LABORATORY DIAGNOSIS

VIRAL HEPATITIS & COVID-19

ilbs

WHOCC on Viral Hepatitis and Liver Diseases

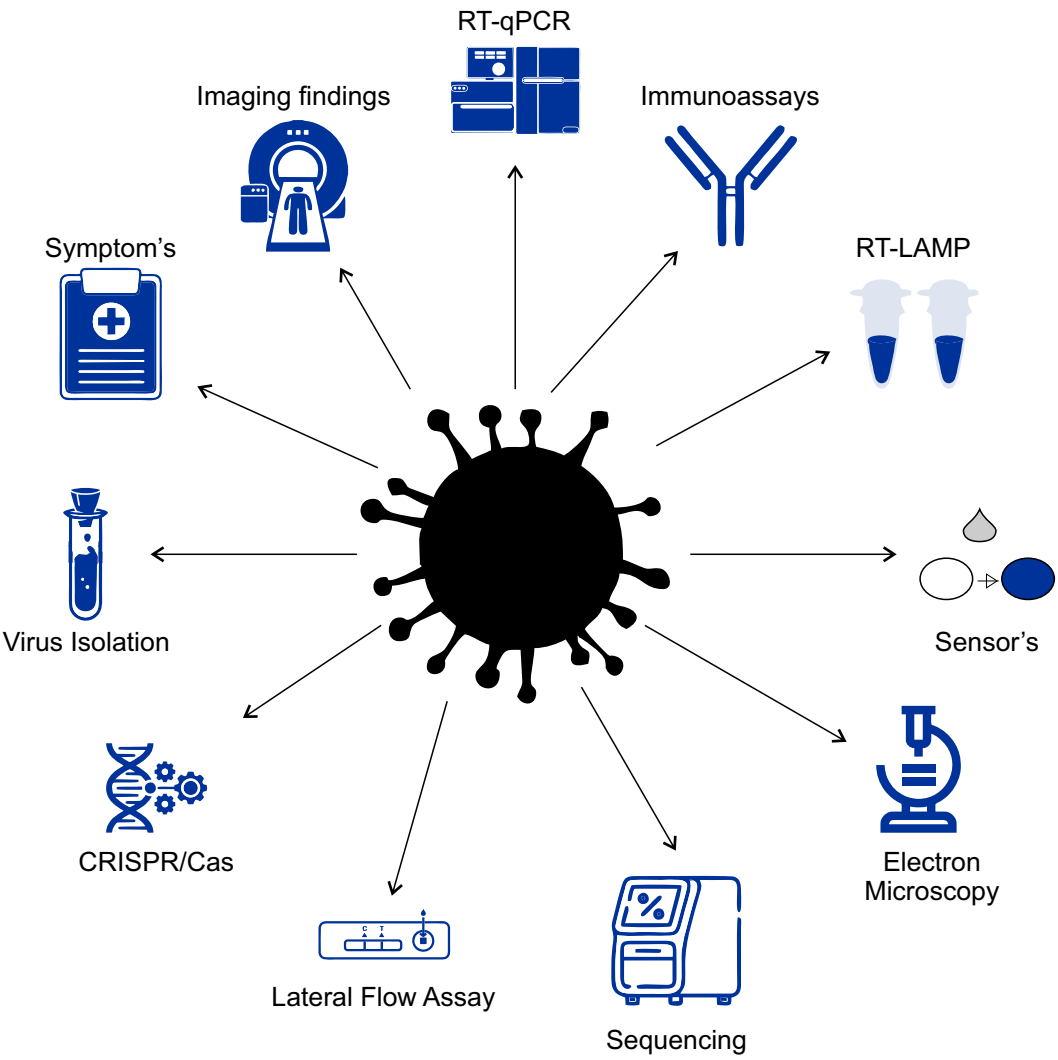
Volume 1

Medical manual for healthcare professionals in laboratory services

An educational initiative by Project PRAKASH, ILBS

LABORATORY DIAGNOSIS

OF VIRAL HEPATITIS & COVID-19



Medical manual for healthcare professionals in laboratory services

LABORATORY DIAGNOSIS OF VIRAL HEPATITIS & COVID-19

Training Organiser: Institute of Liver & Biliary Sciences, New Delhi | www.ilbs.in

Under the Project
PRAKASH: PRogrammed Approach to Knowledge And Sensitization on Hepatitis

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FOREWORD

It gives me immense pleasure to know that the laboratory professionals from across the country would join Institute of Liver and Biliary Sciences (ILBS) to be trained in "Laboratory Diagnosis of Viral Hepatitis & COVID-19". ILBS was started with the vision to not only be a centre of Excellence for care of patients with liver diseases, but also, to be the destination of choice for bright doctors, research scholars and paramedical professionals who intend to further their knowledge and skills.

ILBS is pleased to welcome each and every one of you to this training program on viral hepatitis & COVID-19 diagnosis. We intend to share with you standard protocols as well as new developments in screening and diagnosis of viral hepatitis and COVID-19. We hope that through this course we can help build a cadre of laboratory professionals in India who are trained and may lead the way in the National Program for Control of Viral Hepatitis launched by the Government of India.

The countrywide efforts are being made to build capacity of healthcare workers in diagnosis and management of COVID-19. ILBS is committed to aid in achieving global standards in care of managing COVID-19 pandemic and prepare the existing healthcare workforce through knowledge sharing on the ever-evolving protocols on COVID-19. Development and dissemination of this module is just one step forward in this regard.

I would like to put on record my appreciation for the ILBS team of Project PRAKASH for their untiring efforts. I would also like to congratulate the Department of Clinical Virology for putting up a wonderful course material which would serve as a ready reckoner for the learners.

I do hope that you will enjoy the learning process through Project PRAKASH; and times to come, become trainers to train others and spread the light of knowledge further down the public health system across the nation.

Dr. S.K Sarin
Vice Chancellor, ILBS

PREFACE

Liver diseases are increasing and the full impact has not yet been felt; it was once a minority killer; however liver diseases are now becoming common and are the fifth biggest killer. Viral Hepatitis is a large problem and is on the rising trend with more than 300 million people affected with viral hepatitis B & C globally.

Despite the high prevalence of HBV & HCV, and the availability of effective curative treatment for HCV infection, as well as long-term suppressive antiviral treatment for HBV; most people infected with HBV or HCV globally have never been tested and so remain unaware of their infection. Key reasons for this current very low rate of hepatitis testing in LMICs include: limited laboratory capacity and access to reliable, HCV diagnostics, and lack of testing guidance to the healthcare providers working in the laboratories.

The outburst of COVID-19 has engrossed global consideration, the dramatic spread of COVID-19 has disrupted lives, livelihoods, communities and businesses worldwide. COVID-19 is a grave public health issue affecting millions of people throughout the globe with considerable morbidity and mortality within a short span. Organisations and people around the world are coming together to find ways to minimise the disastrous impact of COVID-19.

The main objective of the program is to inform feasibility of potential recommendations on testing approaches (who and where to test) and how to test (selection of assays) in the viral hepatitis and COVID-19 testing guidelines, and also to assess key perceived barriers/challenges and strategies to address these and so guide implementation of testing and treatment services.

This module on viral hepatitis and COVID-19 for laboratory professionals will describe the knowledge and skills that are required to deliver point of care diagnostic support to patients. The module focuses on patient centred outcomes and will be an indispensable tool for those commissioning, managing and developing the workforce. This program is intended to be used with other local policies and pathways around the scope of practice undertaken.

The program may also be useful for those health care professionals (HCPs) who are working in department of Virology, Microbiology, Pathology and Blood Banks and may be useful as part of their professional learning and development. The document can also be used by students to improve their knowledge, understanding and skill acquisition in laboratory diagnosis of viral hepatitis and COVID-19.

MESSAGE

Department of Clinical Virology at the Institute of Liver & Biliary Sciences, provides full range of diagnostic services for the detection and monitoring of infection caused by hepatitis viruses (A, B, C, D and E). The laboratory takes pride in performing several diagnostic assays for the first time in the country, like drug resistance testing in HCV, testing for occult HBV infection and cccDNA in biopsy samples; screening for various mutations in HBV; HEV RNA Quantification and genotyping. Virology laboratory has been made as one of the testing laboratories by Central Drugs Standard Control Organization for the validation of diagnostic kits. Virology laboratory at ILBS is also nominated by ICMR as one of the Apex viral hepatitis laboratories in the country to look after external quality assurance (EQAS) in various labs across the nation.

