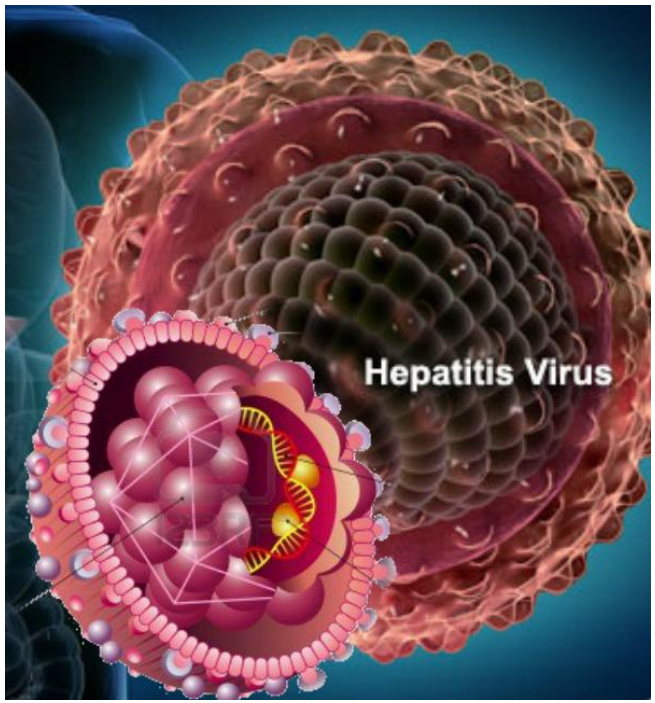
Medimail
August 2020

Viral Hepatitis Diagnostics



Viral Hepatitis

- Systemic infection affecting liver predominantly
- Caused by one of five viral agents: HAV, HBV, HCV, the HBV-associated delta agent or hepatitis D virus (HDV), HEV
- All are RNA viruses, except for hepatitis B, which is a DNA virus but replicates like a retrovirus
- Can lead to acute, chronic or sequel of chronic infection

	A	B	C	D	E
Source of virus	feces	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	feces
Route of transmission	fecal-oral	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	fecal-oral
Chronic infection	no	yes	yes	yes	no
Prevention	pre/post-exposure immunization	pre/post-exposure immunization	blood donor screening; risk behavior modification	pre/post-exposure immunization; risk behavior modification	ensure safe drinking water

CDC

World Statistics

- Viral hepatitis is sixth leading cause of death (2015).
- HBV, along with associated infection by HDV, is one of the most common pathogens afflicting humans. ~2 billion people in the world have evidence of past or current HBV infection, with 240 million chronic carriers of hepatitis B surface antigen (HBsAg). HBV leads to 650,000 deaths annually as a result of viral hepatitis–induced liver disease.
- An estimated 71 million people are chronically infected with HCV worldwide. About 55-85% of these people progress to chronic HCV infection, with a 15-30% risk of developing liver cirrhosis within two decades. Risk of transmission of HCV from a mother to child occurs in 4–8% of births to HCV infected women, and in 10.8–25% of births to women with HIV and HCV co-infection.
- HAV is responsible for an ~1.4 million infections annually.

India Statistics

- ~40 million people are chronically infected with HBV and ~6-12 million people with HCV.
  - HBsAg positivity in general population ranges from 1.1% to 12.2%, with an average prevalence of 3-4%.
  - Anti-HCV antibody prevalence in general population is ~0.09-15%.
  - Chronic HBV infection accounts for 40-50% of hepatocellular carcinoma (HCC) and 20-30% cases of cirrhosis.
  - Chronic HCV infection accounts for 12-32% of HCC and 12-20% of cirrhosis.
- HAV is responsible for 10-30% of acute hepatitis and 5-15% of acute liver failure cases.
- HEV is responsible for 10-40% of acute hepatitis and 15-45% of acute liver failure.

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India Statistics

WHO Global Disease estimates 2016 viral hepatitis could be contributing to early 2.85% of all deaths in India

