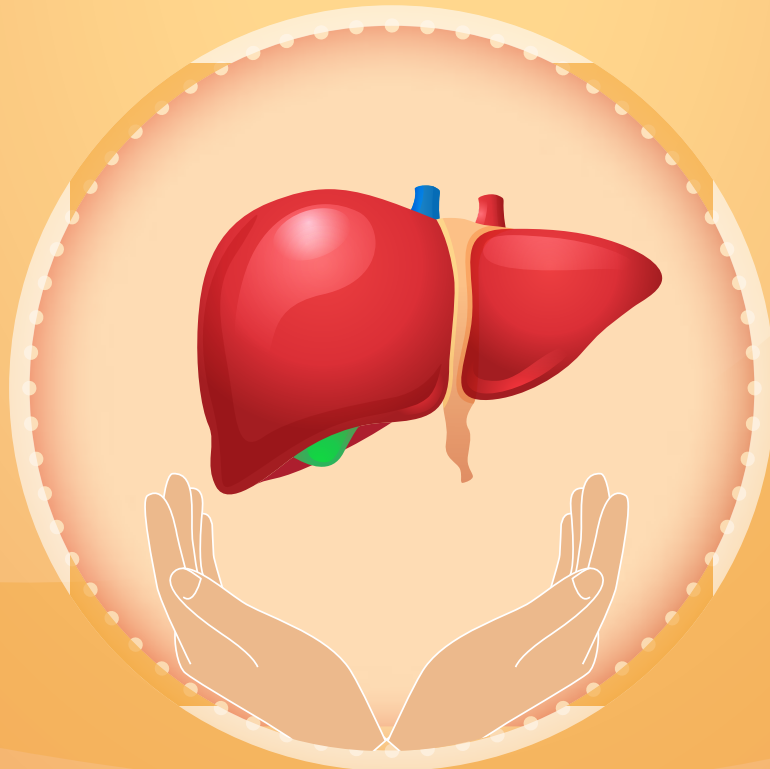




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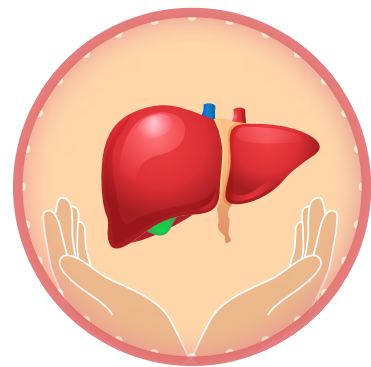


National Laboratory Guidelines for Testing of Viral Hepatitis



2018

*National Laboratory
Guidelines for*
Testing of Viral Hepatitis



डॉ. एस वेंकटेश
Dr S. VENKATESH

DNB, MD, DPH, MPH (Harv) FAMS, FIPHA
Director General of Health Services



सत्यमेव जयते

भारत सरकार
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
निर्माण भवन, नई दिल्ली - 110 108

GOVERNMENT OF INDIA
MINISTRY OF HEALTH & FAMILY WELFARE
NIRMAN BHAWAN, NEW DELHI- 110 108
TEL : 23061063, 23061438 (O), 23061924 (F)
E-mail : dghs@nic.in

दिनांक/Dated.....25.07.2018

Foreword

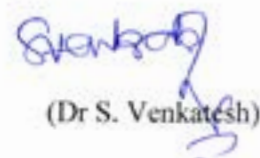
The burden of viral hepatitis constitutes a public health challenge in India. The multiple etiology and similar set of presentations pose significant challenges in diagnosis. Thus, laboratory testing is the key to differentiating the causative organism. This is important since the infections with hepatitis A and E often are acute while those with B and C have high probability to chronicity. With the possible cure for hepatitis C and a treatment for hepatitis B, it becomes even more important to correctly diagnose the etiology with the laboratory confirmation. Testing and diagnosis of viral hepatitis infection is the gateway for access to both prevention and treatment services. Quality assured lab services are also extremely important to classify the disease progression, monitor the response and the outcome of treatment.

The primary audience for this guideline is the laboratory workforce, programme-managers and health-care providers responsible for planning and implementing hepatitis testing, prevention and treatment services under this programme.

The *Guideline*, is intended to serve as a reference manual for laboratorians and practicing physicians and is intended to provide standardized testing protocols and algorithms to ensure consistency in diagnosing viral hepatitis (type specific) across the country in the most scientifically appropriate and cost-effective manner.

I am confident that this guideline will complement the operational guidelines and treatment guidelines for the programme and outline the public health approach to strengthen and expand current testing practices for viral hepatitis across the country.

I hope that this guideline will foster good clinical laboratory practices and contribute to enhanced quality of care and better patient outcomes.


(Dr S. Venkatesh)

Dr. Lalit Dar, M.D.

PROFESSOR
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
DEPARTMENT OF MICROBIOLOGY
Ansari Nagar, New Delhi 110 029, India.



Telegram : MEDINST
Tel: (O) +91-11-26594926
(Mob) +91-9818137078
Fax : +91-11-26588663
E-mail : lalidar@gmail.com



Preface

Viral hepatitis is a global problem of huge dimensions, which also leads to significant morbidity and mortality in India. Infection with the blood-borne hepatitis B and C viruses can result in chronic infection in a proportion of cases, from where it can progress to cirrhosis or even liver cancer. There is an effective preventive vaccine against hepatitis B, while effective antiviral drugs have become available against hepatitis C. Hepatitis A and E viruses are transmitted through the faeco-oral route and have the potential to cause large outbreaks. Infection by hepatitis E may be especially life-threatening in pregnant women.

A clinical suspicion of any of these types of viral hepatitis can only be confirmed by virus-specific laboratory tests. The development of these National Laboratory Guidelines for Viral Hepatitis Testing is an important step towards defining the approach and procedures for the performance of these tests.

A robust, multi-tiered network of testing laboratories is necessary for the success of any national-level programme that seeks to reduce the burden and alleviate the adverse impact of an infectious disease. These laboratories, which are really the driving engines of the programme, should be capable of providing quality-assured and timely reports for the accurate diagnosis of the infection, in order to initiate appropriate treatment if available, and to monitor the response of the patient to treatment.

Detection of the specific viral aetiology of hepatitis is become increasingly crucial, especially since the availability of a diverse group of effective direct-acting antivirals (DAAs) against the hepatitis C virus, which can elicit a sustained virological response (SVR).

The National Laboratory Guidelines for Viral Hepatitis Testing provide background information on the various hepatitis viruses, along with the details of their genomes and antigens, as well as the antibodies produced in response to them, which can all be targeted for detecting these viruses. They also include the principles of the laboratory tests used for this purpose. Most importantly, practical guidance and algorithms for viral hepatitis testing are also included. All stages of the process have been covered, starting from proper sample collection and transport (the pre-analytical stage), to the actual performance of the test and its quality control (the analytical stage), to the generation of reports and safe disposal of biomedical waste (the post-analytical stage). Quality assurance, one of the most important aspects for generating confidence in the results of the laboratories among patients and treating physicians, is also dealt with in adequate detail. Ethical issues like informed consent and ensuring the confidentiality of patient information have been given due importance.

Treating the testing laboratory as the living heart of this programme, these guidelines are intended to help support and sustain the elimination viral hepatitis as a public health threat by 2030 (reducing new infections by 90% and mortality by 65%), as envisaged by the WHO under its Global Health Sector Strategy (GHSS) on Viral Hepatitis 2016–2021. With adequate infrastructure, capacity building and training of manpower for the network of viral hepatitis testing laboratories in concordance with the requirements of these guidelines, India should be well on its way to achieve the target it has set for itself.

ABBREVIATIONS AND ACRONYMS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APRI	Aminotransferase/Platelet Ratio Index
AST	Aspartate Aminotransferase
CHB	Chronic Hepatitis B
CI	Confidence Interval
CLIA	Chemiluminescence Immunoassay
DAA	Direct-Acting Antiviral
DBS	Dried Blood Spot
ECL	Electrochemiluminescence Immunoassay
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EQAS	External Quality Assessment Scheme
HBeAg	Hepatitis B e Antigen
HBIG	Hepatitis B Immunoglobulin
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCVcAg	Hepatitis C Virus Core Antigen
HDV	Hepatitis D Virus
HIV	Human Immunodeficiency Virus
IVD	In Vitro Diagnostic (Medical Device)
LoD	Limit Of Detection
M&E	Monitoring And Evaluation
MSM	Men Who Have Sex With Men
MTCT	Mother-To-Child Transmission
NAT	Nucleic Acid Testing
PCR	Polymerase Chain Reaction
PEG-IFN	Pegylated Interferon
PHC	Primary Health Care
PMTCT	Prevention Of Mother-To-Child Transmission
PPV	Positive Predictive Value
PST	Plasma Separator Tube
PWID	People Who Inject Drugs
QA	Quality Assurance
QC	Quality Control
QI	Quality Improvement
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
SVR	Sustained Virological Response
WHO	World Health Organization

GLOSSARY OF TERMS

1. Hepatitis A total antibody: Measures combined IgG and IgM (Anti-HAV).
2. Hepatitis A IgM: Test diagnosis of acute hepatitis A (IgM Anti-HAV).
3. Hepatitis B surface antigen (HBsAg): HBV envelope protein often produced in excess and detectable in the blood in acute and chronic HBV infection.
4. Hepatitis B core antigen (HBcAg): HBV core protein. The core protein is coated with HBsAg and therefore not found free in serum. It is found freely in the hepatocytes.
5. Hepatitis B e antigen (HBeAg): Viral protein found in the high replicative phase of HBV. HBeAg is usually a marker of high levels of replication with wild-type virus but is not essential for viral replication.
6. Hepatitis B surface antibody (anti-HBs): Antibody to HBsAg. Develops in response to hepatitis B vaccination and during recovery from hepatitis B, denoting past infection and immunity.
7. Hepatitis B core antibody (anti-HBc): Antibody to HBV core (capsid) protein. Anti-HBc antibodies are non-neutralizing antibodies and are detected in both acute and chronic infection.
8. Anti-HBc IgM. Subclass of anti-HBc: Detected in acute/recent HBV infection but can be detected by sensitive assays in chronic HBV infection.
9. HBV e antibody (anti-HBe): Antibody to HBeAg. Detected in persons with lower levels of HBV replication but also in HBeAg-negative disease (i.e. HBV that does not express HBeAg).
10. HBV DNA: HBV viral genomes that can be detected and quantified in plasma by nucleic acid testing (NAT).
11. Anti-HCV antibody: Antibody to HCV, which can be detected in the blood usually within two or three months of HCV infection or exposure. The terms HCV antibody and anti-HCV antibody are equivalent, but in these guidelines, HCV antibody is used throughout.
12. Hepatitis E IgM: IgM antibody to hepatitis E virus (anti HEV IgM).
13. HCV RNA: HCV viral RNA that can be detected and quantified in serum by nucleic acid testing (NAT).
14. HCV core antigen (HCVcAg): Nucleocapsid peptide 22 [p22] of HCV, which is released into plasma during viral assembly and can be detected from early on and throughout the course of infection.
15. Chronic HBV infection: Persistence of HBsAg for at least six months. The persistence of HBsAg in two specimens six months apart is frequently used in clinical practice to confirm chronic hepatitis B infection.
16. Chronic HCV infection: The presence of viraemic HCV RNA or HCVcAg in association with positive serology for HCV antibody.
17. Viraemic infection: Hepatitis B or C infection associated with presence of virus in the blood (as measured by HBV DNA or HCV RNA), and often referred to as active, ongoing or current infection.
18. Occult HBV infection: HBsAg negative but HBV DNA positive, although at very low levels (invariably <200 IU/mL). Most are also anti-HBc positive.

19. Cirrhosis: An advanced stage of liver disease characterized by extensive hepatic fibrosis, nodularity of the liver, alteration of liver architecture and disrupted hepatic circulation.
20. Decompensated cirrhosis: Clinical features are portal hypertension (ascites, variceal haemorrhage and hepatic encephalopathy), coagulopathy, or liver insufficiency (jaundice). Other clinical features of advanced liver disease/cirrhosis may include – hepatomegaly, splenomegaly, pruritus, fatigue, arthralgia, palmar erythema and oedema.
21. Hepatocellular carcinoma (HCC): Primary cancer of the liver arising from the hepatocytes and may be a complication of chronic hepatitis B or C infection.
22. Hepatitis C qualitative PCR: It is a very sensitive assay and will detect the presence of hepatitis C RNA and indicates ongoing viral replication.
23. Hepatitis C quantitative PCR: It is used to measure the viral RNA titres (viral load) and for monitoring response to antiviral therapy.
24. HCV sustained virological response (SVR): Undetectable HCV RNA in the blood at defined time point after the end of treatment, usually at 12 or 24 weeks (SVR12 or 24).
25. HCV non-response: Detectable HCV RNA and no significant drop in HCV RNA titres in the blood throughout treatment.
26. Serological assays: Assays that detect the presence of either antigens or antibodies, typically in serum or plasma but also in capillary/venous whole blood and oral fluid. These include rapid diagnostic tests (RDTs), and laboratory-based immunoassays, e.g. enzyme immunoassays (EIAs), chemiluminescence immunoassays (CLIAs), and electrochemiluminescence immunoassays (ECLs).
27. Rapid diagnostic test (RDT): Immunoassays that detect antibodies or antigens and can give a result in less than 30 minutes. Most RDTs can be performed with capillary whole blood collected by finger-stick sampling.
28. Enzyme immunoassay (EIA): Laboratory-based serological immunoassays that detect antibodies, antigens, or a combination of both.
29. Nucleic acid testing (NAT): A molecular technology, for example, polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) that can detect very small quantities of viral nucleic acid (RNA or DNA), either qualitatively or quantitatively.
30. Multiplex or multi-disease testing: Refers to testing using one specimen in the same test device (or reagent cartridge) that can detect other infections (e.g. HIV, syphilis, hepatitis C, hepatitis B).
31. Testing algorithm: The combination and sequence of specific assays used within hepatitis B and C testing strategies.
32. Key populations: Groups of people who, due to specific high-risk behaviours, are at increased risk for HIV infection irrespective of the epidemic type or local context. This may also apply to HBV and/or HCV infection. Key populations often have legal and social issues related to their behaviors that increase their vulnerability to HIV, HBV and HCV infection. These guidelines refer to the following groups as key populations: men who have sex with men (MSM); people who inject drugs (PWID).

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C H A P T E R

1

Introduction to viral hepatitis

The laboratory plays a crucial role in the diagnosis and management of patients towards combating viral hepatitis. The guideline has been developed to provide a framework for implementing priority activities for strengthening laboratory capacity mandated in the national initiative. The purpose of the national guideline is to ensure the delivery of effective, efficient, accessible, equitable and quality laboratory services towards management of viral hepatitis.

Viral hepatitis

Viral hepatitis is primary inflammation of the liver due to infiltration of hepatocytes with viral infected cells leading to parenchymal necrosis in portal and peri-portal areas. Almost all cases of viral hepatitis are caused by one of five viral agents: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the HBV-associated delta agent or hepatitis D virus (HDV) and hepatitis E virus (HEV). HAV and HEV are transmitted enterically by the fecal-oral route while the others are transmitted usually by per-mucosal or per-cutaneous route. The salient features of viral hepatitis are described in the following table 1:

Table 1: Salient features of viral hepatitis.

Virus	A*	B	C	D	E
Nucleic acid	RNA	DNA	RNA	RNA	RNA
Size	27-32 nm	27-42 nm	50-80nm	35-37 nm	32–34 nm
Family	Picornaviridae	Hepadnaviridae	Flaviviridae	Unclassified	Hepeviridae
Genus	Hepatovirus	Orthohepadna virus	Hepacivirus	Deltavirus	Hepevirus
Genome	Positive sense +ssRNA	Partial dsDNA	Positive sense +ssRNA	Negative sense –ssRNA	Positive sense +ssRNA
Spread					
Faecal-oral	Yes	No	No	No	Yes
Blood/blood products	Rare	Yes	Yes	Yes	No
Vertical (Mother to child)	No	Yes	Rare	Occasional	No
Saliva	Yes	Yes	Yes	? No	?
Sexual	Rare	Yes	Yes (rare)	Rare	No
Incubation period	Short (15-45 days)	Long (30-180 days)	Long (15-160 days)	Long (30-180 days)	Short (14-60 days)
Age	Young	Any	Any	Any	Any
Carrier state	No	Yes	Yes	?	No ^a
Severity/Chronicity	Mild; acute	Occasionally severe; 5–10% chronic	Subclinical; 70% chronic	Exacerbates symptoms of HBV; chronic w/ HBV	Normal patients, mild; pregnant women, severe; acutea
Liver cancer	No	Yes	Yes	Yes	No
Immunization					
Passive	Available	Available:	No	No	No
Active (licensed) Vaccine in India	Available.	Available	None available	None available	None available

*Single serotype.

^a Chronic hepatitis in immunosuppressed patients.

Viruses that are infrequently associated with hepatitis are Epstein Barr virus, herpes simplex virus, cytomegalovirus, measles virus, yellow fever virus etc.

Two different patterns of viral hepatitis are recognized: acute viral hepatitis with rapid onset of infection and, usually, rapid resolution; and chronic viral hepatitis, which is asymptomatic and often detected on routine blood tests or during screening for infection. The enterically transmitted forms of viral hepatitis are self-limited and do not cause chronic hepatitis (rarely, acute hepatitis A serves as trigger for the onset of autoimmune hepatitis in genetically susceptible patients and hepatitis E can cause chronic liver disease in immunosuppressed hosts). HBV, HCV and HDV may cause acute or chronic disease.

Hepatitis A

HAV presents with non-specific constitutional symptoms of low grade fever, anorexia, nausea and vomiting, fatigue, malaise, arthralgia, myalgia, headache, photophobia, pharyngitis, cough and coryza may precede onset of jaundice by 1-2 weeks.

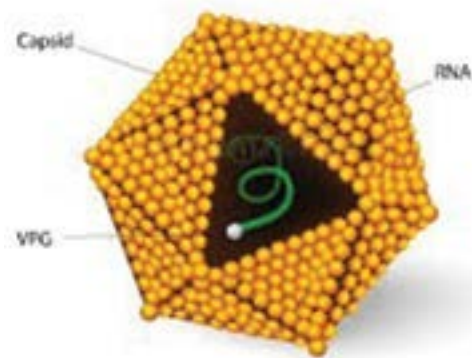


Fig. 1: Structure of hepatitis A virus.

