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RECENT DEVELOPMENTS IN SCREENING HEPATITIS B VIRUS BY MOLECULAR TECHNIQUES

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ABSTRACT
Hepatitis B virus is a sickness that still causes a lot of health problems around the world. It is especially common in countries that are still developing. When doctors find out about this sickness late, people can get very sick or even die. Finding out early if someone has this sickness is very important. This helps doctors give the right treatment, stop the sickness from spreading to others, and work towards completely getting rid of it. This review talks about new and better ways to find the Hepatitis B virus. It explains how these methods work, how they are used, what is good about them, and what problems they might have. Old ways of finding the sickness by testing blood are helpful but sometimes miss the virus, especially when the sickness just started or if the virus is hiding. New tests that look at the virus's tiny parts (like its genetic material) are better at finding even very small amounts of the virus. These tests can also find changes in the virus and check if treatment is working. There are special tools that doctors can use anywhere to test quickly. Some tools use smart technology to help understand the test results better. Comparing these methods shows that we need tests that are not too expensive, are fast, and give correct answers. New ideas like tiny labs on a chip or sensors that people can wear are coming soon and may change how we find this virus. This review also points out what is missing in current tests and suggests ideas for future research to create easy, cheap, and accurate tests that everyone in the world can use.

Keywords: Hepatitis B virus, Molecular diagnostics, PCR, CRISPR, Point-of-care testing

1. INTRODUCTION

Molecular screening (a special way of testing using tiny parts of the virus called molecules) has changed how we find out if someone has the hepatitis B virus (HBV). HBV is a virus that can harm the liver. This new method helps doctors find the virus by directly looking for its nucleic acids (the virus's genetic material, like DNA or RNA that tells the virus how to work). This test is very good at noticing even very tiny amounts of the virus.

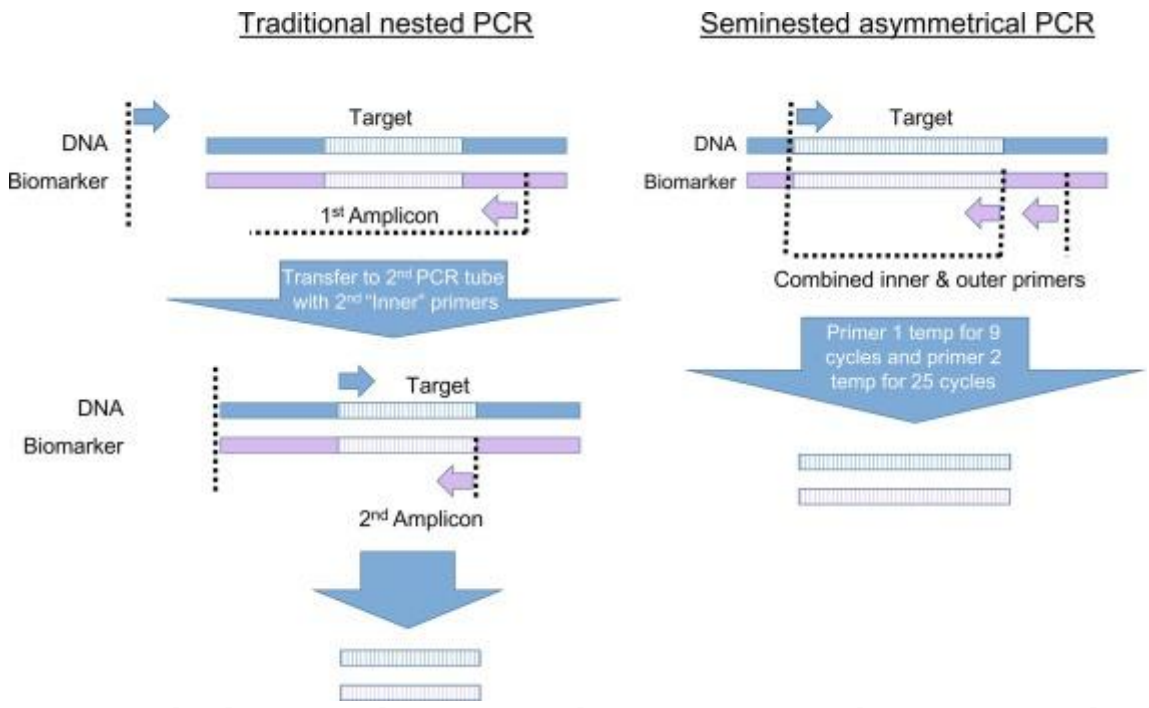
Finding HBV early is very important so that doctors can start antiviral therapy (medicines that fight against viruses) as soon as possible. This helps stop the disease from getting worse and turning into cirrhosis (a serious problem where the liver gets badly damaged and can't work properly) or hepatocellular carcinoma (a dangerous kind of liver cancer).

Older testing methods, called serological assays (blood tests that look for special proteins in the blood), can find viral antigens (tiny pieces of the virus that the body reacts to) and antibodies (special soldiers made by the immune system to fight viruses). But sometimes, these tests don't find the virus when there's only a little of it in the body. This is called low-level viremia (when the virus

is in the blood, but in very small amounts) or occult infections (infections that are hidden and don't show up in usual tests). This problem happens more often in people who are immunocompromised (people whose immune system is weak and cannot fight infections well). [6]. A lot of people in the world still suffer from a long-term disease called chronic HBV infection (chronic HBV infection means a long-lasting illness caused by the hepatitis B virus, which harms the liver). About 296 million people around the world have this illness. Every year, nearly 820,000 people die because of liver disease (liver disease means when the liver, a very important part of your body, gets damaged or stops working properly) caused by this infection [11].

Because this is such a big health problem (public-health imperative means something very important for the health of many people), scientists have worked hard to make new and better ways to find this illness using molecular diagnostics (molecular diagnostics means special scientific tests that look for tiny things like viruses in your body). These tests give results very fast (rapid turnaround times), can find the virus even in very small amounts (greater analytical sensitivity), and can count how much of the virus is in a person's body (quantitative viral load measurement). These features are very important parts (hallmarks) of how doctors today take care of people with HBV (contemporary HBV management means the modern way of treating and managing hepatitis B).

In the middle of the 1990s, a method called conventional polymerase chain reaction (PCR) [polymerase chain reaction (PCR) is a laboratory method used to make many copies of a small piece of DNA, which helps in finding viruses or bacteria] became very important for checking if someone had HBV [HBV means Hepatitis B Virus, which is a virus that affects the liver]. This method helped scientists make more copies of the virus's DNA directly from a person's sample, like their blood [3].



[Figure 1: Nested Polymerase Chain Reaction https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/nested-polymerase-chain-reaction]

A scientist named Naito and his team created a test using something called nested PCR [nested PCR is a more detailed version of PCR that involves doing the PCR test two times to make it more accurate]. This test could find even very tiny amounts of the virus—only about 10 pieces of the virus's DNA—better than the old tests that looked for signs of the virus in the blood, which are called serological tests [serological tests are tests that check for antibodies or proteins in the blood to see if a person has had a virus or infection] [9].

But even though nested PCR was very good at finding small amounts of the virus (very sensitive), it had some problems. It could easily get contaminated [contamination means unwanted things like bacteria or viruses can mix into the test and give wrong results], and it needed many steps to be done, which made it harder to use regularly in hospitals.

Even with these problems, conventional PCR helped scientists build better and faster testing methods later on, like real-time PCR [real-time PCR is a quicker and easier version of PCR that shows results while the test is happening], which are now commonly used in labs that study viruses.

Quantitative real-time PCR (qPCR) further changed how we test for HBV (which is a virus called Hepatitis B Virus) by combining two steps—making many copies of the virus's DNA (this is called amplification) and checking for the virus (this is called detection)—all inside one closed tube. This is good because it stops the sample from getting dirty or mixed with other things (which is called contamination) and helps us measure exactly how much virus is in the sample (this is called viral load) [14].

Carman and others tested a special qPCR test called a TaqMan-based qPCR assay. They showed it could measure virus amounts between 10^2 to 10^8 IU/mL (this means international units per milliliter, a way to count virus particles), and the test results were very reliable with very little variation (less than 5%) when done many times or by different people [3]. This made it easier for doctors to keep track of how patients were doing during treatment and if the virus was coming back, helping them decide when to start or change medicine. Also, qPCR machines can be changed to test for more than one virus at the same time (this is called multiplex formats), like testing for hepatitis C virus (HCV) or HIV along with HBV.

Even though qPCR is very useful, it needs special machines that change temperature many times (this is called thermal cycling equipment), and these machines can be expensive and hard to use in places with fewer resources. To solve this problem, another method called Loop-mediated isothermal amplification (LAMP) was created. LAMP makes many copies of DNA but at a constant temperature, so it does not need those complicated machines (thermocyclers) [4]. LAMP was first made by Notomi and friends in 2000, and later it was changed to test for HBV with similar accuracy to qPCR in about 30–60 minutes [4].