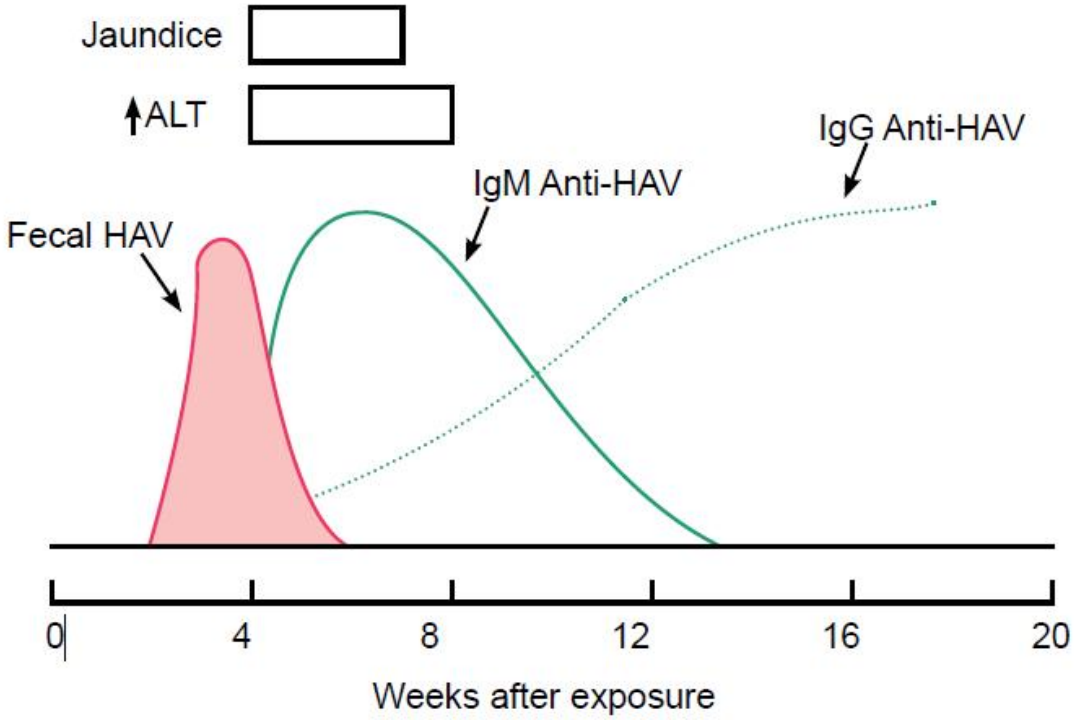
Hepatitis		Numbers in thousands	Total (in thousands)
a.	Acute hepatitis A	5.0	78.7
b.	Acute hepatitis B	43.3	
c.	Acute hepatitis C	1.1	
d.	Acute hepatitis E	29.2	
Liver cancer		Numbers in thousands	Total (in thousands)
a.	Liver cancer secondary to hepatitis B	15.3	20.1
b.	Liver cancer secondary to hepatitis C	4.9	
Cirrhosis of the liver		Numbers in thousands	Total (in thousands)
a.	Cirrhosis due to hepatitis B	141.8	173.6
b.	Cirrhosis due to hepatitis C	31.9	

Estimated Total Deaths (All causes)		9559.1
% of deaths attributed to viral hepatitis		2.85%

Hepatitis A Infection

- It is an outbreak prone disease with an incubation period of around 4 weeks.
- Excretion in the stool occurs for only 7-14 days after the onset of the clinical illness and is diagnostic of an acute HAV infection.
- No carrier state has been identified.

Laboratory Markers of HAV Infection



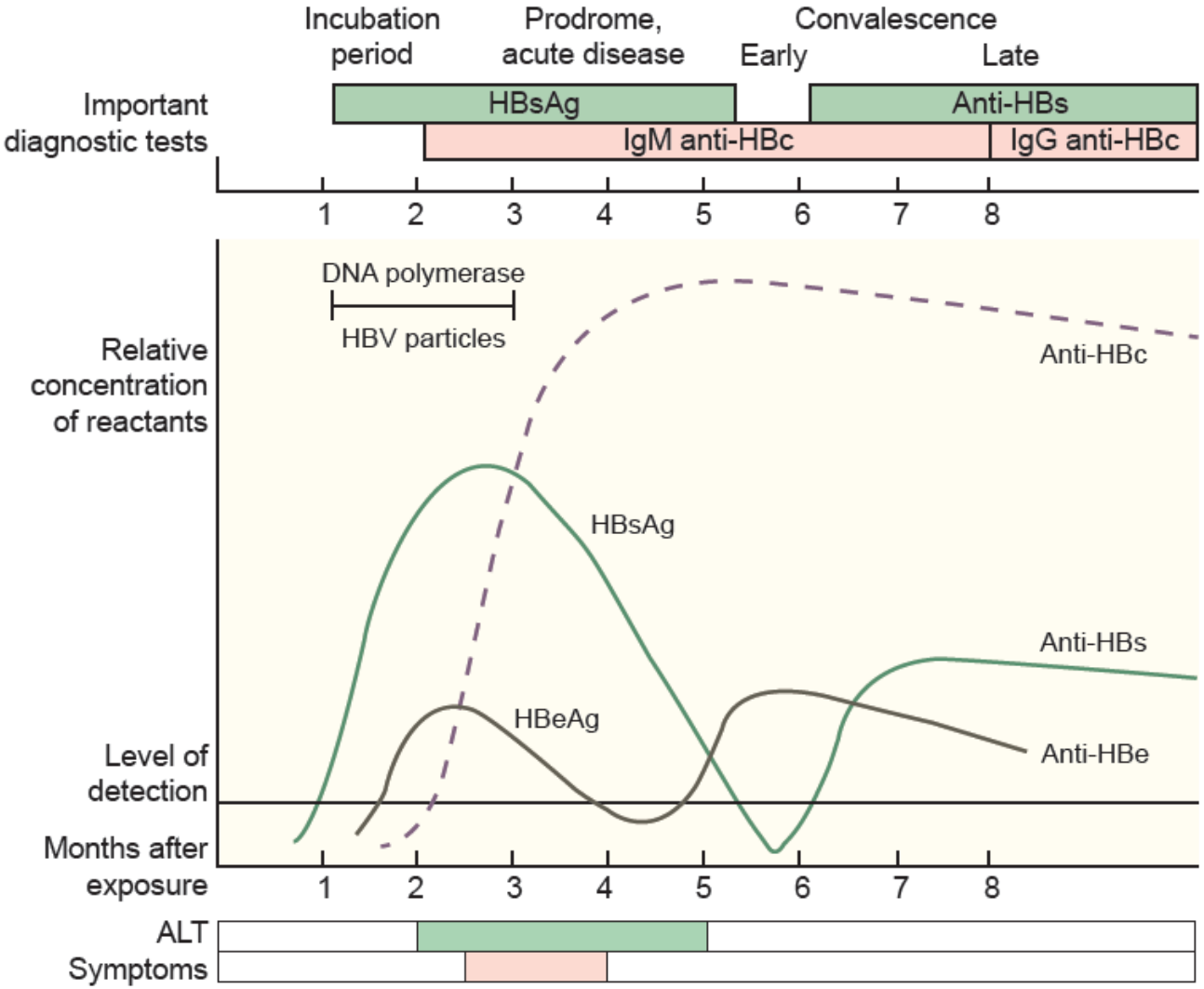
NHM 2018. National Guidelines for Diagnosis & Management of Viral Hepatitis

