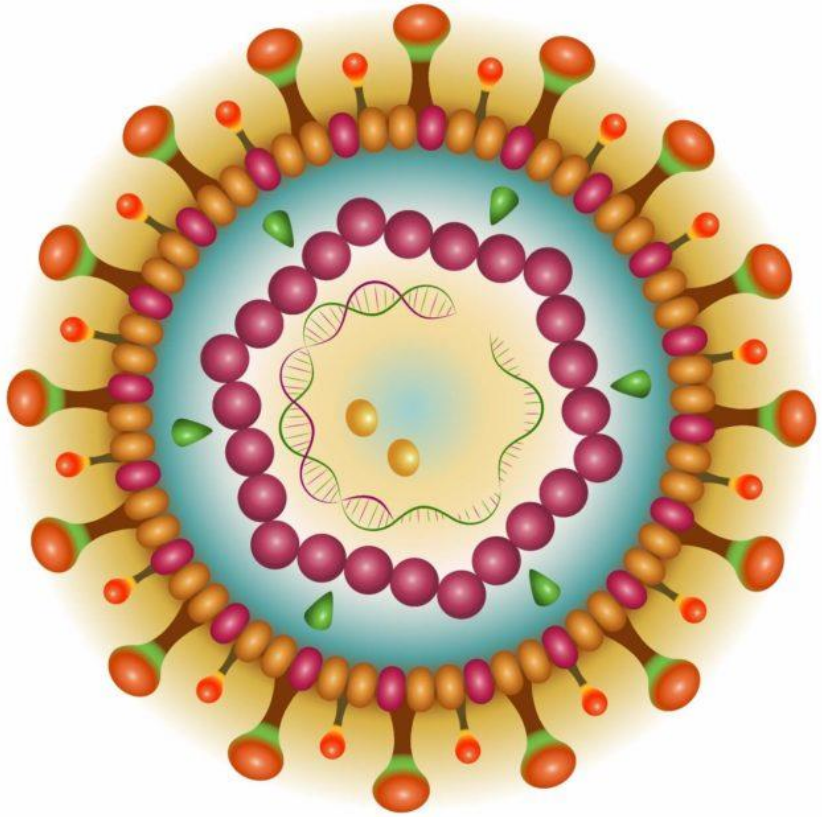


Hepatitis B Virus (HBV)

Prepared by : Lim Teck Wei (104986)

"There is a growing need to make people aware of the severity of the disease in the region. People need to be extra careful, as it is one of the region where infection with hepatitis B virus (HBV) is common." – Dr. RS Verma

Overview of Hepatitis Viruses

- The hepatitis viruses include at least 6 viruses, A → E and G [1]
- The target organ for each of these viruses is the liver and the basic hepatitis symptoms are similar
- However, they differ greatly in their structure, mode of replication, mode of transmission and in the time course and sequelae of the disease they cause [1]
- Each of the hepatitis viruses infects and damages the liver, causing the classic icteric symptoms of jaundice and the release of liver enzymes [1]
- These viruses are readily spread because infected people are contagious before, or even without, showing symptoms. [1]

Reference

1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Comparison and characteristics of different hepatitis viruses

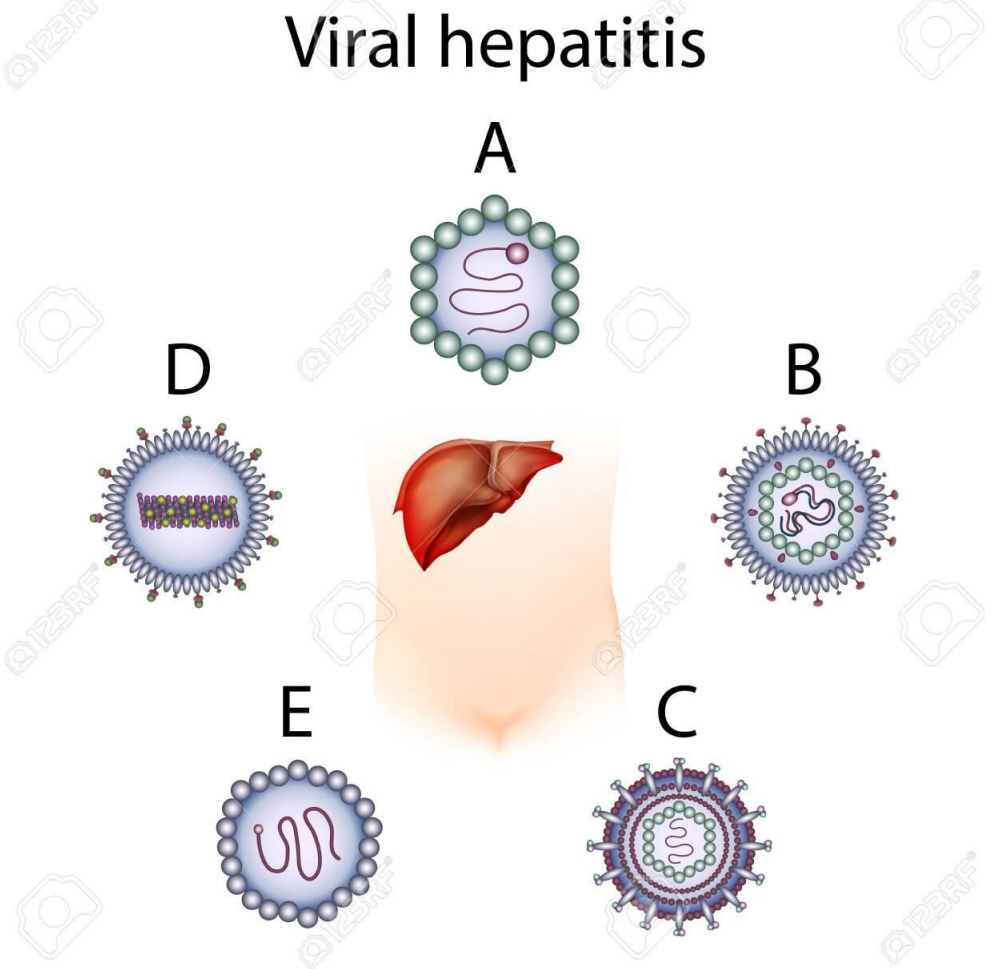
Feature	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Common name	"Infectious"	"Serum"	"Non-A, non-B posttransfusion"	"Delta agent"	"Enteric non-A, non-B"
Virus structure	Picornavirus; capsid, (+) RNA	Hepadnavirus; envelope, DNA	Flavivirus; envelope, (+) RNA	Viroid-like; envelope, circular RNA	Calicivirus-like; capsid, (+) RNA
Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Onset	Abrupt	Insidious	Insidious	Abrupt	Abrupt
Incubation period (days)	15-50	45-160	14-180+	15-64	15-50
Severity	Mild	Occasionally severe	Usually subclinical; 70% chronicity	Co-infection with HBV occasionally severe; superinfection with HBV often severe	Normal patients, mild; pregnant women, severe
Mortality	<0.5%	1%-2%	≈4%	High to very high	Normal patients, 1%-2%; pregnant women, 20%
Chronicity/carrier state	No	Yes	Yes	Yes	No
Other disease associations	None	Primary hepatocellular carcinoma, cirrhosis	Primary hepatocellular carcinoma, cirrhosis	Cirrhosis, fulminant hepatitis	None
Laboratory diagnosis	Symptoms and anti-HAV IgM	Symptoms and serum levels of HBsAg, HBeAg, and anti-HBc IgM	Symptoms and anti-HCV ELISA, genome testing	Anti-HDV ELISA	—

DNA, Deoxyribonucleic acid; *ELISA,* enzyme-linked immunosorbent assay; *HAV,* hepatitis A virus; *HBc,* hepatitis B core; *HBeAg,* hepatitis Be antigen; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HDV,* hepatitis D virus; *IgM,* immunoglobulin M, *RNA,* ribonucleic acid.