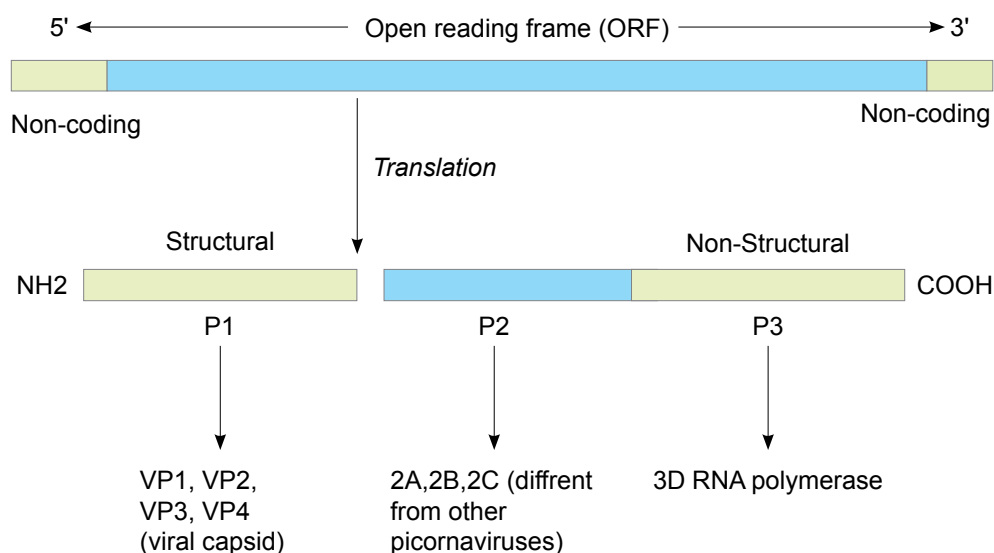


Fig. 2: Genome of hepatitis A virus

Source: Kumar and Clark's Medicine.

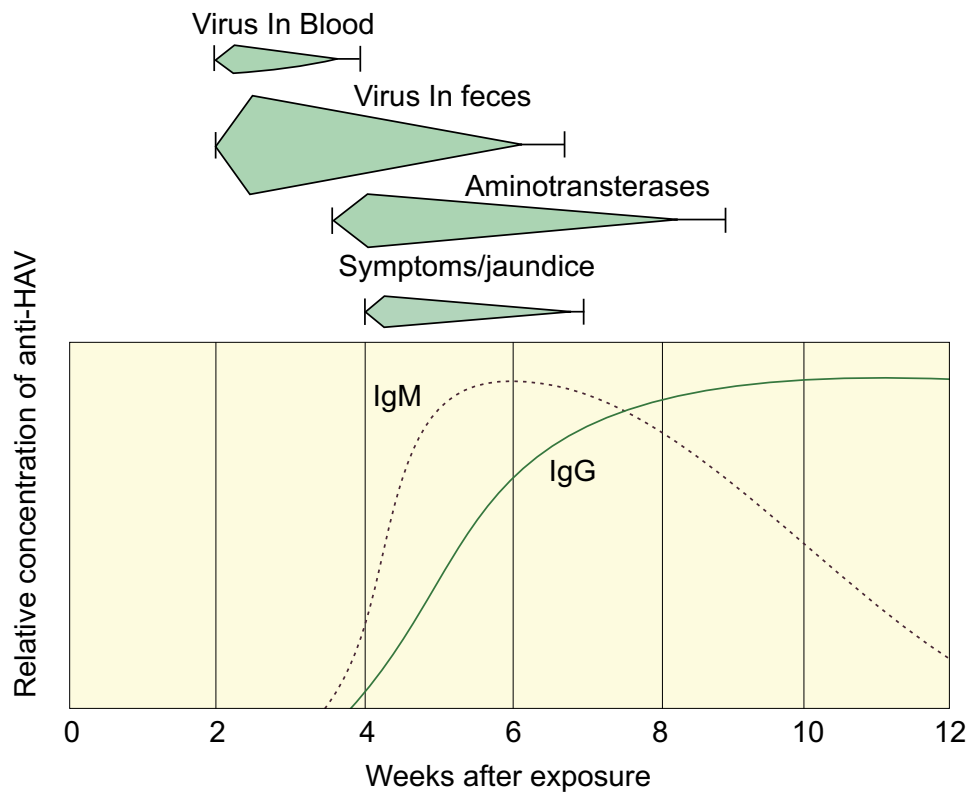


Fig. 3: Summary of immunological and biological events

Source: Karen C. Carroll, Stephen A. Morse, Timothy Meitzner, Steve Miller: *Jawetz, Melnick, and Adelberg's Medical Microbiology, 27th Edition, Mc-Graw Hill Education.*

The early antibody response is predominantly of the IgM class and persists for several (about 3 months) rarely for 6-12 months, followed by IgG. Detection of IgM anti-HAV antibody indicates acute/recent infection whereas the presence of HAV total antibody in the absence of HAV IgM indicates previous infection or immunity. In an infant less than 18 months of age, a positive antibody test result may indicate passive transfer of maternal antibody.

An HAV total antibody test detects both IgG and IgM; when used in combination with the HAV IgM antibody test, it is an effective way to determine current or previous infection and test for immunity before vaccination.

Hepatitis B

The disease can manifest both in acute and chronic forms and varies from asymptomatic to symptomatic progressive disease.

Structure

The small (3.2 kb), partially double-stranded, relaxed circular (rc) DNA features four open reading frames encoding seven proteins: HBeAg (HBV e antigen, secreted dimeric protein), HBcAg (HBV core antigen, viral capsid protein), HBV Pol/RT (polymerase, reverse transcriptase activity), PreS1/PreS2/HBsAg (large, medium, and small surface envelope glycoproteins), and HBx (HBV x antigen, regulator of transcription required for the initiation of infection).

Envelope HBsAg sub-determinants include a common group reactive antigen- 'a' shared by all HBsAg isolates and one of several subtype-specific antigens- d or yw or r -as well as other specificities. Hence the surface protein has four subtypes: adw, adr, ayw and ayr. Hepatitis B isolates fall into one of at least eight subtypes and 10 genotypes (A-J). Geographic distribution of genotypes and subtypes varies.

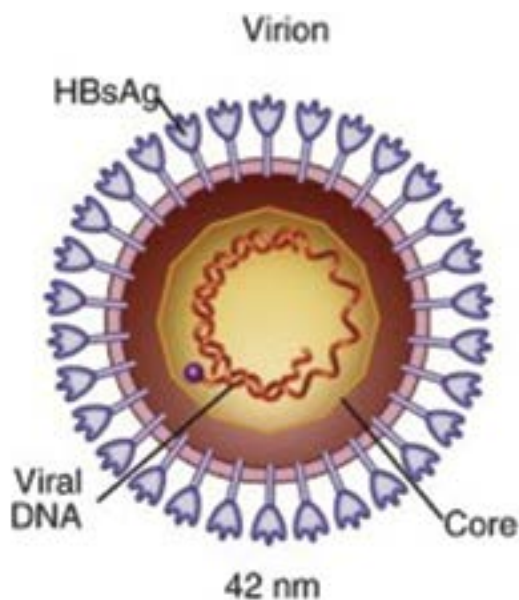


Fig. 4: Structure of hepatitis B virus

Source: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Updated Edition. Thio, Chloe Lynne; Hawkins, Claudia. Published January 1, 2015. Pages 1815-1839.e9. 2015.

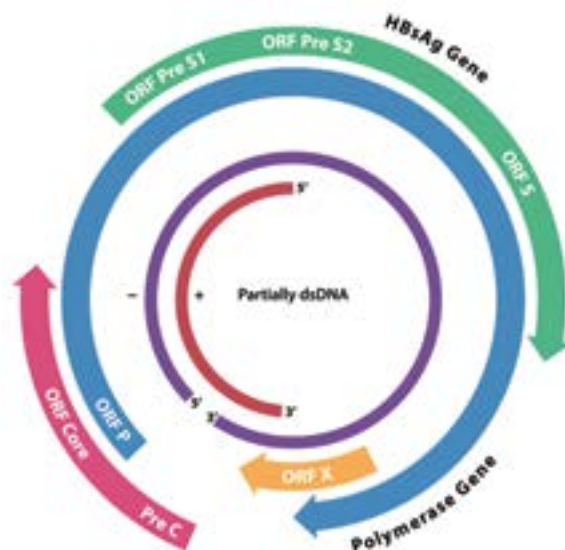


Fig. 5: Genome of hepatitis B virus

The viral DNA is partially double-stranded (red incomplete circle and purple circle). The long strand encodes seven proteins from four overlapping reading frames [S, surface (Pre-S1, Pre-S2, S)-green; c, core (Pre-C, C)-pink; P, polymerase (P)-blue; and X gene (X)]-yellow.

HBV is not usually directly cytopathic (although high replication levels in immunosuppressed individuals can lead to direct toxicity) and liver damage is produced by the host's immune response.

The following figure 6 indicates the clinical progression along with associated serological events in acute HBV infection.

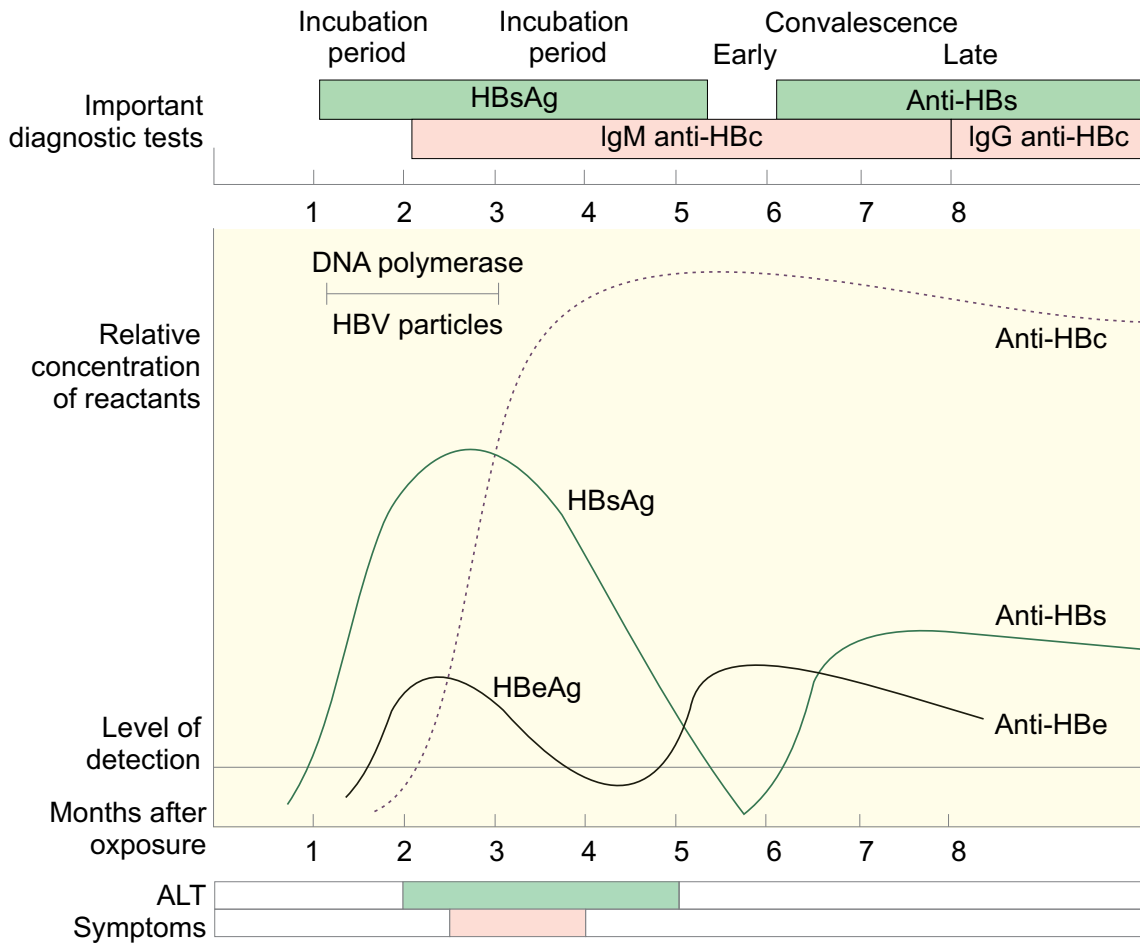


Fig. 6: Clinical progression along with associated serological events in acute HBV infection

Source: Karen C. Carroll, Stephen A. Morse, Timothy Meitzner, Steve Miller: Jawetz, Melnick, and Adelberg's Medical Microbiology, 27th Edition, Mc-Graw Hill Education.

Chronic HBV infection is a dynamic process reflecting the interaction between HBV replication, hepatocytes and the host's immune response and not all patients with chronic HBV infection have chronic hepatitis (CHB). The natural history of chronic HBV infection has been schematically divided into four phases taking into account the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) values and eventually the presence or absence of liver inflammation. The risk of progression to cirrhosis and HCC is variable and is affected by the host's immune response.

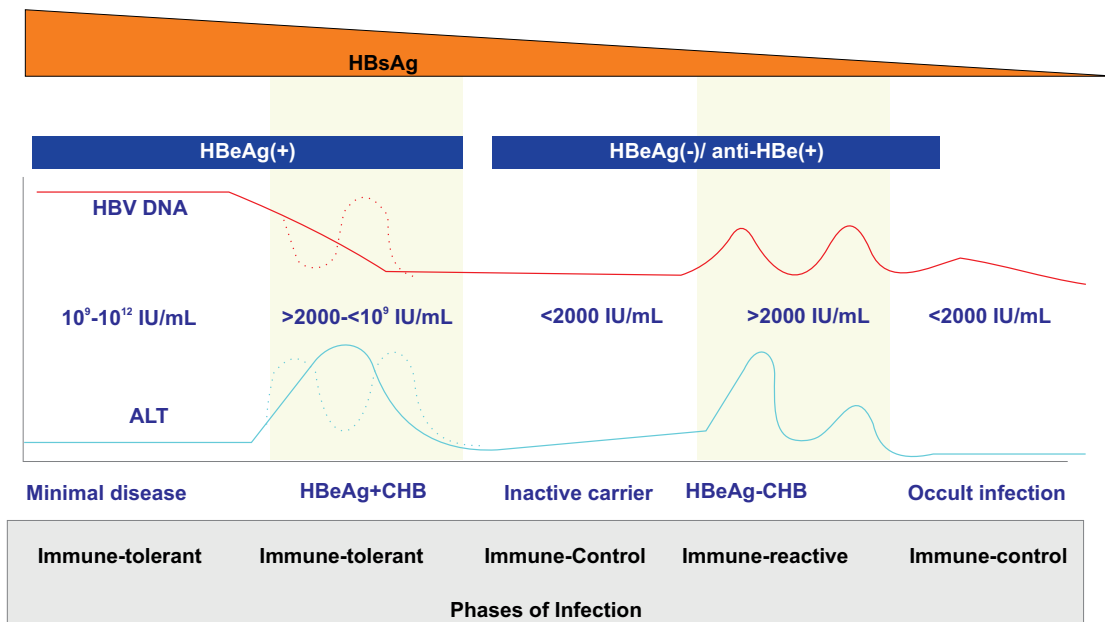


Fig. 7: Serological markers in different phases of chronic hepatitis B virus infection

Source: Santantonio T, Fasano M, Current concepts on management of chronic hepatitis B. <http://dx.doi.org/10.5772/54759>.

Interpretation of HBV markers

Table 2: Commonly encountered serology patterns of hepatitis B infection.

HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Interpretation
+	-	IgM	+	-	Acute hepatitis B , high infectivity *
+	-	IgG	+	-	Chronic hepatitis B , high infectivity *
+	-	IgG	-	+	1. Late acute or chronic hepatitis B , low infectivity 2. HBe Ag negative ("precore- mutant") hepatitis B (chronic or rarely acute)
+	+	+	IgM	+/-	1. HBsAg of one subtype and heterotypic anti-HBs (common) 2. Process of seroconversion from HBsAg to anti-HBs (rare)
-	-	IgM	+/-	+/-	1. Acute Hepatitis B* 2. Anti-HBc "window"
-	-	IgG	-	+/-	1. Low level Hepatitis B carrier 2. Hepatitis B in remote past
-	+	IgG	-	+/-	Recovery from Hepatitis B
-	+	-	-	-	1. Immunization with HBsAg(after vaccination) 2. Hepatitis B in the remote past 3. False Positive

*IgM Anti-HBc may reappear during acute reactivation of chronic Hepatitis B

Source: Harrison's Principles of Internal Medicine, 20th edition

HBV molecular variants

Hepatitis B viral mutants can emerge as a result of selection pressure from either immune response (vaccination) or treatment options (anti- viral therapy).

The commonly seen variants are:

1. Escape mutants
2. Pre-core mutant
3. YMDD (tyrosine-methionine-aspartate-aspartate) mutant.

Thus HBV has significant genomic diversity and are associated with antiviral therapy response, vaccine escape, diagnostic failure, liver fibrosis progression and HCC development.

Hepatitis C

HCV infection usually resolves spontaneously in 15 to 45% patients and progresses to chronic infection in 55 to 85% patients.

Structure

It is a single-stranded, positive sense RNA virus of the Flaviviridae family. The genome is approximately 10 kb in length, encoding a poly-protein product consisting of structural (capsid and envelope) and non-structural viral proteins.

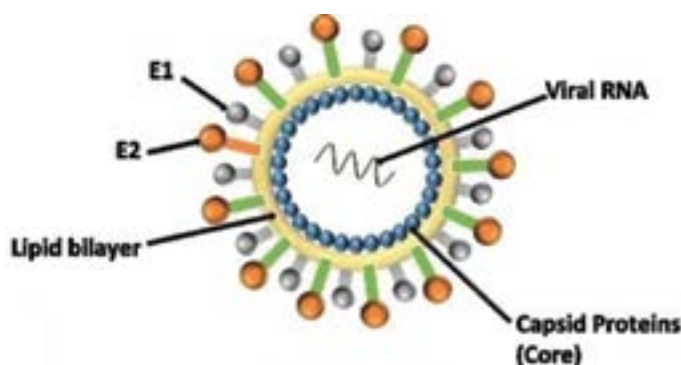


Fig. 8: Structure of hepatitis C virus

Source: *Virology Journal*; December 2017, 14:88 | Cite as; *Hepatitis C virus management: potential impact of nanotechnology.*

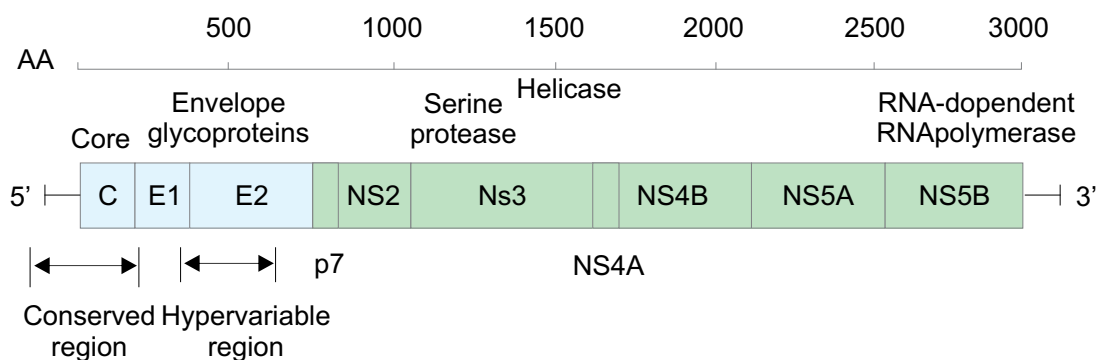


Fig. 9: Organization of the HCV genome and its associated amino acid proteins

Source: *Harrison's Principles of Internal Medicine, 20th edition.*

The HCV genome consists of seven functional regions- the core, the envelope, including the E1 and E2 regions, and the nonstructural region, including NS2, NS3, NS4, and NS5.