Immunoprophylaxis of Hepatitis A

- **Passive immunoprophylaxis**
  - Recommended for several high-risk groups, including travelers to endemic areas, nursing homes and household contacts of index cases with HAV infection.
  - Administration of immune globulin (IG) before exposure and within 1 to 2 weeks of exposure will prevent or attenuate infection in 85% to 95% of exposed persons.

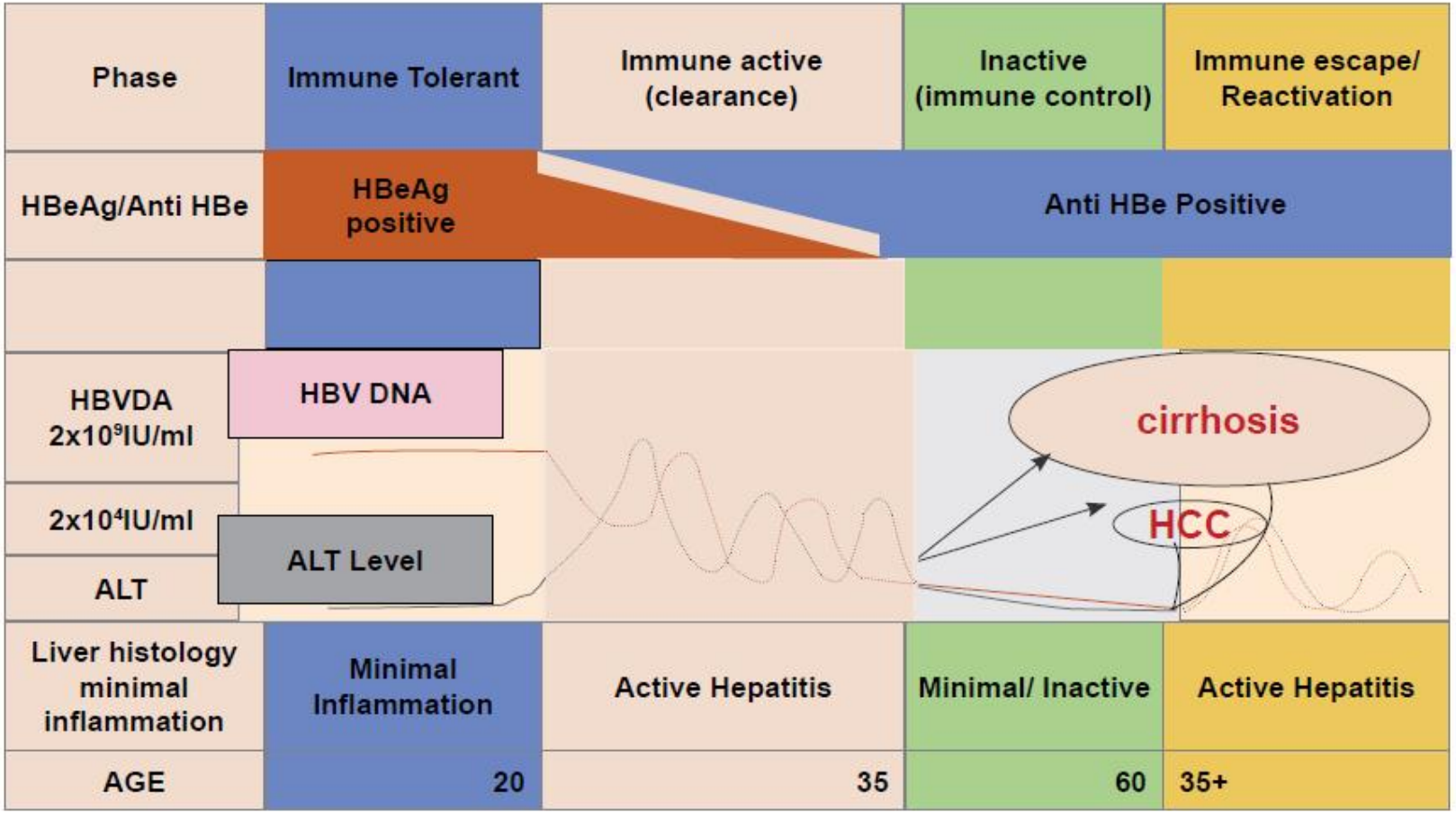
- **Active immunoprophylaxis**
  - Vaccination recommended for members of high-risk groups against HAV has been used to shorten the duration of community outbreaks.
  - Three different strategies have been utilized in vaccine development: a live attenuated vaccine, an inactivated vaccine, and a recombinant polypeptide vaccine.