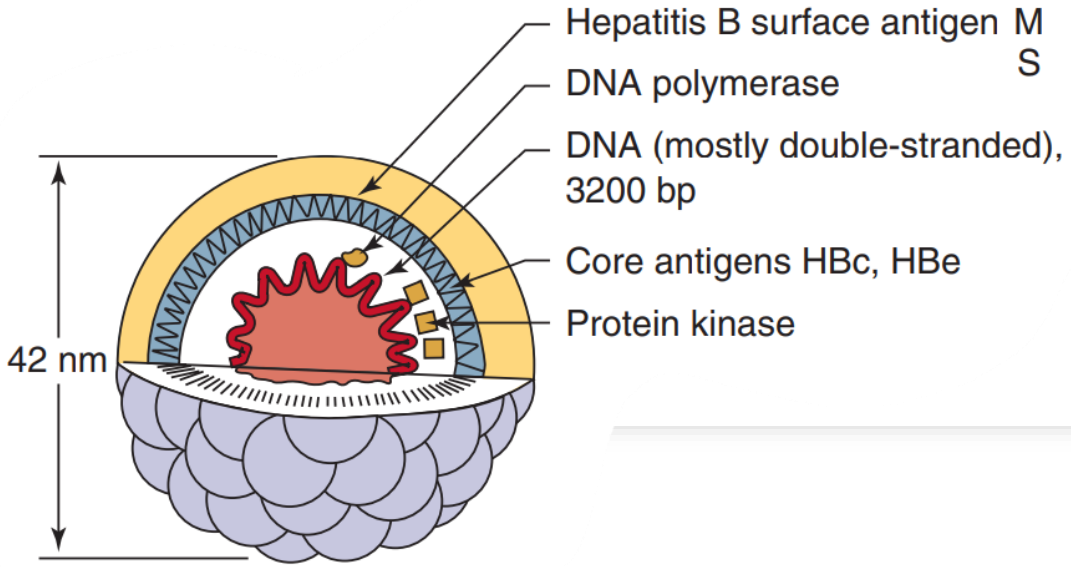
Reference

Adopted from Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

They all sound same but different. They are from different family, phenotypically and genotypically unrelated to each other



HBV – Characteristics



- Major member of the hepadnaviruses [1]
- Have limited tissue tropisms and host ranges
  - Infect liver and, to a lesser extent, the kidneys and pancreas of only humans and chimpanzees
- Enveloped DNA virus with several unusual properties [1]
- Genome is a small, circular, partly double-stranded DNA of only 3200 bases [1]
- Although it is a DNA virus, it encodes a reverse transcriptase and replicates through an RNA intermediate [1]

Reference

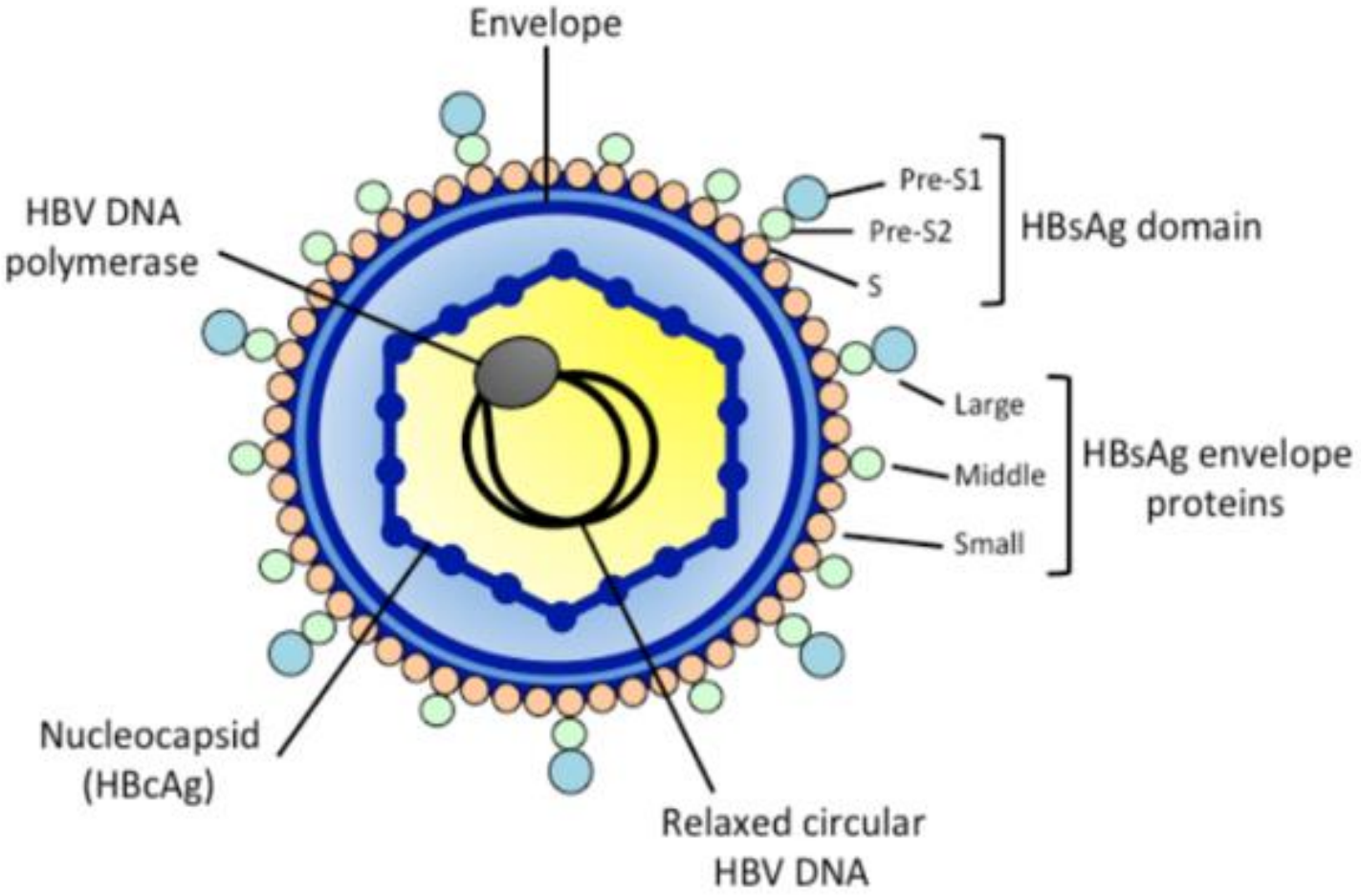
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

HBV – Characteristics (cont.)