Comparisons of sub-genomic regions, such as E1, NS4 or NS5, have allowed variants to be classified into major genotypes with many subtypes. The term genotype refers to different genetic variations or strains of hepatitis C (HCV). The variance in genetic differences is approximately one third between the different genotypes. There are six distinct major genotypes and a minor genotype 7 and more than 50 subtypes within the genotypes of HCV have been identified. Within each genotype are further divisions called subtypes (for example 1a and 1b) and intra-genotypic variations are referred to as quasi-species and differ in sequence homology by only a few percent.

As the HCV virus replicates rapidly, it constantly changes and mutates. The process of constant mutation helps the virus escape the body's immune response, and may lead to the development of chronic HCV disease.

Infections are usually asymptomatic, about 10% of patients having a mild illness with constitutional features, with jaundice and a rise in serum aminotransferases. Most patients will not be diagnosed until they present, years later, with evidence of the disease only being discovered following a routine biochemical test when mild elevations in the aminotransferases (usually ALT) are noticed (50%). The elevation in ALT may be minimal and fluctuating and some patients have a persistently normal ALT (25%), the disease being detected by checking HCV antibodies (e.g. in blood donors).

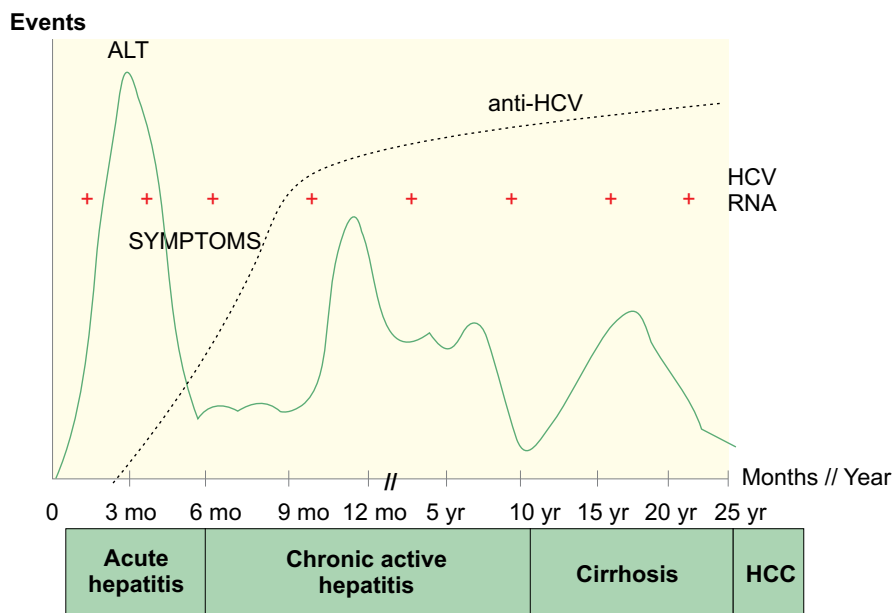


Fig. 10: Clinical and serology events associated with HCV infection

Source: Karen C. Carroll, Stephen A. Morse, Timothy Meitzner, Steve Miller: Jawetz, Melnick, and Adelberg's Medical Microbiology, 27th Edition, Mc-Graw Hill Education.

The diagnosis of HCV infection is made by detection of anti-HCV antibodies using immunoassays followed by detection of HCV RNA in serum or plasma. Current HCV RNA assays express HCV RNA titres in international units per mL (IU/mL).

The assessment of the disease progression and its management takes into consideration platelet count, serum aminotransferases and other non-invasive tests.

A reactive HCV antibody result indicates one of the following:

1. Current HCV infection
2. Past HCV infection that has resolved
3. False positive.

A reactive HCV antibody result should be followed by testing for HCV RNA using NAT (RT-PCR) for confirmation of hepatitis C infection.

If HCV RNA is detected, results indicate current/active HCV infection. If HCV RNA is not detected, results indicate either past, resolved infection or false HCV antibody positivity. Regardless of serology for HCV antibody, patients with detectable HCV RNA should be considered to have active HCV infection and should be referred for further medical evaluation.

HCV RNA can be detected within a few days of exposure. ALT elevation is usually seen after HCV RNA is detectable in blood. Sero-conversion usually occurs after 8-9 weeks of infection and about 90% of patients are

positive for anti-HCV antibodies within 5 months. Anti-HCV antibody test results may be negative in early acute hepatitis C and in profoundly immunosuppressed patients (HCV RNA testing in serum or plasma should be part of the initial evaluation).

Counseling messages for screening test results: All patients should be provided information in language understood by them on the meaning of their test results by the attending clinicians/trained health care workers/peer counselors.

Providing Pre- test information:

Information to be provided through material such as posters, brochures, websites and short video clips shown in waiting rooms. This would include information on viral hepatitis and the benefits of testing for hepatitis B or C; the meaning of a positive and negative test result; a brief description of prevention options; confidentiality of the test result; the practical implications of a positive test result, including the when and where of treatment available.

Post- test counselling and linkages to treatment services for a reactive hepatitis C screening test:

- Explain the meaning of the reactive antibody test and counsel on the need for diagnostic testing (hepatitis C RNA test) to confirm a diagnosis of chronic hepatitis and other tests for staging of liver disease.
- Explain that the patient may be chronically infected or have cleared the virus in the past.
- Provide basic hepatitis C disease, prevention and treatment information. Make an active referral to the viral hepatitis treatment units for confirmation of diagnosis.
- Discuss the importance of minimizing risk behaviors to avoid transmitting hepatitis C infection to others, and encourage notification and screening of needle sharing and sexual partners.
- Encourage and offer HBV and HCV testing for family members, including children, and sexual partners after confirmation.
- Discuss healthy life practices, including stopping or reducing alcohol intake and getting vaccinated against hepatitis A and B, if appropriate.

Adherence counselling by trained pharmacist:

1. Inform patients about common side effects;
2. Pill count- the total number of pills/doses dispensed and the total number of pills/doses returned at monthly visits for each drug for the entire treatment duration for all patients;
3. Patient self-reports, it helps to determine reasons for non-adherence.

Post-test information/counselling for a non-reactive hepatitis C screening test:

- Explain the meaning of the non-reactive antibody test, ensuring that the patient understands a negative antibody test does not protect him/her from future infection in the event of risk taking behaviours.
- Discuss that if the patient was recently exposed (6 months), he/she may be in a window period and recommend repeat screening in 6 months, and provide information on hepatitis C prevention, risk and harm reduction.
- Encourage the patient to make healthy choices and to get vaccinated against hepatitis A and B, if appropriate.

It is important to counsel the patient at every human interface in the initiative. Laboratory workforce, if required, need to impart pre-test information and post-test counselling and therefore need to be trained on the same. Moreover, special trainings need to be conducted for sensitization on confidentiality and respecting the status of a positive patient.

The following table 3 summarizes the interpretation of HCV markers with further actions to be taken:

Table 3: Interpretation of HCV markers

TEST OUTCOME	INTERPRETATION	FURTHER ACTIONS
HCV antibody (Anti-HCV) Non-reactive	No HCV antibody detected	Sample can be reported as non-reactive for HCV antibody. No further action required. If recent exposure in person tested is suspected, NAT test for HCV RNA.*
HCV antibody (Anti-HCV) Reactive	Presumptive HCV infection	A repeatedly reactive result is consistent with current HCV infection, or past HCV infection that has resolved, or biologic false positivity for HCV antibody. NAT Test for HCV RNA to identify current infection.
HCV antibody reactive, HCV RNA detected	Current HCV infection	Provide person tested with appropriate counseling and link person tested to medical care and treatment.
HCV antibody reactive, HCV RNA not detected	No current HCV infection	No further action required in most cases. If distinction between true positivity and biologic false positivity for HCV antibody is desired, and if sample is repeatedly reactive in the initial test, test with another HCV antibody assay. In certain situations [§] , follow up with HCV RNA testing and appropriate counseling.

* If the person tested is immune-compromised, consider testing for HCV RNA.

§ If the person tested is suspected of having HCV exposure within the past 6 months, or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Source: CDC MMWR - Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians (May 2013).

Direct acting antiviral agents (DAAs)

Treatment for HCV infection is undergoing a revolution, with treatments changing from interferon-based regimes to all-oral combination regimes using directly acting antiviral agents. DAAs are molecules that target specific non-structural proteins of the virus and results in disruption of viral replication and infection. There are four classes of DAAs which are defined by their mechanism of action and therapeutic target. The four classes are non-structural proteins 3/4A (NS3/4A) protease inhibitors (PIs), NS5B nucleoside polymerase inhibitors (NPIs), NS5B non-nucleoside polymerase inhibitors (NNPIs) and NS5A inhibitors.

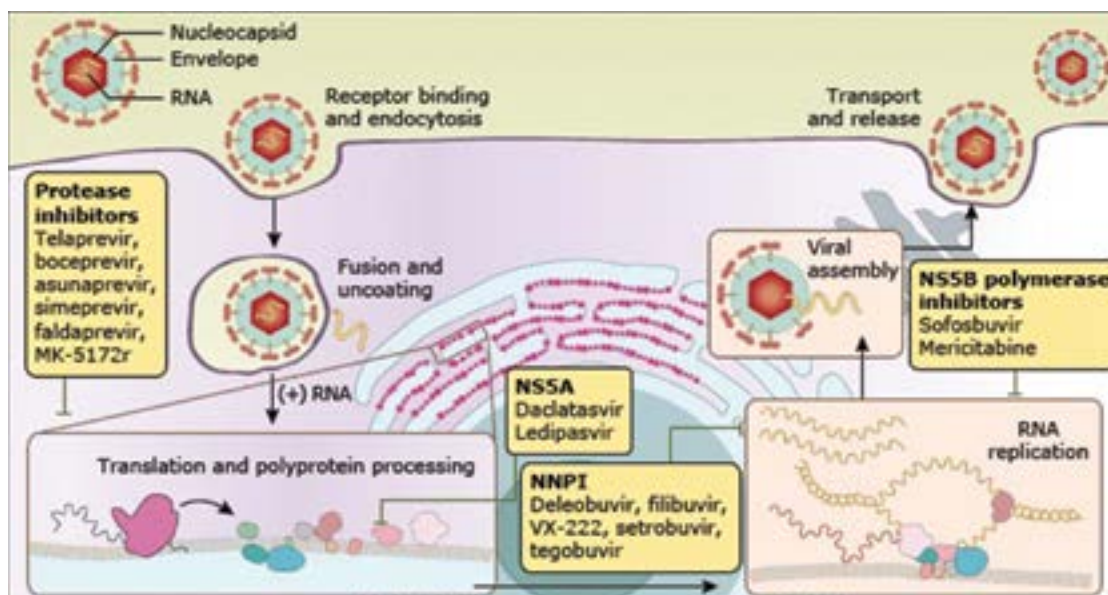


Fig. 11: Mechanism of action of direct acting antivirals for hepatitis C virus.

NS5A: non-structural protein 5A; NS5B: non-structural protein 5B; NNPI: non-nucleoside polymerase inhibitor.
Source: Clinical Pharmacology & Therapeutics. Au J, Pockros PJ. Novel Therapeutic Approaches for Hepatitis C. Clin Pharmacol Ther 2014; 95:78. Macmillan Publishers Ltd. www.nature.com/clpt.

Hepatitis D

Hepatitis D virus (HDV or delta virus) is a satellite virus, which is dependent on HBV for the production of envelope proteins. It is an incomplete RNA particle enclosed in a shell of HBsAg. The virus is unable to replicate on its own but is activated by the presence of HBV. Natural HDV infections occur as either a co-infection with HBV or a super-infection of HBV carriers. HDV can cause severe acute and chronic liver diseases in HBV-infected individuals; most of the HBV carriers super-infected with HDV become carriers of both HBV and HDV.

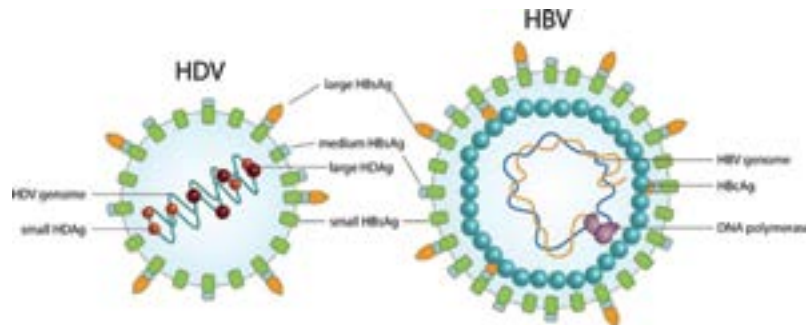


Fig. 12: Structure of hepatitis D virus.

- Co-infection of HDV and HBV is clinically indistinguishable from an acute icteric HBV infection, but a biphasic rise of serum aminotransferases may be seen. Diagnosis is confirmed by finding serum IgM anti-HDV in the presence of IgM anti-HBc. IgM anti-delta appears at 1 week and disappears by 5–6 weeks (occasionally 12 weeks), when serum IgG anti-delta is seen. The HDV RNA is an early marker of infection. The infection may be transient but the clinical course is variable.
- Super-infection results in an acute flare-up of previously quiescent chronic HBV infection. A rise in serum AST or ALT may be the only indication of infection. Diagnosis is made by finding HDV RNA or serum IgM anti-HDV at the same time as IgG anti-HBc. Active HBV DNA synthesis is reduced by delta super-infection and patients are usually negative for HBeAg with low HBV DNA.

Acute hepatic failure can follow both types of infection but is more common after co-infection. HDV RNA in the serum and liver can be measured and is found in acute and chronic HDV infection.

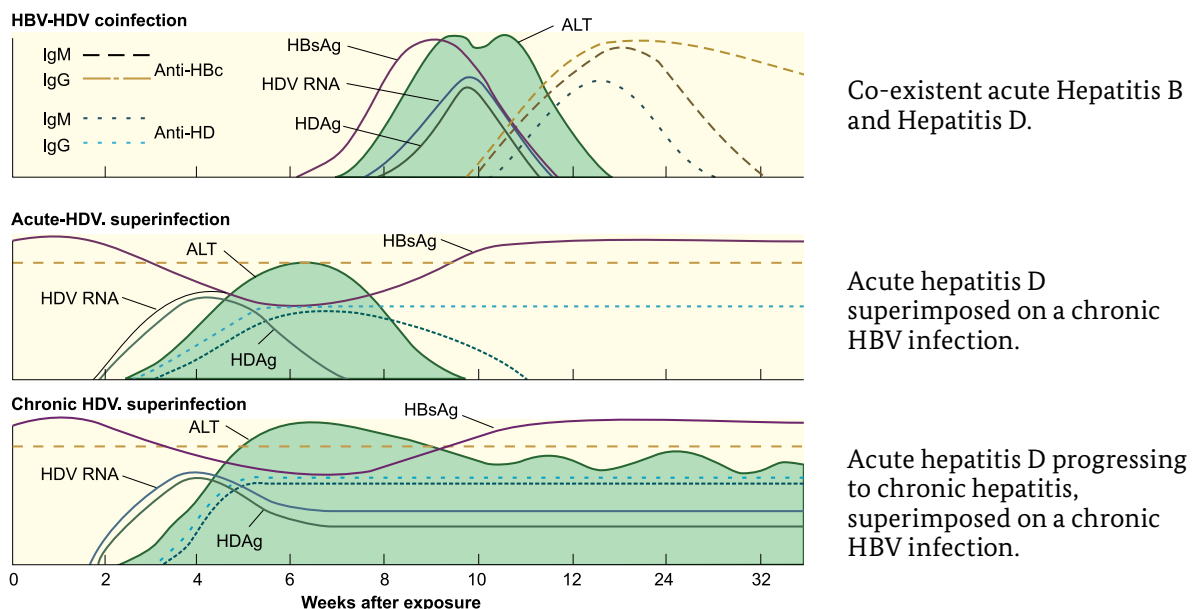


Fig. 13: Serologic patterns of type D hepatitis after co-infection or super-infection of a person with HBV infection.

Source: Karen C. Carroll, Stephen A. Morse, Timothy Meitzner, Steve Miller: *Jawetz, Melnick, and Adelberg's Medical Microbiology, 27th Edition, Mc-Graw Hill Education.*

When HDV infection is suspected in HBV-positive individuals, laboratory testing can be used for diagnosis, differentiating co-infection from super-infection, and determining if the HDV infection is active or resolved. The total HDV antibody assay is generally the initial test.

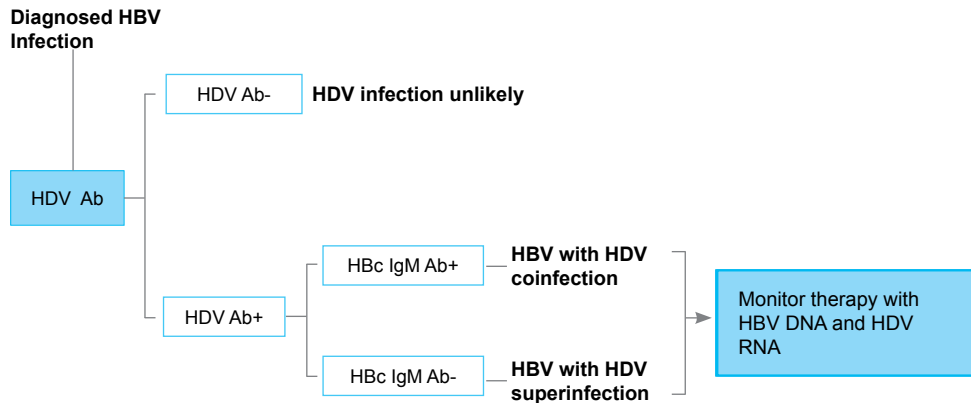


Fig. 14: Approach to detect HDV in HBV diagnosed individual.

Hepatitis E

HEV infection is usually an acute self-limiting disease.

Structure

Hepatitis E virus (HEV) is an RNA virus and is a 27- to 34-nm, icosahedral capsid, non-enveloped, HAV-like virus with a 7200-nucleotide, single-strand, positive-sense RNA genome. HEV has three open reading frames (ORF) (genes), the largest of which, ORF1, encodes nonstructural proteins involved in virus replication. A middle-sized gene, ORF2, encodes the nucleocapsid protein—the major structural protein, and the smallest, ORF3, encodes a structural protein whose function remains undetermined. All HEV isolates appear to belong to a single serotype despite genomic heterogeneity of up to 25% and the existence of four genotypes (genotypes 1 to 4 [HEV1, HEV2, HEV3, and HEV4]). Genotypes 1 and 2 appear to be more virulent, whereas genotypes 3 and 4 are more attenuated and account for subclinical infections.