Hepatitis B Infection (Acute): Clinical Progression along with Associated Serological Events



NHM 2019. Technical Guidelines for Diagnosis & Management of Hepatitis B

Hepatitis B Infection (Chronic): Natural Course and Phases



- This phase seen in HBV transmission at birth/1-2 years of life.
- HBeAg +ve and high viral load (107 IU/mL) but no elevation of transaminases and minimal activity
- in liver as there is no immunological response.

- With increased immune response HBV DNA level decreases.
- Liver enzymes fluctuate.
- Active inflammation in liver ending in HBeAg negative and HBeAb +ve (HBeAg seroconversion)
- Ongoing activity could progress to fibrosis and liver cirrhosis with HCC.

- HBeAg remains negative in 70-85% with low viral load <2 x 103 IU/mL with persistently normal liver enzymes but hepatitis activity may continue in some
- Fibrosis/ cirrhosis noted in those who had progressed in immune active phase

- Progression from HBeAg negative inactive phase to HBeAg negative hepatitis B with mutation in core or core promoter region of HBV genome resulting in HBeAg negative but with continued HBV replication and progression in liver disease.

Assessment and Staging of HBV Chronic infection

- Routine assessment of HBsAg-positive persons is needed to guide management and indicate the need for treatment. This generally includes assessment of:
  1. Serological markers of HBV infection
  2. Measurement of HBV DNA levels
  3. Assessing severity of liver disease by
     a. Liver chemistries, prothrombin time, full blood count
     b. Non-invasive tests such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), , fibrosis-4 (FIB-4), transient elastography (FibroScan)
     c. Liver biopsy, if available
     d. Other routine investigations include ultrasonography and alpha-fetoprotein (AFP) measurement for periodic surveillance for hepatocellular carcinoma (HCC), and endoscopy for varices in persons with cirrhosis.

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Interpretation of Screening Tests for HBV Infection

Screening Test Results				
HBsAg	Anti-HBc	Anti-HBs	Interpretation	Management
+	+	–	Chronic hepatitis B	Additional testing and management needed
–	+	+	Past HBV infection, resolved	No further management unless immunocompromised or undergoing chemotherapy or immunosuppressive therapy
–	+	–	Past HBV infection, resolved or false-positive	HBV DNA testing if immunocompromised patient
–	–	+	Immune	No further testing
–	–	–	Uninfected and not immune	No further testing

Terrault NA. Hepatology, Vol. 67, No. 4, 2018

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Measurement of HBV DNA levels

- Plasma HBV DNA concentrations quantified by real-time polymerase chain reaction (PCR) are used –
  - To correlate with disease progression
  - To differentiate active , hepatitis B e-antigen (HBeAg)-negative disease from inactive chronic infection
  - For decisions to treat
  - For optimal monitoring of response to antiviral therapy; a rise may indicate emergence of resistant variants

- The same assay should be used in same patient to evaluate efficacy of antiviral therapy

Assessment of Degree of Fibrosis

- Liver biopsy has been used to ascertain the degree of necro-inflammation and fibrosis, and to help guide the decision to treat.
- Pathological features of CHB on liver biopsy depend upon stage of disease, host immune response and degree of virus replication.

Metavir Stage	F0	F1	F2	F3	F4
Definition	No Fibrosis	Portal fibrosis without septa	Portal fibrosis with septa	Numerous septa without cirrhosis	Cirrhosis

- Limitations of biopsy include –
  - sampling error
  - subjectivity in reporting
  - high costs
  - risks of bleeding and pneumothorax
  - discomfort to patient
  - need for training and infrastructure.