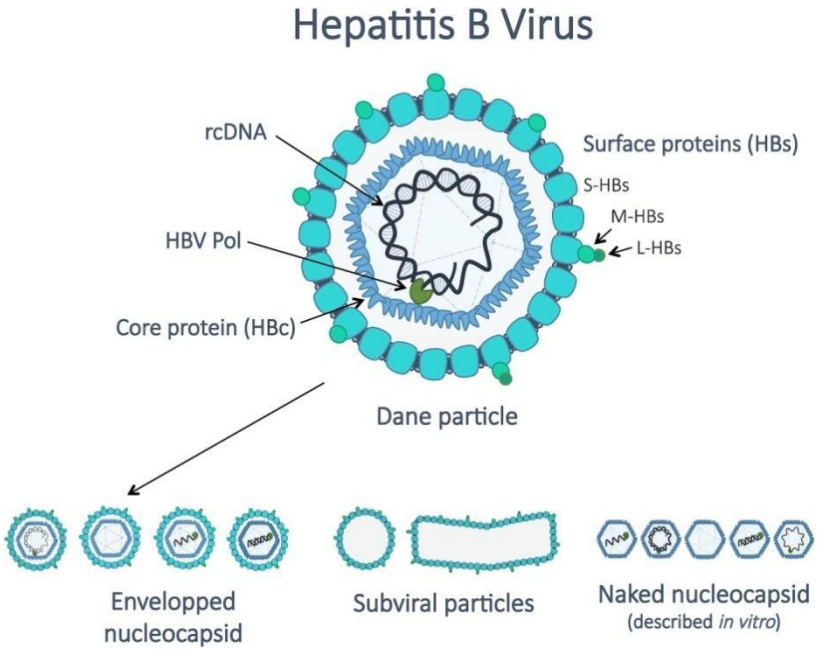
There are easy ways to see LAMP results, like changes in color (colorimetric) or glowing light (fluorescent), so the test can be done right where the patient is (this is called point-of-care testing), without needing big machines. Because LAMP is simple and fast, it can be used for testing many people in places where there are lots of cases (endemic regions) but not many labs.

New tests have been made to be even better at finding small amounts of virus (sensitivity) and correctly identifying the virus (specificity). One is digital PCR, which splits a sample into thousands of tiny parts (microreactors), letting scientists count the exact number of virus pieces without comparing to standards. It can detect very tiny amounts, even less than 1 IU/mL, and is very reliable (less than 2% variation) [15].

At the same time, there are new tests using CRISPR technology, which uses special enzymes called Cas12 and Cas13. These enzymes can cut pieces of DNA or RNA very specifically, helping find tiny amounts (attomolar means extremely small amounts) of virus material [6]. Gootenberg and his team made a test using Cas12a and Cas13a enzymes that can tell different types (genotypes) of HBV in 40 minutes at body temperature (37 °C). This shows it could be used quickly in the field for checking virus types [1].

Finally, next-generation sequencing (NGS) machines can read the entire genetic code (genomes) of HBV, study the variety (quasispecies diversity) of the virus in a patient, and find changes that cause resistance to medicines (drug-resistance mutations) all in one test [17]. These machines now use special steps (target enrichment protocols) to focus on HBV DNA and read it deeply enough to find even rare virus versions that make up less than 1% of the total. Computer programs (bioinformatics pipelines) and smart computer learning (machine learning algorithms) help scientists find these changes more accurately and predict how the disease might act. As NGS becomes cheaper and easier to use, it will help doctors give better, personalized treatment plans for HBV and help efforts to completely get rid of the virus.

1.1 Overview of Hepatitis B Virus (HBV)



[Figure 2: Hepatitis B Virus (HBV) https://www.sciencedirect.com/science/article/pii/S0166354220303399]

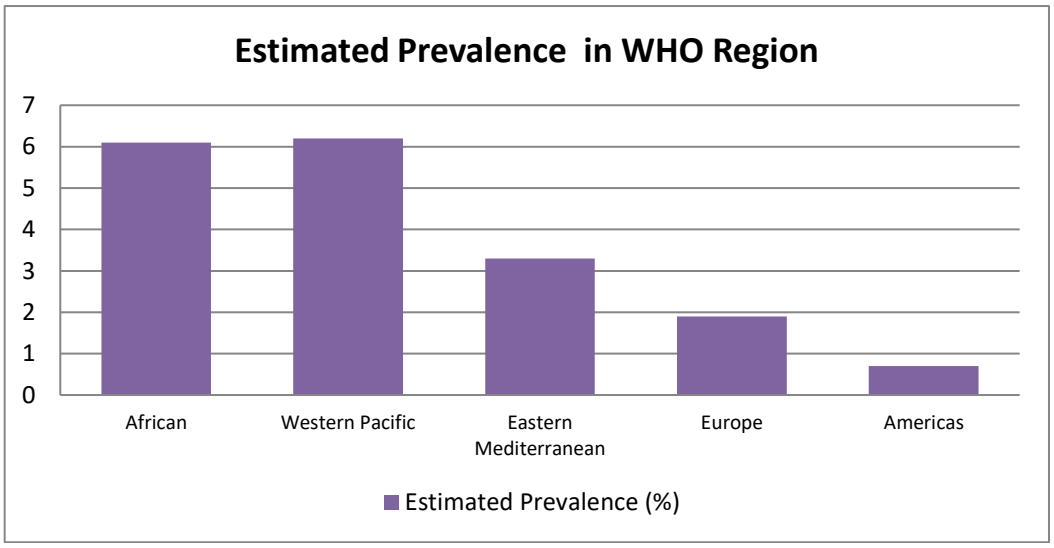
Hepatitis B virus (HBV) is a small, enveloped DNA virus belonging to the *Hepadnaviridae* family, characterized by a partially double-stranded, circular genome of approximately 3.2 kb [8]. The viral particle comprises an inner nucleocapsid core—formed by core (HBc) protein—surrounded by a lipid envelope studded with three forms of hepatitis B surface antigens (small, middle, and large HBs) that mediate host-cell entry via the sodium taurocholate co-transporting polypeptide (NTCP) receptor on hepatocytes [9]. Inside the hepatocyte nucleus, the relaxed circular DNA (rcDNA) is repaired to form covalently closed circular DNA (cccDNA), which serves as the template for all viral transcripts and underlies HBV's persistence and difficulty of complete eradication [18][9].
HBV exhibits at least ten genotypes (A–J), defined by >8 % nucleotide divergence, each with distinct geographic distributions and clinical outcomes [10]. Genotype C, for example, is associated with higher rates of progression to cirrhosis and hepatocellular carcinoma compared to genotype B, which often presents with earlier HBeAg seroconversion [11]. Mutations within the basal core promoter and precore regions can abrogate HBeAg expression, giving rise to "e" antigen–negative variants that complicate serological diagnosis and correlate with more aggressive liver disease [10][11]. The complex interplay between viral genetic diversity and host immune responses underscores the need for precise molecular tools to characterize infection dynamics and guide individualized patient management.

1.2 Global and Regional Epidemiology

Chronic HBV infection remains a major global health challenge, with an estimated 296 million people living with chronic HBV at the end of 2019, corresponding to a prevalence of 3.9 % worldwide [12]. Although universal childhood vaccination programs have dramatically reduced incidence in many high-income countries, regions such as the WHO African and Western Pacific areas continue to bear the greatest burden [13]. Mother-to-child transmission during birth remains the predominant route of chronic infection in these high-endemicity settings, necessitating robust screening of pregnant women and timely administration of birth-dose vaccine and hepatitis B immunoglobulin.

WHO Region	Estimated Prevalence (%)	Key Notes
African	6.1	Predominantly perinatal and early childhood transmission [12]
Western Pacific	6.2	High rate of genotype C infections [3]
Eastern Mediterranean	3.3	Variable prevalence; significant migrant populations [13]
Europe	1.9	Reduced by vaccination; pockets in Eastern Europe [15]
Americas	0.7	Low endemicity; outbreak clusters in indigenous communities [13]

Table 1. Estimated chronic HBV prevalence by WHO region (2019). **[12][13]**



[Figure 3: Estimated Prevalence in WHO Region]

Even though we have made progress, there are still some problems. Some pregnant women (antenatal means "before birth") do not get tested (screening) or vaccinated properly, especially when the first vaccine dose is not given soon after birth (timely birth-dose administration). This lets the disease keep spreading, especially in poor areas (low-resource settings). The way the disease spreads and appears (epidemiological heterogeneity) is different in different places. This shows why it is very important to choose the right testing methods (molecular screening strategies) based on how common the disease is in the area (regional prevalence), the types of the virus there (genotype distribution), and the local health system (healthcare infrastructure).