The lab has been active in initiating teaching modules and programs in clinical virology. NABL accreditation was awarded to the lab in 2013 and is the one of the very few NABL accredited molecular virology labs in India. The Virology Lab has made an exhaustive protocol for Needle Stick Injuries, Post exposure prophylaxis for healthcare professionals. As a part of World Health Organization Collaborating Center (WHO-CC) in Viral Hepatitis, laboratory is actively involved in imparting capacity building training to microbiologists and other laboratory staff across the country.

Virology laboratory at ILBS was one of the first in Delhi state to be considered by ICMR as COVID-19 testing lab. We are also ICMR site for kit validation for COVID-19. 5th and 6th sero surveys for COVID-19 antibody prevalence in Delhi dtate has been done exclusively by the department. We are also one the sites for performance of whole genome sequencing for SARS CoV2 for variant detection.

I hope this training program will enrich healthcare professionals in the laboratory diagnosis of viral hepatitis and COVID-19 infection.

Dr.Ekta Gupta,
Professor & Head, Clinical Virology
Nodal Officer, WHO CC
Institute of Liver & Biliary Science

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ACKNOWLEDGEMENTS

The outcome of this program was possible by the guidance and support from many people. We are incredibly privileged to have got this along with the successful implementation of the program. It required a lot of effort from each individual involved and we would like to thank each one of them.

We would first like to thank **Dr. S. K. Sarin**, Vice Chancellor, ILBS whose expertise was invaluable in formulating and implementing this project and its methodology. Your insightful feedback pushed us to sharpen our thinking and brought our work to a higher level.

We are particularly grateful to **Dr. Anil Agarwal**, Head, Administration, ILBS for his patient support and guidance throughout the tenure of the project.

We would like to acknowledge **Cipla Foundation** for providing financial support to carry out this project. Thank you for this wonderful collaboration.

We are grateful to **Dr. Ekta Gupta**, Professor, Virology, ILBS for her continuous guidance and support in formulating training program for laboratory professionals.

We would also like to thank all the **speakers** for delivering lectures and sharing knowledge and learnings with the trainees. The preparation of this module consumed huge amount of work, research and dedication which would not have been possible without extensive contribution from **Dr. Reshu Agarwal**, Associate Professor, Virology, ILBS. Therefore, we would like to extend our sincere gratitude to her.

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ABBREVIATIONS & ACRONYMS

ACLF	Acute on Chronic Liver Failure
ALT	Alanine Transaminase
Anti-HAV	Antibody to Hepatitis A Virus
Anti-HBc	Hepatitis B Core Antibodies
Anti-HBe	Hepatitis B e Antibody
Anti-HBs	Hepatitis B Surface Antibody
Anti-HCV	Antibody to Hepatitis C Virus
Anti-HDV	Antibody to Hepatitis D Virus
Anti-HEV	Antibody to Hepatitis E Virus
AST	Aspartate Aminotransferase
AVH	Acute Viral Hepatitis
BMW	Bio-Medical Waste
BSCs	Bio-Safety Cabinets
BSL	Bio-Safety Level
CBWTF	Common Bio-Medical Waste Treatment and Disposal Facility
cccDNA	Covalently Closed Circular Deoxyribonucleic Acid
cDNA	Complementary Deoxyribonucleic Acid
CHB	Chronic Hepatitis B
CLIA	Chemiluminescence Immunoassay Analyzer
CMA	Chemiluminescent Microparticle Immunoassay
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic Acid

ECLIA	Electrochemiluminescence Immunoassay Analyzer
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immuno Sorbent Assay
EQA	External Quality Assurance
HAV	Hepatitis A Virus
HBeAg	Hepatitis B e Antigen
HBIg	Hepatitis B Immune Globulin
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepato-Cellular Carcinoma
HCP	Healthcare Personnel/Professional
HCV	Hepatitis C Virus
HCW	Healthcare Worker
HDAg	Hepatitis D Antigen
HDV	Hepatitis D Virus
HE	Hemagglitinin Esterase
HEPA	High-Efficiency Particulate Air
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
ICMR	Indian Council of Medical Research
IDP	Internally Displaced Populations
IgG	Immunoglobulin G
IgM	Immunoglobulin M

ILBS	Institute of Liver & Biliary Sciences
ILI	Influenza Like Illness
IM	Intramuscular
INR	International Normalized Ratio
IQC	Internal Quality Control
IV	Intravenous
LFIA	Lateral Flow Immunoassays
LOD	Limit of Detection
MERS	Middle East Respiratory Syndrome
MSM	Men Who Have Sex with Men
NAAT	Nucleic Acid Amplification Test
NABL	National Accreditation Board for Testing & Calibration Laboratories
NS	Non-structural Protein
NSI	Needle Stick Injury
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PPE	Personnel Protective Equipment
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
RBD	Receptor Binding Domain
RdRp	RNA-Dependent RNA Polymerase
RDTs	Rapid Diagnostic Tests

RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOPs	Standard Operating Procedures
SPCB	State Pollution Control Board
STD	Sexually Transmitted Disease
STIs	Sexually Transmitted Infections
ULBs	Urban Local Bodies
VTM	Viral Transport Media
WHO	World Health Organisation
WHOCC	World Health Organisation Collaborative Center

ILBS

Institute of Liver & Biliary Sciences (ILBS) was started in 2010 as an autonomous institution under Government of National Capital Territory of Delhi (GNCTD) as a teaching hospital of liver and biliary diseases.

The vision of the institute is:

ILBS is committed to the highest levels of patient satisfaction, healthcare, staff and patient safety through continual improvement by ensuring:

- Evidence-based clinical practices of highest standard.
- Transparent management processes, facilitating patient satisfaction & ensuring dignity and rights of patients.
- Safe and conducive work environment for staff, and
- Establishing a dedicated centre of excellence in healthcare, teaching/training & research in the field of liver & biliary diseases.

The mission of the institute is:

To develop a facility with international standards, which could provide a comprehensive and most modern set up for the diagnosis and treatment, an advanced centre for dedicated research and resource for advanced training in the field of liver diseases, including liver transplantation, gall bladder and biliary diseases and allied specialties.

To serve as a torch-bearer model of health care in the country by amalgamating the skills and structure of academic universities, clinical and research acumen of the super-specialists and the managerial skills of the corporate world.

Our Values underpins the way we integrate values of clinical practice, education, research and skill development. Our core value focuses on:

- Integrity & ethical values for proper conduct.
- Focussed thinking by adopting a holistic approach employing various methods for liver-based therapies.
- Excellence in both clinical and research.
- Team work for enabling best patient care and collaborative initiative for capacity building.
- To be the torchbearers and pioneers to take on new challenges and developing solutions in both patient care and academic pursuit.

ILBS – WHOCC

ILBS has achieved the distinction of being a **World Health Organization Collaborating Centre (WHOCC) on liver disease and second on viral hepatitis.**

The collaboration between ILBS and WHO would enable systematic collection and analysis of community and hospital-based data on hepatitis A, B, C and E, including various aspects of transmission, prevention, and treatment specific to low resource settings in India. Such data would help WHO in developing guidelines and recommendations on these aspects of diseases and formulate policies accordingly. The collaborating centre will also serve as a resource center for training of different categories of healthcare workers in relation to viral hepatitis and liver diseases.

1. Generate data, evidence-based policies
2. Capacity through quality training
3. Prevent transmission,
4. Increased access to treatment

BACKGROUND

Viral hepatitis is a major public health problem in need of an urgent response

Viral hepatitis caused 1.34 million deaths in 2015, a number comparable to deaths caused by tuberculosis and higher than those caused by HIV. However, the number of deaths due to viral hepatitis is increasing over time, while mortality caused by tuberculosis and HIV is declining. Most viral hepatitis deaths in 2019 were due to chronic liver diseases like hepatocellular carcinoma and liver cancer (8,20,000 deaths due to hepatitis B and 2,90,000 deaths due to hepatitis C). Globally, in 2019, an estimated 296 million people were living with chronic HBV infection, and 58 million people with chronic HCV infection.

The road to elimination by 2030 requires a comprehensive public health approach taken to scale.