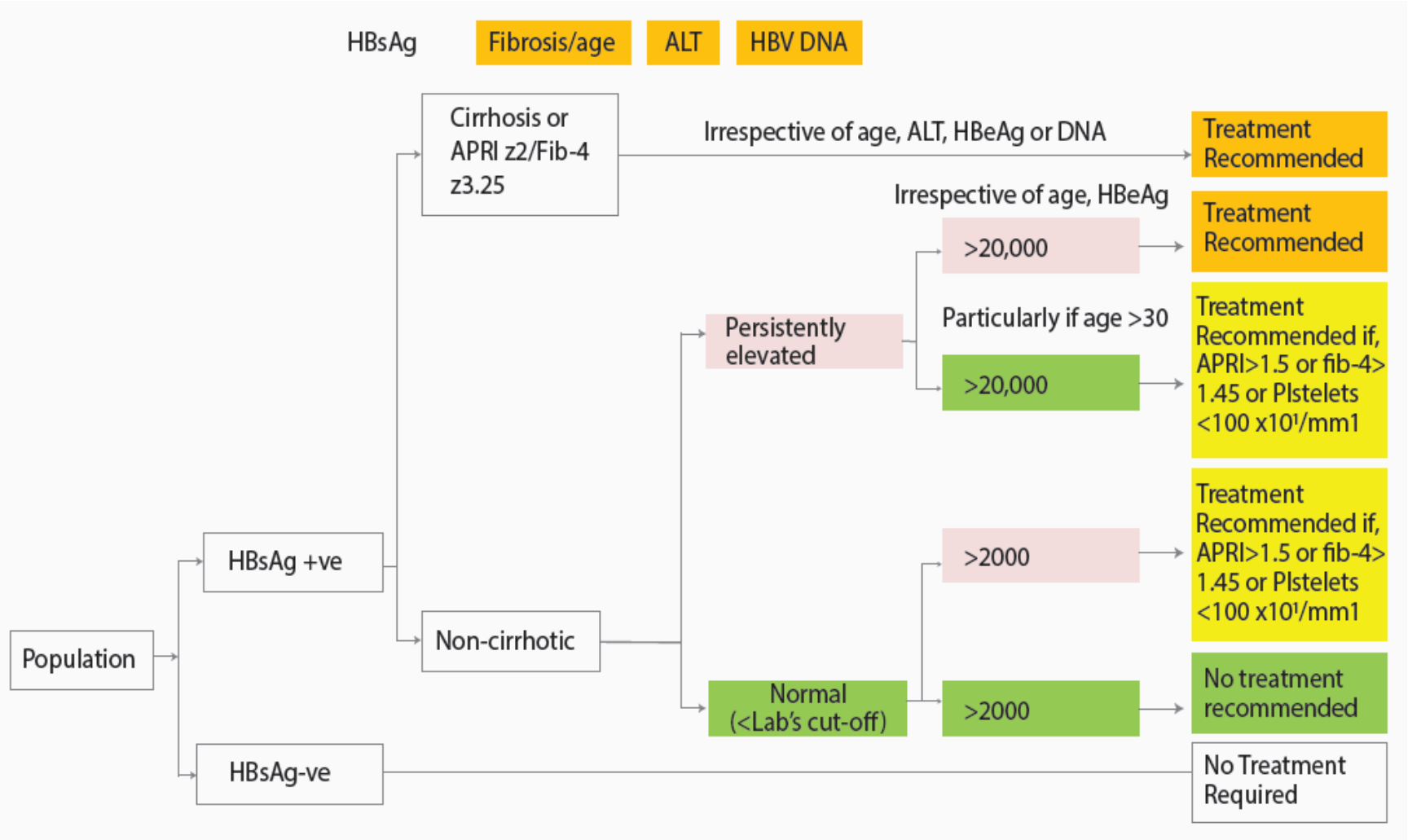
Non-invasive Tests (NITs)

- Though liver biopsy remains gold standard, NIT methods for assessing stage of liver disease are supplanting it due to limited availability and accessibility to liver biopsy to rule out advanced fibrosis.
- APRI (AST-to-platelet ratio index) and FIB 4 are recommended as preferred NIT to assess for the presence of cirrhosis (APRI score >2: FIB 4 >3.25 in adults).
- Transient elastography (e.g. FibroScan) may be the preferred NITs in settings where they are available and cost is not a major constraint.

APRI and FIB-4 can be readily calculated by the following formulae

APRI = * (AST/ULN) x 100) / platelet count (10^9/L)
FIB-4 = (age (yr) x AST (IU/L)) / (platelet count (10^9/L x [ALT (IU/L)1/2])

Chronic Hepatitis B (CHB) Infection: Whom To Treat



HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e-Antigen; APRI, AST to platelets ratio index; FIB-4, fibrosis-4

NHM 2019. Technical Guidelines for Diagnosis & Management of Hepatitis B

Monitoring: Parameters and Frequency

The disease is complex and has sequelae, resolution as well as drugs side effects. Hence, three types of monitoring is necessitated –

- Monitoring for disease progression and treatment response in persons with CHB prior to, during and post-treatment
- Monitoring for tenofovir or entecavir side-effects
- Monitoring for HCC

Interval (Months)	3 Months			6 Months			9 Months			12 Months		
HBeAg/Anti HBe		√	√	√	√	√	√	√	√	√	√	√
HBVDNA		√	√	√	√	√	√	√	√	√	√	√
ALT	√	√	√	√	√	√	√	√	√	√	√	√
AST										√		√
CBC (Platelet)										√	√	√
APRI/FIB4/ Fibro Scan										√	√	√
USG				√						√	√	√
Serum Creatinine											√	
eGFR											√	
Phosphate											√	
Urine Protein: Creatinine Ratio											√	