1.3 Importance of Early Detection and Screening

Finding out if someone has HBV infection early using special tests on the virus's genes (molecular techniques) is very important. It helps stop the disease from getting worse and stops it from spreading to others. Measuring the amount of virus's DNA (HBV DNA) in the body tells doctors how serious the infection is and when to start medicine (antiviral therapy). According to rules from AASLD (American Association for the Study of Liver Diseases), treatment should start if:

HBV DNA is more than 2 000 IU/mL in people without a certain virus protein (HBeAg-negative) but who have high levels of an enzyme called alanine aminotransferase (ALT) (this enzyme shows liver damage), or if HBV DNA is above 20 000 IU/mL in people who have the virus protein (HBeAg-positive), no matter what their ALT levels are [16].

Special tests (molecular assays) can find even very low amounts of virus in the blood (< 20 IU/mL) that other blood tests (serological tests) often miss. This helps find hidden infections (occult infections) in people who donate blood and those with weak immune systems

(immunocompromised patients) [17]. Starting medicine on time can reduce liver damage (liver fibrosis), stop serious liver disease (cirrhosis), and lower the chances of liver cancer (hepatocellular carcinoma) by up to 50% in ten years [16][12].
Using quick molecular tests (point-of-care molecular diagnostics) during pregnancy care (antenatal care) and when checking blood can greatly lower the chance of the virus passing from mother to baby at birth (perinatal transmission) or through blood transfusions [18]. Fast methods like LAMP (Loop-mediated Isothermal Amplification), which is a way to copy the virus's genetic material at a steady temperature, let doctors check virus amount quickly on the spot, even in far or poor places where usual machines (qPCR) are not available [11][19]. To meet world goals to almost stop the disease in young children (≤ 0.1 % prevalence in children under five by 2030), stopping the virus from spreading and having early testing is very important. These molecular screening methods are the main tools for controlling HBV.

1.4 Objectives of the Review

1. To give an updated summary of HBV molecular biology (study of the virus's molecules) important for making tests.
2. To carefully look at old and new molecular screening methods for HBV.
3. To check the good and bad points and how well these tests work in places with different resources.
4. To study recent research and future ideas in molecular HBV testing.
5. To find missing parts in current testing methods and suggest areas to improve and research more.

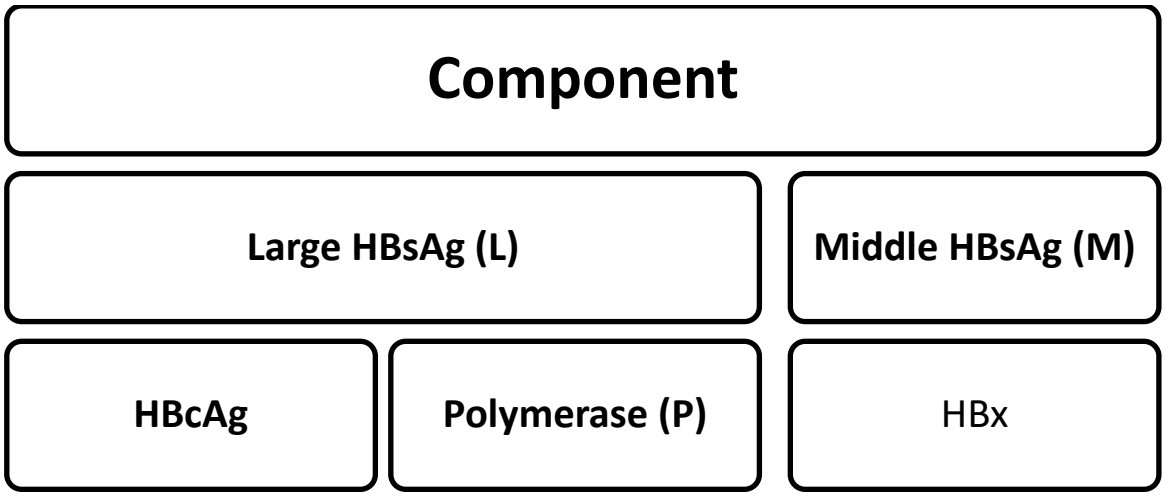
2. HEPATITIS B VIRUS: STRUCTURE AND GENOME

2.1 Viral Structure

Hepatitis B virus (HBV) is a virus with an outer fat layer (enveloped virus) that protects it. Inside, it has a partly double-stranded, circular DNA (a type of genetic material) about 3.2 thousand base pairs (kb) long. This DNA is inside a tiny shell called a nucleocapsid shaped like a 20-sided shape (icosahedral) about 27 nanometers (nm) wide [14]. On the outside fat layer (lipid envelope), the virus has three similar proteins (glycoproteins) called large (L), middle (M), and small (S) hepatitis B surface antigens (HBsAg). These proteins help the virus stick to and enter liver cells (hepatocytes) by attaching to a special receptor called NTCP on those cells [5]. Inside the nucleocapsid is a core protein (HBcAg) that holds the circular viral DNA and an enzyme called viral polymerase. This enzyme can copy the virus's DNA in a special way (reverse transcriptase) and cut RNA (RNase H), which is important for the virus to multiply [14]. The small DNA contains four overlapping sections called open reading frames (S, C, P, and X), which means the virus uses the same DNA parts for different functions to save space and control its activities.

Component	Gene Region	Function
Large HBsAg (L)	preS1/preS2	Receptor binding, virion assembly
Middle HBsAg (M)	preS2	Immune modulation
Small HBsAg (S)	S	Particle secretion, immune decoy
HBcAg	C	Nucleocapsid formation, genome packaging
Polymerase (P)	P	Reverse transcription, DNA synthesis, RNase H activity
HBx	X	Transcriptional regulation, viral persistence, oncogenesis

Table 2. Major structural and functional components of HBV **[14][15]**



[Figure 4: Major structural and functional components of HBV]

2.2 HBV Genotypes and Mutations

HBV is short for Hepatitis B Virus, which is a virus that affects the liver. Scientists have found at least ten different types of HBV, called genotypes (A–J). These genotypes are grouped based on differences in their genetic material (DNA) — if the DNA is more than 8% different, they are called different genotypes. Each genotype is more common in different parts of the world and can cause different kinds of health problems (clinical correlates) [16].

Genotypes	Serotypes	Subtypes	Geographic location
A	adw	A1	Sub-Saharan Africa and India
		A2	Northern Europe and India
		A3	Western Africa
B	adw, ayw	B1	Japan
		B2–5	East Asia, Taiwan, China, Indonesia, Vietnam, and the Philippines
		B6	Alaska, Northern Canada, and Greenland
C	adw, ayr, adr	C1–3	Taiwan, China, Korea, and Southeast Asia
		C4	Australia
		C5	The Philippines and Vietnam
		C6–11	Indonesia
D	ayw	D1–6	Africa, Europe, Mediterranean countries, India, and Indonesia
E	ayw		Restricted to West Africa
F	adw	F1–4	Central and South America
G	adw		France, Germany, and the United States
H	adw		Central America
I	adw		Vietnam and Laos
J			Japan

[Figure 5: Geographic distribution of hepatitis B virus genotypes and subtypes https://www.researchgate.net/figure/Geographic-distribution-of-hepatitis-B-virus-genotypes-and-subtypes_tbl1_276473537]

For example, genotypes B and C are found mostly in East Asia. Genotype C is linked with a delay in the body's process of stopping a protein called HBeAg (this process is called HBeAg seroconversion) and with a higher chance of getting liver cancer (hepatocellular carcinoma). On the other hand, genotype A is mostly found in Europe and Africa and is usually easier to treat [17].

Sometimes the virus changes its genetic code. These changes are called mutations. Some mutations happen in a part of the virus called the precore and basal core promoter (BCP) regions — for example, G1896A in the precore region and A1762T/G1764A in the BCP. These changes stop the

virus from making the HBeAg protein and can lead to a more harmful form of the disease called "e-antigen–negative" chronic hepatitis B [16].
There are also changes called "escape" mutations that happen in another part of the virus called the HBsAg "a" determinant. For example, the G145R mutation can stop antibodies (disease-fighting proteins) made by vaccines from working. This can make it harder to protect people through vaccines and to test for the disease in blood tests (serological screening assays) [7].