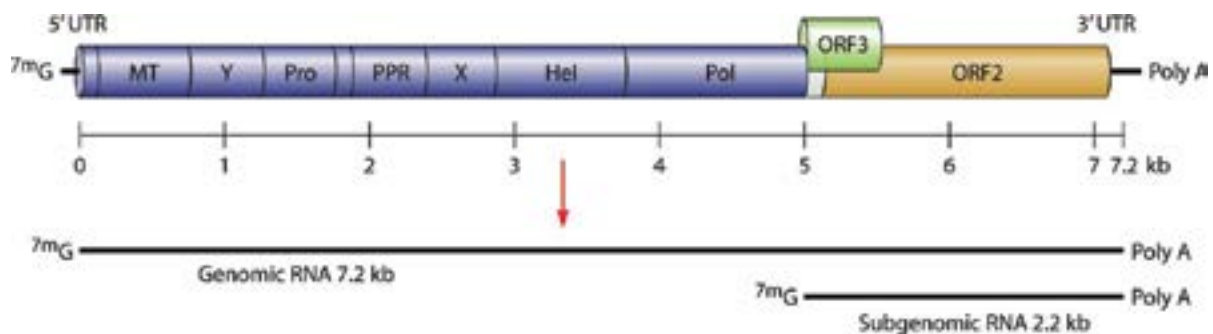


Fig. 15: Hepatitis E virus genome.

Source: Nassim Kamar et al. *Clin. Microbiol. Rev.* 2014; 27: 116-138.

Human infection with HEV has two distinct epidemiological patterns. In areas of poor sanitation and hygiene, genotypes HEV1 and HEV2 are transmitted between humans by the fecal-oral route, usually via contaminated water, is usually a self-limiting illness which last a few weeks and results in frequent sporadic cases and occasional large outbreaks.

In developed countries, genotypes HEV3 and HEV4 are transmitted zoonotically from animal reservoirs, with sporadic cases. Moreover, HEV 3 infection in immune-compromised patients in developed countries causes chronic infection with rapidly progressive cirrhosis (in organ transplant recipients, patients with hematological malignancy requiring chemotherapy, and individuals with HIV). HEV also causes extra-hepatic manifestations, including a number of neurological syndromes and renal injury.

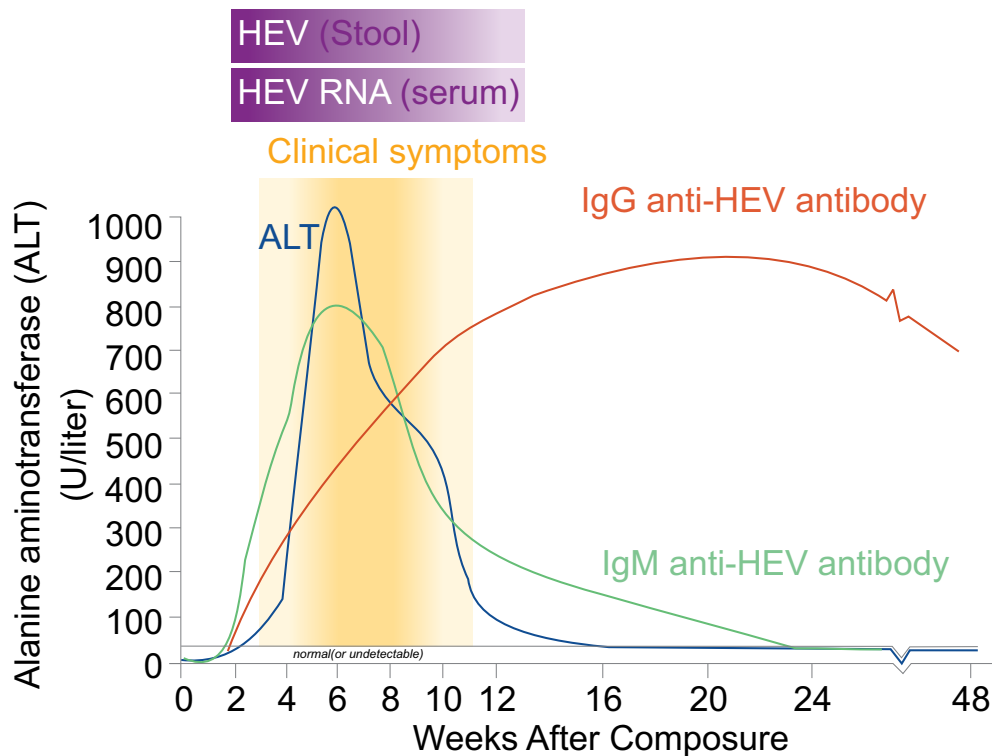


Fig. 16: Course of acute hepatitis E virus infection.

Source: Lisa J Krain et al. *Clin. Microbiol. Rev.* 2014;27:139-165.

HEV infections can be diagnosed by measuring anti-HEV antibodies, HEV RNA or viral capsid antigen in blood or stool.

HEV infection can be diagnosed either indirectly by detecting serum anti-HEV antibodies or directly by detecting the HEV RNA in blood or other body fluids. Following an incubation period of 2 to 6 weeks, an initial short-lived IgM response is followed by longer-lasting IgG antibodies. The presence of anti-HEV IgM is a marker of acute infection. The presence of anti-HEV IgG alone is a marker of past infection. HEV RNA becomes detectable in early phase of the disease and is undetectable in blood in about 3 weeks after the onset of symptoms but can be detected in feces for another 2 weeks.

C H A P T E R

2

Organization of laboratory services for diagnosis of viral hepatitis

A variety of tests are required to establish a diagnosis of viral hepatitis and its further management. These include platelet count, estimation of liver enzymes and specific serological tests and molecular tests (HBV DNA and HCV RNA). The initiative envisages a tiered network of existing laboratories taking into account their existing competencies and capacities in order to attain a quality assured test result.

The specific tests for viral hepatitis offered in the initiative across public health laboratories are summarized below.

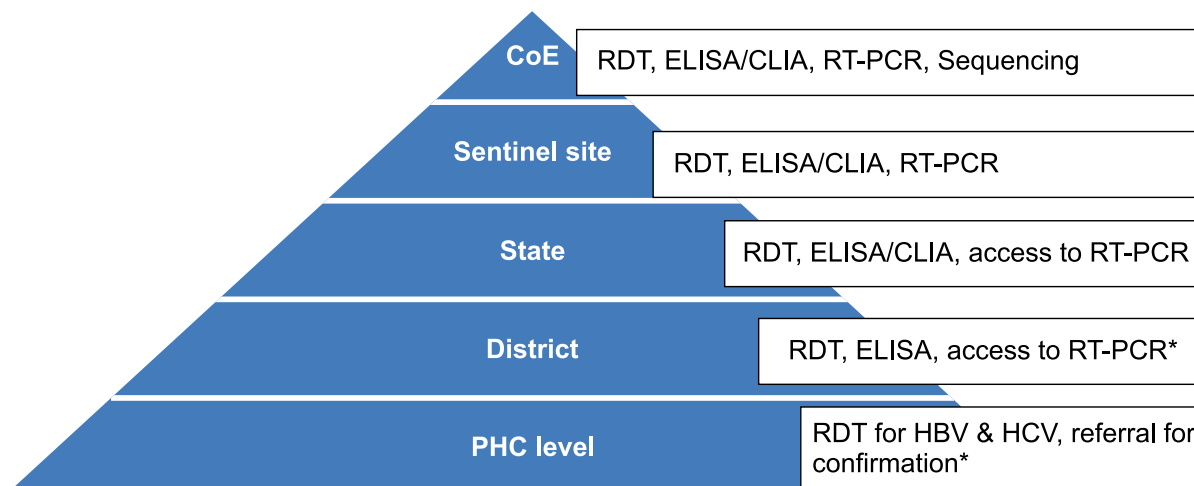


Figure17 : Tiered laboratory network and the available tests.

**If samples are to be transported, they need to be collected, packaged and transported within six hours of collection under suitable environmental conditions.*

To effectively deliver the services, the following pattern of assistance will be provided to the state laboratories under the initiative-

Table 4: Pattern of assistance for state laboratories.

Budget head	Number	Total (Annual), in INR	Remarks
Human Resource			
Coordinator (Microbiologist)	1	Regular cadre	From Regular cadre
Technical officer	1		As per state NHM norms for each personnel. To be transferred from SVHMU of NHM
Data Entry operator	1		
Laboratory technician	1		
Equipment (computer, printer, scanner)		100,000	To be transferred from SVHMU to state lab
Meeting/ Training	4	600,000	
Consumables		500,000	

For district laboratories under the initiative, there is provision for only one laboratory technician subject to projection from the state in PIP.

C H A P T E R

3

**Approach to diagnosis
of viral hepatitis**

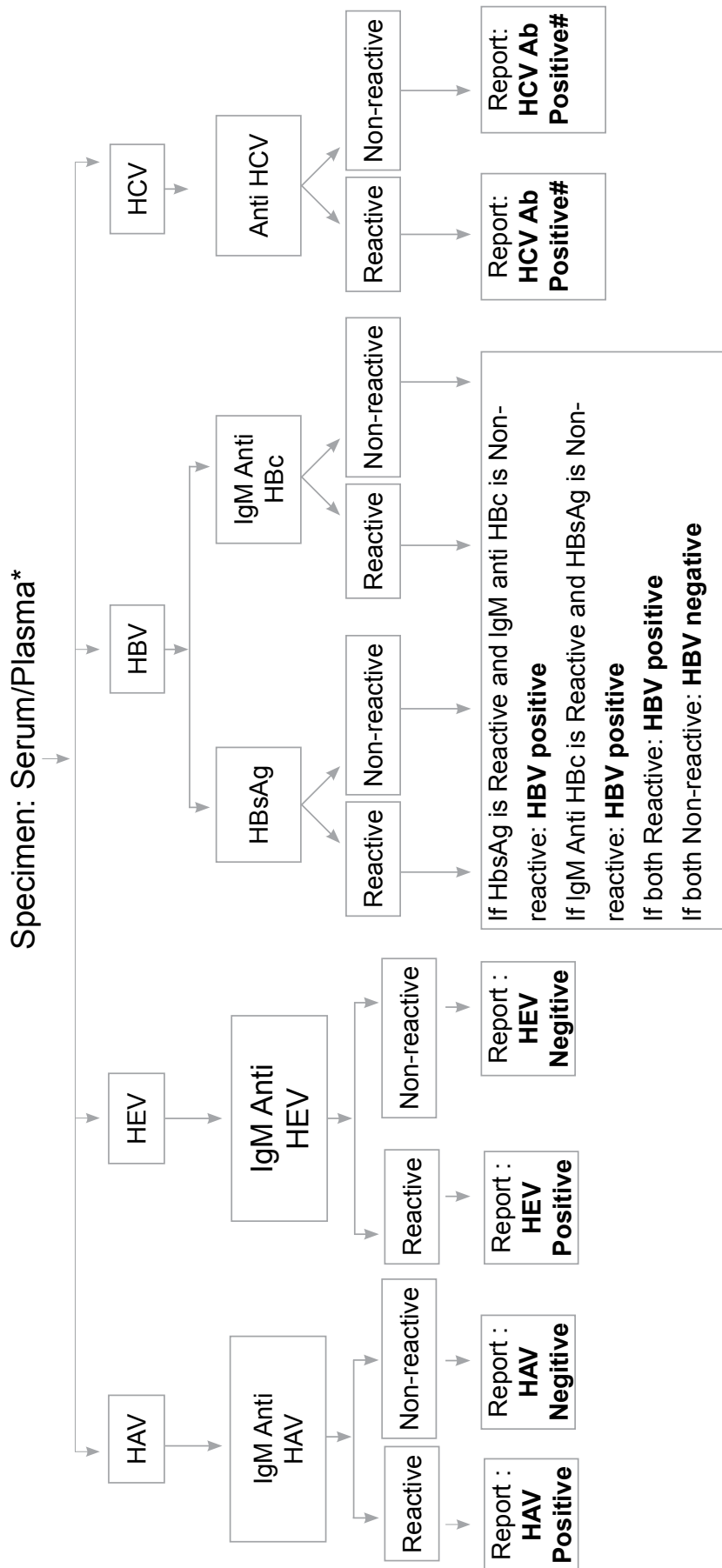
A patient with acute or chronic viral hepatitis infection may present at a health care setting with or without jaundice. The patient may be referred by a treating doctor for investigations after taking a written informed consent (Annexure 1) with a complete test requisition form.

Testing for HBV in pregnant women: In states where institutional deliveries are less than 90%, screening of all pregnant women should be carried out for HBsAg detection. Institutional delivery of HBsAg positive pregnant women must be mandated to prevent transmission to the child by giving birth dose hepatitis B vaccine.

Self-presenting asymptomatic individuals at high risk may be provided access to testing by a defined mechanism in the health care facility.

The algorithms to be followed for diagnosis are in the following pages:

Testing algorithm for the diagnosis of viral hepatitis in jaundiced patients.

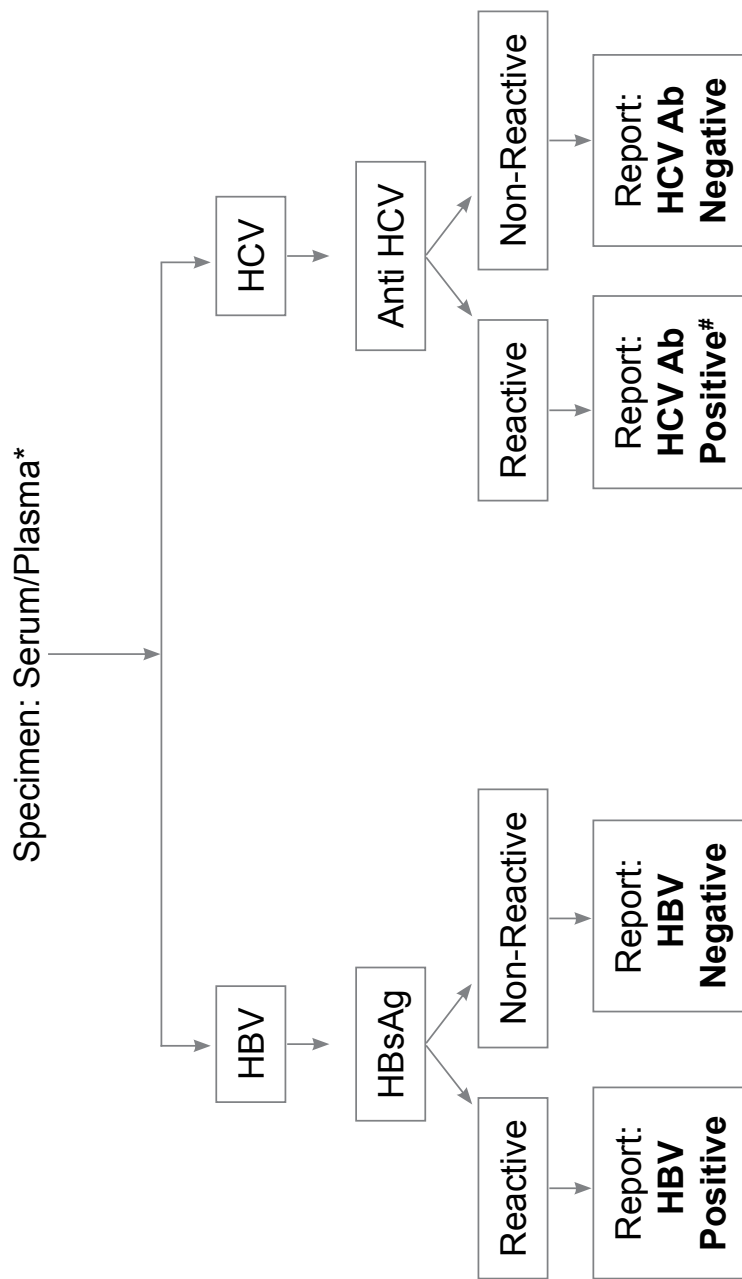


* Serum samples to be used for serological and biochemical testing, to be aliquoted and stored at -20 ° C for retesting for quality purposes, dispute etc.

#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 -80 ° C for quality assurance

Fig. 18a: Testing algorithm for the diagnosis of viral hepatitis in jaundiced patients.

Testing algorithm for the diagnosis of viral hepatitis in patients without jaundice.



* Serum samples to be used for serological and biochemical testing, to be aliquoted and stored at -20 ° 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 -80 ° C for quality assurance

Fig. 18b: Testing algorithm for the diagnosis of viral hepatitis in patients without jaundice.

C H A P T E R

4

Laboratory tests

Immunoassays – detect antibodies to the virus or a viral antigen in the host.

Immunoassays may be available as rapid diagnostic tests (RDTs) which are simple, rapid, low cost and do not require extensive laboratory infrastructure. They are less technically intensive and equipment independent. In comparison, enzyme linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA) are useful for high-volume clinical laboratories due to high throughput but technically intensive, equipment dependent and require appropriate laboratory infrastructure.

Rapid diagnostic tests

Rapid diagnostic tests (RDTs) are single-use disposable assays that are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and can give a simple qualitative result ≤ 30 minutes.

Most RDTs can be performed using venous/capillary whole blood, serum or plasma. They detect antigens and/or antibodies by often using an in-vitro diagnostics (IVD) device. They are based on immunologic principles like particle agglutination, lateral flow immunoassay, immune-filtration etc. Positive test result is indicated by clumping/dot/line visible to the naked eye.

The lateral flow immunoassay (LFI) is a test based on the principles of immune-chromatography for the qualitative detection of antibody/antigen.

- i. The required volume of whole blood/serum/plasma is added to the specimen well. This is the sample-loading pad/ adsorbent pad, which acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample.
- ii. Then the analyte to be detected reacts with the conjugate (particle coated with antibody/antigen). If the analyte is present, the immobilized conjugated antibodies and labels (visible indicator system) will bind to the target and continue to migrate along the test. LFI utilize colloidal gold nanoparticles, latex microspheres, carbon, or coloured latex nanoparticles.
- iii. The complex with the analyte then migrates upward along the membrane by capillary action, and reacts with polyclonal antibodies, which are pre-coated on the test line region in the reaction membrane/detection membrane.
- iv. Ensure validity of the test by observing the control as per kit literature

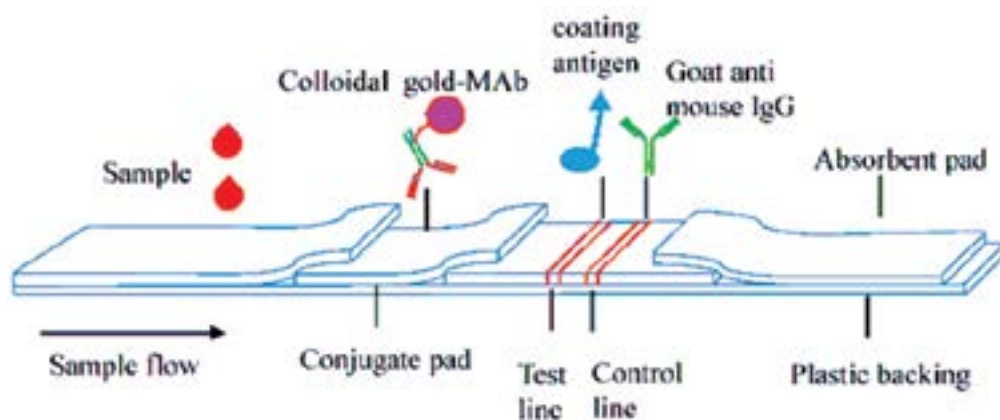


Fig. 19: Schematic representation of lateral flow immunoassay.

