Combinations of the above

The latest generation HBV quantification assays utilize real-time PCR and have improved analytical performance characteristics, including low limits of detection, broad linear ranges ,excellent precision, better

Commercially available assays and reagents for HBV DNA quantification

Test or reagent (manufacturer)	Method	sensitivity IU/ml	
COBAS TaqMan Roche Diagnostics)	Real-time PCR	3.5	(Europe, CE)
Artus HBV PCR (QIAGEN Diagnostics)	Real-time PCR	$2*10^1$	Europe, CE)
Real-Time HBV PCR (Abbott Molecular)	Real-time PCR	10	(Europe, CE)

HBV Genotyping (Genotype A-H)

The HBV genotype is a variable that could potentially influence the outcome of chronic hepatitis B

An HBV genotype-dependent response to antiviral therapy

None of the current guidelines have advocated a role for genotyping in counseling patients on the outcome of chronic hepatitis B

Given its limited utility, HBV genotype testing has not yet been widely adopted in clinical laboratories

genotyping assays

A variety of methods have been used including

* whole- or partial-genome sequencing

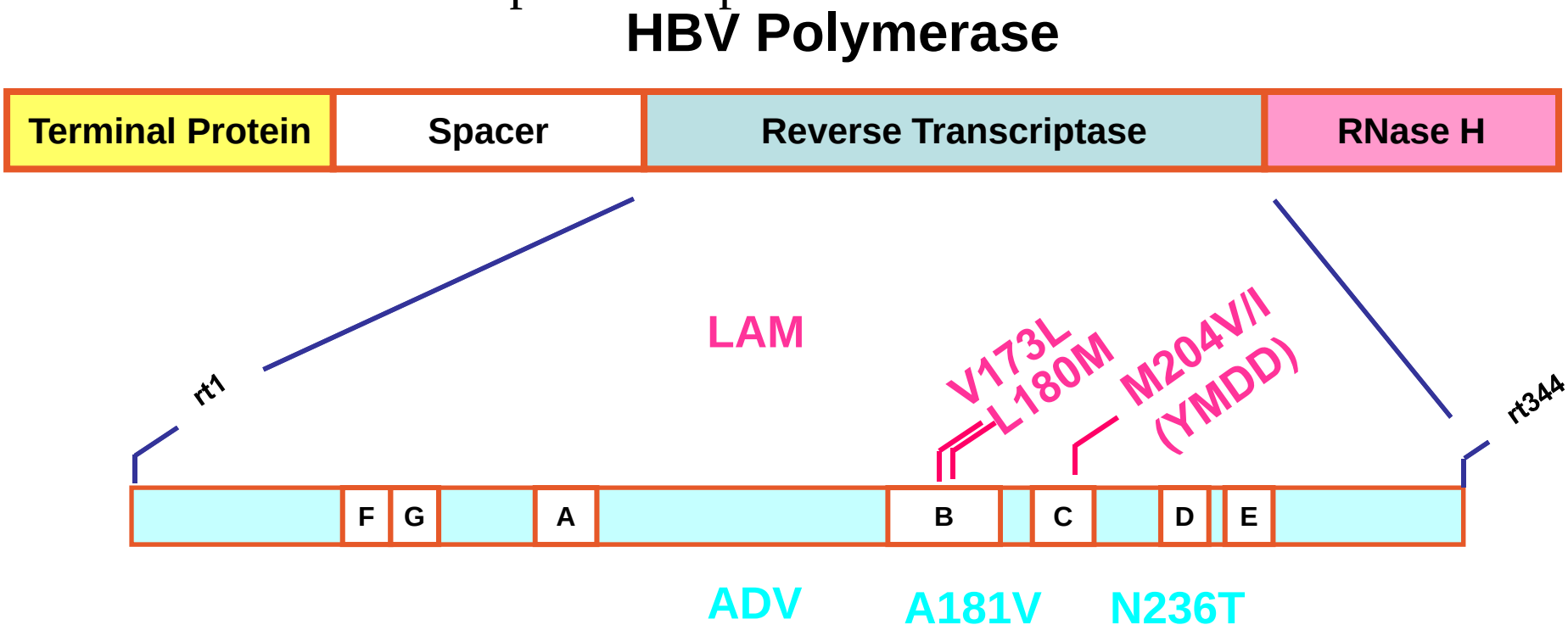
Whole-genome sequencing is the “gold standard,” and it is particularly accurate for detecting recombinant viruses

* Restriction fragment length polymorphism (RFLP)
* Genotype-specific PCR amplification

* PCR plus hybridization PCR has been adapted into a commercial product (INNO-LiPA; Innogenetics)
*Serology

Drug Resistance Mutations

The emergence of drug-resistant HBV is related with a 10-fold increase in viral load compared to patient with documented therapeutic response



Drug resistant mutation assays

- Direct sequencing can identify known and potential new resistance mutations

- PCR plus hybridization PCR that the second-generation product (INNO-LiPA DR, version2.0) has a refined, expanded lamivudine resistance panel (codons 80, 173, 180, and 204) and also detects adefovir resistance mutations (codons 181 and 236)

Hepatitis Delta Virus - HDV

HDV antibody tests

- Anti-HDV, IgG antibody :

- the first diagnostic screening test and should be performed in all HBsAg –positive patients
- Usually, only chronic cases demonstrate IgG antibody

- anti-HDV IgM antibody:

- can be used to determine disease activity in acute/chronic HDV infection
- Acute HDV infections are associated with anti-HDV IgM antibody

- HDV antigen (HDsAg):

In acute co infections, HDsAg appears early, after HBV virus surface antigen, and disappears with convalescence

- HDV RNA qualitative:

Gold standard to determine HDV replication and active infection

- HDV RNA quantification tests :

can be useful for antiviral treatment

are not yet standardized; viral load results do not necessarily correlate with disease

HDV genotyping:

At least eight different HDV genotypes have been described and each has a characteristic geographic distribution and a distinct clinical course.

Type 3 associated with more severe disease

HBsAg quantitative :

determines the level of HBsAg in the blood that is associated with HDV level and HBsAg monitoring can be useful during antiviral treatment