A strategic information system based on surveillance and programme data is needed to direct policy change and implementation. Second, service coverage of testing and treatment needs to be rapidly scaled up. Third, hepatitis services need to be delivered through a public health approach to benefit all. Fourth, sustainable financing is required to enable universal health coverage, the overarching framework for health in the 2030 Agenda for Sustainable Development. Fifth, innovations are necessary; new diagnostics, treatments, cure and vaccines need to be developed, tested and delivered urgently to transform the hepatitis response and attain the elimination targets.

To achieve the elimination of viral hepatitis by 2030, strategic training of healthcare professionals is a key step.

The overall aim of the PRAKASH training program is in harmony with the National Viral Hepatitis Control Program (NVHCP) i.e. capacity building of existing human resources in management of viral hepatitis and its complications. Both the programs are in line with each other with the intention of raising the awareness level about hepatitis infection across the nation thereby aligning the program with global mandate of viral hepatitis elimination by 2030 set by WHO.

ILBS being the only liver specialist institute has under taken this initiative with an aim to reduce the burden of viral hepatitis by conducting nationwide training of healthcare fraternity in management of viral hepatitis.

PRAKASH

There are many challenges to prevent and eradicate viral hepatitis from the country. Health professionals in the country needs to join hands towards achieving the target of viral hepatitis elimination by 2030, a global call for action by WHO. The above can only be achieved by building capacity in the existing healthcare delivery system by imparting knowledge of screening, diagnosis, and management of viral hepatitis amongst healthcare providers.

It is being felt that the knowledge of viral hepatitis, especially B and C is necessary for our doctors, nurses and laboratory technicians for better discharge of their duties to protect the patients and themselves from viral hepatitis infection.

Project PRAKASH (**PR**ogrammed **A**pproach to **K**nowledge **A**nd **S**ensitization on **H**epatitis), has been conceptualised and a delivery mechanism has been formalised so that comprehensive knowledge sharing among technical experts from ILBS and healthcare professionals across India could be done at a common platform. It is a training program for primary care physicians, and paramedical professionals to provide comprehensive training in screening, diagnostic and therapeutic services for viral hepatitis to general and high-risk population of the country.

Since its inception in 2017 the program has successfully trained more than 8,000 healthcare professionals from 500 healthcare institutions across 25 states and union territories of the country.

The program has two major components in line with National Viral Hepatitis Control Program:

1. Hepatitis Induction Program
2. Hepatitis Update Program

OBJECTIVE

WHO has recognised, capacity building of the existing healthcare workforce as a fundamental response measure to see through the ever-magnifying demand of viral hepatitis and COVID-19. This course is providing latest standardized information that is known about the identification and diagnosis of viral hepatitis and COVID-19.

This document provides technical guidance for laboratory professionals and other key stakeholders to understand and test viral hepatitis and COVID-19 in laboratory settings. For healthcare facilities, this document can also serve as a checklist to identify any remaining gaps.

This document is intended to guide the diagnosis of viral hepatitis and COVID-19 and to ensure that patients can access appropriate testing support without compromising safety of healthcare workers.

This medical medium is designed to assist as ready reference material to equip laboratory professionals towards:

- Understanding diagnosis of viral hepatitis and COVID-19, newer testing techniques in laboratory settings.
- Understanding the basics of molecular and serological testing.
- Reaffirms standard precautions as the foundation for preventing transmission of infectious agents in laboratory settings.
- To be thoroughly equipped with personal protective equipment's, post exposure prophylaxis, and bio-medical waste management.
- Understand the principals of quality control and good laboratory practices.

SCIENTIFIC SCHEDULE

VIRAL HEPATITIS | DAY 1

Laboratory diagnosis of viral hepatitis (A-E)

Overview and comparative features of viral hepatitis A-E and testing methods available for viral hepatitis; serological and molecular viral markers of hepatitis A-E and its interpretation.

Serological methods in viral hepatitis testing & quality control

Interpretation of ELISA, CLIA, RDTs, immunochromatography tests (lateral flow & flow through)

Molecular methods in viral hepatitis testing

Basics of molecular testing and its principles; types of PCR & its principles

Needle Stick Injury & Post Exposure Prophylaxis

Introduction to NSI, PPE, PEP; First aid, do's and don'ts and reporting in NSI; PEP for HBV & HCV

COVID-19 | DAY 2

Overview of COVID-19

What is coronavirus; its transmission and symptoms; diagnosis and advice for laboratory professionals

Laboratory diagnosis of COVID-19

Whom to test; types of specimen; sample collection, packaging and transportation; types of molecular and serological testings; antibody and antigen detection

Bio-safety & waste management in viral hepatitis & COVID-19

Bio-medical waste management rules; its segregation, treatment and disposal.

Good laboratory practices in clinical virology

Standard & universal precautions; donning & doffing of PPE; bio-safety levels of infectious agents; types of bio-safety cabinets; infrastructure of a good laboratory, personnel, equipments and standard operating procedures.

VIRAL HEPATITIS

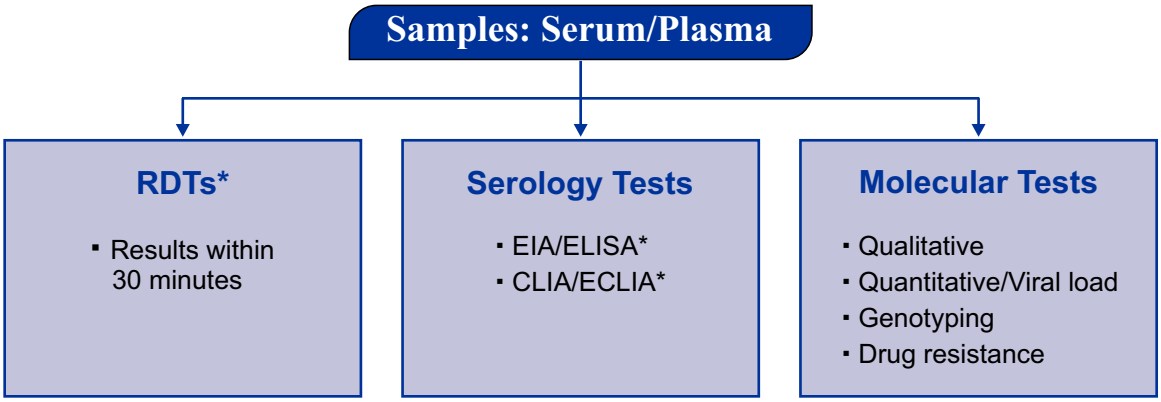
OVERVIEW OF VIRAL HEPATITIS

Viral hepatitis is a systemic disease with primary inflammation of the liver caused by any one of a heterogenous group of 'hepatitis viruses' , which currently consists of types A, B, C, D, and E. Hepatitis A and E are transmitted enterically and hepatitis B, C and D are parenterally transmitted. Infections caused by them can be either acute or chronic. Hepatitis A always and E usually causes acute infection. Hepatitis B, C and D may cause acute or chronic infection.

Table 1: Comparative features of viral hepatitis

Features	HAV	HBV	HCV	HDV	HEV
Genome	RNA	DNA	RNA	RNA	RNA
Family	Picornaviridae	Hepadnaviridae	Flaviviridae	Deltavirus	Hepeviridae
Incubation (days)	15-45	30-180	15-160	90-180	14-60
Onset	Acute	Insidious or acute	Insidious	Insidious or acute	Acute
Transmission					
Fecal-oral	+++	-	-	-	+++
Percutaneous	Unusual	+++	+++	+++	-
Perinatal	-	+++	±	+	-
Sexual	±	++	+	++	-
Clinical					
Severity	Mild	Severe	Moderate	Occasionally severe	Mild
Chronicity	None	Occasional	Common	Common	None
Carrier	None	0.1-30%	1.5-3.2%	Variable	None
Hepatocellular carcinoma	None	+	+	±	None
Antigen in blood	HAV	HBsAg, HBeAg	HCV	HDAg	HEV
Antibodies in blood	Anti-HAV	Anti-HBs, Anti -HBe, Anti-HBc	Anti-HCV	Anti-HDV	Anti-HEV
Prognosis	Excellent	Worse with age, debility	Moderate Chronic: poor	Acute: good	Good
Prophylaxis	Immunoglobulin, Inactivated vaccine	HBIG, Recombinant vaccine	None	HBV vaccine	Unknown

Figure 1: Currently available testing methods for viral hepatitis



*EIA - Enzyme immunoassays; ELISA - Enzyme-linked immunosorbent assay; CLIA - Chemiluminescent immunoassay; ECLIA - Electro chemiluminescent immunoassay