The visual readout is interpreted as specified in the kit literature. The invisibility of the control line indicates insufficient specimen volume or incorrect procedure techniques. The presence of one coloured (red/blue) line in the control region indicates a negative result, and the presence of two distinct lines in both the control and test regions indicates a positive result. In case of absence of control the test is invalid and needs to be repeated.

Immuno-filtration assays (IFAs) also known as immune-concentration or flow through assays use nitrocellulose (NC) membranes or glass fiber filters as a solid phase with large surface that allows the flow of the sample. The sample passing through the membrane gets concentrated and accelerates the binding of antigens and antibodies. The solid support immobilizes or captures the analyte to be detected, along with a proper signal reporting system in a typical sandwich reaction. The testing process involves multiple steps that include drop-wise addition of buffer, samples, and conjugates. Recombinant and synthetic antigens are used to provide better, stable, and sensitive test systems. The assay includes a built-in control to verify the correct protocol. The control mainly uses antihuman immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added appropriately.

It is important to determine the extent to which the tests are able to identify the likely presence or absence of a disease/condition of interest so that their findings encourage appropriate decision making. Adequacy and usefulness of screening tests are determined and described by sensitivity, specificity and predictive value of these tests. All four metrics should be regarded as important when describing and assessing a screening test's adequacy and usefulness. (Annexure 2)

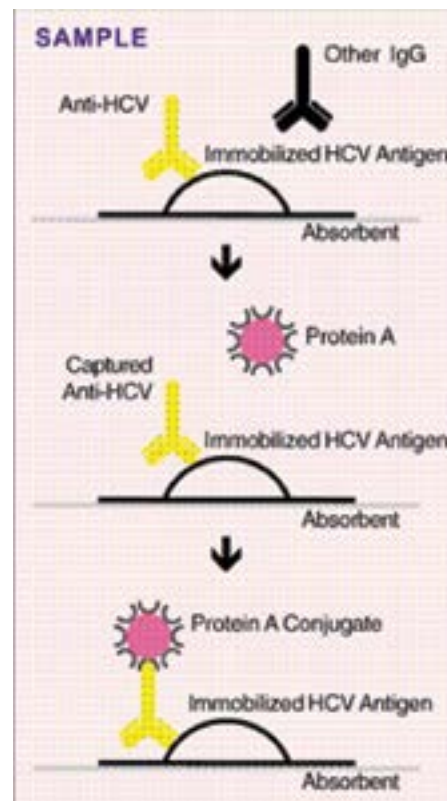


Fig.20: Schematic presentation of Immuno-concentration technique.

Enzyme linked immunosorbent assay (ELISA) / Enzyme immunoassay (EIA)

ELISA is a plate based assay technique which is used for detecting and quantifying substances such as peptides, proteins, antigens, antibodies and hormones. An enzyme conjugated with an antibody reacts with colourless substrate to generate a coloured product. Such substrate is called chromogenic substrate. A number of enzymes have been used for ELISA such as glucose oxidase, alkaline phosphatase, and beta galactosidase. Specific substrates such as TMB (for peroxidase); paranitrophenyl phosphate (for alkaline phosphatase); are used, which are hydrolyzed to give coloured end product.

Materials needed in ELISA testing:

- A. Pipettes, washer system, ELISA plate reader: Readers, washers and pipette are available as manual or automated system. One of the main factors affecting equipment selection is the number and types of test samples being run.
- B. Reagents needed for the testing: Concluded in the kit (coated plates, sample diluents, controls, wash concentrate, conjugate, substrate, stop solution).

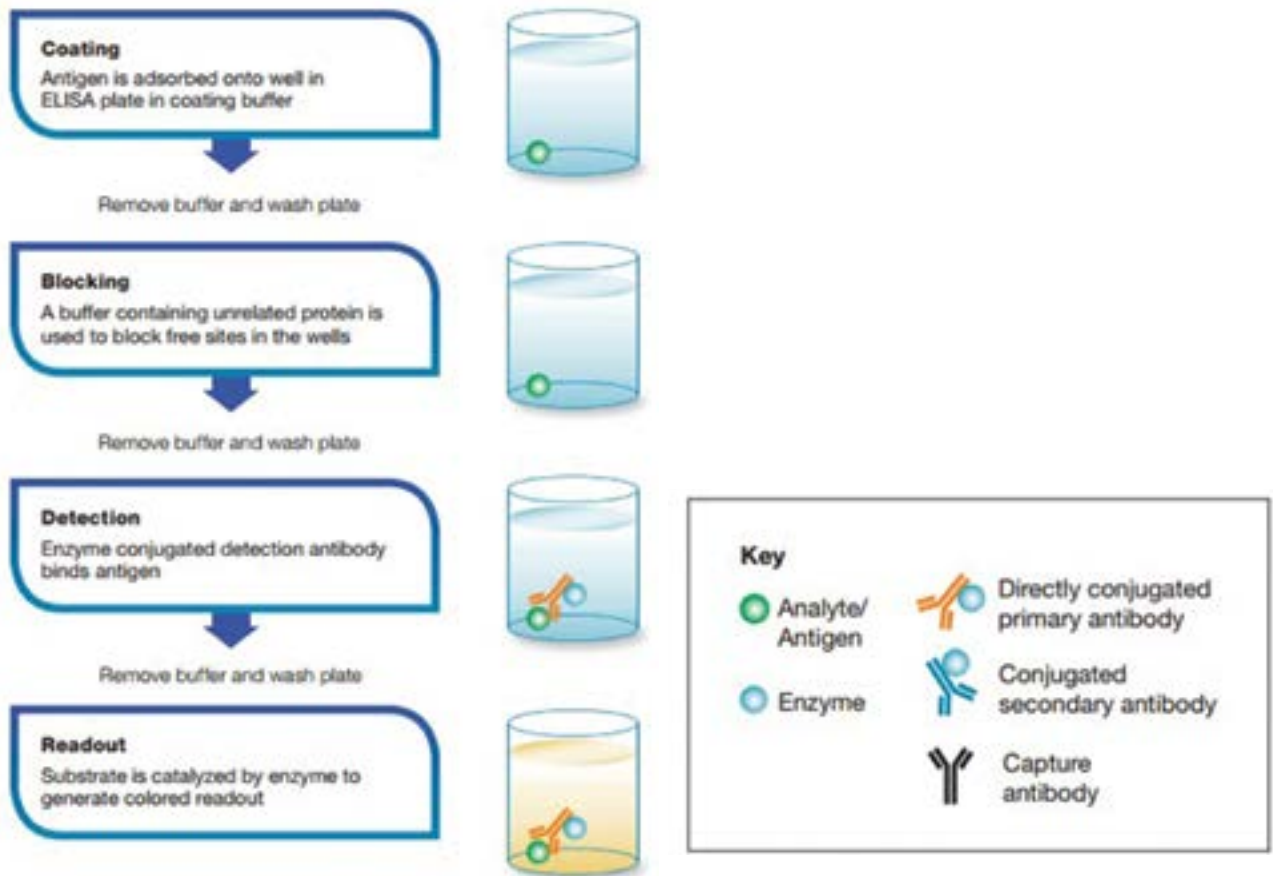


Fig.21: General procedure of ELISA.

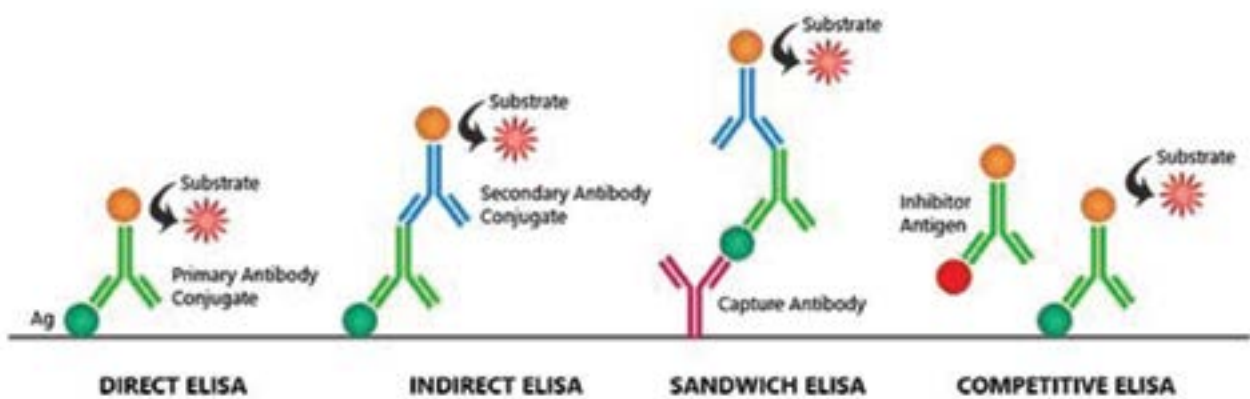


Fig. 22 : Types of ELISA.

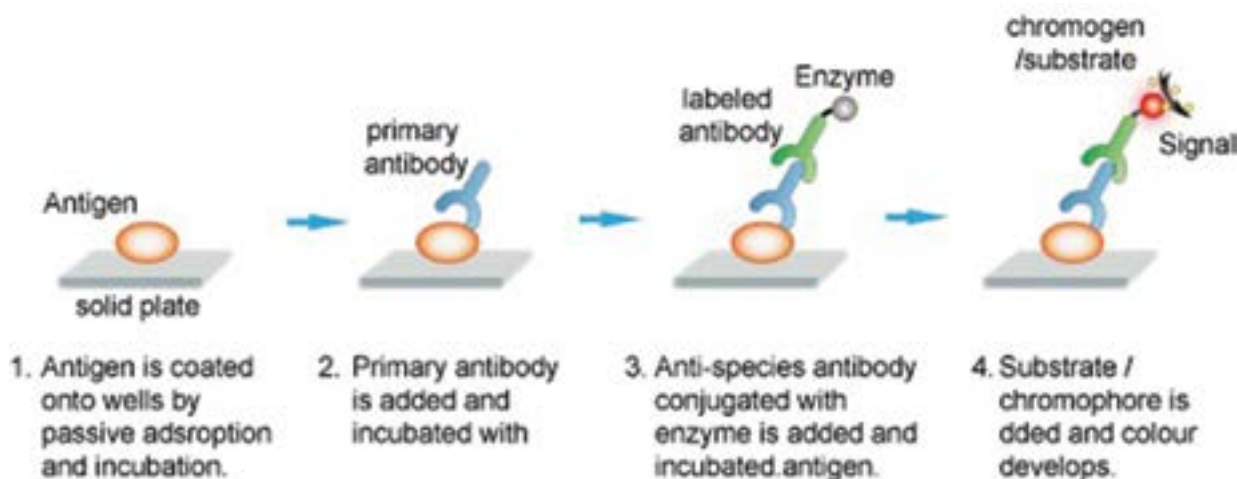


Fig. 23: Indirect ELISA.

Advantages	Disadvantages
High sensitivity - more than one labeled secondary antibody can bind the primary antibody	Possibility of background noise - secondary antibody may be cross-reactive
Economical - fewer labeled antibodies are needed	Longer procedure than direct ELISA technique - additional incubation step for secondary antibody needed
Greater flexibility - different primary antibodies can be used with a single labeled secondary antibody	

Best for: determining total antibody concentration in samples.

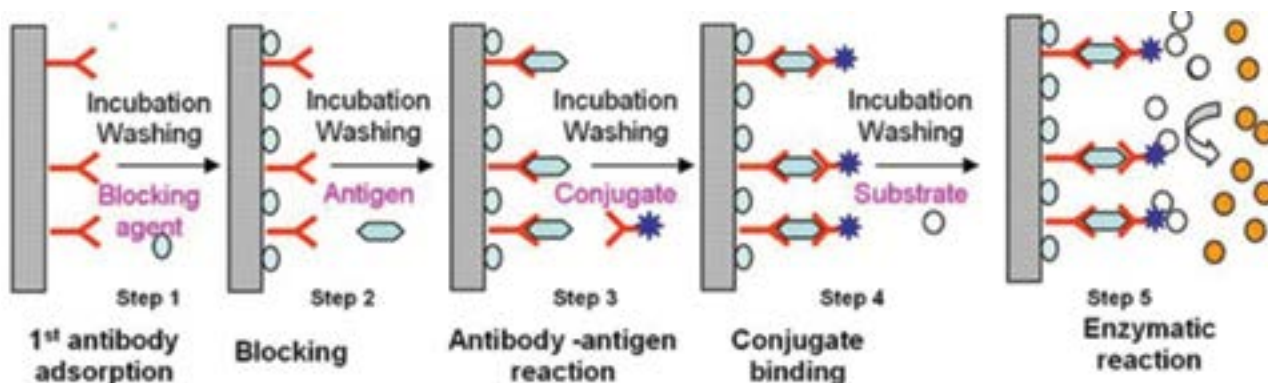


Fig. 24: Sandwich ELISA.

Advantages	Disadvantages
High sensitivity - 2-5 times more sensitive than direct or indirect ELISA	Antibody optimization can be difficult - cross-reactivity may occur between the capture and detection antibodies. Needs a standardized ELISA kit or tested antibody pair.
High specificity - two antibodies are involved in capture and detection	
Flexibility - both direct and indirect detection can be used	

Best for: analysis of complex samples, since the antigen does not need to be purified prior to measurement.

Antibody capture ELISA is similar to sandwich ELISA but in the first step, anti-Ig (M or G) is coated on the plate. Antibody capture ELISAs is particularly sensitive in demonstrating IgM responses early in illness. Reasons for this increase in sensitivity are: specific antibody isotypes in the patient's specimen are bound to the solid phase by the capture antibody allowing the specific antibody-antigen reaction to occur in the absence of competing isotypes.

• Ag Detection

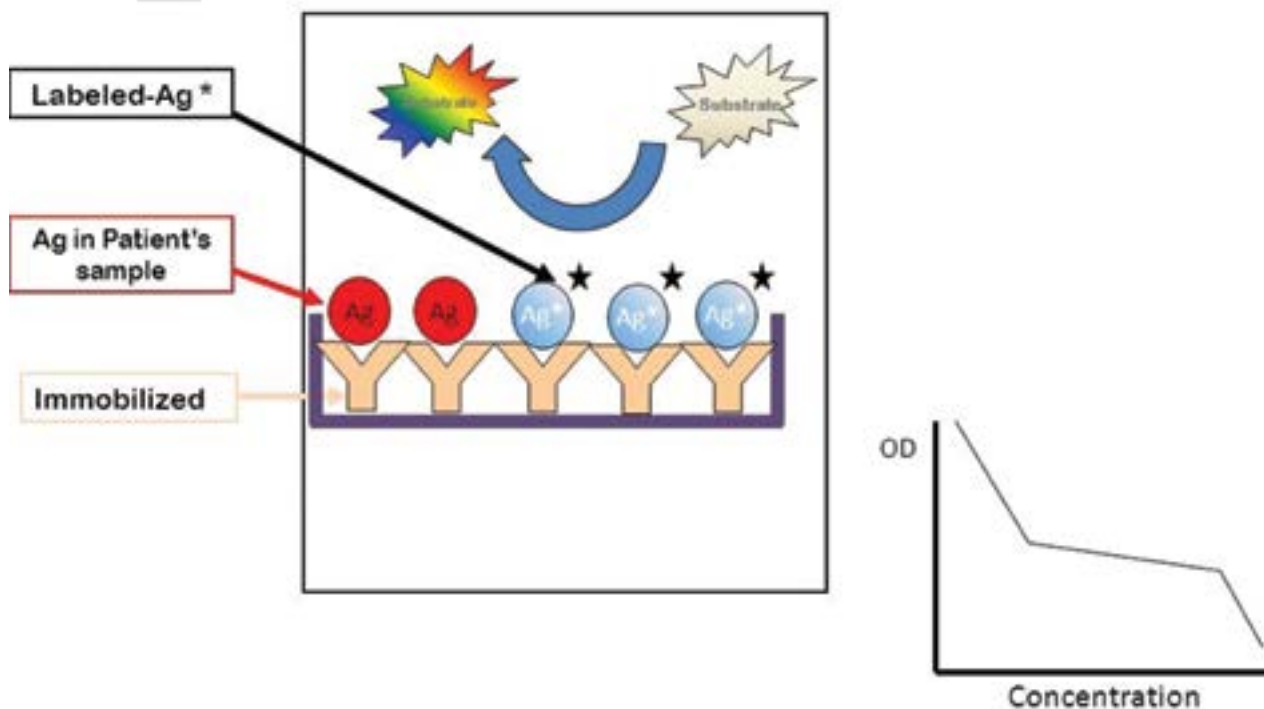


Fig. 25: Competitive ELISA (for antigen detection).

Advantages	Disadvantages
Main advantage - no sample processing is required and crude or impure samples can be used	Same limitations as base ELISA - as each ELISA technique can be adapted to a competitive format
More robust - less sensitive to sample dilution and sample matrix effects than the sandwich ELISA	
More consistent - less variability between duplicate samples and assays	
Maximum flexibility - it can be based on direct, indirect or sandwich ELISA	

Best for: commonly used when only one antibody is available for the antigen of interest. It is also suitable for detecting small antigens that cannot be bound by two different antibodies such as in the sandwich ELISA technique.

The EIA results have also been interpreted in the form of signal (or sample) to cut-off ratio (s/co ratio) to express the results quantitatively. S/co ratio of EIA for HCV could serve as an important tool in notifying the blood donors of their HCV status in resource poor setting where there is absence of supplemental testing. Algorithms derived on the basis of s/co ratio could also be used for guiding the blood donors for further referral and for re-entry purposes.

Newer techniques

It may use fluorogenic, electro-chemiluminescent and quantitative PCR reporters to create quantifiable signals. These new reporters can have various advantages, including higher sensitivities and multiplexing.

Chemiluminescence immunoassay (CLIA)

CLIA is an immunoassay technique where the label, i.e. the true “indicator” of the analytic reaction, is a luminescent molecule. The resultant potential energy in the atom gets released in the form of light, which is measured in terms of Relative Light Units (RLU). In spectrophotometry, luminescence has an advantage over absorbance in that the former is an absolute measure whereas the latter is relative.