- The virion, also called the Dane particle, is 42 nm in diameter [1]
- The virions are unusually stable for an enveloped virus [1]
  - Resist treatment with ether, low pH, freezing and moderate heating
- The virion is surrounded by an icosahedral capsid formed by the hepatitis B core antigen (HBcAg) and an envelope containing three forms of glycoprotein hepatitis B surface antigen (HBsAg) [1]
- HbsAg- containing particles are released into the serum of infected people and outnumber the actual virions [1]
- They are immunogenic and were processed into the first commercial vaccine against HBV [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Structure of HBV



Reference

Adopted from Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Replication of HBV

- HBV replicates through an RNA intermediate and produces and releases antigenic decoy particles (HBsAg) [1]
- Attachment
  - HBV attaches to hepatocytes mediated by the HBsAg glycoproteins [1]
  - The mechanism of entry is not known, but HBsAg binds to polymerized human serum albumin and other serum proteins, and binding and uptake of these proteins may facilitate virus uptake by the liver [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Replication of HBV (cont.)

- Penetration
  - Nucleocapsid delivers the genome to the nucleus, where the partial DNA strand of the genome is completed to form a complete dsDNA circle [1]
  - Transcription of the genome is controlled by cellular transcription elements found in hepatocytes [1]
  - The 3500-base mRNA encodes the HBc and HBe antigens, the polymerase, and a protein primer for DNA replication and acts as the template for replication of the genome [1]
  - The 2100-base mRNA encodes the small and medium glycoproteins [1]
  - The 2400-base mRNA encodes the large glycoproteins overlaps the 2100-base mRNA [1]
  - The 900-base mRNA encodes the X protein, which promotes viral replication as a transactivator of transcription and as a protein kinase [1]

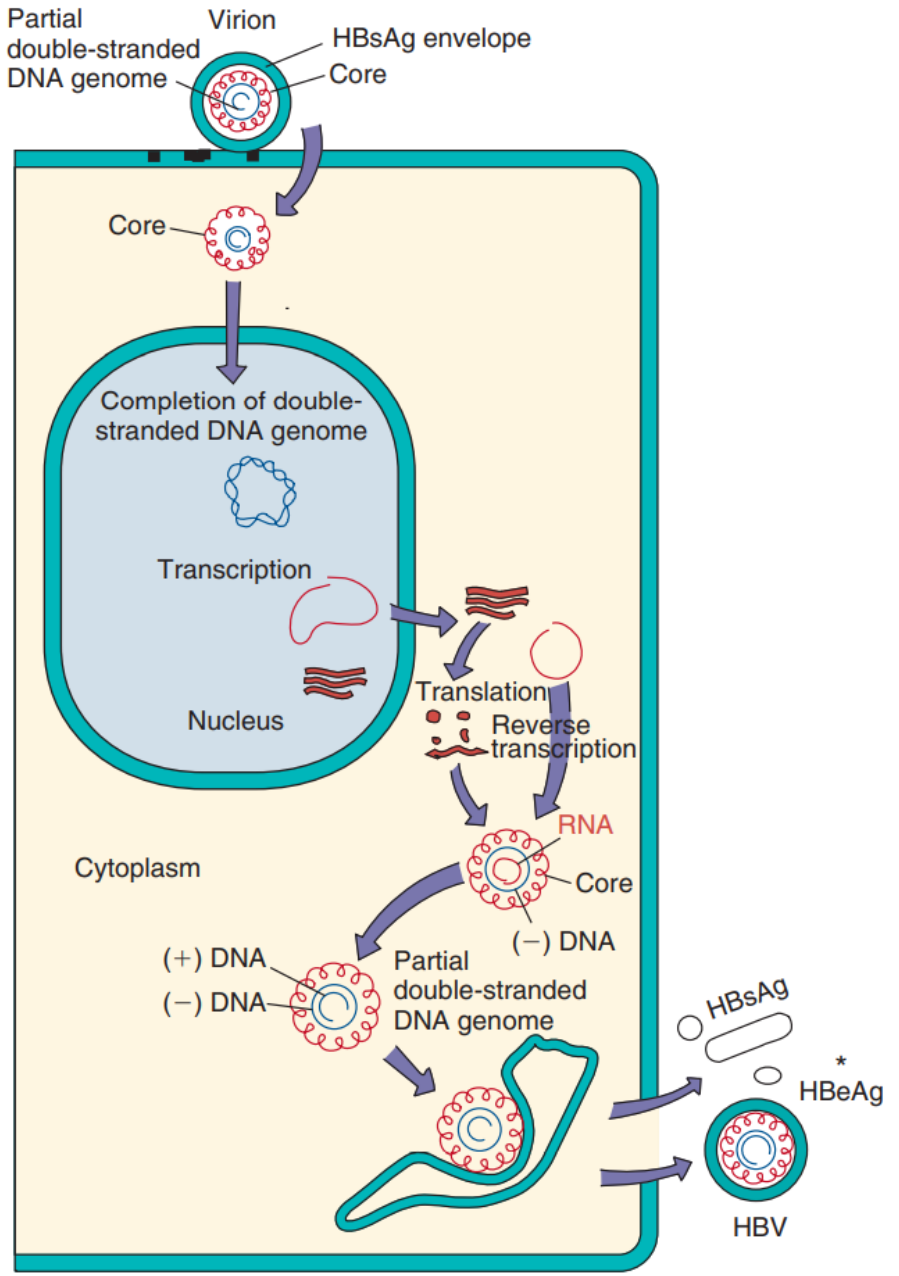
Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Replication of HBV (cont.)

- Replication
  - Replication of the genome utilizes the 3500-base mRNA [1]
  - This is packaged into the core nucleocapsid that contains the RNA-dependent DNA polymerase [1]
  - This polymerase has reverse transcriptase and ribonuclease H activity, but HBV lacks the integrase activity of the retroviruses [1]
  - The 3500-base RNA acts as a template, and (-) strand DNA is synthesized using a protein primer [1]
  - The RNA is degraded by the ribonuclease H activity as the (+) strand DNA is synthesized from the (-) strand DNA template [1]
  - However, this process is interrupted by envelopment of the nucleocapsid at the HBsAg-containing endoplasmic reticulum membrane, thereby capturing genomes containing a complete circular and incomplete DNA strand [1]
  - The virion and HBsAg-containing particles are then released from the hepatocyte by exocytosis, without killing the cell [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Overview of replication pathway of HBV



Reference

Adopted fom Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Pathogenesis

- HBV can cause acute or chronic, symptomatic or asymptomatic disease [1]
- The major source of infectious virus is blood, but HBV can be found in semen, saliva, milk, vaginal and menstrual secretions, and amniotic fluid [1]
- The virus starts to replicate in hepatocytes of the liver within 3 days of its acquisition [1]
- Symptoms may not be observed for 45 days or longer because they are primarily caused by immunopathology [1]
- The infectious dose, the route of infection and the immune system of the person determine the incubation period [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Pathogenesis (cont.)