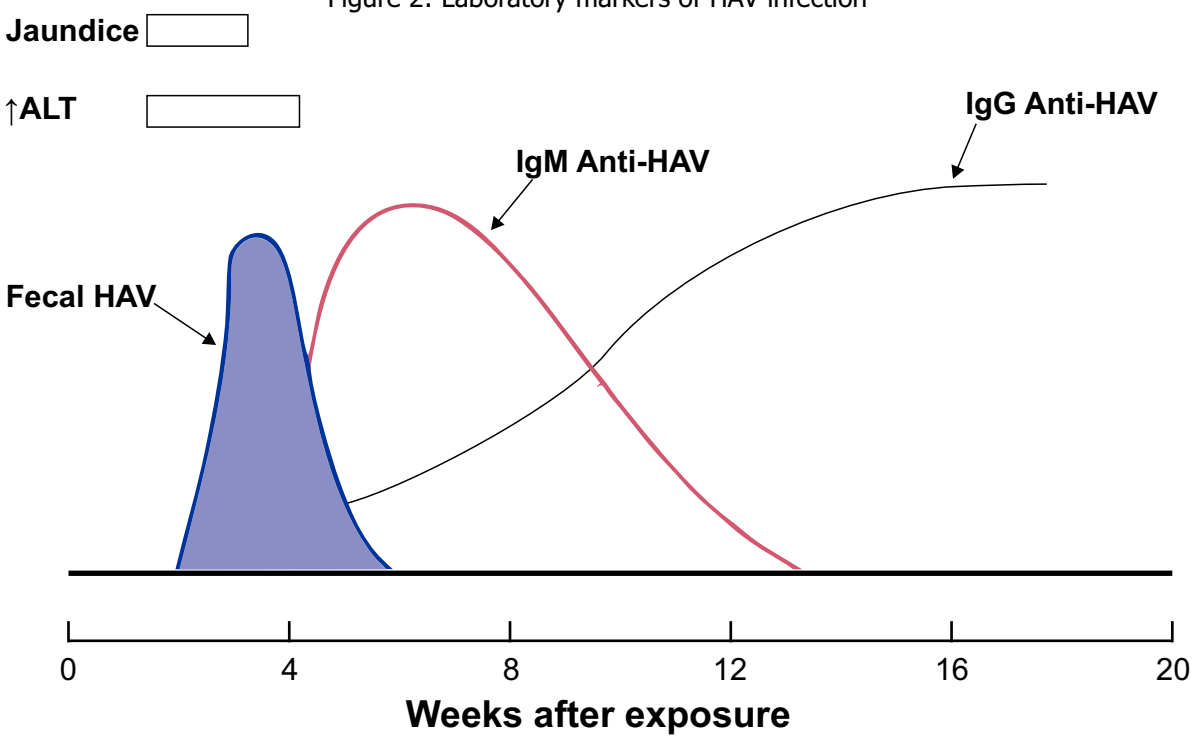
LABORATORY DIAGNOSIS OF HAV

Hepatitis A is the most common acute viral hepatitis occurring worldwide, often in epidemics. Hepatitis A is RNA virus belonging to the family Picornaviridae. The spread of infection is by feco-oral route arising after the consumption of contaminated food and water. The incubation period ranges from 2-3 weeks with no carrier state.

Table 2: Serological viral markers for Hepatitis A

Serological viral markers	Importance
Anti-HAV IgM antibodies	Confirms diagnosis of acute hepatitis A Rapidly increases in titre over 4-6 weeks Declines to non-detectable within 3-6 months
Total Anti-HAV antibodies	In the absence of either IgM anti-HAV or an abnormal ALT level, it is suggestive of previous infection with HAV or successful vaccination and protection against future infection. These antibodies appear shortly after the onset of symptoms and confer long-term (usually lifelong) immunity.

Figure 2: Laboratory markers of HAV infection



Source: Faud AS, Kasper DL, Braunveld E, Hauser SL, Longo DL, Jameson JL, Loscalzo J, Harrison's Principles of Internal medicine, 19th Edition: http://www.accessmedicine.com

LABORATORY DIAGNOSIS OF HBV

Hepatitis B, a DNA virus belonging to Hepadanaviridae family is prevalent worldwide with estimated 200 million carriers. The incubation period ranges from 1-6 months. Both horizontal and vertical transmission may lead to infection. The vertical transmission i.e. from mother to child, in utero, during parturition or soon after birth is the most common route of transmission in India. Horizontal transmission includes spread via intravenous route (e.g. transfusion of infected blood or blood products, or contaminated needles) or by sexual intercourse, particularly in men who have sex with men.

The virus can be found in semen and saliva. Minor abrasions or close contact with other children lead to spread of infection in children. The spectrum of clinical manifestations of hepatitis B virus (HBV) infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations can also occur with both acute and chronic infection. Diagnosis of HBV infection is based on two categories of laboratory tests, serological and molecular.

Table 3: Serological viral markers for diagnosis of Hepatitis B

Viral Markers	Description
Hepatitis B surface antigen (HBsAg)	• The hallmark of HBV infection and first serological marker to appear in acute hepatitis • The persistence of HBsAg for more than 6 months is referred to chronic HBV infection. • HBsAg is the antigen used to make hepatitis B vaccine.
Hepatitis B surface antibody (anti-HBs)	• Anti-HBs is neutralizing antibody indicating protective immunity against HBV infection. • It is the only detectable serological marker in those with successful hepatitis B immunization.
Total hepatitis B core antibody (Total anti-HBc / IgG anti-HBc)	• Appears at the onset of symptoms in acute hepatitis B and persists for life. • The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined time frame. • Can be found in window period of acute HBV, in recovered patients or in those with CHB or as an isolated marker in occult or silent HBV infection (HBsAg –ve and HBV DNA +ve).

IgM antibody to hepatitis B core antigen (IgM anti-HBc)	• First antibody to be detected in acute HBV infection; 2-4 weeks after the detection of HBsAg and usually disappears by 6 months. • Positivity indicates recent infection with HBV (≤6 months).
Hepatitis B e antigen (HBeAg)	• Its presence indicates that the virus is replicating and the infected person has high levels of HBV.
Hepatitis B e antibody (HBeAb or anti-HBe)	• The seroconversion from HBeAg to anti-HBe is usually associated with disease remission, increased likelihood of HBsAg seroconversion and lower levels of HBV.

Table 4: Interpretation of Hepatitis B serological markers:

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. HBeAg negative (precore mutant) hepatitis B
+	+	+	+/-	+/-	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
-	+	-	-	-	Immunization with HBsAg (after vaccination)
-	-	-	-	-	Susceptible to Hepatitis B infection

Table 5: Molecular test for Hepatitis B diagnosis

Viral load testing	Genotyping	Drug resistance
Most HBV DNA assays used in clinical practice are based on polymerase chain reaction (PCR) amplification. Recently, HBV DNA assays that utilize real-time PCR technology with improved sensitivity (5-10 IU/ml) and wider dynamic range (up to 8-9 log10 IU/ml) are also available and commonly used.	At least 10 different genotyping techniques have been developed but the gold standard methods commonly used for genotyping are sequencing and hybridization techniques (Line Probe Assay).	Direct sequencing and reverse hybridization are currently the main methods for detecting drug-resistance mutations of hepatitis B virus (HBV).

Figure 3.1: Acute HBV infection with recovery

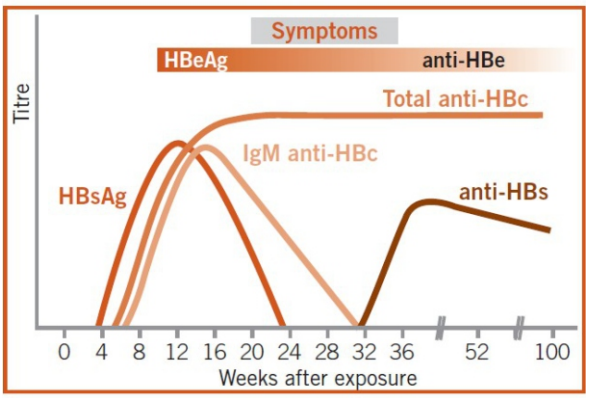
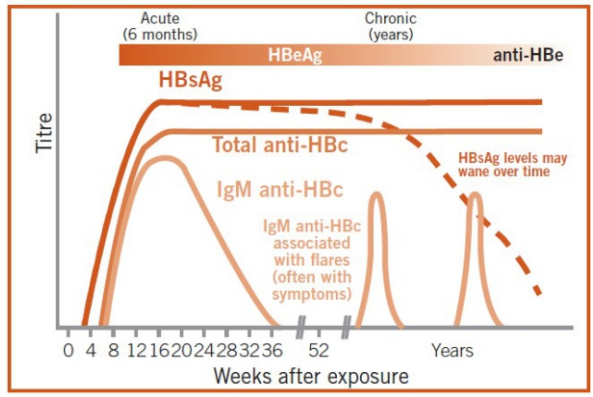


Figure 3.2: Chronic HBV infection



Source: WHO guidelines for hepatitis B & C testing.