	Not on Treatment
	Treatment
	Discontinue Treatment

NHM 2018. National Guidelines for Diagnosis & Management of Viral Hepatitis

Immunoprophylaxis of Hepatitis B

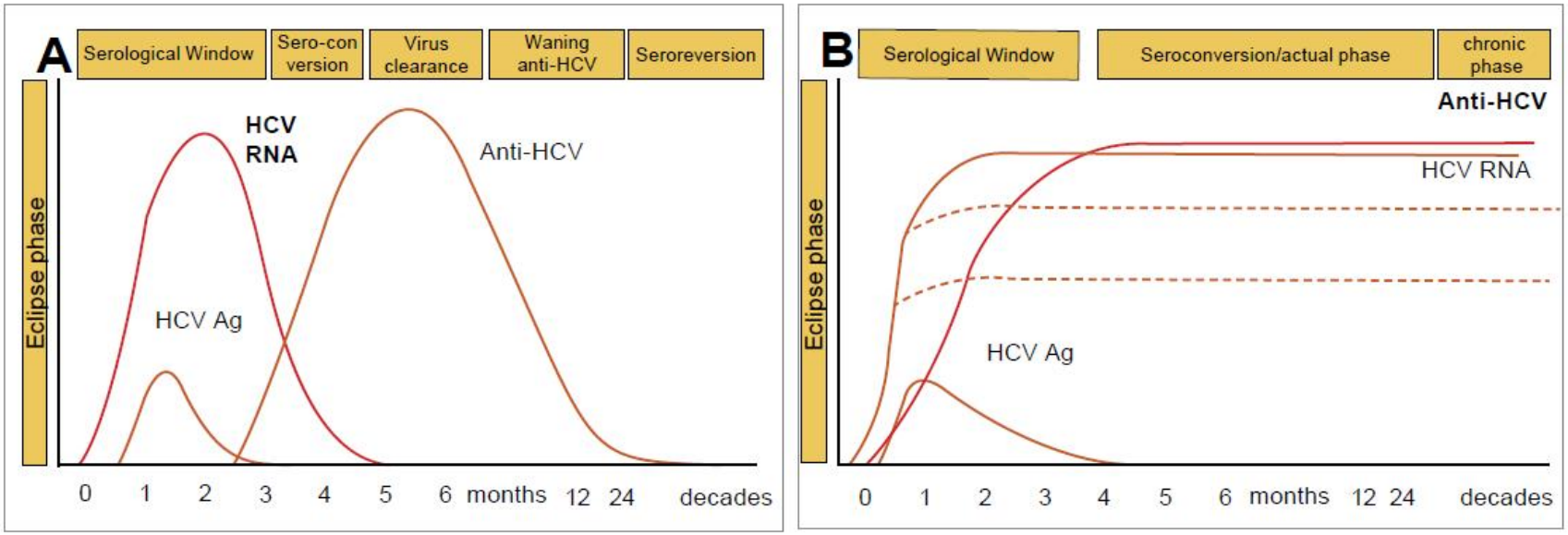
- **Passive immunoprophylaxis**
  - Hepatitis B immune globulin (HBIG) is efficacious in postexposure passive prophylaxis of HBV infection in clinical settings, such as accidental needle-stick contact, sexual contact, perinatal exposure, and recurrence of hepatitis B after liver transplantation.
  - HBIG provides protection for only 3 to 6 months. If prolonged or repeated exposure is expected, such as medical occupation, living in a household with a chronic HBV carrier, homosexual or promiscuous sexual behavior, or an ongoing requirement for blood transfusions, prophylaxis should include hepatitis B vaccine.
- **Active immunoprophylaxis**
  - Prevents chronic HBV infections which result in chronic liver disease later in life.
  - HBV vaccines have a protective efficacy of 90-95%.

Hepatitis C Infection

- Approximate time course of virological and immunological markers of HCV infection with –

  (A) Self-resolving HCV infection

  (B) Chronic HCV infection



Monitoring Schedule Framework for HCV Treatment

Time	Regimen: Only DAAs (non-cirrhotic usually)			Regimen: DAAs and Ribavirin (cirrhotic usually)		
	CBC, S.Creatinine, LFT	Adherence and side effects	HCV RNA	CBC, S.Creatinine , LFT	Adherence and side effects	HCV RNA
Baseline	Yes		Yes	Yes		Yes
Week 1				Yes	Yes	
Week 2				Yes	Yes	
Week 4	Yes	Yes		Yes	Yes	
Week 8				Yes	Yes	
Week 12				Yes	Yes	
Week 12 after completion of treatment (SVR-12)			Yes	Yes		Yes

Hepatitis D

- HDV uses HBsAg as its envelope protein; thus, HBV coinfection is necessary for packaging and release of HDV virions from infected hepatocytes.
- HDV is believed to infect approximately 5% of the world's HBsAg carriers (ie, about 15 million with chronic HBV/HDV). Fewer than 5% of these patients develop chronic HDV infection.
- Superinfection may give HBsAg-positive patients appearance of a sudden worsening or flare of hepatitis B and may also result in fulminant hepatic failure.
- 70-80% of these patients have evidence of chronic liver disease with cirrhosis, compared with only 15-30% of patients with chronic HBV alone.