- Infection proceeds for a relatively long time without causing liver damage or symptoms [1]
- Copies of the HBV genome integrate into the hepatocyte chromatin and can remain latent [1]
- Intracellular build-up of filamentous forms of HBsAg can produce the ground-glass hepatocyte cytopathology characteristics of HBV infection [1]
- HBsAg particles continue to be released into the blood even after virion release has ended and until the infection is resolved [1]
- An individual is infectious when both HBsAg and the HBeAg components of the virion can be detected in the blood [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Pathogenesis (cont.)

- Cell-mediated immunity and inflammation are responsible for causing the symptoms and effecting resolution of the HBV infection by eliminating the infected hepatocyte [1]
- An insufficient T-cell response to the infection generally results in the occurrence of mild symptoms, an inability to resolve the infection, and development of chronic hepatitis [1]
- Chronic infection also exhausts CD8 T cells, preventing them from killing infected cells [1]
- Antibody (generated by vaccination) can protect against initial infection by preventing delivery of the virus to the liver [1]
- Later in the infection, the large amount of HBsAg in serum binds to and blocks the action of neutralizing antibody, which limits the antibody's anility to resolve an infection [1]
- Immune complexes formed between HBsAg and anti-HBs contribute to the development of hypersensitivity reactions, leading to problems such as vasculitis, arthralgia, rash and renal damage [1]

Reference

1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Epidemiology

- In the US, more than 12 million people have been infected with HBV (1 out of 20), with 5000 deaths per year [1]
- In the world, 1 out of 3 people have been infected with HBV, with approximately a million deaths per year [1]
- More than 350 million people worldwide have chronic HBV infection [1]
- In developing nations, as many as 15% of the population may be infected during birth or childhood [1]
- In Malaysia, the incidence rate of HBV has increased from 2.26 per 100,000 in 2010 to 12.65 per 100,000 populations in 2015 [2]
- Up till 2017, 35,861 cases of HBV had been notified to the Ministry of Health (MOH) [2]
- The many asymptomatic chronic carriers with virus in blood and other body secretions foster spread of the virus [1]

Reference

1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.
2. National Strategic Plan for Hepatitis B and C, 2019 – 0223, https://www.moh.gov.my/moh/resources/Penerbitan/Pelan%20Strategik%20/NSP_Hep_BC_2019_2023.pdf

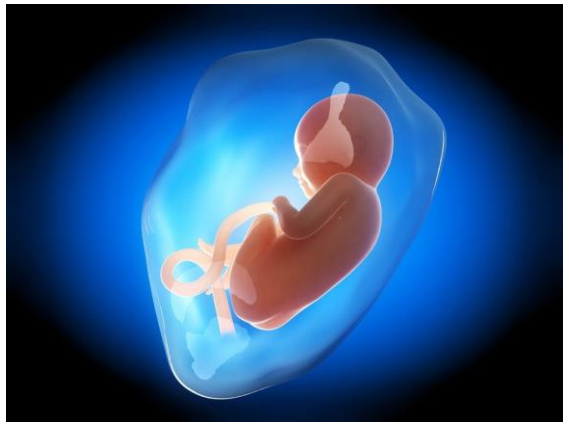
Epidemiology (cont.)

- The virus is spread by sexual, parenteral, and perinatal routes [1]
- Transmission occurs through contaminated blood and blood components by transfusion, needle sharing, acupuncture, ear piercing, or tattooing and through very close personal contact involving the exchange of semen, saliva, and vaginal secretions [1]
- Medical personnel are at risk in accidents involving needle-sticks or sharp instruments [1]
- Sexual promiscuity and drug abuse are major risk factors for HBV infection [1]
- HBV can be transmitted to babies through contact with the mother's blood at birth and in the mother's milk [1]
- Babies born to chronic HBV-positive mothers are at highest risk for infection [1]

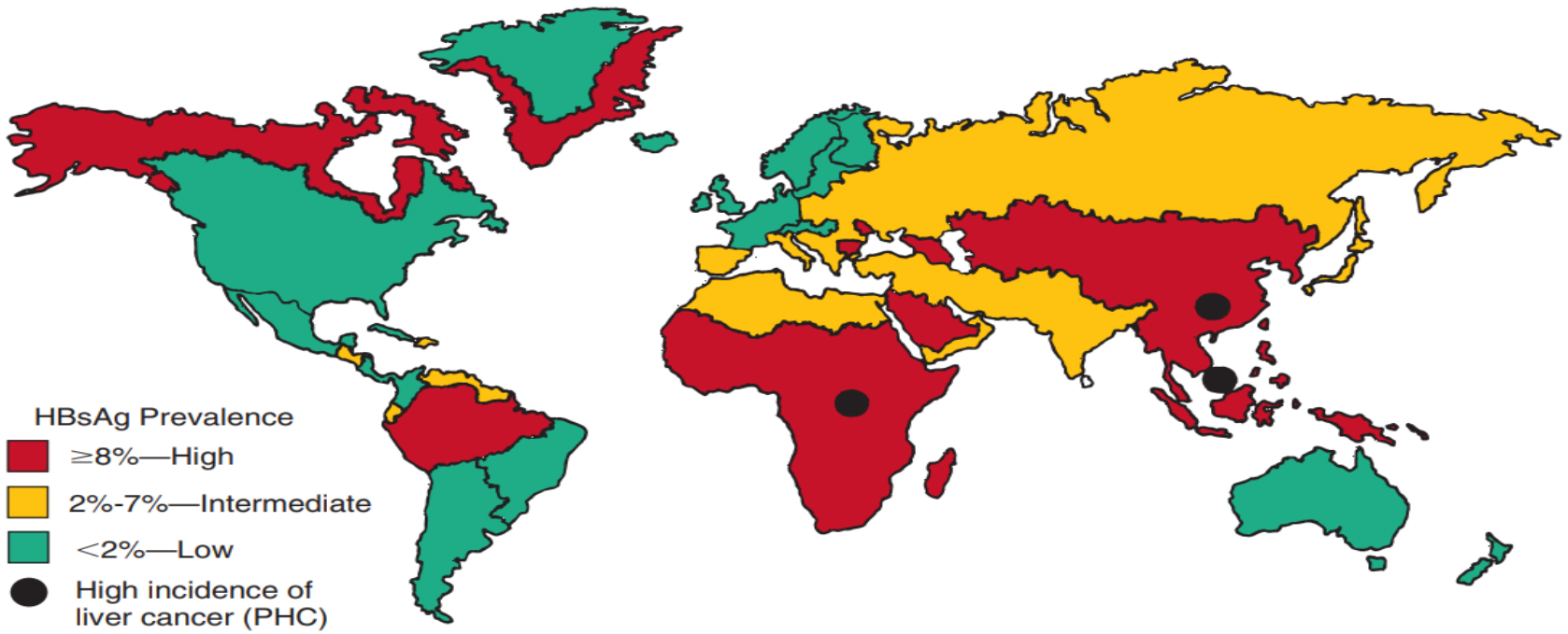
Reference

1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Sources and Routes of Transmission of HBV



Worldwide prevalence of HBV (HBsAg) carriers



Reference

Adopted from Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Overview of Laboratory Diagnosis

- The initial diagnosis of hepatitis can be made on the basis of the clinical symptoms and the presence of live enzymes in the blood [1]
- However, the serology of HBV infection describes the course and nature of the disease [1]
- Acute and chronic HBV infections can be distinguished by the presence of HBsAg and HBeAg in the serum and the pattern of antibodies to the individual HBV antigens [1]
- HBsAg and HBeAg are secreted into the blood during viral replication [1]
- Detection of HBeAg is the best correlate to the presence of infectious virus [1]

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Overview of Laboratory Diagnosis (cont.)