2.3 Replication Cycle and Pathogenesis

When HBV enters the body, it first attaches weakly (low-affinity attachment) to sugar-based molecules on cells called heparan sulfate proteoglycans. Then it attaches more strongly (high-affinity binding) using a special part of itself called the L-HBs preS1 domain to a doorway on the liver cell called NTCP. This helps the virus get inside the cell (endocytosis of the nucleocapsid) [8].
Inside the cell's nucleus, the virus's DNA, which is shaped like a relaxed circle (relaxed circular DNA), is changed into a stable form called covalently closed circular DNA (cccDNA). This cccDNA acts like a copy machine that helps the virus make more of its genetic material (all viral RNAs), including one called pregenomic RNA (pgRNA) [18].
The pgRNA joins with a viral enzyme called polymerase and forms new virus cores (nucleocapsids). Inside these cores, the pgRNA is turned into a type of DNA called minus-strand DNA, and then partly into plus-strand DNA to make relaxed circular DNA again [8].
These new virus cores can either go back to the nucleus to make more cccDNA or move into a part of the cell called the endoplasmic reticulum, where they get a coating and are released from the cell as new virus particles (progeny virions).
The virus can stay in the body for a long time because of cccDNA and because some of its DNA gets added to the person's own DNA (integration of HBV DNA into the host genome). This can cause a long-term infection and sometimes liver cancer (hepatocarcinogenesis), through things like causing changes in the cell's functions (insertional mutagenesis and HBx-mediated dysregulation of cellular pathways) [19].

3. CONVENTIONAL METHODS OF HBV DETECTION

3.1 Serological Assays (ELISA, Rapid Tests)

Serological assays are tests done on blood to check for signs of the HBV virus. These tests look for proteins made by the virus (viral antigens) or proteins made by your body to fight the virus (host antibodies) to understand if someone is infected and how far the infection has gone. One such test is called ELISA (Enzyme-linked immunosorbent assay). This test is very accurate (sensitivity ≥ 99 %) and can test many samples quickly. It is often used in blood banks and labs [20].
If the HBeAg protein is found, it means the virus is actively making copies of itself. Other blood tests can detect total and IgM anti-HBc antibodies, which help doctors know if the infection is new (acute), long-term (chronic), or already cured (resolved) [20].
There are also quick tests called rapid lateral-flow assays. These can give results in about 20 minutes and are used outside big labs (point-of-care). They can work almost as well as ELISA, depending on the test maker and the quality of the sample [15].

Marker	Diagnostic Window	Clinical Interpretation
HBsAg	1–10 weeks post-exposure	Active infection (acute or chronic)
HbeAg	Transient in acute, persists in high-replicative chronic	High infectivity, active replication
Anti-HBc IgM	4–6 months post-infection	Acute or recent infection
Anti-HBc Total	Persists for life	Past or current infection
Anti-HBs	4–6 months post-infection/vaccination	Immunity (recovery or vaccine-induced)

Table 3. Key serological markers used in HBV diagnosis **[20][21]**

3.2 Limitations of Traditional Techniques

Despite being used a lot, serological tests (serological assays) have some important problems. One big issue is the "window period" (the time gap between when a person no longer has HBsAg [a virus marker found early in infection] and when they start making anti-HBs [a protein the body makes to fight the virus]). During this time, the test might wrongly say the person doesn't have the virus (this is called a false-negative result), which can be dangerous if that person's blood is used for a transfusion (giving blood to someone else) [22].

Also, serology (blood tests to detect disease) cannot tell how much virus is in the body (this is called viral load), and sometimes it misses certain infections called occult HBV infections (these are cases where HBsAg [a virus marker] is not found, but the virus is still there in small amounts). This is especially a problem for people whose immune systems are weak (immunosuppressed individuals) [31].

Another issue is that sometimes the body reacts to other similar viruses (this is called serological cross-reactivity), or the virus changes slightly (called "escape" mutations in the HBsAg region), so the test might not find the virus, giving another false-negative result. This can make it hard to know if vaccines (shots to prevent disease) are working well.

Lastly, these tests cannot tell if the virus has changed in a way that makes it resist medicines (this is called drug-resistance mutations), or what type of virus it is (called viral genotypes), which makes it hard for doctors to choose the right treatment [22][35].

4. MOLECULAR TECHNIQUES IN HBV SCREENING

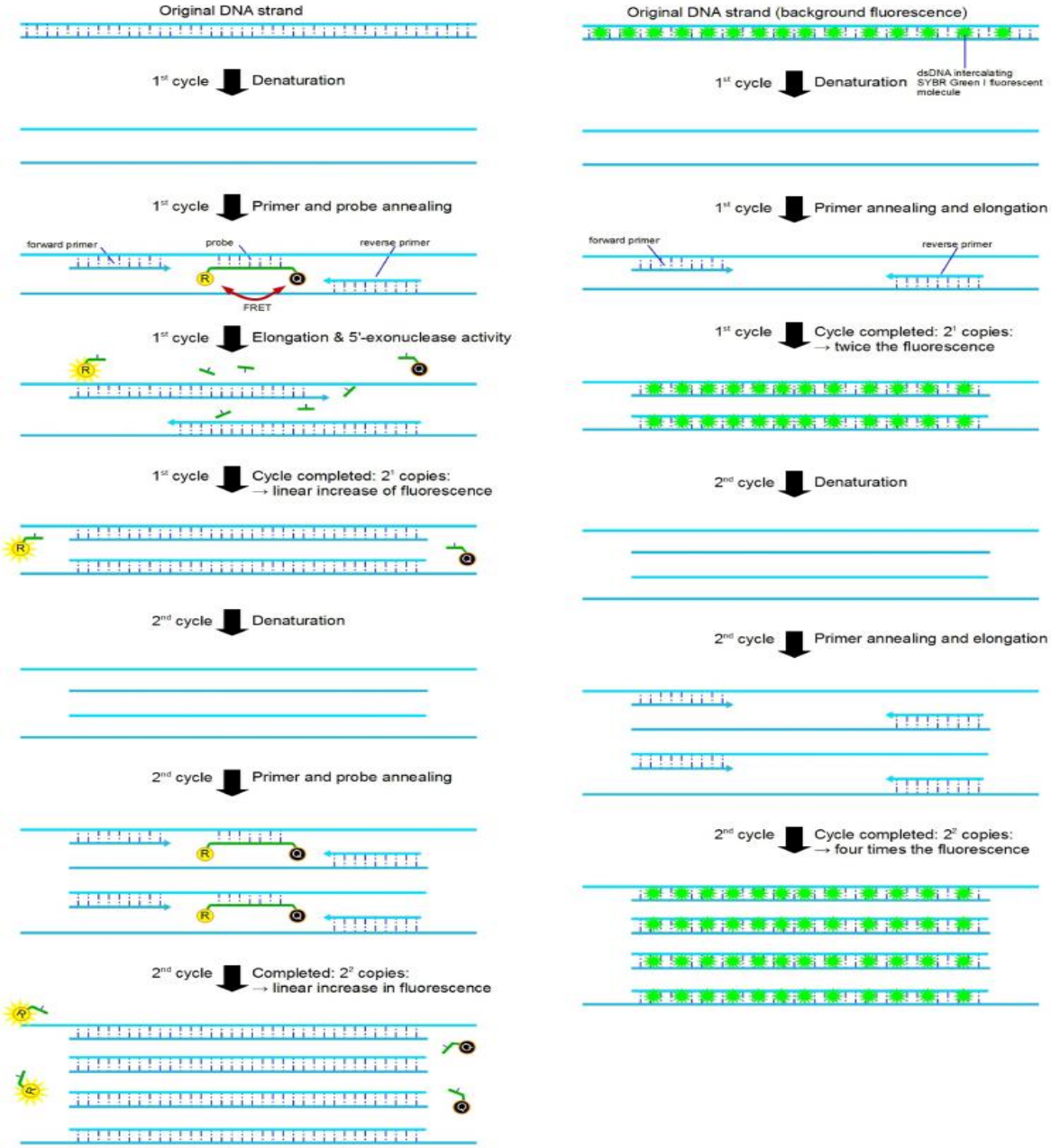
4.1 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a basic and very useful test used to find the hepatitis B virus (HBV). It works by copying the virus's DNA (the virus's instructions) many times so it can be seen easily. It does this by going through three steps again and again: heating to separate the DNA (denaturation), cooling so the matching pieces stick (annealing), and making new DNA (extension) [21].

In PCR tests for HBV, doctors look at certain parts of the virus's DNA, like the S or C genes. They usually copy pieces that are 100 to 400 base pairs long (bp means "base pairs," which are building blocks of DNA) so the test can work well for different types of the virus [24]. Normal PCR tests can find very tiny amounts of the virus (as little as 10 to 100 copies in one test). But after the copying is done, the results must be looked at using gel electrophoresis (a lab method to separate DNA pieces), which takes extra time and can cause mistakes if the samples get mixed up [24].

Doctors use blood plasma or serum (the liquid part of blood) for this test. Before doing the test, they need to take out the DNA using special methods like silica-column or magnetic-bead methods (ways to clean and collect the DNA). This helps make sure there is enough good-quality DNA for the test [29].