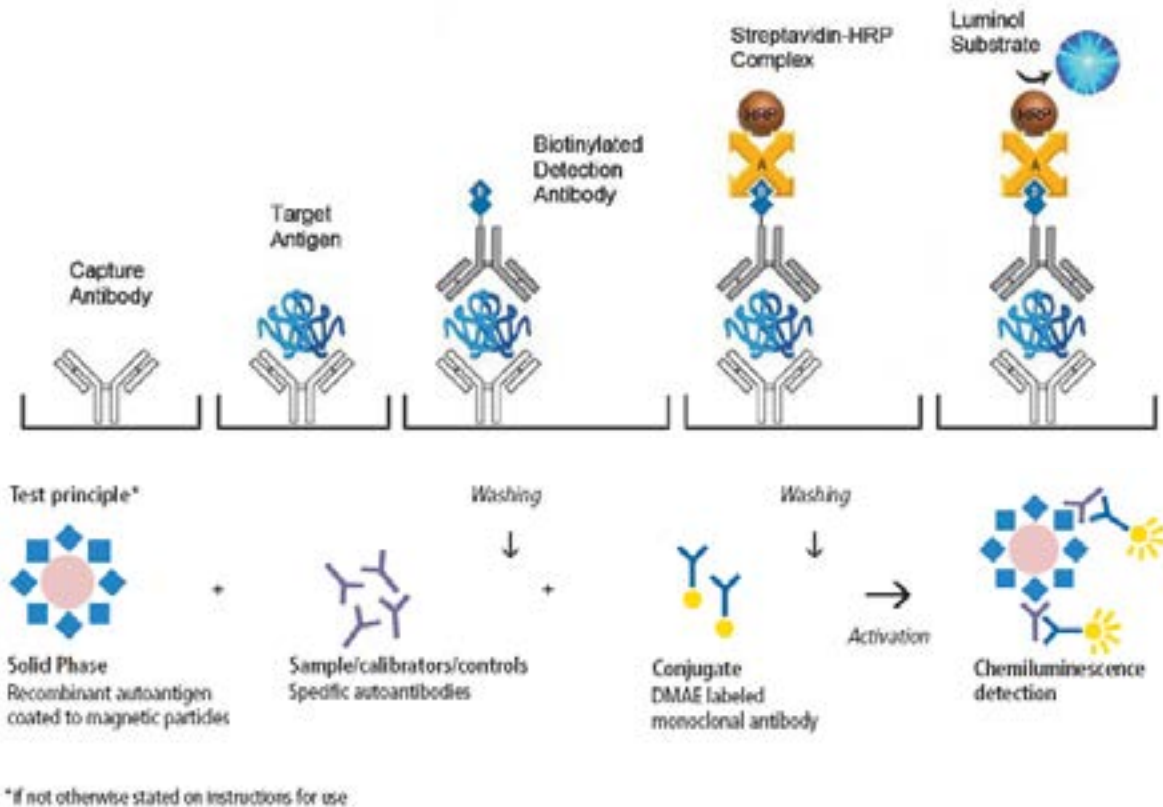


Fig. 26: Chemiluminescence Assays.

Chemiluminescent methods can be direct—using luminophore markers—or indirect—using enzyme markers. Either method may be competitive or non-competitive.

CLIA analysers are useful for the detection of serological markers of hepatitis viruses B virus (HBV), hepatitis C virus (HCV), hepatitis A virus (HAV) and hepatitis E virus (HEV). CLIA has advantages of being more reliable, precise, technically simple, shorter execution times (30–40 minutes) and high-speed throughput. It provides univocal recognition of patient and quality control samples and of specific and common reagents resulting in complete control of the analytical process. CLIA has higher analytical sensitivity and improved diagnostic sensitivity and specificity as compared to conventional EIAs.

Table 5: Interpretation of individual serology test results in the diagnosis of acute and chronic viral hepatitis.

MARKER	INTERPRETATION
IgM Antibody to hepatitis A (Anti-HAV IgM or HAV IgM Ab)	<ul style="list-style-type: none"> • Positive result indicates a current or recent HAV infection • A negative result indicates absence of infection. May be negative in early infection (if collected within five to seven days after onset of symptoms) • Present for three to six months after onset of acute infection
Total Antibody to hepatitis A (IgM and IgG) (Anti-HAV or HAV Ab)	<ul style="list-style-type: none"> • Positive result indicates past infection and immunity to HAV (in the absence of HAV IgM antibody) • Individuals given serum immune globulin for HAV prophylaxis may test as positive for at least six months
Hepatitis B surface antigen (HBsAg)	<ul style="list-style-type: none"> • Presence indicates that a person has HBV infection and is infectious • First marker to appear in an acute infection • Disappearance indicates recovery from infection • Used to diagnose an acute or chronic infection • Persistence for > 6 months indicates chronic infection (carrier) • Individuals tested within 72 hours after administration of the vaccine may test as positive (see anti-HBs, anti-HBc IgM and HBeAg.)
Antibody to hepatitis B surface antigen (Anti-HBs or HBs Ab)	<ul style="list-style-type: none"> • Presence indicates resolution and immunity against HBV infection or response to vaccination. • Levels of 10MIU/mL (10 IU/L) are usually considered protective • Routine monitoring of levels in individuals who have received the complete course of vaccine is not considered necessary • Some individuals, e.g., healthcare workers, who are believed to have been exposed to the virus by a needle injury, should have their anti-HBs levels tested to determine whether they require administration of hepatitis B immune globulin (HBIG) and hepatitis B vaccine booster • Positive result in individuals with recent acute HBV infection Indicates convalescence • Usually NOT detected when HBsAg is also present • In some cases of chronic hepatitis B infection, both HBsAg and anti-HBs can be detected. These antibodies are heterotypic and likely not protective • Antibody levels may decline with time
IgM antibody to hepatitis B core antigen (Anti-HBc IgM or HBc IgM Ab)	<ul style="list-style-type: none"> • Primarily be used if there is a high index of suspicion to indicate that the patient is in the early convalescence “window period” (two to 16 weeks post infection) when HBsAg has disappeared and anti-HBs levels are not yet detectable • Positive result usually indicates HBV infection within the preceding 4 to 6 months (ie, acute infection). • Usually detectable for three to 12 months.
Hepatitis B e antigen (HBeAg)	<ul style="list-style-type: none"> • Presence indicates active viral replication and high infectivity. Marker of active HBV replication and of infectivity. However, the absence of HBeAg in a person who is HBsAg-positive does not imply that the individual is NOT infectious. • Can be used to monitor therapy of patients with chronic HBV infection

Antibody to hepatitis B e antigen (Anti-HBe or HBe Ab)	<ul style="list-style-type: none"> • Appears as HBeAg disappears • In chronic hepatitis B infection, a positive result indicates resolving, or response to therapy or minimal liver disease • However, individuals who are HBsAg-positive and have anti-HBe present must still be considered infectious
Total antibody to hepatitis B core antigen (Anti-HBc or HBc Ab)	<ul style="list-style-type: none"> • A positive result indicates past or current HBV infection • Usually persists for life • This antibody is absent in individuals who are immune solely as a result of vaccination • Up to 10% false-positive rate has been described in individuals with no documented infection to HBV. If uncertain, presence of one other marker, e.g., anti-HBs or anti-HBe would confirm previous exposure with HBV. Alternatively a negative repeat test later may indicate an earlier false-positive result.
Antibody to hepatitis D virus (Anti-HDV or HBV Ab) HDV Ab, total	<ul style="list-style-type: none"> • Presence coincident with the presence of HBsAg indicates past or current HBV/HDV co-infection or super-infection. • Antibodies appear late during the course of acute infection
HDV IgM	<ul style="list-style-type: none"> • Presence coincident with the presence of HBsAg indicates past or current HBV/HDV co-infection or super-infection. A negative result coincident with the presence of HDV total antibody indicates resolved infection.
Antibody to hepatitis C (Anti-HCV or HCV Ab)	<ul style="list-style-type: none"> • Presence of antibody can be due to acute or chronic infection. It may represent only evidence of an infection with HCV • Presence of antibody does not imply immunity to HCV • Presence (with detectable HCV RNA) indicates current infection. A positive result coincident with a negative HCV RNA test may indicate a resolved infection or a false-positive antibody screening test.
IgM Antibody to hepatitis E virus (Anti-HEV IgM or HEV IgM Ab)	<ul style="list-style-type: none"> • Positive result indicates a current or recent HEV infection. • A negative result indicates absence of infection. May be negative in early infection (if collected within five to seven days after onset of symptoms) Present for three to six months after onset of acute infection

Nucleic acid testing

Nucleic acid testing (NAT) are highly sensitive and specific in detecting the presence of viral nucleic acid (HBV DNA or HCV RNA). It is an important tool to confirm diagnosis, identify individuals with high viral loads, which may suggest high infectivity, to monitor disease progression and the efficacy of antiviral therapies, to detect drug resistant mutants, and to identify relapse after the discontinuation of an antiviral therapy.

Laboratory-based technologies for NAT require sophisticated equipment, rigorous laboratory conditions and specimen collection, and highly trained staff who can perform precision steps and avoid contamination. In addition to NAT assays that target a single virus, multiplex NAT screening assays have been developed, which can detect DNA or RNA from multiple viruses simultaneously.

Currently, viral load is measured using international units per milliliter (IU/mL). However, in the past it was measured in copies per milliliter (copies/mL). In order to convert copies into international units, there are about 5.6 copies in one international unit, so 5 000 copies/mL equals about 893 IU/mL.

Hepatitis B quantitative DNA PCR plays a critical role in determining the phase of infection, deciding the treatment, and detecting responses to antiviral therapy. Assay range - 10 IU/mL to 1.0 x 10⁹ IU/mL, HBV DNA detected below 10 IU/mL will be reported as "<10 IU/mL". Reported in two formats: IU/mL and Log₁₀ IU/mL.

Methods to detect HCV RNA are based on an adaptation of the reverse-transcriptase polymerase chain reaction (RT-PCR). Transcription mediated amplification (TMA) or a signal amplification strategy that involves the use of enzymatically labeled branched nucleotide (bDNA) are available.

Genotyping

Genotyping is useful for investigating outbreaks and for understanding the epidemiology and virological features of this virus. Accurate classification of genotypes and subtypes of HCV is important for correct stratification of groups and accurate analysis of data related to efficacy and resistance of new HCV drugs. It is also essential for the implementation of therapeutic procedures, the production of effective vaccines, and the improvement of diagnostic tests.

Many genotyping methods targeting different regions of the HCV genome have been developed, such as restriction fragment length polymorphism, line probe assay, TaqMan PCR, liquid microarray, sequencing and solid-phase electrochemical array.

Genotyping by Nucleotide Sequencing: A few sequencing-based and several non-sequencing-based HCV genotyping assays are available and are used for routine determination of the HCV genotype and selected subtypes. The non-sequencing-based HCV genotyping assays are mainly founded on reverse hybridization or real-time PCR. Since both assay groups belong to DNA probe-based technologies, they occasionally fail to provide unambiguous results, particularly when the genetic diversity of the target is high, as in the case of HCV. Even with the last versions of commercial non-sequencing-based HCV genotyping assays, the HCV genotype/subtype fails to be assigned in 5 to 10% of patients and sometimes misclassifies the HCV genotypes/subtypes with clinical consequences such as treatment failure.

Laboratories may use the more accurate, Sanger sequencing of a carefully selected, usually relatively short part of the HCV genome using sequencing-based assays or in-house sequencing protocols. This is very labour-intensive and time-consuming, however, it is the preferred method for population screening.

Next generation sequencing

Next-generation sequencing (NGS) is a high-throughput genome sequencing which has revolutionized the study of genomics and molecular biology. NGS platforms perform sequencing of millions of small fragments of DNA in parallel. Bioinformatics analyses are used to piece together these fragments by mapping the individual reads. For example, by using NGS, an entire human genome can be sequenced within a single day.

It is a rapid and cost-effective method for generating the whole HCV genome to accurately and simultaneously determine HCV genotypes/subtypes, RASs (resistance associated substitutions), and quasi species diversity and to allow comprehensive viral strain analysis.

C H A P T E R

5

**Specimen collection, storage,
transportation**

Appropriate specimen management impacts patient care in several very important ways. Nothing is more important to the effectiveness of a laboratory than a specimen that has been appropriately selected, collected, and transported. It influences therapeutic decisions, hospital infection control, patient length of stay, overall hospital costs and directly affects patient care and patient outcome.

All standard precautions must be followed during specimen collection, storage and transport.

Test requisition form

The Test Requisition Form (TRF) – printed or computer-generated form, should be dated and provide the following information:

1. Patient's full name
2. Patient's age and gender
3. Patient's hospital/medical record number (inpatient or outpatient identification number)
4. The department or location where the specimen was collected
5. Ordering physician information
6. Type of specimen provided
7. A unique identification number
8. An accessioning number
9. Date and time of specimen collection (critical information)
10. Specific test(s) required
11. Written informed consent taken
12. Any other information, if needed

Supplemental information, if it can be provided, would be helpful for the laboratory:

- Relevant clinical information regarding patient's condition; clinical diagnosis, relevant patient history e.g. H/o repeated blood transfusion, dialysis, thalassemia
- Special procedures used in obtaining the specimen
- Drugs/Antimicrobial agents, if any, that the patient is receiving

All primary specimen containers are labeled with at least two patient-specific identifiers. Examples of acceptable identifiers include, but are not limited to: patient's name, date of birth, hospital number, requisition number, accession number, unique random number, and the date and time of collection

A location (e.g. hospital room number) is not an acceptable identifier. Identifiers may preferably be in a machine readable format, such as a barcode.

Aseptically collected fresh serum/plasma sample that is clear, non-haemolysed, or non-lipemic is the preferred specimen for testing. Serum can be stored at room temperature if the assay is performed within 8 hours of collection. If the assay cannot be completed within 8 hours, the specimen should be refrigerated at 2–8 °C. The specimens can be stored up to 7 days at 2–8 °C, and for 1 month at -20 °C. In case the specimen is to be stored beyond 30 days, temperature of storage should be at least -80 °C. Repeated freeze-thaw of specimens should be avoided.

The following equipment are needed for routine venipuncture:

- Evacuated collection tubes - the tubes are designed to fill with a predetermined volume of blood by vacuum. The rubber stoppers are colour coded according to the additive that the tube contains. Various sizes are available. Blood should NEVER be poured from one tube to another since the tubes can have different additives or coatings.
- Needles - the gauge number indicates the bore size: the larger the gauge number, the smaller the needle bore. Needles are available for evacuated systems and for use with a syringe, single draw or butterfly system.
- Holder/Adapter – use with the evacuated collection system.
- Tourniquet – Wipe off with alcohol and replace frequently.
- Alcohol wipes – 70% isopropyl alcohol. (Povidone-iodine wipes/swabs - used if blood culture is to be drawn).
- Gauze sponges – for application on the site from which the needle is withdrawn.
- Adhesive bandages/tape – protects the venipuncture site after collection.
- Needle disposal unit – needles should NEVER be broken, bent, or recapped. Needles should be placed in a proper disposal unit IMMEDIATELY after their use.
- Gloves – can be made of latex, rubber, vinyl; worn to protect the patient and the phlebotomist.
- Syringes – may be used in place of the evacuated collection tube for special circumstances.

Phlebotomy: Procedural steps

Before carrying out phlebotomy, technicians must wash their hands with soap and water and then dry with a single-use towel. An alternative would be to cleanse the hands with 3 mL of alcohol rub, starting with the palm of the hand, rubbing it into the fingertips and all over the hands until the alcohol dries out.

Put on well-fitting latex, rubber or vinyl gloves. Then

1. Patient ID
2. Explain the procedure and purpose to the patient.
3. Assess patient's condition and position the patient sitting or lying down. (Never allow the patient to sit upright on a high stool or standing due to the possibility of syncope).
4. Check the requisition form for requested tests, patient information and any special requirements.
5. Select suitable site for venipuncture
6. Assemble and prepare equipment required (order of draw)
7. Prepare patient and venipuncture site. Apply the tourniquet: clean the site with a swab soaked with 70% alcohol for 30 seconds, then allow it to dry. The cleaning is done in concentric circles starting from the site of puncture.
8. Perform venipuncture.
9. Collect sample/s in the appropriate container/s.
10. While the tube fills, remove the tourniquet and apply a dry swab/cotton on the puncture wound with pressure to contain the bleeding.
11. Assess for any possible complications.
12. Tubes with anticoagulants should be gently and completely inverted (end over end) four to six times after collection.
13. Label collection tubes.
14. Send samples to lab with requisition form immediately.

Phlebotomy– infection prevention and control practices:

- MUST carry out hand hygiene (use soap and water or alcohol rub), and wash/rub carefully, including wrists and spaces between the fingers for at least 30 seconds (follow WHO hand hygiene procedure)
- MUST use one pair of non-sterile gloves per procedure or patient
- MUST use a single-use device for blood sampling and drawing
- MUST disinfect the skin at the venipuncture site
- MUST discard the used device (a needle and syringe is a single unit) immediately into a puncture proof sharps container
- MUST seal the sharps container with a tamper-proof lid
- MUST place laboratory sample tubes in a sturdy rack (before injecting into the rubber stopper)
- MUST immediately report any incident or accident linked to a needle or sharp injury, and seek assistance; start PEP as soon as possible.

Table 6: Recommended order of draw for plastic vacuum tubes.

Order of use ^a	Type of tube/ usual colour ^b	Additive ^c	Mode of action	Uses
1	Blood culture bottle (yellow-black striped tubes)	Broth mixture	Preserves viability of micro-organisms	Microbiology – aerobes, anaerobes, fungi
2	Non-additive tube			Serum
3	Coagulation tube ^d (light blue top)	Sodium citrate	Forms calcium salts to remove calcium	Coagulation tests (prothrombin time), requires full draw
4	Clot activator (red top)	Clot activator	Blood clots, and the serum is separated by centrifugation	Chemistries, immunology and serology, blood bank (cross-match)
5	Serum separator tube (red-grey tiger top or gold)	None	Contains a gel at the bottom to separate blood from serum on centrifugation	Chemistries, immunology and serology
6	Sodium heparin (dark green top)	Sodium heparin or lithium heparin	Inactivates thrombin and thromboplastin	For lithium level use sodium heparin, for ammonia level use either
7	PST (light green top)	Lithium heparin anticoagulant and a gel separator	Anticoagulants with lithium, separates plasma with PST gel at bottom of tube	Chemistries
8	EDTA (purple top)	EDTA	Forms calcium salts to remove calcium	Haematology, Blood bank (cross-match) requires full draw
9	Blood tube (pale yellow top)	Acid-citrate-dextrose (ACD, ACDA or ACDB)	Complement inactivation	HLA tissue typing, paternity testing, DNA studies
10	Oxalate/fluoride (light grey top)	Sodium fluoride and potassium oxalate	Antiglycolytic agent preserves glucose up to five days	Glucoses, requires full draw (may cause haemolysis if short draw)

a “1” indicates draw first, and “10” draw last (if used).

b Verify with local laboratory in case local colour codes differ.

c Gently invert tubes with additives to mix thoroughly; erroneous test results may be obtained when blood is not thoroughly mixed with the additive.

d If a routine coagulation assay is the only test ordered, then a single light blue top tube may be drawn. If there is a concern about contamination by tissue fluids or thromboplastins, then a non-additive tube can be drawn before the additive tube. The PST tube contains lithium heparin anticoagulant and a gel separator; if used, draw in the order shown.

Table 7: Elements of quality assurance in phlebotomy.