LABORATORY DIAGNOSIS OF HCV

Hepatitis C virus (HCV), belonging to Flaviviridae family can cause both acute and chronic hepatitis. The acute phase is self-limited, rarely causes hepatic failure, and usually leads to chronic infection. Chronic HCV infection often follows a progressive course over many years and can ultimately result in cirrhosis, hepatocellular carcinoma, and the need for liver transplantation. The incubation period ranges from 15-160 days. Most patients infected with HCV acquired the disease through intravenous drug use or blood transfusion. Injectable drug users, transplant recipients and immunocompromised population are at higher risk. Sexual transmission is probably less common and vertical transmission from mother to child may take place.

Serological tests for anti-HCV antibody detection

- Enzyme Linked Immunosorbent Assay (ELISA):

Table 6: Generations of ELISA for anti-HCV

Generation	Antigens used
First Generation	c100-3 – a part of the NS4 region of HCV genome
Second Generation	c100-3 + c22-3: Nucleocapsid protein encodes an immunodominant epitope c200: Protein derived from NS3 and NS4 regions/ c33c from Ns3
Third Generation	Additional antigen used: Recombinant NS5 protein

- Chemiluminescent microparticle immunoassay (CMA)
- HCV Rapid antibody test: These assays are based on recombinant antigens derived from core, NS3, NS4, and NS5 proteins in an immunochromatographic format which exhibit a high specificity of >99%, and sensitivity ranging from 86% to 99%.

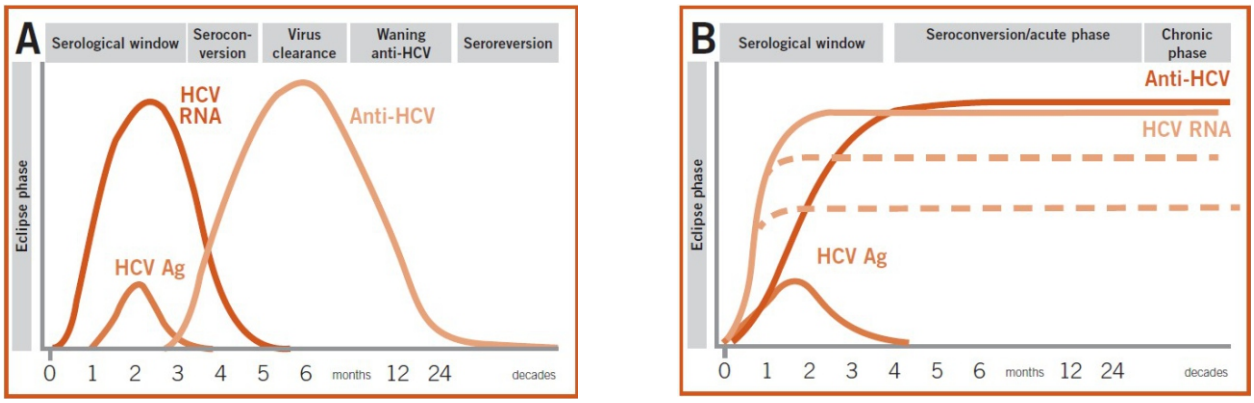
Molecular test for HCV RNA

- HCV RNA is a confirmatory test for hepatitis C virus infection.
- HCV RNA testing is done by real-time RT-PCR(Reverse transcriptase polymerase chain reaction). HCV RNA levels are quantitated and expressed as IU/ml.
- HCV-RNA is detectable in plasma and in serum 1 to 3 wk after infection, about 1 month before the appearance of anti-HCV antibody, and is a hallmark of ongoing viral replication and also helps determine the length of treatment needed.

HCV genotyping testing

- Genotype refers to the genetic structure or makeup of living organisms. The hepatitis C virus has seven different genotypes, which are numbered in the order of their discovery.
- It is an optional test which is done if HCV RNA is positive but it is important to find out which hepatitis C genotype you have, because it determines both the type of treatment and the length of treatment.

Figure 4: Approximate time course of virological and immunological markers of HCV infection with (A) Self-resolving HCV infection, and (B) Chronic HCV infection



Source: WHO guidelines for hepatitis B & C testing.

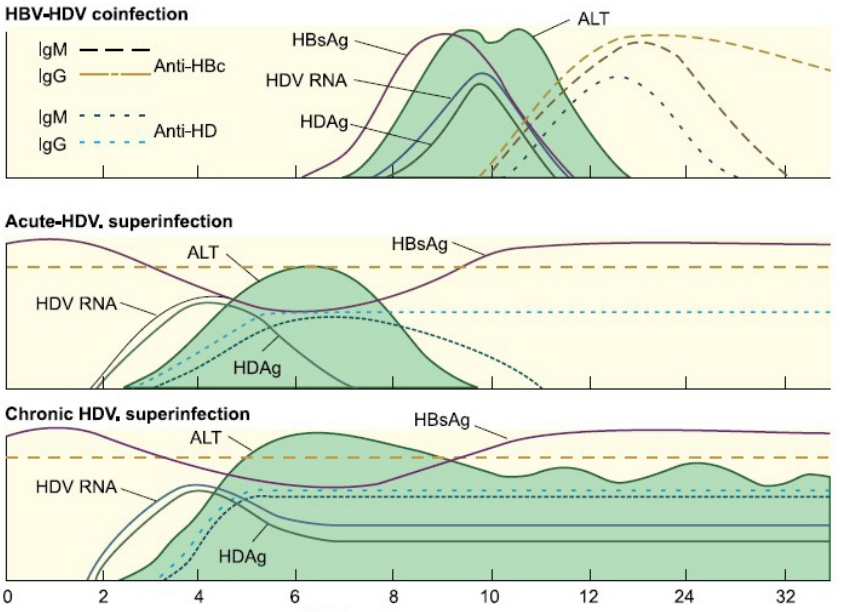
LABORATORY DIAGNOSIS OF HDV

Hepatitis D only occurs among people who are infected with the HBV because HDV is an incomplete virus that requires the helper function of HBV to replicate.The outcome of disease largely depends on whether the two viruses infect simultaneously (coinfection), or whether the newly HDV-infected person is a chronically infected HBV carrier (superinfection). Its mode of transmission is same as of HBV.

Laboratory diagnosis of HDV

- Serology (antibody detection)- anti-HDV-total and IgM anti-HDV
- Antigen detection- HDAg- ELISA
- NAAT –RT-PCR

Figure 5: Serologic patterns of type D Hepatitis after co-infection or super-infection of a person with HBV infection.



Co-existent acute Hepatitis B and Hepatitis D.

Acute hepatitis D superimposed on a chronic HBV infection.

Acute hepatitis D progressing to chronic hepatitis, superimposed on a chronic HBV infection.