4.2 Quantitative Real-Time PCR (qPCR)



[Figure 6: A graphical representation of qPCR amplification (the first two cycles) as it happens in the PCR tube https://biosistemika.com/blog/qpcr-technology-basics/]

Quantitative real-time PCR (qPCR) is an improved version of PCR. It does the copying and checks the result in the same closed tube, which is faster and cleaner. It uses light signals to measure how much virus DNA is there. This light is made using intercalating dyes (like SYBR Green that glows when it joins with DNA) or fluorogenic probes (like TaqMan that lights up when the DNA is copied) [25].

qPCR can measure a wide range of virus levels, from 10^2 to 10^9 IU/mL (IU means "international units per milliliter," which is a way to count how much virus is there). It can even find very small amounts, like 10 IU/mL. This is very helpful for doctors to decide how to treat the patient [37].

qPCR uses special probes called dual-labeled hydrolysis probes. These are very good at finding even small changes in the virus's DNA (like single-nucleotide polymorphisms and escape mutations) that could affect the test [25].

In big labs, machines can automatically do the DNA extraction and qPCR test, checking hundreds of samples every day. But the machines are expensive and need steady electricity and controlled temperature, so they are hard to use in poor areas [41].

4.3 Nested PCR and Multiplex PCR

Nested PCR is a more detailed type of PCR. It does the copying process two times using special inner primers (short DNA pieces that help start the copying). This helps find even very small amounts of virus—less than 10 copies. But it takes more time and has a higher chance of contamination (mixing up test materials) [26].

Multiplex PCR is a smart method that allows doctors to check for many parts of the virus (like surface, precore, or polymerase regions) in one single test. It uses different sets of primers (short DNA pieces) with unique sizes or glowing colors (fluorescent labels), so each part can be seen clearly [35].

Multiplex PCR can also check for more than one virus at the same time (like HBV/HCV/HIV) or look for changes in the virus linked to different types (genotype-specific mutations). This makes it useful for studying the virus completely [20]. But it's very important to design the primers carefully so they don't stick to each other (primer–dimer formation) and all targets get copied well [16].

Method	Sensitivity (copies/reaction)	Turnaround Time	Contamination Risk	Multiplexing Capability
Conventional PCR	10–100	4–6 hours	Moderate	Low
Nested PCR	<10	6–8 hours	High	Low
Multiplex PCR	50–100	5–7 hours	Moderate	High

Table 4. Comparison of PCR-based methods for HBV detection **[35][26][16]**

4.4 Reverse Transcription PCR (RT-PCR) for HBV RNA

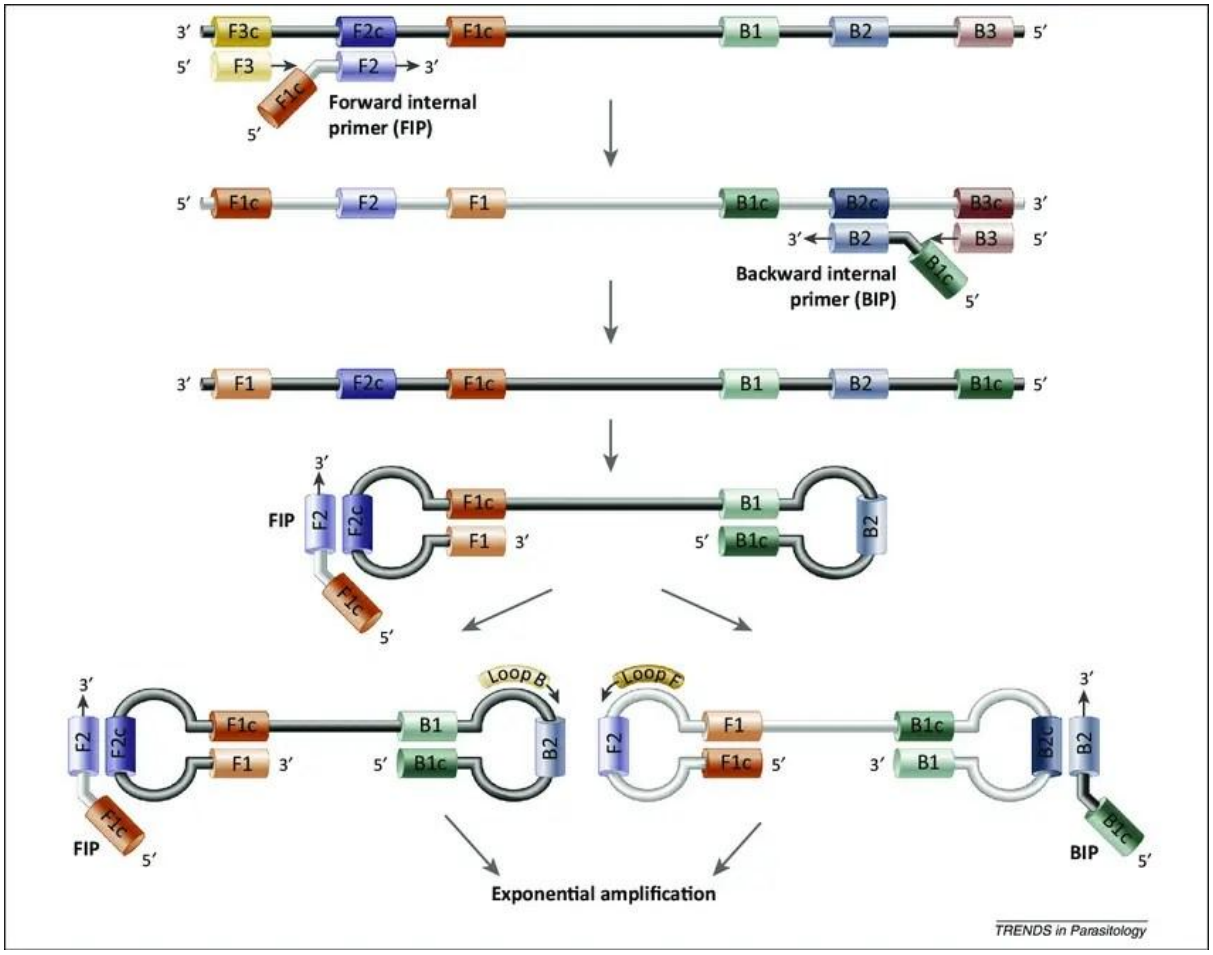
Hepatitis B virus (HBV) is usually known as a DNA virus, but sometimes scientists study its RNA (a type of genetic material like DNA) to understand if the virus is still actively working inside the body. Some types of RNA they check are called pregenomic RNA (pgRNA) and subgenomic RNAs. These help scientists know if the virus is making copies of itself and using something called cccDNA to do that [18].

In Reverse Transcription PCR (RT-PCR), scientists first change the virus's RNA into something called complementary DNA (cDNA) using an enzyme called reverse transcriptase. Then, they use a method called PCR amplification to make many copies of the cDNA. This helps them measure how much pgRNA is present. The amount of pgRNA shows how much cccDNA is in the body [15].

Studies show that if there is more pgRNA, there's more cccDNA inside the liver (this is called intrahepatic cccDNA) and this can tell us if the virus might come back after stopping treatment [28].

In RT-PCR, scientists often use something called DNase to remove any leftover HBV DNA so it doesn't affect results. RT-PCR can be done in one step (RT and PCR done together) or in two steps (RT and PCR done separately). It can find as few as 100 virus copies in one milliliter (mL) of blood [11].

4.5 Loop-Mediated Isothermal Amplification (LAMP)



[Figure 7: Loop-Mediated Isothermal Amplification (LAMP) https://i0.wp.com/microbeonline.com/wp-content/uploads/2022/06/Loop-mediated-isothermal-amplification-LAMP.png?ssl=1]

Loop-mediated isothermal amplification (LAMP) is a fast method to find DNA from HBV. It works at a constant temperature (about 65 °C), which means the temperature doesn't need to change during the test. It uses 4 to 6 short DNA pieces called primers and a special enzyme called strand-displacing DNA polymerase to make many copies of the DNA [29].

LAMP tests for HBV can give results in less than 60 minutes. You can see the result with your eyes by looking for cloudiness (turbidity) or a color change (for example, with a dye like hydroxynaphthol blue), so no machines are needed [29]. LAMP tests can be just as sensitive as qPCR, which means they can detect even very small amounts of virus (down to 10–50 copies per test) and reduce chances of samples getting mixed up (cross-contamination) because the tubes stay closed [32]. Because LAMP is simple, it is now being used in paper-based tests and tiny lab devices (called microfluidic devices) that can be used even in rural or remote areas [30].