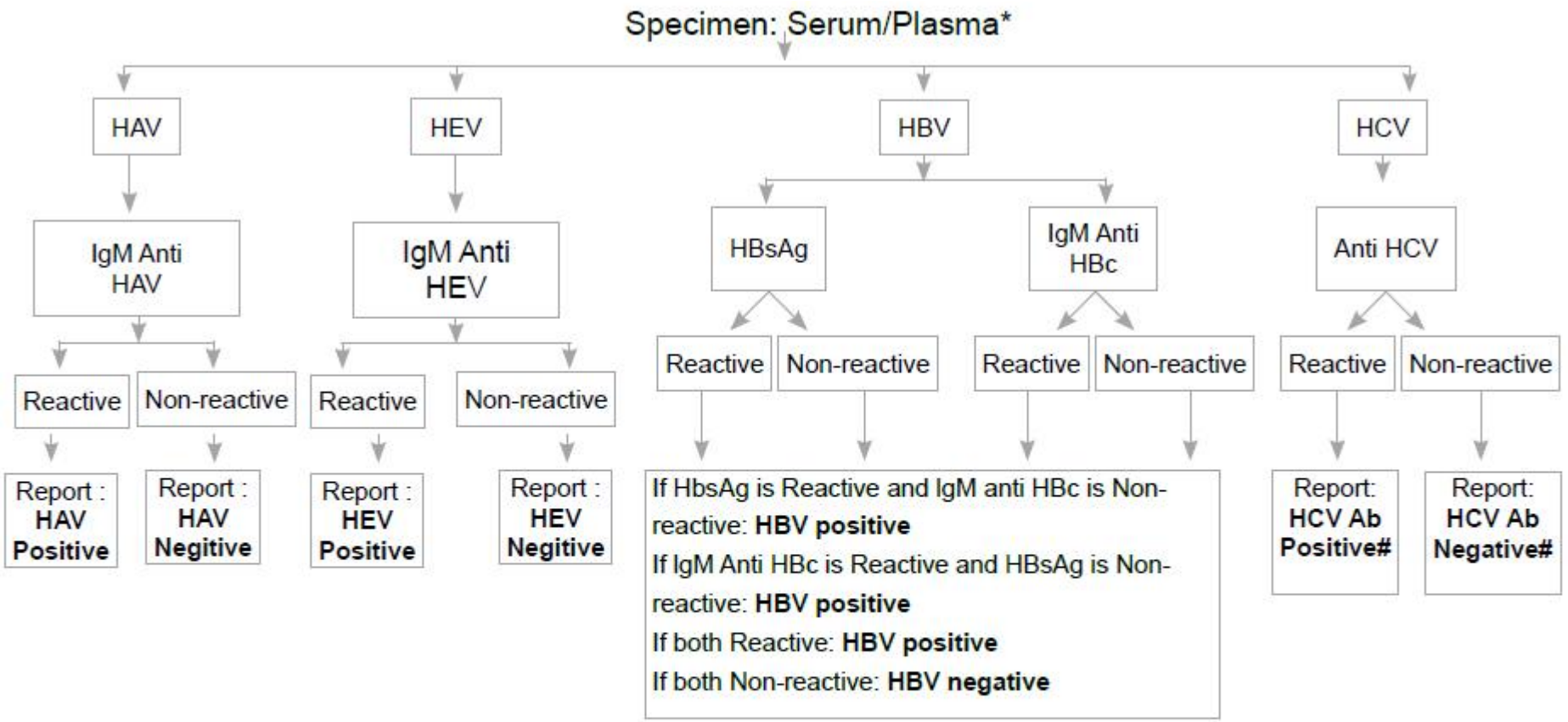
Laboratory Diagnosis of HDV Infection

- Serologic diagnosis of HDV infection is made by using IgM antibody to HDV (anti-HDV) and IgG anti-HDV tests.
- IgM antibody to hepatitis B core antigen (anti-HBc) should be used to help distinguish between coinfection (positive for IgM anti-HBc) and superinfection (negative for IgM anti-HBc).
- Detecting HDV RNA in serum is also possible.
- Assays to detect IgM antibody to HDV do not need to be routinely performed in all patients with suspected hepatitis.

Hepatitis E

- Definitive diagnosis of hepatitis E infection is based on detection of specific IgM antibodies to the virus in a person's blood.
- In acute hepatitis with clinical jaundice, the serum bilirubin levels are above 2.5mg/dL and serum ALT is more than 10 times the upper limit of normal.
- Serologic diagnosis of HEV infection is made by using IgM antibody to HEV (anti-HEV) and IgG anti-HEV.
- HEV RNA can be detected in serum and stool of infected patients.