- A chronic or unresolved infection can be distinguished by the continued finding of HBeAg, HBsAg or both and a lack of detectable antibody to these antigens
- Antibody to HBsAg (anti-HBs or HBsAb) indicates resolution of infection or vaccination
- Antibody to HBcAg indicates current or prior infection by HBV, and IgM anti-HBc is the best way to diagnose a recent acute infection, especially while the infection is being resolved and when neither HBsAg nor anti-HBs can be detected (window)
- The amount of virus in blood can be determined by quantitative genome assays using PCR and related techniques
- Knowing the viral load can help in following the course of chronic HBV infection and antiviral drug efficacy

Reference
1. Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.

Specimen Type/Handling

- Serologies should be performed on serum or plasma separated from whole blood within 24 hours [1]
- These samples may be stored for up to 5 days at 2 – 8 ^{o}C o frozen indefinitely at -70 ^{0}C. [1]
- Collection tubes should not contain heparin, as this compound can interfere with the performance of the assays [1]
- Repeated freeze/thaw cycles should be avoided
- For nucleic acid assays, blood samples should be collected in tubes containing EDTA (lavender top) or citrate dextrose (yellow top) [1]
- Processing should occur within 6 hours; testing should be performed within 24 hours or the sample should be frozen at -70 ^{o}C [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.

Blood collected in EDTA tube (lavender top)

Serum/Plasma is used in testing

Liver Profile/Liver Function Test

- Frequently, chronic viral hepatitis is diagnosed because routine “liver function tests” (total bilirubin, alanine aminotransferase (ALT), aspartine aminotransferase (AST), alkaline phosphatase, and gamma glutamyl transferase) are abnormal [1]
- Both ALT and AST are intracellular enzymes that are released when hepatocytes die
- Because the ALT is made primarily by hepatocytes, an elevated level is most specific for inflammation of the liver [1]
- Despite the misnomer “liver function test”, high levels of AST and ALT represent hepatocyte destruction [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette’s Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.

Normal Range of Liver Profile Test

Panel 1: Typical adult reference ranges for liver function tests

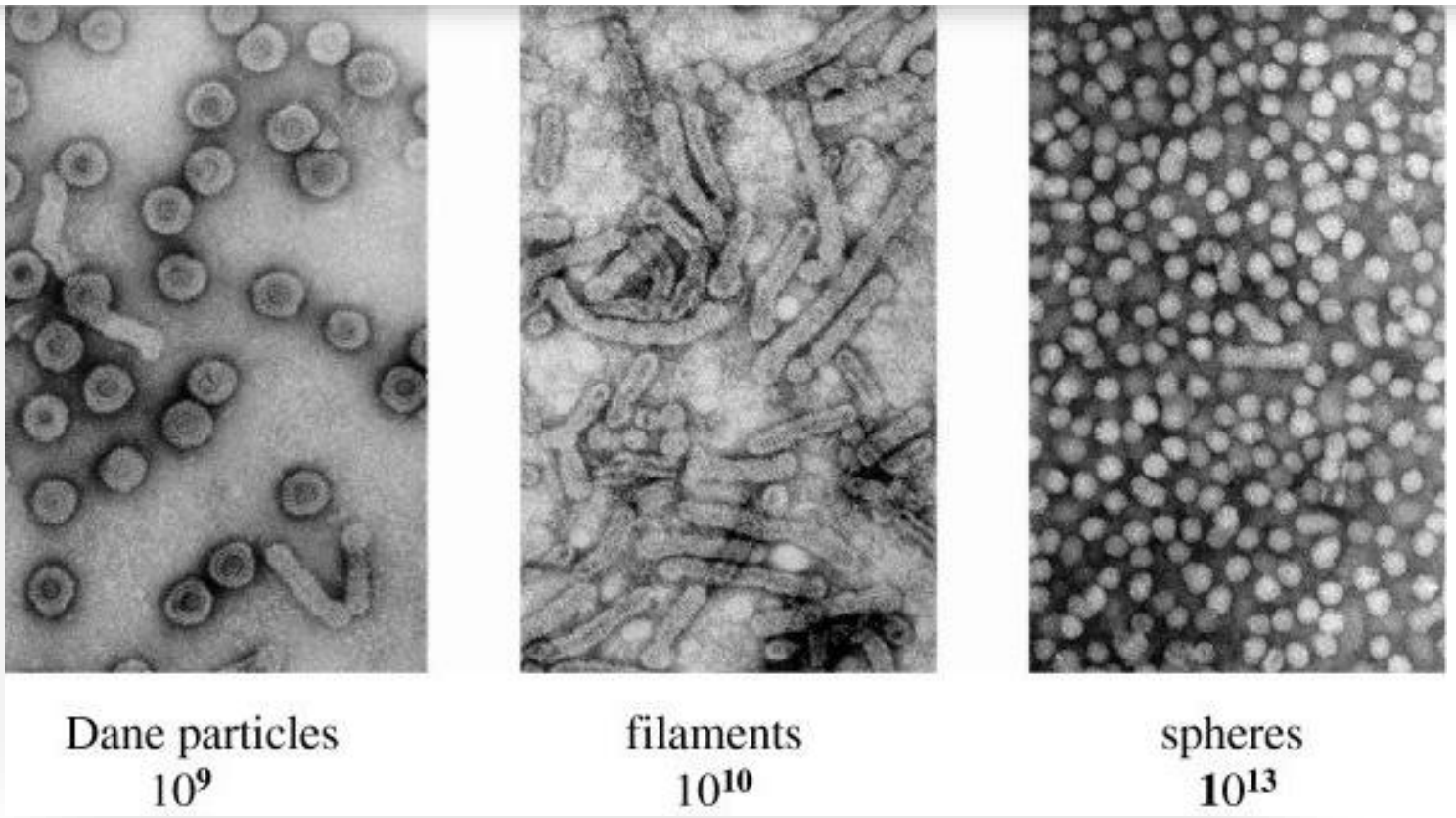
Albumin	35—55 g/L
Total bilirubin	3—20μmol/L
Conjugated bilirubin	0—14μmol/L
Alanine aminotransferase	0—45IU/L
Aspartate aminotransferase	0—50IU/L
Gamma-glutamyl transferase	0—70IU/L ♂
	0—40IU/L ♀
Alkaline phosphatase	90—300IU/L

Reference
Adopted from: https://pharmaceutical-journal.com/article/ld/blood-tests-used-to-investigate-liver-thyroid-or-kidney-function-and-disease

Direct Examination - Microscopy

- HBV can be detected by electron microscopy
- Although this is expensive and laborious, and is therefore only performed in research settings [1]
- Three distinct morphologic entities can be visualized
  - Spherical surface antigens [1]
  - Filamentous form of surface antigens [1]
  - Double-shelled spherical particle containing the HBV virion (Dane particle) [1]
- HBsAg is produced in both acute and chronic infections [1]
- HBeAg is indicative of active replication and has been associated with a higher risk of hepatocellular carcinoma (HCC) [2]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.
2. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002; 347:168–174



Electron microscopy images (negative staining) and approximate numbers of HBV associated particles in 1 ml of the serum from a highly viremic chronically infected HBV carrier.