5. ADVANCED MOLECULAR DIAGNOSTICS

5.1 Digital PCR

Digital PCR (dPCR) is a very accurate method to count DNA. It splits the DNA sample into thousands of tiny parts, where each part has either zero or one copy of the target DNA. This helps scientists count the exact number of virus DNA pieces without needing to compare with a standard [30].

dPCR can find very small amounts of HBV DNA (even less than 1 International Unit (IU) per mL) and is very accurate, with a small chance of error (less than 1.5%) [36]. It is especially useful to

detect very low amounts of virus in the blood (low-level viremia) and tiny changes in the virus that can cause it to resist medicines (these are called drug-resistance mutations and can be present in less than 1% of the virus) [30]. But dPCR is expensive and cannot test as many samples at once compared to qPCR, so it is usually used in special labs [34].

5.2 CRISPR-Based Detection Systems

CRISPR-based tests use a special system called CRISPR-Cas. When the Cas12 or Cas13 enzymes find the virus's DNA or RNA, they cut other molecules called fluorogenic reporters that give off light or signal [37].

CRISPR-Cas12a tests for HBV can find DNA at very tiny amounts (called attomolar concentrations) in 30–40 minutes at a temperature of 37 °C. These tests often use another method called recombinase polymerase amplification (RPA) or RPA-LAMP to help find small amounts of virus [31]. CRISPR is special because scientists can quickly change the test to find new types of the virus (called viral variants). It can also find different types (genotypes) of HBV or detect more than one infection at the same time (co-infections) [41]. These CRISPR tests can be made into small, cheap devices with test strips (called lateral-flow strips) that can be used even in villages or small clinics [48].

5.3 Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) is a powerful method to read the complete genetic code of the HBV virus. It helps scientists see all the small differences (called quasispecies diversity) and any changes that may cause the virus to resist treatment (drug-resistance mutations) [20].

Some methods like hybrid capture or tiled amplicon panels help focus only on HBV during sequencing. These methods can read the virus's DNA more than 10,000 times to find even the smallest changes (as low as 1%) [32]. Special computer programs (called bioinformatics pipelines) help scientists put together the full virus DNA, find new types (recombinant strains), and understand all the changes in the virus. This information is useful for studying how the virus spreads (epidemiology) and choosing the right treatment for each person (personalized therapy) [32]. Although NGS is cheaper now, it still needs strong computers and experts to study the results, which can make it hard to use in every lab [18].

5.4 Biosensor and Nanotechnology-Based Approaches

Biosensors are tiny devices that can detect HBV quickly. They work by using special molecules (like aptamers, antibodies, or CRISPR proteins) that can catch the virus, and other parts called signal transducers (which change this catching into a signal you can measure) [41]. These signals can be electrical (electrochemical), seen with light (optical), or based on vibration (piezoelectric).

Nanotechnology helps make biosensors better. For example, gold-nanoparticle probes and graphene-based field-effect transistors are used to make the signal stronger and detect even very tiny amounts of virus (as low as femtomolar levels) [46]. One type of test called electrochemical aptasensor can find HBV DNA in just 20 minutes with great accuracy and not react to wrong targets [51]. These small biosensors are useful for fast and accurate testing at a patient's bedside or in far-away clinics. However, scientists still need to test them more to use them widely in hospitals [33][34].

Technique	Detection Limit	Turnaround Time	Equipment Needs	Primary Application
Digital PCR	<1 IU/mL	3–4 hours	dPCR system	Low-level viremia, resistance
CRISPR-Cas	~10 copies/reaction	30–40 minutes	Portable incubator	Rapid field screening
NGS	~1 % variant frequency	2–3 days	Sequencer + compute	Genotyping, quasispecies, R-

				resistance
Nanobiosensor	0.5 fM	20 minutes	Handheld device	Point-of-care detection

Table 5. Comparison of advanced molecular diagnostics for HBV screening **[41][46][51][33][34]**

6. RECENT RESEARCH AND TECHNOLOGICAL DEVELOPMENTS

6.1 Recent Breakthroughs and Case Studies

In the last two years, many important studies have helped us understand how to test for HBV (Hepatitis B Virus) very carefully and quickly. One big study was done in many places at once (a multicenter trial). They tested a new method called CRISPR-Cas13a assay (a tool that uses special proteins to find tiny pieces of virus DNA) combined with microfluidic sample preparation (a technique that uses very small amounts of liquid to prepare the blood sample). This method could find as few as 5 copies of the virus in one milliliter of blood and gave almost the same results (98.5% agreement) as the usual method called qPCR (quantitative Polymerase Chain Reaction, a common lab test that copies and measures virus DNA) when testing 1,200 samples from places in Southeast Asia where HBV is common [35]. The test uses a small cartridge (like a small container) that can give results in 45 minutes without needing special cold chemicals, which means it can be used in many places, not just big labs.

At the same time, another study called a prospective cohort study (a study that follows a group of people over time) in Africa tested a combined LAMP-CRISPR approach (a fast way to copy and detect virus DNA using special methods called LAMP and CRISPR). This helped pregnant women by quickly finding the virus and giving treatment during the same visit, which reduced the chance of babies getting HBV by 40% [39]. This shows how fast testing can help even in places with fewer resources.

In Brazil, a hospital did a case study (a detailed example) using nanopore sequencing (a method that reads virus DNA directly from blood plasma) to learn more about the different virus types and if they resist medicines. This helped find rare virus changes (less than 1% of the virus) that normal methods like Sanger sequencing (an older way to read DNA) missed, allowing doctors to change treatment early and stop the virus from growing in 15% of patients [52].

Similarly, a hospital in Germany used digital droplet PCR (ddPCR) (a very sensitive version of PCR that divides samples into tiny droplets to find low amounts of virus) to find hidden HBV in donors whose immune system was weak. This test could find as low as 0.2 IU/mL (international units per milliliter, a way to measure virus amount) and prevented three infections from donated organs. This helped change rules about who can donate [16].

These new methods show how combining new tools and medical practice helps detect the virus better and faster. It is important to test these tools in real life and check if they are worth the cost. In the future, studies may combine several methods like multiplexed CRISPR diagnostics (tests that can find many virus types at once using CRISPR) and portable sequencing (small devices to read DNA on the spot) to give complete HBV test results in one step [37][39].

6.2 Point-of-Care Molecular Diagnostics

Point-of-care (POC) molecular diagnostics (tests done near the patient, not in a distant lab) for HBV have improved from early versions to systems that can be used in real places. One good example is the GeneXpert HBV viral load cartridge (a small device that does automatic virus copying and measuring inside a single box). It needs very little training to use. In rural India, tests using this device were finished 90% of the time and agreed 95% with the lab's usual qPCR results for 500 pregnant women, reducing waiting time from 7 days to under 2 hours, which helped doctors make quick decisions [39].

Another test uses a paper-based LAMP assay (a fast virus copying test done on paper) with a phone app that sees glowing signals (fluorescence detection) and could find 20 IU/mL virus. In remote Amazon villages, it tested 300 people on-site, matched lab tests 100%, and saved 30% of costs per test [57].

New POC devices use tiny chips called microfluidic “lab-on-a-chip” technologies (small devices that do many lab steps like preparing samples and copying DNA all in one cartridge about the size of a credit card). One device uses magnetic bead extraction (tiny magnetic balls to pull out virus parts) followed by RPA-CRISPR detection (another fast way to copy and detect virus DNA). It shows color changes that people can see without special machines in 60 minutes and only needs a small heater [41]. In West Africa, this test was 97% sensitive (able to detect positive cases) and 99% specific (able to avoid false alarms), proving it works well for big testing programs. Also, solar-powered heaters and dried chemicals (lyophilized reagents) allow testing where there is no electricity [21].

These POC tests help reduce the problem of not enough testing in places where HBV is common. But problems remain like making sure all tests are good quality, keeping a steady supply of cartridges and chemicals, and connecting test results to electronic health records and disease monitoring systems [39][42].

6.3 Integration with AI and Machine Learning

Artificial intelligence (AI) and machine learning (ML) (computer methods that learn from data to make decisions) are being used to improve HBV tests, from designing the tests to understanding the results. For example, deep learning models (AI that uses many layers of computers to understand patterns) can tell if a small amount of virus is really there or just background noise in qPCR tests, reducing wrong positive results below 15 copies/mL and improving test accuracy by 5–10% [43]. Another ML method predicts how well test parts called primer–probe (small pieces of DNA used to find the virus) will work on different HBV types and changes, helping design better tests faster, from months to weeks [41].

In DNA sequencing for monitoring, AI looks at NGS (Next Generation Sequencing, a method that reads lots of DNA pieces quickly) data to find mixed virus types and new mutations, warning about vaccine or drug resistance early. In China, a cloud-based AI system processed HBV data from 1,000 patients and made automatic reports about 50 known resistance changes and virus family trees (phylogenetic clustering), helping doctors choose the best treatment within 2 days [45]. AI with POC devices also lets phone apps read test pictures and use convolutional neural networks (a type of AI that sees images) to give objective results, reducing mistakes from users [31].