Element	Notes
Education and training	Education and training is necessary for all staff carrying out phlebotomy. It should include an understanding of anatomy, awareness of the risks from blood exposure, and the consequences of poor infection prevention and control.
Standard operating procedures (SOPs)	SOPs are required for each step or procedure. They should be written and be readily available to health workers.
Correct identification of the patient	Identification should be through matching to the laboratory request form. After samples have been taken from a patient, a system of identification and tracking is essential to ensure that the sample is correctly matched with the result and with the patient or donor.
The condition of the sample	The condition of the sample should be such that the quality of the results is satisfactory.
Safe transportation	Making safe transportation of blood or blood products part of best practices will improve the quality of results from laboratory testing.
An incident reporting system	A system is required for reporting all adverse events. A log book or register should be established with accurate details of the incident, possible causes and management of adverse events.

Specimen collection for NAT testing

Specimen type: Plasma is the standard specimen type used for the detection and quantitation of HBV DNA or HCV RNA. The volume of plasma required varies by test and platform used. Methods that use automated extraction platforms may require a larger sample volume. Although serum may be an approved specimen type for certain platforms, testing of serum using most quantitative assays generally results in viral loads significantly less than those measured in plasma. Therefore, plasma is generally recommended for quantitative NAT and results are reported as plasma viral load.

Collection tubes and anticoagulants: Nearly all methods require that plasma specimens be collected in tubes containing specific anticoagulants. In general, EDTA is the anticoagulant of choice; acid citrate dextrose (ACD) is acceptable in some situations. The higher volume of anticoagulant in ACD tubes results in a viral load decrease of 15%.

Sample collection for quantitative detection of plasma HCV RNA levels-

- Collect 4-5 mL whole blood in EDTA, ACD or EDTA evacuated tube with clot activator gel
- Centrifuge within 6 hours of draw and transfer 2 mL plasma to a sterile, screw top tube.
- If the specimen was collected in PPT, the entire tube can be shipped frozen following centrifugation.
- If shipped (at 2 –8 °C), separated plasma fraction must arrive within 24 hours of draw.

Sample collection for quantitative detection of serum HCV RNA levels

- Collect 4-5 mL whole blood in red-top or SST.
- Centrifuge within 6 hours of draw and transfer 2 mL serum to a sterile, screw top tube.
- If the specimen was collected in SST, the entire tube can be shipped frozen following centrifugation.
- If shipped ambient, separated serum fraction must arrive within 24 hours of draw.

This is not the preferred method for HCV RNA estimation.

Storage of Specimen in laboratory and during transport

For immunoassays, separated serum/plasma should remain at room temperature for no longer than eight hours. After that they need to be stored at 2 – 8 °C. If assays are not completed within 48 hours or the separated serum/plasma need to be stored beyond 48 hours, store at - 20 °C for up to one month and below - 80 °C for long durations of storage. Repeated freeze-thaw should be avoided as it causes marked reduction in values.

For NAT testing, the laboratory should follow the directions in the assay manufacturer’s product insert for specimen collection, transport and storage. When EDTA is used, whole blood can be collected in tubes with or without a gel separator. For RNA targets such as HCV, whole blood samples should be centrifuged and, in case of a non-gel separator tube, the plasma removed to a secondary tube within six hours of phlebotomy. Plasma separated in a gel separator tube may be transported to the laboratory in situ. Plasma samples are stable for up to five days at 2 – 8 °C and longer if frozen at -20 °C or -80 °C or lower. The laboratory should validate the effects on analytical results of in situ freezing of the plasma sample in gel separator tubes and freeze thaw cycles of plasma stored in secondary tubes.

Blood scheduled for DNA analysis can be stored at room temperature for up to 24 hours or at 2– 8 °C for up to 72 hours prior to DNA extraction.

Polypropylene and polyethylene tubes are associated with DNA adsorption. Polyallomer tubes and some specially designed polypropylene tubes have been shown to be appropriate for storing DNA.

“Frost free” freezers are not suitable for the storage of serum/plasma samples. Freeze/thaw cycles allow the temperature of the sample to increase and then drop (is cycled several times per day in this variety of freezer – allowing the sample to refreeze), causing degradation of nucleic acid targets and other analytes.

Specimen labeling and requisition submission for NAT

1. Collection time: The laboratory should determine a mechanism to ensure that the time of collection is accurately recorded.
2. Specimen labeling: Unique ID number – 15-digit Unique Infant code, to be allocated on the following basis:
 - » First three digits: RNA/DNA
 - » Next two digits: state code
 - » Next three digits: district code
 - » Next two digits: testing centre number
 - » Next two digits: year
 - » Next three digits: serial number of the patient at the testing laboratory
3. Written informed consent must be obtained.

Specimen Transport/Shipment

Diagnostic samples are shipped as “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.

Refer to the following for regulated shipping instructions:

- a) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, (World Health Organization)

b) The IATA Dangerous Goods Regulations, (International Air Transport Association)

1. The culture or specimen should be placed in a screw-capped tube or leak-proof container that is clearly labeled.
2. The lid is then sealed with waterproof tape. This tube is wrapped in absorbent packing material and inserted in a secondary shipping tube/ziplock bag that has soft packing in the bottom to protect the specimen tube from breakage.
3. The requisition form or paperwork accompanying the specimen is wrapped around the outside of this secondary container or is written on the outside of the secondary container.
4. The secondary container is then placed in a sturdy shipping container and labeled for mailing and marked with a biosafety notice.
5. Both the shipper and consignee's name, address, and telephone number must be on the outer package and should also be on the inner containers.

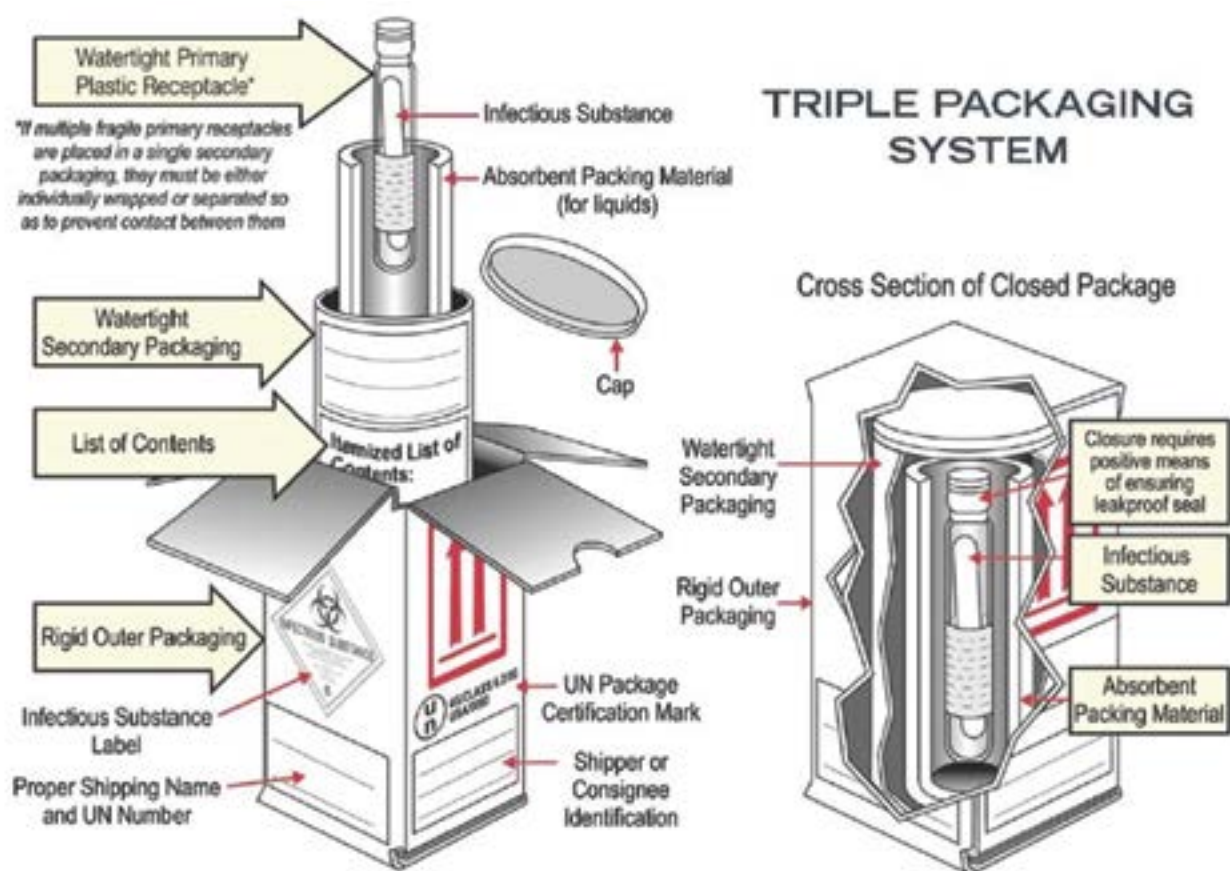


Fig. 27: Packaging and shipping of specimens.

Source: Image courtesy of Centres for Disease Control and Prevention.
<http://www.cdc.gov/vhf/ebola/pdf/ebola-lab-guidance.pdf>.

Unacceptable samples (Specimen rejection criteria)

The following criteria are used to consider a sample is unacceptable and will be rejected, as it may lead to erroneous results.. The laboratory staff will notify the clinician who has requested the tests.

- Incompletely filled or no specimen identify on the request form
- Specimen without accompanying request form
- Inappropriate specimen
- Specimen without any label/ inadequate labeling
- Discrepancy in patient's identity between the request form and specimen label
- Inappropriate specimen containers
- Inappropriate volume of plasma/blood
- Using the wrong collection tube, for example, wrong use of container/preservative/anticoagulant
- Specimens not meeting the stability or storage requirements
- Specimen is haemolysed, lipaemic or contaminated

Specimen referral network is a coordinated system that allows a health facility or laboratory lacking capacity to perform tests to safely send a patient's specimen to another or higher-level laboratory with capacity to perform the requested tests. The aim is safe, efficient handling and analysis of specimens to obtain reliable results without delays to provide optimal care to the patients at the referring facility. Prior to initiating the specimen transport system, workers must be trained on specimen referral and biosafety, and standard transportation containers with packaging be provided.

In the "hub-and-spoke" design/model, the patients' samples (blood, serum, plasma) are shipped/ couriered from "spoke" collection facilities (DH, state) to the core testing laboratory placed at the "hub" (regional/CoE) of the network. The integrated specimen referral and transport system is designed to serve multiple disease programs (such as Hepatitis, HIV, TB etc.) and in order to further improve the delivery of laboratory services, the testing capacities of the specimen transport hubs are being strengthened to allow them to conduct testing (as immunoassays and NAT testing) for multiple health programmes. An electronic reporting system is established to deliver real-time notification of test results to the treating clinician, regional focal person, and the national programme/CoE. In this manner the patient does not move but the specimen moves and test results can be available easily at lesser equipped labs.

CHAPTER



Quality management system

A laboratory consists of numerous processes in which inputs are turned into outputs through one or more process steps. The core process of the laboratory is the primary process consisting of three stages: the pre-analytical stage (the sample is collected, received at the laboratory, registered and processed), the analytical stage (the actual laboratory test is performed and the result is recorded), and the post-analytical stage (the result is authorized, reported and archived and the sample is discarded/archived).

A Quality Management System (QMS) affects each single process of the laboratory and consists of several layers. A QMS can be described as a set of building blocks, called quality system essentials (QSEs) needed to control, assure and manage the quality of the laboratory's processes. Quality can be assured by ensuring that all the processes related to the QSEs perform correctly.



The laboratory should have a written quality management/quality control (QM/QC) programme. The programme must ensure quality throughout the pre-analytic, analytic, and post-analytic (reporting) phases of testing, including patient identification and preparation; specimen collection, identification, preservation, transportation, and processing; and accurate, timely result reporting. The programme must be capable of detecting problems in the laboratory's systems, and identifying opportunities for system improvement. The laboratory must be able to develop plans of corrective action based on data from its QM system.

Specimen collection and handling

Specimen collection manual: There are written procedures describing methods for patient identification, patient preparation, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing, consistent with good laboratory practice.

There should be written criteria for the rejection of unacceptable specimens, instructions for the special handling of sub-optimal specimens, and records of disposition of all unacceptable specimens in the patient report and in the quality management records. If there is a problem with a specimen, there must be a mechanism to notify clinical personnel responsible for patient care. If the treating physician desires the result, then the laboratory must note the condition of the specimen on the report.

Standard operating procedure (SOP) manual: The SOP should be used by personnel at the workbench and must include the following elements, when applicable to the test procedure:

1. Principle and clinical significance
2. Requirements for patient preparation; specimen collection, labeling, storage, preservation, transportation, processing, and referral; and criteria for specimen acceptability and rejection
3. Step-by-step performance of the procedure, including test calculations and interpretation of results
4. Preparation of, solutions, calibrators, controls, reagents and other materials used in testing
5. Calibration and calibration verification procedures
6. The analytic measurement range for test results for the test system, if applicable. The analytic measurement range may not apply to qualitative or semi-quantitative tests.
7. Quality Control (QC) procedures
8. Corrective action to take when calibration or quality control results fail to meet the laboratory's criteria for acceptability
9. Limitations in the test methodology, including interfering substances
10. Reference intervals (normal values)
11. Critical or urgent test results
12. The laboratory's system for entering results in the patient record and reporting patient results including, when appropriate, the procedure for reporting critical results
13. Pertinent literature references
14. Description of the course of action to take if a test system becomes inoperable

Electronic (computerized) manuals may also be used. There is no requirement for paper copies to be available for the routine operation of the laboratory, so long as the electronic versions are readily available to all personnel. However, procedures must be available to laboratory personnel when the electronic versions are inaccessible (e.g. during laboratory information system or network downtime); Electronic versions of procedures must be subjected to proper document control (i.e. only authorized persons may make changes, changes are dated/signed (manual or electronic), and there are records of the review.

Inventory/Commodities/Reagents

Reagents, controls, calibrators and test kits must be stored and handled as recommended by the manufacturer to prevent environmentally induced alterations that could affect reagent stability and test performance. If there are multiple components of a reagent kit, the laboratory must use components of reagent kits only within the kit lot unless otherwise specified by the manufacturer.

If the manufacturer defines a required storage temperature range, the temperature of storage areas must be monitored and recorded daily (at least twice in a day). The identity of the individual recording the temperature(s) must be recorded (initials of the individual are adequate). Prepared reagents must be properly stored, mixed, when appropriate, and discarded when stability parameters are exceeded.

If the laboratory identifies a problem with a reagent that was used for patient testing (e.g. expired vial or reagent subjected to unacceptable storage conditions, etc.), the laboratory must evaluate the potential impact on patient test results and maintain records of the evaluation and actions taken. If ambient storage temperature is indicated, there must be records that the defined ambient temperature is maintained and corrective action taken when tolerance limits are exceeded.

Records of the commodities (reagents/test kits, calibrators, controls, chemicals, and consumables must be maintained in a log (paper or electronic), with the following elements:

1. Content and quantity, concentration or titer
2. Storage requirements
3. Date received, prepared, or reconstituted by laboratory
4. Expiration date
5. Batch/Lot number

New reagent lots and shipments must be checked against old reagent lots or with suitable reference material before or concurrently with being placed in service. The purpose of this check is to confirm that the use of new reagent lots and shipments do not affect patient results.

For qualitative tests, minimum cross-checking includes retesting at least one positive and negative sample with known reactivity against the new reagent lot. A weakly positive sample should also be used in systems where patient results are reported in that method. Examples of suitable reference materials for qualitative tests include:

1. Positive and negative patient samples tested on a previous lot;
2. Previously tested proficiency testing materials;
3. External QC materials tested on the previous lot.

For quantitative tests, patient specimens should be used to compare a new lot against the old lot. Manufactured materials, such as proficiency testing (PT) or QC materials may be affected by matrix interference between different reagent lots, even if results show no change following a reagent lot change. The use of patient samples confirms the absence of matrix interference. Other than patient samples, the following materials may be used:

1. Reference materials or QC products provided by the manufacturer with method specific and reagent lot specific target values;
2. Proficiency testing materials with peer group established means;
3. QC materials with peer group established means based on inter-laboratory comparison that is method specific and includes data from other laboratories;
4. Third party general purpose reference materials. If the material is referenced to, in the package insert, it has to be run along with the patient specimens

5. QC material used to test the current lot is adequate alone to check a new shipment of the same reagent lot, as there should be no change in potential matrix interactions between the QC material and different shipments of the same lot number of reagents.

Instruments and equipment

Instrument/equipment performance verification: The performance of all instruments and equipment should be verified upon installation and after major maintenance or service to ensure that they run according to expectations.

There must be written procedures for start-up, operation and shutdown of instruments and equipment, as applicable and should include a procedure for emergency shutdown and for handling workload during instrument downtime. These procedures must readily be available to the operator in the immediate vicinity of the instrument. Instructions are provided for minor troubleshooting and repairs of instruments (such as manufacturer's service manual).

Appropriate maintenance and function checks should be performed and records maintained for all instruments (e.g. analysers) and equipment (e.g. centrifuges) following a defined schedule, at least as frequent as specified by the manufacturer. These may include (but are not limited to) cleaning, electronic, mechanical and operational checks. Function checks should be designed to detect drift, instability, or malfunction, before the problem is allowed to affect test results. The defined tolerance limits must follow the manufacturer's specified limits. Function checks must be within the defined tolerance limits prior to use for testing patient samples. For equipment that has no standard frequency or requirement for maintenance and function checks, each laboratory should establish a schedule and procedure that reasonably reflects the workload and specifications of its equipment.

Calibration is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures are usually specified in the manufacturer's instructions, but may also be established by the laboratory.

Calibration verification denotes the process of confirming that the current calibration settings for each analyte remain valid for a test system. If the manufacturer provides a calibration validation or verification process, it should be followed. Other techniques include: 1) assay of the current method calibration materials as unknown specimens, and determination that the correct target values are recovered, and 2) assay of matrix-appropriate materials with target values that are specific for the test system.

The laboratory must follow the manufacturer's instructions for calibration, calibration verification, and related functions. Calibration must be performed, at minimum, following the manufacturer's instructions, including the number, type, and concentration of calibration materials and criteria for acceptable performance.

Materials for calibration verification must have a matrix appropriate for the clinical specimens assayed by that method, and target values appropriate for the measurement system. Suitable materials include:

1. Calibrators used to calibrate the analytical system
2. Materials provided by the vendor for the purpose of calibration verification
3. Previously tested unaltered patient specimens
4. Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method
5. Proficiency testing material or PT validated material with matrix characteristics and target values appropriate for the method

In general, routine control materials are not suitable for calibration verification, except in situations where the material is specifically designated by the test manufacturer as suitable for verification of the calibration process.

Required frequency of calibration verification

The Laboratory must calibrate a test system when it is first placed in service and perform calibration verification as follows:

1. A change of reagent lots
2. If QC materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem
3. After major maintenance or service
4. When recommended by the manufacturer
5. At least every six months

The system must be recalibrated when calibration verification fails to meet the established criteria of the laboratory.