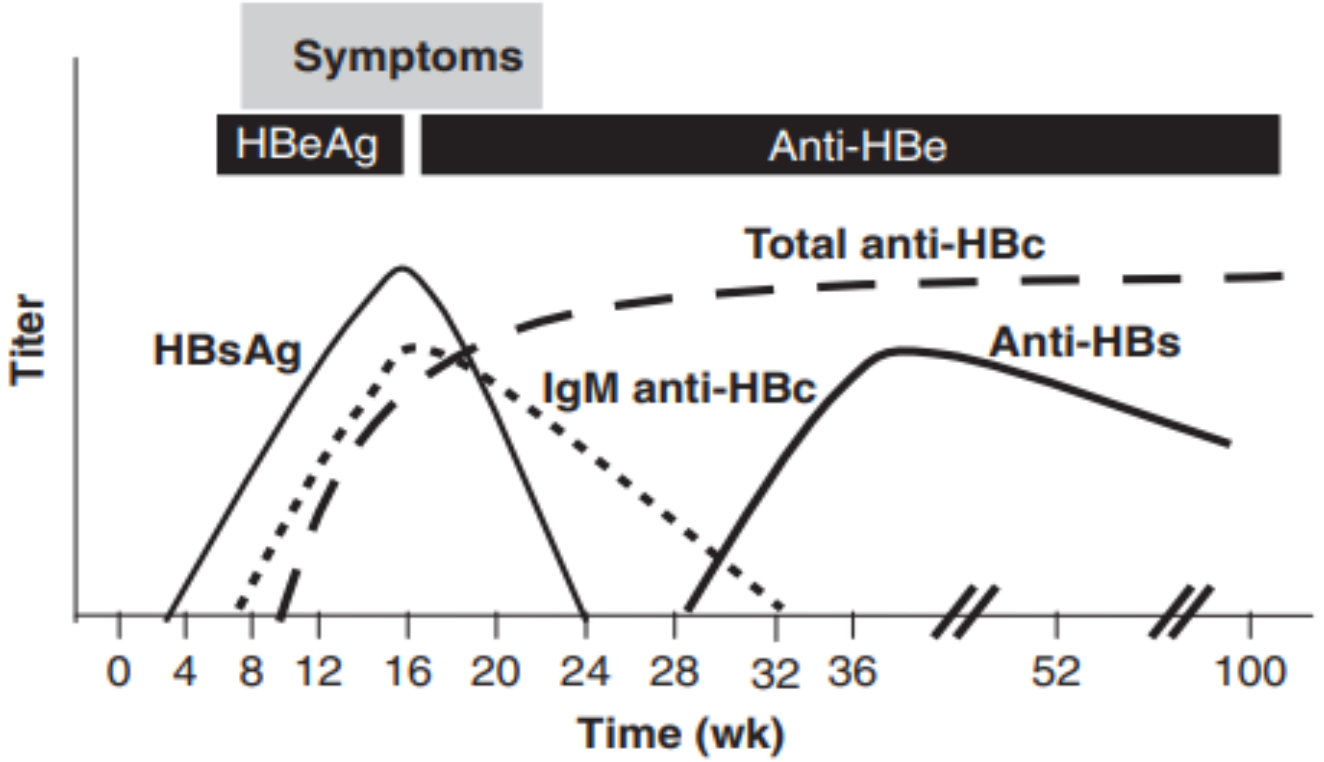
Reference
Adopted from: Gerlich, Wolfram H.. (2013). Medical Virology of Hepatitis B: How it began and where we are now. Virology journal. 10. 239. 10.1186/1743-422X-10-239.

Serological Testing

- The HBsAg is a protein on the surface of HBV, which is produced in both acute and chronic HBV
- The total core antibody (HBcAb) is indicative of past or current infection [1]
- Core IgM is produced in acute infections and thus is a marker of acute HBV
- Core IgG indicates past exposure to HBV [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.

The typical sequence of serologic markers for a patient who has acute HBV and resolution



Reference

Adopted from Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.

Serological Testing (cont.)

- The HBsAg is the first serologic marker of infection, appearing as early as one-week postexposure, but usually between 6 and 10 weeks [1]
- Shortly thereafter, the HBeAg is found in the blood [1]
- Several weeks after the HBeAg and HBsAg appear, the aminotransferases (AST and ALT) will peak [1]
- Approximately 10 weeks after exposure, the IgM core antibody typically becomes positive [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.

Molecular Assays

- The level of HBV DNA has become increasingly important in the natural history and outcome of treatment [1]
- In one Taiwanese study, researchers found that HBV DNA levels were highly predictive of developing HCC
  - Patients with baseline HBV DNA levels > 1 million copies/ml were 10 times more likely to develop HCC over the next decade compared to those with <300 copies /ml [2]
  - A similar study by the same group showed that patients with higher viral loads were more likely to develop cirrhosis [3]
- The viral level is also used for determining whether treatment should be initiated and for monitoring the response to therapy [4]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.
2. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295:65–73. 25.
3. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. JAMA 2006; 130:678–686. 26. ‘
4. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007; 45:507–537. 27.

Molecular Assays (cont.)

- There are 3 commonly used methods for detecting HBV DNA:
  - PCR [1]
  - Branched chain DNA amplification (bDNA) [1]
  - Hybrid capture [1]
- Real-time PCR quantification of HBV DNA is the most popular assay because of its improved sensitivity and simplicity compared to bDNA (Versant v 3.0, Bayer) [2, 3]
- Using the TaqMan platform, real-time PCR quantification is sensitive and linear from a range of 1.7 – 8.0 $\log_{10}$ IU/ml and is equally efficacious for genotypes A-H [4]
- Using specifically designed RNA probes that bind target DNA and then amplify the signal, the hybrid capture technology can detect DNA levels to 10^3 copies/ml, but it is limited to a 4 log range (Ultrasensitive HBV Hybrid Capture II) [5]
- The only FDA-approved assay for HBV DNA is the qualitative PCR test for the screening of HBV from blood donors (HBV AmpliScreen, Roche Molecular Systems, Pleasanton, CA) [1]
- HBV DNA quantification by real-time PCR should incorporate the WHO International Standard as an internal standard, reporting of results in international units per millilitres (IU/mL) ensures better reproducibility and comparability between labs [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.
2. Garbuglia AR, Angeletti C, Lauria FN, et al. Comparison of Versant HBV DNA 3.0 and COBAS AmpliPrep-COBAS TaqMan assays for hepatitis B DNA quantification: Possible clinical implications. J Virol Methods 2007; 146:274–280. 29.
3. Ronsin C, Pillet A, Bali C, et al. Evaluation of the COBAS AmpliPrep-total nucleic acid isolationCOBAS TaqMan hepatitis B virus (HBV) quantitative test and comparison to the VERSANT HBV DNA 3.0 assay. J Clin Microbiol 2006; 44:1390–1399. IHBK053-19 IHBK053-Jerome January 19, 2010 15:37 Char Count= 326 SCOTT ET AL. 30.
4. Chevaliez S, Bouvier-Alias M, Laperche S, et al. Performance of the COBAS AmpliPrep/COBAS TaqMan real-time PCR assay for hepatitis B virus DNA quantification. J Clin Microbiol 2008; 46:1716– 1723. 31.
5. Konnick EQ, Erali M, Ashwood ER, et al. Evaluation of the COBAS Amplicor HBV Monitor assay and comparison with the ultrasensitive HBV Hybrid Capture 2 assay for quantification of hepatitis B virus DNA. J Clin Microbiol 2005; 43:596–603