While AI and ML can greatly help, they need strong rules about data use, standards to work well with other systems, and clear tests to make sure they are fair and accurate. Groups of scientists are sharing anonymous HBV data to create AI standards and keep improving as the virus changes and new tests come out [43][46].

7. COMPARATIVE ANALYSIS OF TECHNIQUES

7.1 Sensitivity and Specificity

When comparing HBV tests, we look at how sensitive they are (analytical sensitivity or limit of detection, LoD — the smallest amount of virus they can find) and how specific they are (diagnostic specificity — how well they avoid false positives) in different types of samples.

qPCR usually detects 10–20 IU/mL virus and is over 99% specific, so it is the best standard test [25][47].

Digital PCR can find less than 1 IU/mL and is more than 99.5% specific, great for detecting very low virus amounts but costs more [30][47].

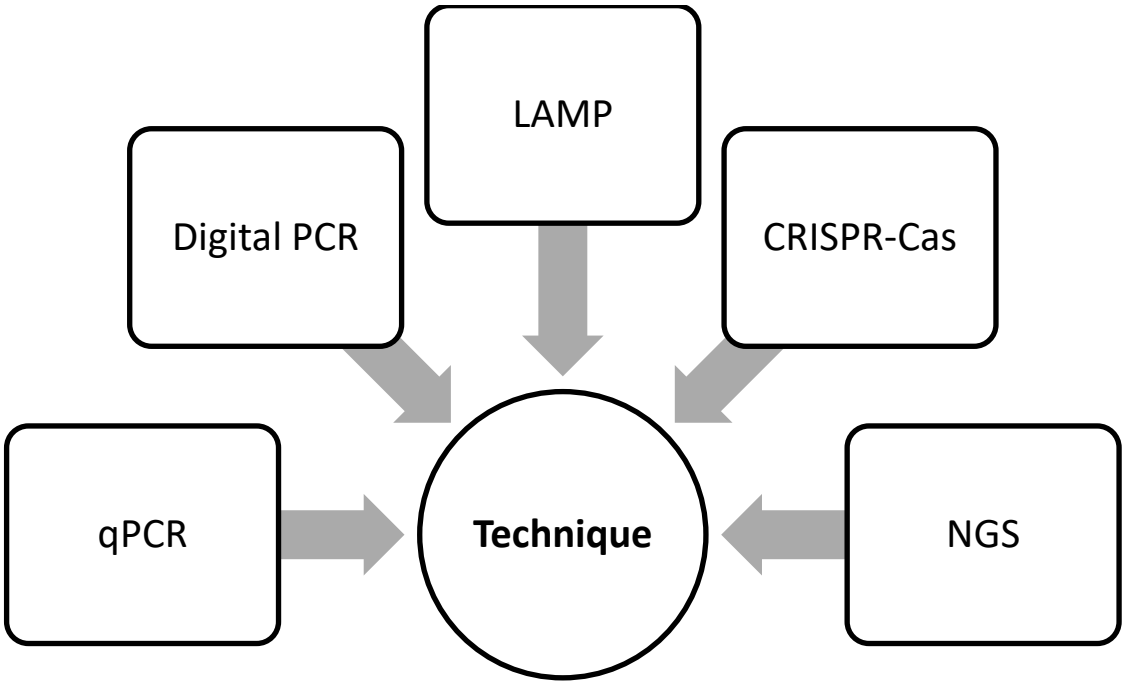
LAMP assays are less exact but detect 10–50 copies per reaction and are 95–98% specific; they can give false positives sometimes, but this can be fixed with sequence-specific probes (tools that only react to the exact virus DNA) [29][48].

CRISPR-based systems are very sensitive (attomolar sensitivity means about 1–10 copies per reaction) and over 99% specific, using a special cutting action (collateral cleavage) that happens only when the exact virus DNA is found [31][48].

NGS-based methods vary but can detect virus variants present at 1% or more and have over 99% specificity if errors are corrected well [32][49].

Technique	Limit of Detection	Specificity	Quantitative?	Multiplex Capacity
qPCR	10–20 IU/mL	> 99 %	Yes	Moderate
Digital PCR	< 1 IU/mL	> 99.5 %	Yes	Low
LAMP	10–50 copies/reaction	95–98 %	Semi-quant	Low
CRISPR-Cas	~1–10 copies/reaction	> 99 %	No	High
NGS	~1 % allele frequency	> 99 %	Yes	Very high

Table 6. Analytical performance comparison of HBV molecular screening techniques **[25][31][47][49]**



[Figure 8: Technique]

7.2 Cost-Effectiveness

This means checking and comparing how much money is needed for different testing methods.

Cost analyses must consider instrument amortization (instrument amortization means dividing the total cost of a machine over the number of years it will be used), reagent costs (reagents are the chemicals used in tests), technician time (this means the time a person takes to do the test), and sample throughput (sample throughput means how many samples can be tested in a certain amount of time).

qPCR stands for quantitative Polymerase Chain Reaction, a method used to find tiny amounts of genetic material.

qPCR setups incur capital expenditure (capital expenditure means money spent to buy machines or equipment) of $50,000–$100,000 per instrument with per-test reagent costs (cost of chemicals used in one test) of $15–$25, yielding cost per result (final cost of doing one full test) of $20–$30 in high-volume laboratories [50][57].

Digital PCR is another type of test that also looks for small amounts of genetic material but is more exact.

Digital PCR platforms cost $80,000–$120,000, with per-test costs of $35–$50 due to specialized consumables (special things that are used up in a test like cartridges or chips), translating to $40–$55 per sample [50].

LAMP stands for Loop-mediated Isothermal Amplification. It is a simple test to detect genetic material and does not need much equipment.

LAMP assays require minimal equipment (heat block $500—a heat block is a small machine that keeps samples warm) and reagents $2–$5 per test, making per-test costs as low as $5 in batch runs (batch runs mean testing many samples at once), though labor (money paid to the person doing the test) and quality control (checking that the test works properly) add $3–$5 [40][52].
CRISPR-Cas is a special tool used to find genetic material with high accuracy.
CRISPR-Cas assays have reagent costs of $10–$15 per test and low instrument needs (portable incubator $2,000—a portable incubator is a small machine that keeps the sample at the right temperature), yielding $12–$20 per result; costs may decline with mass manufacturing of lateral-flow strips (lateral-flow strips are paper-like tools that show test results, like pregnancy test strips) [31][53].
NGS stands for Next Generation Sequencing. It is a very advanced method to read and study genes.
NGS workflows show the greatest variability (variability means the cost can change a lot): small targeted panels (targeted panels mean only some parts of the genes are checked) can cost $100 per sample, while high-throughput runs (high-throughput means testing many samples quickly) reduce cost to $20–$30 per sample but require batched sequencing (testing many samples at once) and bioinformatics overhead (bioinformatics overhead means needing computers and experts to study and understand the gene results) [32][54].
In resource-limited settings, LAMP and CRISPR-Cas POC tests (POC means Point-of-Care, which is testing near the patient without needing a big lab) often emerge as the most cost-effective (cost-effective means giving good results for less money) despite lower multiplexing (multiplexing means testing many things in one go) and quantitative capabilities (quantitative capabilities mean measuring exact amounts).

7.3 Applicability in Low-Resource Settings

In areas where there isn't much money or equipment (called low-resource settings), doing medical tests can be difficult. Some of the problems are power cuts (intermittent power), not enough cold storage for items (limited cold chain), and not enough trained workers (scarcity of trained personnel). Two test types—LAMP and CRISPR-Cas—work well in such areas because they don’t need special machines to keep the temperature steady (isothermal operation) and their materials can be stored without a fridge (lyophilized reagents). These tests have worked well in clinics in villages in Asia and Africa, helping find illnesses more than 90% of the time [29][31][40][42].
Some portable machines for qPCR (which is a type of test that makes copies of DNA quickly) have been tested in such areas, but they have problems. The special materials used in these tests (called reagents) don’t last long, and the machines need regular checking and fixing (called calibration) [39].
Other mobile labs use a small device called MinION (a nanopore device) to check for diseases like HBV during outbreaks. But analyzing the test results needs internet (cloud connectivity) and skilled people (computational expertise), which are not always available in remote places [37].
To make things better, we need to connect these tools to systems where doctors can help from far away (telemedicine platforms). Also, governments and companies need to work together (public–private partnerships) to reduce the costs of materials (consumables) and fixing machines (maintenance) [55][51].

8. CHALLENGES AND FUTURE PERSPECTIVES

8.1 Limitations of Current Molecular Methods

Even though today's medical tests have improved a lot, they still have problems. First, most tests that look at genetic material—like qPCR and digital PCR (both are types of tests that increase DNA or RNA to find viruses)—need advanced labs with steady power, good temperature control, and expensive machines. So, they can't be used in villages or places without these things [57].
Second, some viruses like HBV hide inside liver cells in a very strong form called covalently closed circular DNA (cccDNA), which current tests can't find. So, doctors look for other signs like pgRNA

(a type of RNA that is made when the virus is active), but these don't always show the real amount of virus inside the liver [60].