Temperature-dependent equipment (e.g. refrigerators, freezers, incubators) containing reagents and/or patient specimens must be monitored daily, as equipment failures could affect accuracy of patient test results. Items such as water baths and heat blocks used for procedures need only be checked on days of patient testing. If specific instruments, equipment, kits, or supplies have specified ambient temperature ranges for proper operation or use, there must be records that the specified ambient temperature is maintained and corrective action taken when tolerance limits are exceeded. Acceptable ranges must be defined for all temperature-dependent equipment and environments in accordance with the manufacturer's instructions. There must be evidence of corrective action taken if acceptable temperature ranges are exceeded. Stored reagents, controls, calibrators etc. must be checked to confirm the accuracy or quality of the material before use and records maintained.

Appropriate thermometric standard device of known accuracy (certified to meet NPL/International Standards or traceable to NPL/international Standards) if used must be recalibrated, recertified, or replaced prior to the date of expiration of the guarantee of calibration. All non-certified thermometers that are in use must be checked against a certified calibrated thermometric standard device before initial use and as defined by laboratory policy (at least annually). If digital or other displays of temperatures on equipment are used for daily monitoring, the laboratory must verify that the readout is accurate. The display must be checked initially and periodically thereafter as per the manufacturer's instructions.

Automatic and adjustable pipetting devices must be checked at defined intervals (at least annually) for accuracy and reproducibility, and results recorded. Pipette checks may be done gravimetrically. This consists of transferring a number of measured samples of water from the pipette to a balance. Each weight is recorded, the weights are converted to volumes, and then means (for accuracy) and standard deviation/coefficient of variation (SD/CV - for imprecision) are calculated.

Spectrophotometer including ELISA plate readers wavelength calibration, absorbance and linearity must be checked at least annually (or as often as specified by the manufacturer), with appropriate solutions, filters or emission line source lamps, and the results recorded. For procedures using calibration curves, all the curves are rerun at defined intervals and/or verified after servicing or recalibration of instruments.

Instrument/Equipment records: Instrument and equipment maintenance, function check, performance verification, and service and repair records (or copies) must be available to, and usable by, the technical staff operating the equipment to detect trends or malfunctions. Retention period of equipment records is for at least five years after the equipment has been decommissioned/replaced.

Quality control

Quality Control (QC) in the laboratory is a procedure that verifies the attainment of the intended quality of results. QC materials are processed similar to a patient sample to monitor the ongoing performance of the entire analytic process. Control specimens are tested in the same manner, at the time of and by the same personnel as patient samples. It is implicit in quality control that patient test results will not be reported when controls yield unacceptable results.

The laboratory must define the number and type of quality control used and the frequency of testing in its quality control procedure. Control testing is not required on days when patient testing is not performed.

Controls must be run prior to reporting patient results, after a change of analytically critical reagents, major preventive maintenance, or change of a critical instrument component. Daily quality control must be run as follows:

1. Quantitative tests – two controls at different concentrations at least daily, with each run (of which one must be a low positive).
2. Qualitative tests – a negative control and a positive control at least daily and with each run. For RDT they are run with each new kit or may be run every week if the kit is used beyond a week.

For immunoassays, appropriate controls must be used in each run or batch of samples. Appropriate controls for screening assays should consist of at least one positive control. If a single calibrator is used, the control must be at or near the declared cutoff value(s). Controls must be run with each batch to verify the calibration.

Controls should verify assay performance at relevant decision points. The selection of these points may be based on clinical or analytical criteria. If an internal quality control process (e.g. electronic/procedural/built-in) is used then an external control material must be used to meet daily quality control requirements, as per a documented individualized quality control plan (QCP) approved by the laboratory in charge. Acceptability limits must be defined for all control materials and standards. These controls must be appropriate for the range of sensitivities tested and should, ideally, focus on result ranges that are near clinical decision points.

For quantitative tests, a valid acceptable range must be established or verified for each lot of control material. For unassayed controls, the laboratory must establish a valid acceptable range by repetitive analysis in runs that include previously tested control material. The laboratory must use statistical methods such as calculating SD and CV monthly to detect problems, evaluate analytic imprecision/ variance and to monitor trends over time in numeric QC data (quantitative data should be plotted as Levey-Jennings charts. Testing and supervisory staff must review quality control data on days when controls are run prior to reporting patient results. The laboratory in charge must review QC data for omissions, outliers, trends and their follow-up, at least monthly, as specified in the laboratory QC policy. There must be evidence of corrective action when control results exceed defined acceptability limits.

For single use test devices, appropriate QC materials (both positive and negative) are analyzed with each:

- Change of reagent lot number
- New shipment
- Change in storage conditions
- Replacement of a critical part or following any major preventive maintenance in cartridge based equipment

Controls for molecular testing must assess adequacy of extraction and amplification, e.g., positive and negative controls that go through the entire testing process.

1. An extraction control must be used for each run (positive controls fulfill this requirement).
2. If the samples from an extraction batch are tested over multiple amplification runs, each amplification run (as defined by the laboratory) must have its own amplification control. A single extraction control need only be tested in one of the amplification runs.

Qualitative cut-off: For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially when the test is placed in service, and verified every six months thereafter. If the value of a calibrator or calibration verification material is near that of the cut-off, then the process of calibration or calibration verification is satisfied. Verification of the cut-off should also be performed at changes of lots of analytically critical reagents; after replacement of major instrument components, after major service to the instrument, and when QC materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of accessing and correcting unacceptable control values fail to identify and correct the problem. Appropriate materials for establishment and verification of the cut-off are identical to those recommended for calibration verification. A low-positive control that is close to the limit of detection (cut off) can satisfy this requirement, but must be external to the kit (e.g. weak-positive patient sample or reference material prepared in appropriate matrix).

Quality Control materials may be procured commercially or prepared in-house. Positive/Reactive, weakly reactive and negative/nonreactive controls are all used in test systems. In general, calibrators should not be used as QC materials. If calibrators are used as controls, then different preparations should be used for these two functions. If a calibrator obtained from an outside supplier is used as a control, it must be a different lot number from that used to calibrate the method.

The results of all controls must be recorded. When a QC result is unacceptable, patient test results obtained since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include re-testing patient samples, depending on the circumstances. Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results.

The laboratory must have a written procedure for investigation and corrective action when data from QC precision statistics change significantly from previous data. Records of investigation and corrective actions taken must be maintained.

Quality assurance

External assessment of quality assurance is an essential component of each clinical laboratory's overall quality assurance programme.

Laboratories must participate in the appropriate required proficiency testing (PT) / EQA (when available) for all patient tests. The laboratory should have written procedures for proficiency testing including procedures for the proper handling, analysis, review and reporting of proficiency testing materials. There must be written procedure(s) for investigation and correction of problems that are identified by unacceptable proficiency testing results. The laboratory should also have procedure(s) for investigation of results that, although acceptable, show bias or trends suggesting a problem. The laboratory must integrate all proficiency testing samples within the routine laboratory workload, and those samples are analysed by personnel who routinely test patient/client samples, using the same primary method systems as for patient/client/donor samples. If the laboratory uses multiple methods for an analyte, proficiency samples should be analysed by the primary method.

For tests for which PT/EQA is not available, the laboratory must implement, at least semi-annually an alternative assessment procedure for the affected analytes. (Split sample analysis with reference or other laboratories, split samples with an established in-house method, assayed materials, clinical validation by chart review, or other suitable and documented means.) It is the responsibility of the laboratory in-charge to define such alternative assessment procedures and the criteria for successful performance in accordance with good clinical and scientific laboratory practice.

In summary, for the validity, reproducibility, and reliability of an assay, blank reagents, cut-off, calibrators, and controls (high positive, low positive, and negative) must be tested in parallel with patient samples with every assay. Most commercially available kits provide these reagents; however, additional controls may be tested. Additional external or in-house-pooled standard controls with known titers add to the rigorousness and validity of the testing conditions, method and test results. In order to ensure the validity of in-house controls, they should be tested over a period of several assay runs to establish a laboratory reference range that could be used to validate a test run. Every kit has its own reference range for all of its blank reagents, cut-off calibrators, and controls. Patient test results should not be released until the blank reagent, control, and cutoff calibrator parameters are within acceptable ranges. If any parameters are outside of the acceptable range, the test run should be deemed invalid and repeated. Each new lot of kits should be tested (in tandem) and validated before routine use. Periodic review of test results will help to identify, investigate, and troubleshoot any testing anomaly or unusual result patterns.

Reporting of results

The laboratory must report reference (normal) intervals or interpretations with patient results, where such exist. This is essential to allow proper interpretation of patient data. Age- and/or gender-specific reference ranges (normal values) or interpretive ranges must be reported with patient test results, as applicable. In addition, the use of high and low flags is recommended.

The laboratory must have written procedures for immediate notification of a clinician when results of designated tests exceed established "critical" values that are important for prompt patient management decisions. Critical results with their values should be defined by the laboratory in charge, in consultation with the clinicians served. Records of notification should be maintained. These records must include: date, time, responsible laboratory individual, person notified and test results. Any problem encountered in accomplishing this task should be investigated to prevent recurrence.

In summary, QMS requires laboratories to:

1. Maintain optimal patient specimen integrity and identification throughout testing process
2. Specify responsibilities and qualification for personnel performing the test
3. Establish and follow written Quality Control (QC) procedures
4. Have comprehensive Quality Assurance (QA) programme in place
5. Participate in proficiency testing programme for each analyte, or test

REFERENCES

1. CDC Morbidity Mortality Weekly Report, Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians. Weekly, May 10, 2013 / 62(18); 362-365. <http://www.cdc.gov/mmwr>).
2. Clin. Microbiol. Rev. January 2014 vol. 27 no. 1 139-165. Host Immune Status and Response to Hepatitis E Virus Infection (American Society for Microbiology) Lisa J. Krain et al. Clin. Microbiol. Rev. 2014;27:139-165
3. Clinical relevance of hepatitis B virus variants Shan Gao, Zhong-Ping Duan, and Carla S Coffin. World Journal Hepatol. 2015 May 18; 7(8): 1086–1096. Published online 2015 May 18. doi: 10.4254/wjh.v7.i8.1086
4. CLSI guidelines – Quality management system: A model for laboratory services; Approved guideline (Fourth edition)
5. Detecting Hepatitis B Surface Antigen Mutants; Paul F. Coleman; Clinical implications of hepatitis B virus mutations: Recent advances. Ivana Lazarevic. World Journal Gastroenterol. 2014 June 28; 20(24): 7653–7664. Published online 2014 Jun 28. doi: 10.3748/wjg.v20.i24.7653
6. Diagnosis and Management of Hepatitis C Virus Infection. Ronita Mukherjee, Andrew Burns, Diane Rodden, et al. Journal of Laboratory Automation 2015, Vol. 20(5) 519– 538
7. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection* (*European Association for the Study of the Liver) Journal of Hepatology 2017 vol. 67 j 370–398
8. EASL Recommendations on Treatment of Hepatitis C 2018* (*European Association for the Study of the Liver) journal of hepatology (2018), <https://doi.org/10.1016/j.jhep.2018.03.026>
9. Expanded Classification of Hepatitis C Virus Into 7 Genotypes and 67 Subtypes: Updated Criteria and Genotype Assignment Web Resource Donald B Smith, Jens Bukh, Carla Kuiken, A Scott Muerhoff, Charles M Rice, Jack T Stapleton, and Peter Simmonds Emerg Microbes Infect. 2017 Nov; 6(11): e95. Published online 2017 Nov 1. doi: 10.1038/emi.2017.77
10. Global health sector strategy on viral hepatitis 2016-2021, Authors: WHO Publication date: June 2016.
11. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection Authors: WHO Publication date: March 2015
12. Guidelines for the screening care and treatment of persons with chronic hepatitis C infection. Updated version, April 2016, WHO
13. Guidelines on hepatitis B and C testing Authors: WHO, Publication date: February 2017
14. Harrison's Principles of Internal Medicine, Twentieth Edition by J. Larry Jameson), Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser , Dan L. Longo , Joseph Loscalzo. New York: McGraw Hill Education, 2018.
15. Hepatitis Delta: Epidemiology, Diagnosis and Management 36 Years after Discovery Mazen Nouredin and Robert Gish, Current Gastroenterology Report 2014; 16(1): 365. Published online 2013 Nov 30.
16. ISO 15189:2012 Medical laboratories - Requirements for quality and competence
17. Jawetz, Melnick, & Adelberg's. Medical Microbiology. 27th edition by Karen C. Carroll, Jeffery A. Hobden, Steve Miller, Timothy A. Mietzner, Barbara Detrick, Thomas G. Mitchell et al. New York: McGraw Hill Education, 2016.
18. Kumar and Clark's Clinical Medicine 9th edition, edited by Parveen Kumar, Michael Clark. Elsevier Edinburgh 2017
19. Lateral Flow Immunoassay Raphael C. Wong l Harley Y. Tse Editors e-ISBN: 978-1-59745-240-3
20. Mandell, Douglas, and Bennett's Infectious Disease- Essentials by John E. Bennett, Raphael Dolin, Martin J. Blaser. 2017 by Elsevier Inc..
21. NABL 112: Specific criteria for accreditation of medical laboratories issue number 4 (May, 2016)
22. World Health Organization media centre. Hepatitis A fact sheet in world health organization media centre. [Online] 2016. [Http://www.who.int/mediacentre/](http://www.who.int/mediacentre/).

Annexure 1: Consent form

INDIVIDUAL'S CONSENT FORM FOR TESTING AND MANAGEMENT OF VIRAL HEPATITIS

I, _____ (full name), daughter/son of _____
_____ (full name) age _____ resident of (address) _____
_____ have read/have been read over and explained (circle appropriate) the accompanying guidance and have understood the information provided to me related to the investigations and proposed management required (if available)

I understand that the purpose of these tests is to:

- Establish my Viral Hepatitis status,
- Evaluate the presence of liver disease which may be associated with Hepatitis infection.
- I can allow the program to archive my specimen for further molecular testing related to viral hepatitis only in the interest of public health, provided that any information/data/detail relating to or emanating from my molecular sample shall not be divulged to any third party under any circumstances. A breach of this condition shall automatically forfeit my consent and the program's right to retain such information and shall further render them liable to penal action and compensation.

I understand that if a diagnosis of Chronic Hepatitis B/C is confirmed, I will be offered treatment as per the provisions in the initiative. I give my consent to the proposed management offered by the initiative subject to strict protection of my information.

Patient's Signature: _____ DATE: _____

Staff member name obtaining consent: _____

Staff signature: _____ DATE: _____

Annexure 2: Parameters in the test kit for quality assured test result

It is important to determine the extent to which tests are able to identify the likely presence or absence of a disease/condition of interest so that their findings encourage appropriate decision making. Adequacy and usefulness of screening tests are determined and described by the sensitivity, specificity, and predictive values of these tests. All four metrics should be regarded as important when describing and assessing a screening test's adequacy and usefulness.

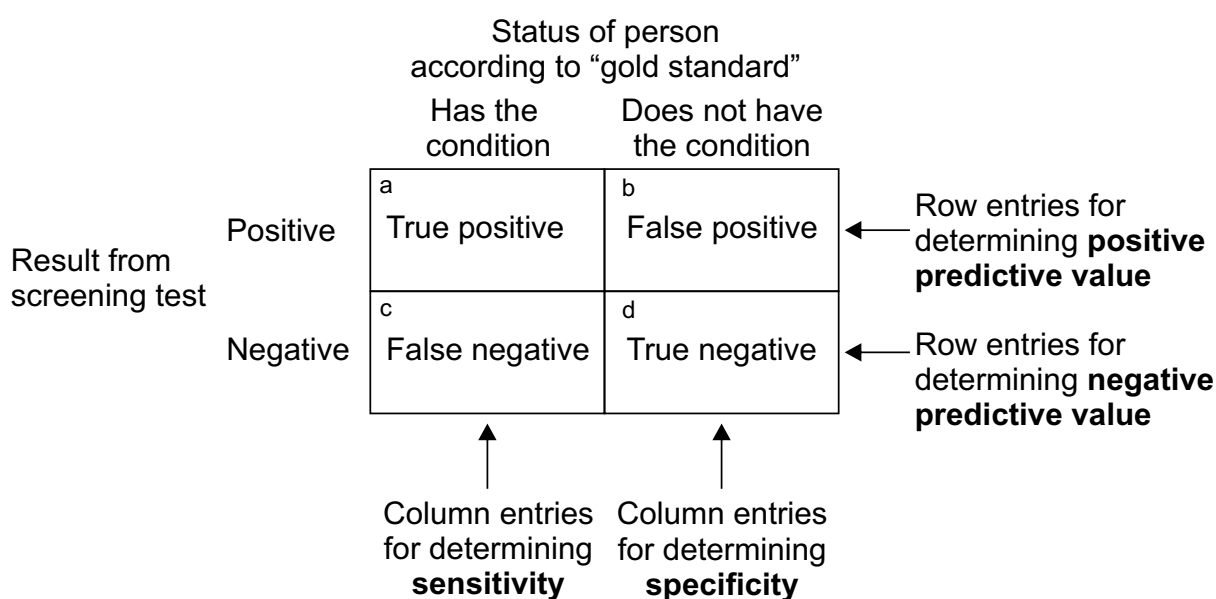


Fig. A1: Diagram demonstrating the basis for deriving sensitivity, specificity, and positive and negative predictive values.

$$\text{Sensitivity} = [a/(a+c)] \times 100$$

$$\text{Specificity} = [d/(b+d)] \times 100$$

$$\text{Positive predictive value (PPV)} = [a/(a+b)] \times 100$$

$$\text{Negative predictive value (NPV)} = [d/(c+d)] \times 100$$

When kit inserts refer to sensitivity, specificity, and predictive values to describe the characteristics of a screening test, they are cited as percentages or as decimal fractions, and preferably with accompanying 95% confidence interval.

Sensitivity and specificity indicate the concordance of a test/effectiveness of a test with respect to a chosen referent, while PPV and NPV, respectively, indicate the likelihood that a test can successfully identify whether people do or do not have a target condition, based on their test results. Predictive values are more relevant than sensitivity and specificity when people are being screened.

List of Contributors

1. Dr Lalit Dar, AIIMS, Delhi
2. Dr Priya Abraham, CMC, Vellore
3. Dr Ekta Gupta, ILBS, Delhi
4. Dr Srikala Baliga, Kasturba Medical College, Mangalore
5. Dr Sujata Baveja, Lok Manya Tilak Municipal Medical College, Mumbai
6. Dr Thasneem Banu S., Madras Medical College, Chennai
7. Dr Rekha Jain, Microbiologist, Mumbai
8. Dr Margaret Yhome, Naga Hospital Authority, Kohima
9. Dr Anita Desai, National Institute of Mental Health & Neurosciences, Bangalore
10. Dr Praveen Malhotra, PGIMS, Rohtak
11. Mr Mehmood Pracha, Sr. Advocate
12. Dr Sandhya Kabra, NCDC, Delhi
13. Dr Partha Rakshit, NCDC, Delhi
14. Dr Preeti Madan, EIS Officer, NCDC, Delhi
15. Dr Vimlesh Purohit, NPO, WHO Country Office, India
16. Dr Hema Gogia, NCDC, Delhi
17. Dr Daniel Garcia, CDC, India
18. Dr Mayank Dwivedi, CDC, India
19. Dr Indranil Roy, CDC, India
20. Mr Sella Senthila, CDSCO, India
21. Dr Sujeet Sinha, NHSRC