Geno-Typing System

- HBV has been classified into 8 major genotypes (A-H), which differ by > 8% in the whole viral sequence [2]
- Genotypes B and C are most common in Asia [1]
- Detection of genotype is performed by identifying nucleotide sequences in the most highly conserved region of the genome, usually the pre-S or S region [1]
- Genotyping can be performed by line probe analysis (Inno-Lipa HBV DRv2, Innogenetics, Gent, Belgium, or Quest Diagnostics) or in-house DNA sequencing [1]

Reference
1. Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.
2. Norder H, Courouce AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes, and HBsAg subtypes. Intervirology 2004; 47:289–309.

Why is DNA Quantification important?

- Quantification of HBV DNA, which reflects the viral load, could also aid in measurement and management of HBV infections [1]
- HBV DNA levels change during different phases of infection in chronically HBV infected individuals [1]
- HBV DNA measurements play a critical role in determining the phase of infection, deciding the treatment, and detecting responses to the antiviral therapy [2]
- According to the guidelines for the prevention, care, and treatment with persons with chronic hepatitis B virus from the WHO and China, HBV DNA quantification is recommended in the treatment of chronic HBV infections [3, 4]

Reference

1. Liu, C., Chang, L., Jia, T. *et al.* Real-time PCR assays for hepatitis B virus DNA quantification may require two different targets. *Virol J* **14,** 94 (2017). https://doi.org/10.1186/s12985-017-0759-8
2. European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167–85.
3. WHO Guidelines Approved by the Guidelines Review Committee. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization; 2015.
4. Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association, Hou JL, Lai W. The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. Zhonghua Gan Zang Bing Za Zhi. 2015;23:888–905.

Roche cobas® 4800 System for HBV

- cobas® HBV is an in vitro nucleic acid amplification test for the quantitation of HBV DNA in human EDTA plasma or serum of HBV-infected individuals [1]
- The test is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy [1]
- The test can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment [1]

Reference
1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual

cobas® X 480 instrument and cobas® Z 480 analyzer

Reference
1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual

Principles of cobas® HBV

- cobas® HBV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection [1]
- Consists of the cobas x 480 instrument and the cobas z 480 analyzer [1]
- Automated data management is performed by the cobas® 4800 software which assigns test results for all tests as target not detected, <LLoQ (lower limit of quantitation), >ULoQ (upper limit of quantitation) or HBV DNA detected, a value in the linear range [1]
- Nucleic acids from patient samples, external controls and added lambda phage DNA QS molecules are simultaneously extracted [1]

Reference
1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual

Principles of cobas® HBV (cont.) – Extraction and Amplification

- Viral nucleic acids are released by addition of proteinase and lysis reagent to the sample [1]
- The released nucleic acids bind to the silica surface of the added magnetic glass particles [1]
- Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash buffer steps and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature [1]
- Selective amplification of target HBV nucleic acids from the patient sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HBV [1]
- Selective amplification of DNA QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HBV genome [1]
- A thermostable DNA polymerase is used for PCR amplification [1]

Reference

1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual

Principles of cobas® HBV (cont.) – AmpErase Decontamination System

- The master mix includes deoxyuridine triphosphate (dUPT), is incorporated into the newly synthesized DNA [2, 3, 4]
- Any contamination amplicon from previous PCR runs are inactivated as PCR templates by AmpErase, which is present in the master mix, prior to the first denaturation step of PCR [1]
- AmpErase catalyzes the removal of uracil from DNA, but has no activity on naturally occurring DNA, which does not contain uracil [1]
- Amplicon formed during subsequent cycles of PCR are not inactivated since AmpErase is inactive at the annealing and denaturation temperatures of PCR [1]

Reference

1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual
2. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Research. 1996; 6: 986-994.
3. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNAglycosylase. Nature. 1995;373:487-493.
4. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase:structural basis for specificity and catalysis. Cell. 1995;80:869-878.

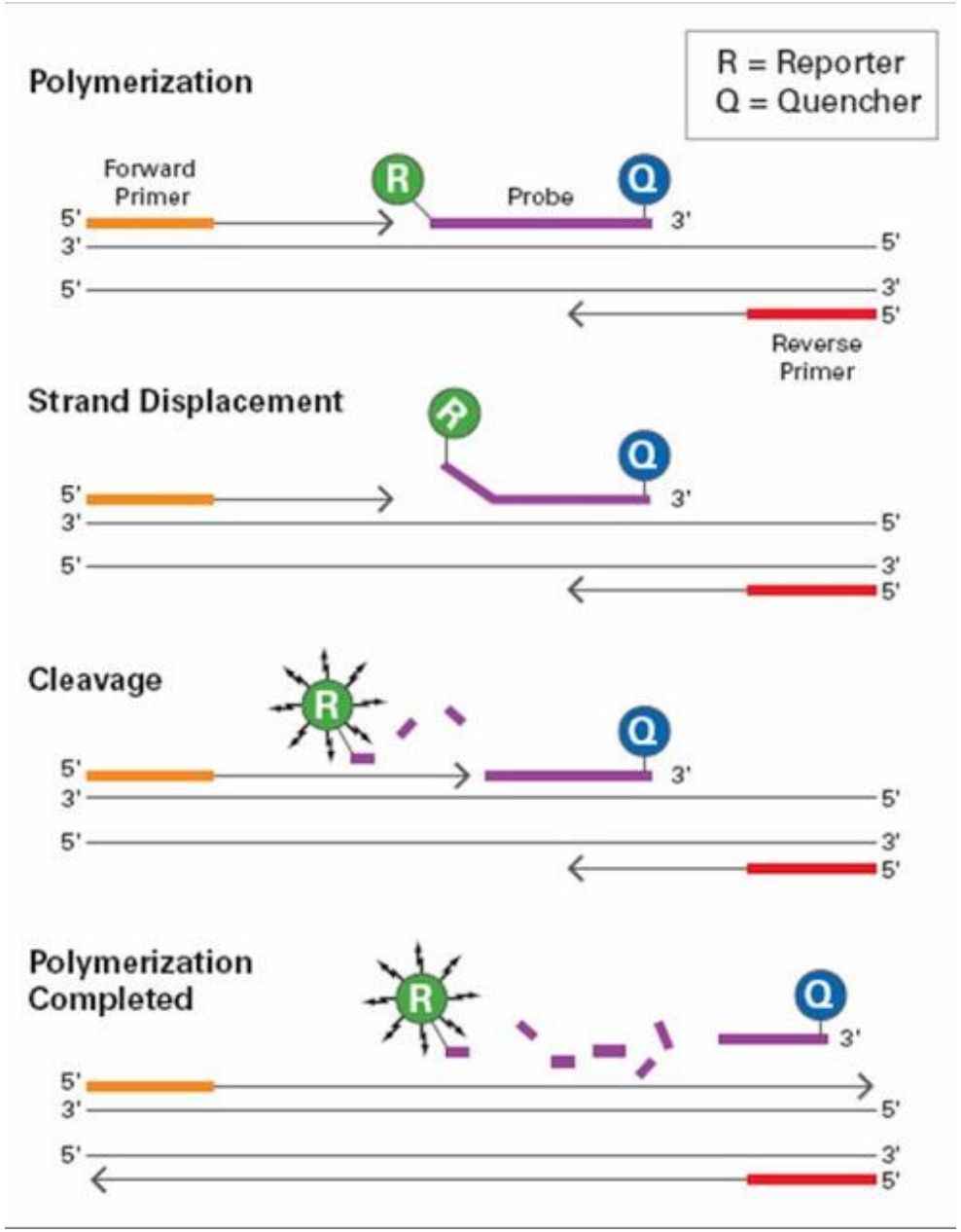
Principles of cobas® HBV (cont.) – Detection of Signal (TaqMan & Quencher System)