Third, when tests are done in many steps, like nested PCR (a kind of test with more than one round of copying), there's a chance of mixing up samples, which can give wrong positive results (false-positive results). So, more safety checks (quality controls) are needed, which makes the tests more expensive and complicated [21].

Finally, the HBV virus changes a lot (high genetic variability). These changes can make it hard for the test to find the virus if it doesn't match the part of the virus the test looks for (primer- or probe-binding regions). So, the tests need to be updated regularly to work well [27].

8.2 Emerging Trends and Innovations

New ideas are coming to fix these problems. One idea is microfluidic "lab-on-a-chip" platforms, which are small devices that can do the whole test—from preparing the sample to finding the virus—all in one small chip. This lowers the risk of mixing up samples and makes testing easier [11].

Another exciting development is CRISPR-based diagnostics. These use special proteins like Cas14 (a type of Cas effector protein) to spot changes in the virus, like whether it resists medicine or vaccines. It can do this all in one step (one-pot format) [70].

New types of tests like recombinase-aided amplification (RAA) and helicase-dependent amplification (HDA) can work faster and with fewer materials. These are helpful for simple, quick testing near the patient (point-of-care use) [64].

Some new methods even combine rolling-circle amplification (a way of copying circular DNA) with nanopore sensing (reading the DNA directly). This might help us find the hidden virus DNA (cccDNA) and measure how much of it is there—without using regular PCR tests [60].

8.3 Future Scope in Clinical and Field Applications

In the future, we will include these tests in everyday doctor work and public health. One idea is wearable biosensors. These are like smartwatches that can check for virus levels all the time, even in very small amounts (sub-IU/mL levels). They use tiny electric parts like graphene-based field-effect transistors and multiplexed microelectrode arrays to find signs of the virus in sweat or body fluids [61].

Also, with telemedicine systems and special phone tools (smartphone-based assay readers), doctors can get results immediately. These can be sent to online systems (cloud-based AI analytics), which help track disease outbreaks and adjust treatment quickly [62].

Lastly, combining different types of data—like virus genes (HBV genomics), how the person's body responds (host transcriptomics), and how genes are turned on or off (epigenetic profiling)—can help doctors predict how the disease will behave and choose the best treatment. This is called precision medicine [61].

To reach the World Health Organization's (WHO's) goal to eliminate HBV by 2030, we need solutions that mix smart technology with strong healthcare systems.

Challenge	Impact	Emerging Solution
Infrastructure dependence	Limited access in remote/resource-limited settings	Microfluidic lab-on-a-chip with all-in-one cartridges [59]
Undetectable cccDNA reservoirs	Incomplete assessment of viral persistence	Rolling-circle amplification plus nanopore sensing [60]
Contamination and workflow complexity	False positives; high quality-control demands	One-pot isothermal CRISPR assays with lyophilized reagents [60]
Genotypic and escape mutation drift	Reduced assay sensitivity and specificity	Programmable Cas14-based allele-specific detection [60]
Lack of continuous	Inability to detect rapid viral	Wearable graphene biosensors

monitoring	load fluctuations	linked to AI analytics [61][62]

Table 7. Key limitations of current HBV molecular methods and emerging solutions

9. CONCLUSION

Scientists have learned a lot about how the hepatitis B virus (HBV) works. This has helped them create better ways to test and find the virus in people. The virus is small and has an outer covering (enveloped virion). Its genetic material is a mix of two types of DNA strands (partially double-stranded DNA genome). The virus can hide inside the body for a long time in a strong circular form (covalently closed circular DNA or cccDNA), which makes it hard to detect. So, doctors need special tests (assays) that can find even small amounts of the virus in the blood (low-level viremia) and can tell if the virus is actively growing or just hiding (distinguish active replication from latent infection).

Scientists have also studied the virus's outer and inner parts (HBV surface and core proteins), and how it makes copies of itself (replication cycle). This copying happens in a special way using an earlier form of genetic material (reverse transcription of pregenomic RNA). These studies have helped design better testing tools like those used in PCR-based tests. PCR means "polymerase chain reaction," a method used to make many copies of a small part of DNA. New kinds of tests like isothermal and CRISPR-based methods are also being developed, which may work faster and with simpler tools.

Old methods like standard PCR, quantitative real-time PCR (qPCR), nested PCR, and reverse transcription PCR are very good at finding the virus. These tests are very sensitive (can find even small amounts of virus) and specific (won't confuse it with something else). They help doctors measure how much virus is in the body (viral load quantification), which is very important to treat the disease. Nested PCR and multiplex PCR can find very tiny amounts of virus and can test for more than one infection at a time (like HBV and HIV together), but they need a clean lab because they can easily get contaminated. They are also hard to do.

Some newer methods like loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) work faster and don't need big machines. Another method, digital PCR, can count exactly how much virus is there without needing a comparison (without calibration curves) \[64]. All these tests have good and bad sides depending on how well they work, how many tests can be done at once (throughput), and how easy they are to use.

Different places have different access to these tests. Richer countries have machines that do automatic qPCR and digital PCR, which give very accurate results (down to <1 IU/mL), but these machines are expensive. In poorer or village areas, LAMP, CRISPR-Cas tests, and simple paper devices (paper-based microfluidic devices) can be used. These give results in about an hour and don't need much equipment. These tests use freeze-dried chemicals (lyophilized reagents) that are easy to carry and store \[65]. Even though the newer methods are more accurate, the simpler and cheaper tests may help more people, especially in places where the disease is very common (endemic regions), if used in local clinics (point-of-care workflows).

Recent studies have made big improvements. Some examples are: ready-to-use qPCR cartridges, CRISPR-Cas13a tests that work with small fluid-handling chips (microfluidic integration), fast genetic reading using nanopore sequencing, and a very sensitive test called digital droplet PCR that can find hidden virus (occult HBV detection). Smart computer systems (artificial intelligence and machine learning) are now used to help design better tests, understand the results, and watch for drug resistance in real time. Small, portable testing machines (lab-on-a-chip) and the use of mobile phones to read the results are helping us move toward easy-to-use tools that can test for HBV and find out which type of the virus a person has (genotyping) right where the patient is.

Even with all these new ideas, some problems still exist. Right now, there is no test that can directly measure the hidden form of the virus in the liver (intrahepatic cccDNA). We use other signs (surrogate markers) like pregenomic RNA, but these are not perfect and need more testing to prove how helpful they really are. The virus can also change its genes (genetic diversity), which may let it escape from tests or treatments. So, we have to keep updating our test designs.

To make sure these new tests work well in poorer areas, we need a steady supply of materials (supply chains), rules for checking test quality (quality-control frameworks), and to connect the test results with digital health systems. Future scientists should work on easier, one-step tests (novel amplification chemistries for one-pot assays), sensors that can keep checking a patient's health (biosensor platforms for continuous monitoring), and using many types of data (multi-omics approaches) to guess how the disease will develop and how people will respond to treatments.
If we fix these problems, we will be closer to what the World Health Organization (WHO) wants: to remove HBV completely by giving everyone early, correct, and affordable tests.

Recommendations for Future Research

Future research should focus on making tests that are very good at finding the virus (highly sensitive), very accurate (specific), and not too expensive. These tests should work both in fancy labs and in small, local clinics. One big goal is to make tests that can directly measure the strong, hiding form of the virus (covalently closed circular DNA or cccDNA). Right now, we depend on less direct signs (surrogate markers) like pregenomic RNA (pgRNA), but they don't always show the full picture of how much virus is still in the body or how well treatment is working. That's why we need tests that can find cccDNA.
We should also work on tests that can check for many things at once (multiplexed detection), like which type of HBV a person has (HBV genotypes), if the virus is resistant to medicine (drug resistance mutations), and if the person also has other infections like HIV or HCV. This will help doctors treat each patient in the best way.
We also need to make these tests easier to use in remote places. Simple paper-based sensors (paper-based biosensors), small lab systems (lab-on-chip systems), and tests that can show results on phones (smartphone-enabled detection platforms) should be improved for use in villages and faraway areas.
Using smart computer tools (artificial intelligence or AI and machine learning) in the testing process can also help. These tools can better understand the patterns in test results (amplification curves), help explain what the results mean, and even guess how the disease might grow in the future.
Also, we need long-term studies (longitudinal studies) to check how well new virus markers like HBV RNA and core-related antigens help in choosing the best treatments.
Lastly, we need research that looks at how much these new tests cost, if they can be made for many people (scalability), and whether people like using them (user acceptability). This will help doctors and governments use these tests not only in labs but also in the real world, like clinics and hospitals.

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