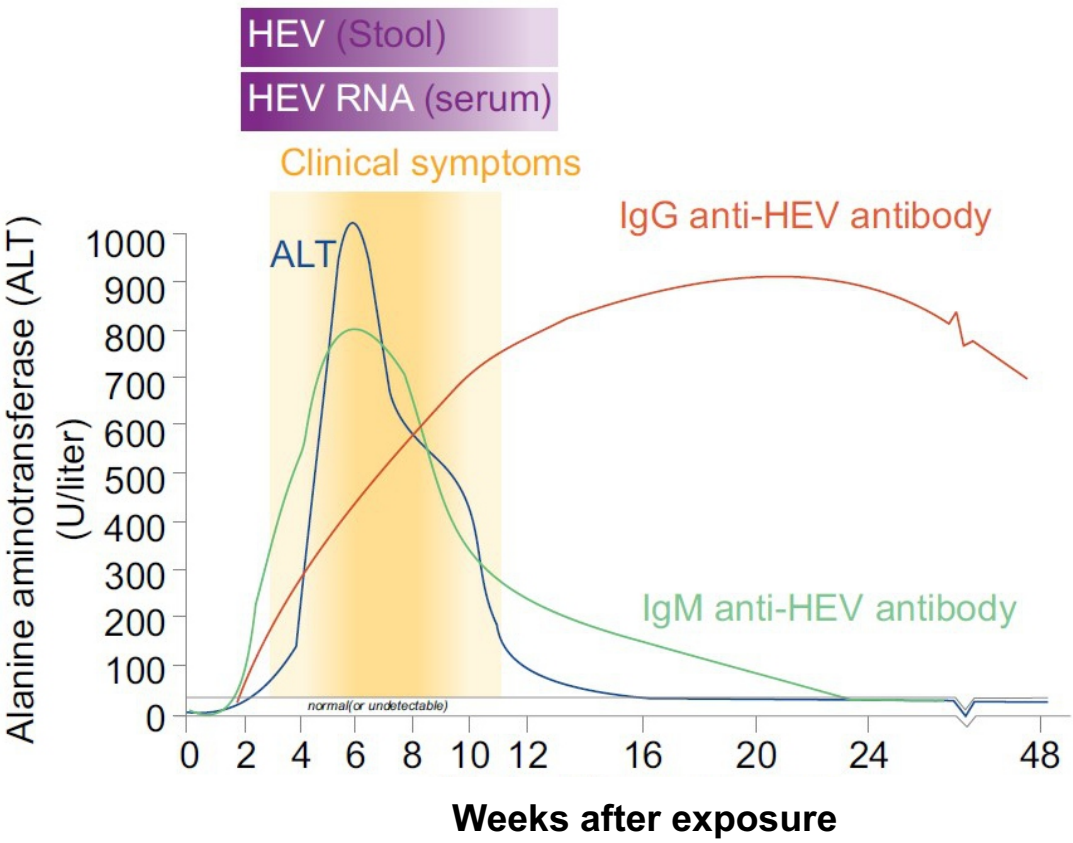
Source: Karen C. Carroll, Stephen A. Morse, Timothy Meitzner, Steve Miller: Jawetz, Melnick, and Adelberg's Medical Microbiology, 27th Edition, Mc-Graw Hill Education.

LABORATORY DIAGNOSIS OF HEV

Hepatitis E belonging to family Hepeviridae manifests clinically very similar to hepatitis A. Transmission of hepatitis E virus (HEV) can occur through contaminated food and water, blood transfusions, and through mother-to-child transmission. Although person-to-person transmission is uncommon, patients are infectious during fecal shedding. The incubation period of HEV infection ranges from 15 to 60 days. Mortality of 1-2% have been reported due to fulminant hepatic failure which increases to 20% in pregnant women. There is no carrier state and infection does not progress to chronic liver disease, except in some immunosuppressed population.

The methods available of detection of antibodies are ELISA.

Figure 6: Course of acute Hepatitis E virus infection



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

Laboratory diagnosis of HEV

A. Serological methods

Table 7: Serological viral markers for Hepatitis E

Serological viral markers	Importance
Anti-HEV IgM antibodies	Current tests are capable of detecting IgM anti-HEV in up to 90% of acute infections if a serum sample is obtained 1 to 4 weeks after the onset of disease. Peaks during first 4 weeks and undetectable after 3 months in 50% patients
IgG Anti-HEV antibodies	Peaks between 2 and 4 weeks after onset of hepatitis and diminishes rapidly thereafter. A rising titer of IgG anti-HEV is also diagnostic.

B. Molecular methods

Real Time PCR (quantitative/qualitative)

COVID-19

CORONA VIRUS DISEASE AND ITS MANAGEMENT

1. Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus known as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2)
2. In humans, corona viruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as MERS and SARS. The most recently discovered coronavirus causes coronavirus disease (COVID-19).
3. Human to human transport occurs primarily through respiratory droplets.
4. Incubation period ranges from 2-14 days (average 5 days).
5. More severe among elderly, and co-morbidities like diabetes mellitus, cancer, heart disease.
6. Symptomatic refers to an individual having fever/cough/shortness of breath.
7. Direct and high-risk contacts include those who live in the same household with a confirmed case and HCP who examined a confirmed case.
8. WHO recommends resampling and testing for multiple respiratory tract sites if initial testing is negative but suspicion for COVID-19 remains.
9. Clinical syndromes associated with COVID - 19 infection include Uncomplicated illness, Mild pneumonia, Severe pneumonia, Acute Respiratory Distress Syndrome, Sepsis and Septic shock.

Figure 7: Coronavirus structure

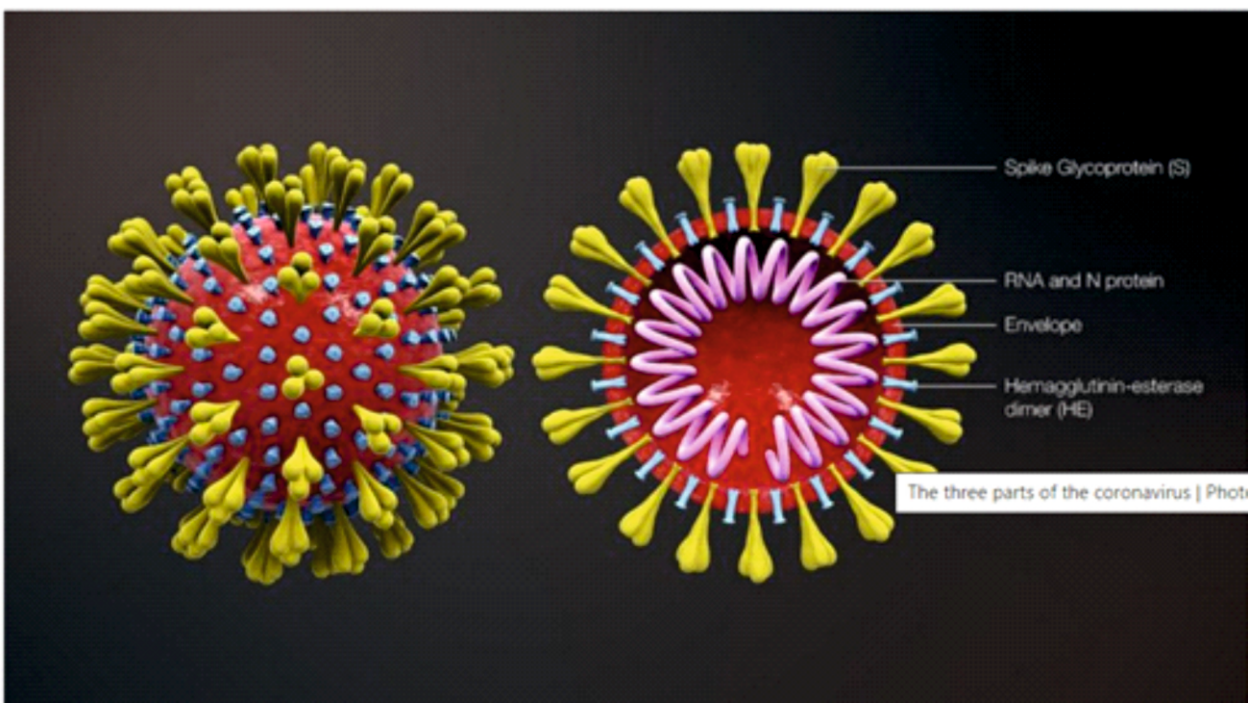


Image source: theprint.in

Figure 8: COVID-19 transmission routes: droplets, direct contact, and indirect contact

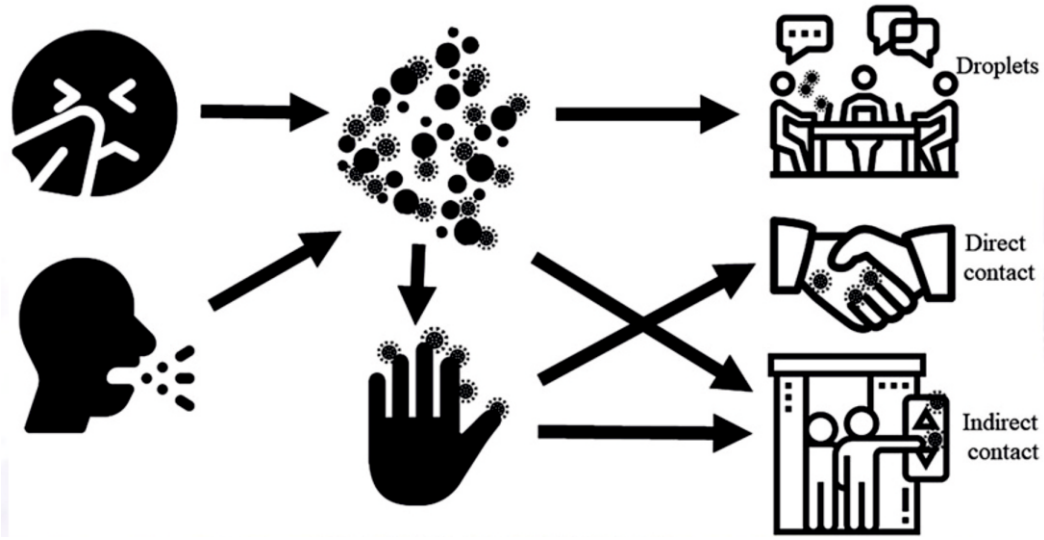


Image Source: https://acsjournals.onlinelibrary.wiley.com/doi/10.1002/cncy.22280

LABORATORY DIAGNOSIS OF COVID-19

Whom to test?