- The cobas® HBV master mix contains detection probes which are specific for the HBV target sequences and the QS nucleic acid, respectively [1]
- The specific HBV and DNA-QS detection probes are each labelled with one of two unique fluorescent dyes which act as a reporter [1]
- Each probe also has a second dye which acts as a quencher [1]
- The 2 reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HBV target and the DNA-QS [2, 3]
- When not bound to the target sequence, the fluorescent signal of the intact probe is suppressed by the quencher dye [1]
- During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by exonuclease activity of the DNA polymerase, resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal [1]
- With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly [1]

Reference

1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual
2. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination inpolymerase chain reactions. Gene. 1990;93:125-128.
3. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences.Bio/Technology. 1992;10:413-417.

Overview of Principles of Taqman Assay



Advantages of cobas® HBV

cobas® HBV performance summary

Parameter	Performance	
Sample type	EDTA plasma, serum	
Minimum amount of sample required	Please refer to the **cobas**® 4800 Systems Operator's Manual for **cobas**® HBV	
Sample processing volume	400 µL or 200 µL	
Analytical sensitivity	EDTA plasma: 4.4 IU/mL (400 µL) 7.6 IU/mL (200 µL)	Serum: 2.8 IU/mL (400 µL) 5.5 IU/mL (200 µL)
Linear range	400 µL: 10.0 IU/mL – 1.0E+09 IU/mL 200 µL: 10.0 IU/mL – 1.0E+09 IU/mL	
Specificity	100.0% (one-sided 95% confidence interval: 99.5%)	
Genotypes detected	HBV genotypes A-H, precore mutant	
Cross Contamination	0.0% (one-sided 95% confidence interval of 1.3%)	

- Save time (automated which frees lab personnel for other tasks)
- Reduce human errors and contamination
- Fast and accurate thermal cycling
- Reliable results
- High specificity

Reference
1. Cobas® HBV, better information for patient management

Limitations of cobas® HBV

- Reliable results are dependent on adequate specimen collection, transport, storage and processing [1]
- Validated only for use with EDTA plasma and serum. Testing of other sample types may result in inaccurate results [1]
- Quantitation of HBV DNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors and stage of infection [1]
- Mutations within the highly conserved regions of a viral genome may affect primers and probe binding, resulting in the under-quantitation of virus or failure to detect the presence of virus [1]
- Not intended for use as a screening test for the presence of HBV in blood or blood products or as a diagnostics test to confirm the presence of HBV infection [1]

Reference

1. Cobas® HBV, Quantitative nucleic acid test for use on the cobas® 4800 system Manual

References

- Murray, Patrick R. (2016). *Medical Microbiology* (8th ed.). Elsevier.
- National Strategic Plan for Hepatitis B and C, 2019 – 0223, https://www.moh.gov.my/moh/resources/Penerbitan/Pelan%20Strategik%20/NSP_Hep_BC_2019_2023.pdf
- Lennette, Edwin H. (2010). *Lennette's Laboratory Diagnosis of Viral Infections* (4th ed.). informa healthcare.
- Liu, C., Chang, L., Jia, T. *et al.* Real-time PCR assays for hepatitis B virus DNA quantification may require two different targets. *Virol J* **14,** 94 (2017). https://doi.org/10.1186/s12985-017-0759-8
- Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002; 347:168–174.
- Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295:65–73. 25.
- Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. JAMA 2006; 130:678–686. 26.
- Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007; 45:507–537. 27.

References (cont.)

- Sum SS, Wong DK, Yuen JC, et al. Comparison of the COBAS TaqMan HBV test with the COBAS Amplicor monitor test for measurement of hepatitis B virus DNA in serum. J Med Virol 2005; 77: 486–490. 28.
- Garbuglia AR, Angeletti C, Lauria FN, et al. Comparison of Versant HBV DNA 3.0 and COBAS AmpliPrep-COBAS TaqMan assays for hepatitis B DNA quantification: Possible clinical implications. J Virol Methods 2007; 146:274–280. 29.
- Ronsin C, Pillet A, Bali C, et al. Evaluation of the COBAS AmpliPrep-total nucleic acid isolationCOBAS TaqMan hepatitis B virus (HBV) quantitative test and comparison to the VERSANT HBV DNA 3.0 assay. J Clin Microbiol 2006; 44:1390–1399. IHBK053-19 IHBK053-Jerome January 19, 2010 15:37 Char Count= 326 SCOTT ET AL. 30.
- Chevaliez S, Bouvier-Alias M, Laperche S, et al. Performance of the COBAS AmpliPrep/COBAS TaqMan real-time PCR assay for hepatitis B virus DNA quantification. J Clin Microbiol 2008; 46:1716– 1723. 31.
- Konnick EQ, Erali M, Ashwood ER, et al. Evaluation of the COBAS Amplicor HBV Monitor assay and comparison with the ultrasensitive HBV Hybrid Capture 2 assay for quantification of hepatitis B virus DNA. J Clin Microbiol 2005; 43:596–603
- Norder H, Courouce AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes, and HBsAg subtypes. Intervirology 2004; 47:289–309.

References (cont.)

- CDC. http://www.cdc.gov/hepatitis/Resources/Professionals/Training/Serology/training. htm#one. Accessed December 7, 2009. 72.
- http://www.cdc.gov/hepatitis/HBV/HBVfaq.htm#general. Accessed December 7, 2009
- Gerlich, Wolfram H.. (2013). Medical Virology of Hepatitis B: How it began and where we are now. Virology journal. 10. 239. 10.1186/1743-422X-10-239
- https://pharmaceutical-journal.com/article/ld/blood-tests-used-to-investigate-liver-thyroid-or-kidney-function-and-disease
- Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Research. 1996; 6: 986-994.
- Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNAglycosylase. Nature. 1995;373:487-493.
- Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase:structural basis for specificity and catalysis. Cell. 1995;80:869-878.
- Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination inpolymerase chain reactions. Gene. 1990;93:125-128.
- Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences.Bio/Technology. 1992;10:413-417.