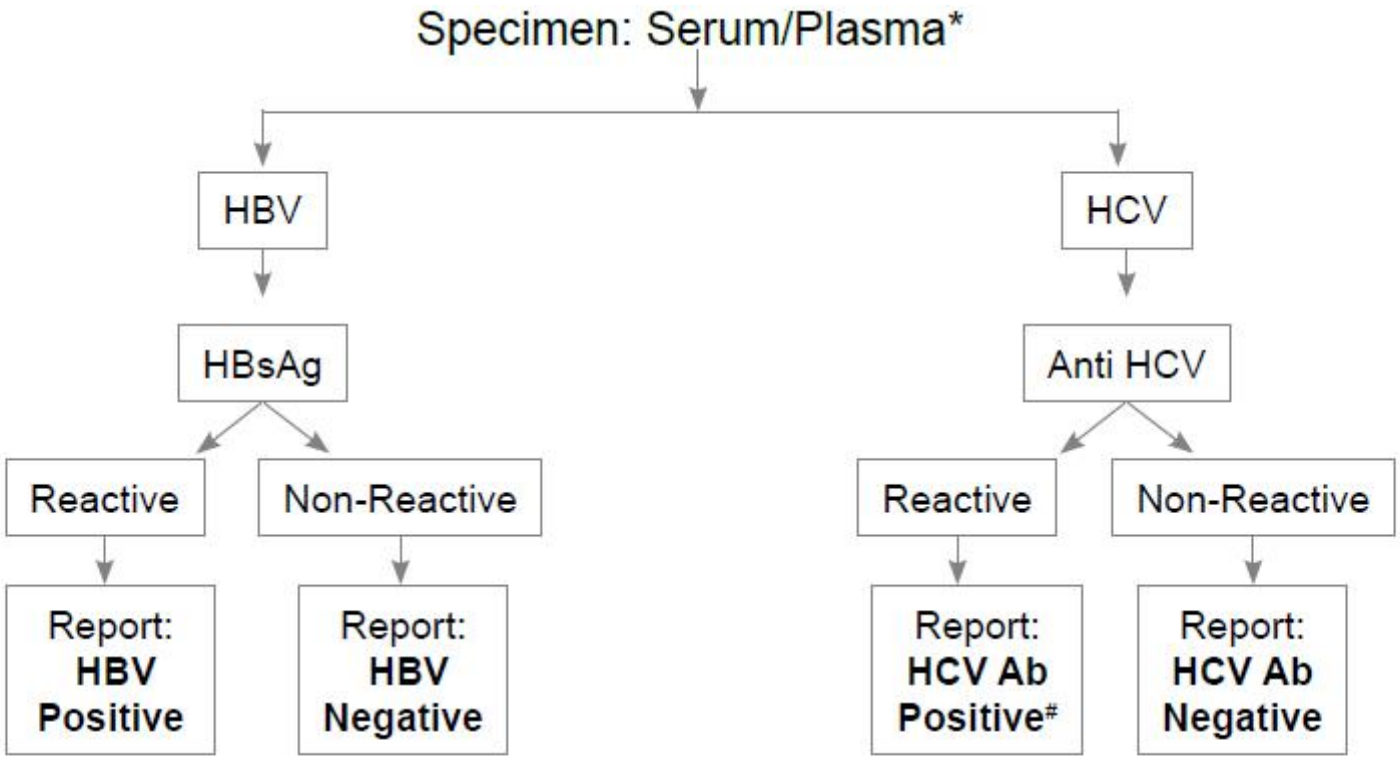
Algorithm for Diagnosis of Viral Hepatitis in Jaundiced Patients



** Serum samples to be used for serological and biochemical testing, to be aliquoted and stored at -20⁰ C for retesting for quality purposes, dispute etc.*

#All HCV antibody (Ab) positive to be referred to treatment centre. Plasma samples to be collected and aliquoted in 3 sterile cryo vials. One vial to be used for quantitative hepatitis C RNA estimation and two archived at -80⁰ C for quality assurance

Algorithm for Diagnosis of Viral Hepatitis in Suspected Patients (Without Jaundice)



** Serum samples to be used for serological and biochemical testing, to be aliquoted and stored at -200 C for retesting for quality purposes, dispute etc.*
#All HCV antibody (Ab) positive to be referred to treatment centre. Plasma samples to be collected and aliquoted in 3 sterile cryo vials. One vial to be used for quantitative hepatitis C RNA estimation and two archived at -800 C for quality assurance

HIV Coinfection with HBV and HCV

- Reduced diagnostic performance and sensitivity of HBsAg assays is seen in HIV-infected persons but EIAs perform well.
- Performance of anti-HCV RDTs is high, but there have been high rates of false positives and false negatives, particularly using earlier generation HCV serology assays.

Recommendations of WHO 2017 Testing Guidelines:

- Use of a single quality-assured serological assay (i.e. either a laboratory-based EIA or RDT to detect HBsAg and anti-HCV).
- Following reactive anti-HCV serology, a quantitative or qualitative RNA NAT is recommended as preferred testing strategy to diagnose viraemic infection.
- Detection of core HCV antigen, in which the assay has comparable clinical sensitivity with NAT technologies, may be considered as an alternative.
- Use of HBV DNA NAT following reactive HBsAg serology is recommended to help further guide who to treat or not treat if there is no evidence of cirrhosis and to monitor for treatment response based on existing recommendations from 2015 WHO HBV management guidelines.

Easterbrrok PJ. Curr Opin HIV AIDS 2017, 12:302–314

Thank You