As per ICMR advisory dated 04/09/2020, testing should be offered:

Routine surveillance in containment zones and screening at points of entry:

- All symptomatic (ILI symptoms) cases including health care workers and frontline workers.
- All asymptomatic direct and high-risk contacts (in family and workplace, elderly ≥ 65 years of age, immunocompromised, those with co-morbidities etc.) of a laboratory confirmed case
- to be tested once between day 5 and day 10 of coming into contact.
- All asymptomatic high-risk individuals (elderly ≥ 65 years of age, those with co-morbidities etc.) in containment zones.

Routine surveillance in non-containment areas:

- All symptomatic (ILI (influenza like illness) symptoms) individuals with history of international travel in the last 14days.
- All symptomatic (ILI symptoms) contacts of a laboratory confirmed case.
- All symptomatic (ILI symptoms) health care workers / frontline workers involved in
- containment and mitigation activities.
- All symptomatic ILI cases among returnees and migrants within 7 days of illness.
- *All asymptomatic high-risk contacts in family and workplace, elderly ≥ 65 years of age, those with co-morbidities etc.

In hospital settings:

- All patients of Severe Acute Respiratory Infection (SARI).
- All symptomatic (ILI symptoms) patients presenting in a healthcare setting.
- Asymptomatic high-risk patients who are hospitalized or seeking immediate hospitalization such as immunocompromised individuals, patients diagnosed with malignant disease, transplant patients, patients with chronic co-morbidities, elderly ≥ 65 years.
- Asymptomatic patients undergoing surgical / non-surgical invasive procedures (not to be tested more than once a week during hospital stay).
- All pregnant women in/near labor who are hospitalized for delivery.
- All symptomatic neonates presenting with acute respiratory / sepsis like illness.
- (Features suggestive of acute respiratory illness in a neonate are respiratory distress or apnea with or without cough, with or without fever. Neonates may also manifest with only non-respiratory symptoms like fever, lethargy, poor feeding, seizures or diarrhea).

Testing on demand (State Governments to decide simplified modalities):

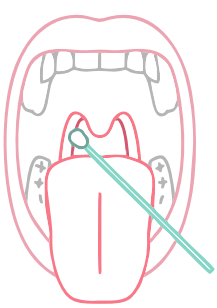
- All individuals undertaking travel to countries/Indian states mandating a negative COVID-19 test at point of entry.
- All individuals who wish to get themselves tested.

Types of specimen

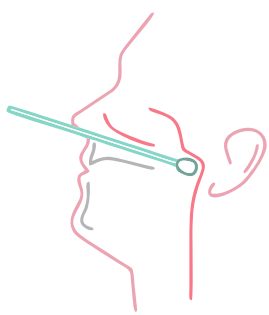
Table 8: Types of specimens for COVID-19 diagnosis

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Not applicable
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	Autopsy sample collection preferably to be avoided

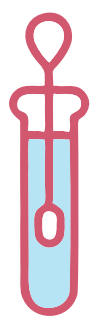
Figure 9: Oropharyngeal & nasopharyngeal swab test for COVID-19



OROPHARYNGEAL SWAB



NASOPHARYNGEAL SWAB

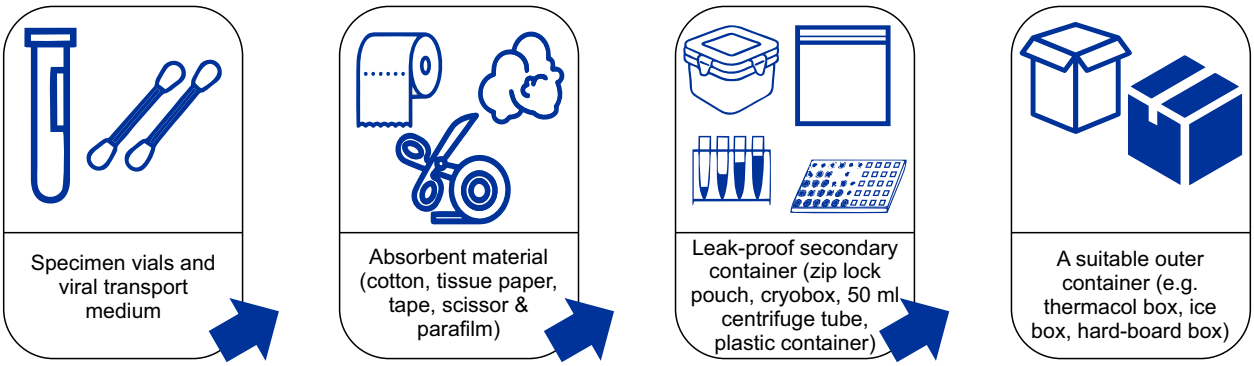


COVID-19 SAMPLE

Specimen collection and transport

The COVID-19 collected samples should have triple layer packaging and to be transported on ice. Below diagram indicates the process in detail.

Figure 10: Packaging of COVID-19 samples



Various diagnostic modalities

Diagnostic modalities

Direct methods

Detection of nucleic acid
Real Time PCR
Near point of care molecular assays
Isothermal nucleic acid amplification
Sequencing
CRISPR based methods

Antigen detection

Viral isolation

Indirect methods

Antibody detection

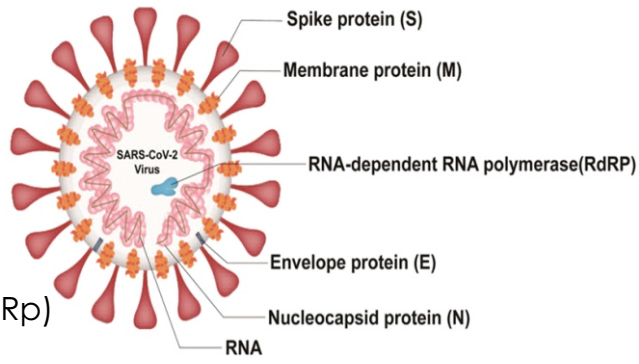
Molecular methods

A. Molecular targets for PCR assays

Structure genes
- Spike (S)
- Envelope (E)
- Transmembrane (M)
- Nucleocapsid (N)

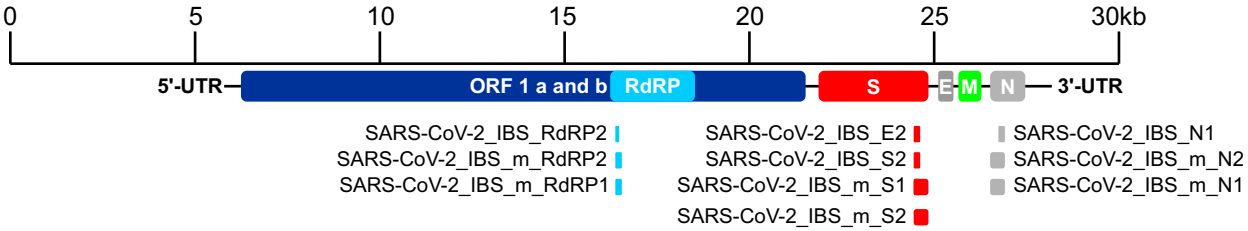
Species pecific accessory genes
- RNA-dependent RNA polymerase (RdRp)
- Hemagglitinin-esterase (HE)
- Open reading frames ORF Ia and ORF1b

Figure 11: Structure of SARS-CoV-2



WHO- Two genes E gene (screening), RdRp gene (confirmatory)

Figure 12: Structure of SARS-CoV-2



B. Near point of care molecular assays.

- Less infrastructural demanding
- Decentralized testing
- Comparable less trained staff
- Shorter turn around time (approx 1-2 hours)
- Lesser Biosafety issues

Table 9: True NAT vs Gene Xpert

True NAT	Gene Xpert
Chip-based assay	Cartidge-based assay
Target: E, RdRp gene	E gene, N2 gene
Battery operable	Power operable
No need of air-conditioned rooms	Need of air-conditioned rooms
Cost-effective	More risk of aerosol generation
Less risk of aerosol generation, as viral lysis media lysis most infectious material	
Two-step process	Single-step process
Limit of detection -468 copies/ml.	Limit of detection -250 copies/ml.

C. Abbott ID NOW™ COVID-19 Assay

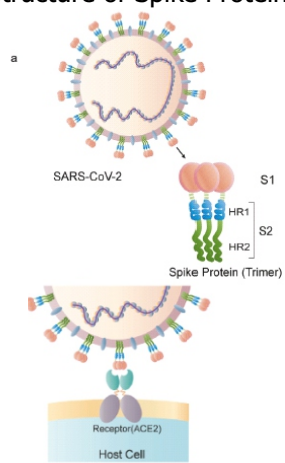
- Rapid, instrument-based isothermal test
- Specimen type: Nasal, nasophayrngel and throat swabs
- Turnaround time: 13 minute or less
- Test swab directly without elution in VTM
- Test as soon as possible: upto 1 hours at room temperature
- Target: RdRp segment
- LOD-125 genomes equivalent/ml
- Senstivity 78%-93%
- Specificty 94.3%

Antigen Detection

- Point of care test
- Qualitative detection by immunochromatography
- Target-Nucleoprotein antigen
- Sample of choice: Nasopharyngeal swab
- Turn around time 20-30 minutes
- Sensitivity ranges from 50-70% depending on viral load, specificity 98-99.5%
- All symptomatic, antigen negative → RT-PCR

Antibody Detection

Figure 13: Structure of Spike Protein



Spike Protein (1273aa)

- Most exposed viral protein
- S1 subunit (14-685)- less conserved and highly specific
- S2 subunit (686-1273)
- Recepetor binding domain (RBD)- S1 subunit
- more conderved than S/S1
- less cross reactivity with other CoVs
-

Nucleocapsid antigen (N)

- Abundantly expressed during infection
- More conserved than S antigen

Antibodies detected by various methods

Figure 14: Various methods used for anti-bodies detection

ELISA	LFIA	CLIA
Enzyme Linked Immunosorbent Assays	Lateral Flow Immunoassays	Chemiluminescent Immunoassays

Pooled sensitivity(%)	83.7(74.1 to 92.5)	72.4(37.9 to 93.6)	89.7(61.3 to 98.7)
Pooled specificity(%)	99.3(98.7 to 99.7)	96.4(88.3 to 99.3)	86.5(80.3 to 99.4)

Source: Lisboa Bastos M et al, BMJ, 2020 Jul 1;370 :m2516

Role of serological testings

NOT FOR DIAGNOSTIC PURPOSE

- Epidemiology/sero-prevalence studies
- Identification of potential convalescent plasma donors
- Evaluation of immune response to candidate vaccines

TECHNIQUES AND TESTINGS

COMMON SEROLOGICAL TECHNIQUES

Serological techniques are those which make use of an antigen antibody reaction for diagnosis. Diagnostic virology relies largely upon use of serological techniques for detection of virus specific antigen or antibodies. The common serological methods used are discussed below.

ELISA

Enzyme linked immunosorbent assay is one of the commonest techniques used in serology. It is a plate-based technique whereby antigens or antibodies can be identified by enzymatic reactions that produce colour change. The intensity of the colour produced is measured spectrophotometrically. There are many variations or types of ELISA assays.

- Indirect
- Competitive
- Sandwich
- Capture

Table 10: Indirect vs sandwich ELISA

Indirect ELISA	Sandwich ELISA
Well is precoated with antigen	Well is precoated with capture antibody
↓	↓
Test serum is added-antibodies bind	Test serum is added-antigens bind
↓	↓
Labeled antiglobulin is added	Detection antibody is added
↓	↓
Enzyme substrate is added	Labeled antiglobulin is added
↓	↓
Color change	Enzyme substrate is added
	↓
	Color change

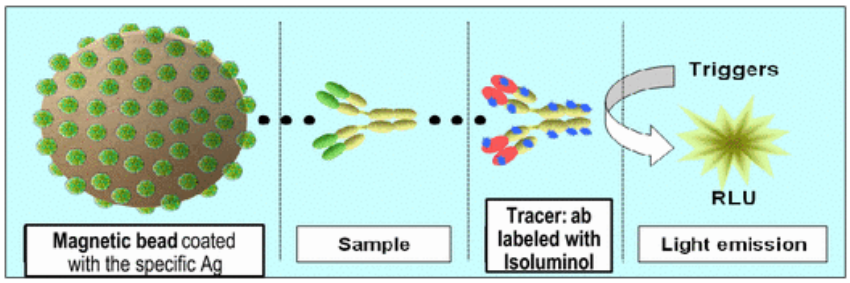
vs

CLIA

Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. Chemiluminescence immunoassay has a similar principle like ELISA but uses a chemiluminescent substrate. The light produced by the reaction indicates the amount of analyte present in the sample. Magnetic microparticles can be used as the supporting phase, over which antibodies or antigens are coated.

The advantage of CLIA over ELISA is a significantly increased sensitivity & dynamic range, which allows detection of lower analyte concentrations & earlier diagnosis of disease. It has a smaller incubation period and does not need addition of stopping reagent. Both of them are semi quantitative tests.

Figure 15: Principle of Chemiluminescence immunoassay



Source: Cinquanta, L., Fontana, D.E. & Bizzaro, N. Chemiluminescent immunoassay technology: what does it change in autoantibody detection?. Autoimmun Highlights 8, 9 (2017).

Rapid tests

Rapid tests refer to qualitative tests that provide prompt detection of HBsAg and Anti-HCV on serum/whole blood (fingerprick blood collected with anticoagulant), and plasma. These tests do not require specialised equipments, and serve as point of care tests.

The two most common formats of rapid tests used in testing for viral hepatitis ar as follows:

A. Immunochromatography (lateral flow) tests

These are one step tests wherein both antigen and signal reagent are incorporated into a nitrocellulose strip. The specimen is applied onto an absorbent pad. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where antigen/antibody has been applied. A procedural control line is usually applied to the strip beyond the control line. A visual line at both the test and control sites indicates a positive test result, a line only at the control location indicates a negative test result, and the absence of a line at the control site means the test is invalid.
Examples include Hepacard for HBsAg detection and SD Bioline (Anti-HCV).

B. Immunoconcentration (flow through) assays

These devices employ solid-phase capture technology, which involves the immobilization of antigens on a porous membrane. The antibodies if present in (serum, plasma, whole blood) the specimen flow through the membrane during the performance of test and are absorbed on the antigen into an absorbent pad. A dot or a line visibly forms on the membrane when developed with a signal reagent. Tests usually include a procedural control dot or line. These tests usually require several steps for the addition of specimen, wash buffers, and signal reagent. They can usually be performed in 5 to 15 minutes.

BASICS OF MOLECULAR TECHNIQUES

Molecular diagnostic assays are invaluable in the management of chronic viral hepatitis. They are used to diagnose active infection, guide treatment decisions, assess virological response to therapy and establish prognosis. They are based on detection of virus specific nucleic acids, ie, DNA or RNA, and are thus very sensitive. These techniques are expensive, require sophisticated equipments, trained laboratory personnel and are prone to contamination.

A variety of nucleic acid detection techniques exist, and are broadly classified as target amplification assays (polymerase chain reaction, ligase chain reaction, transcription-mediated amplification) or signal amplification assays (branched chain DNA assay, hybrid capture assay). The most widely used of all these techniques is the polymerase chain reaction or PCR.

Principle of PCR

PCR is a rapid way of amplifying minute quantities of nucleic acid at specific targets using the enzyme polymerase.

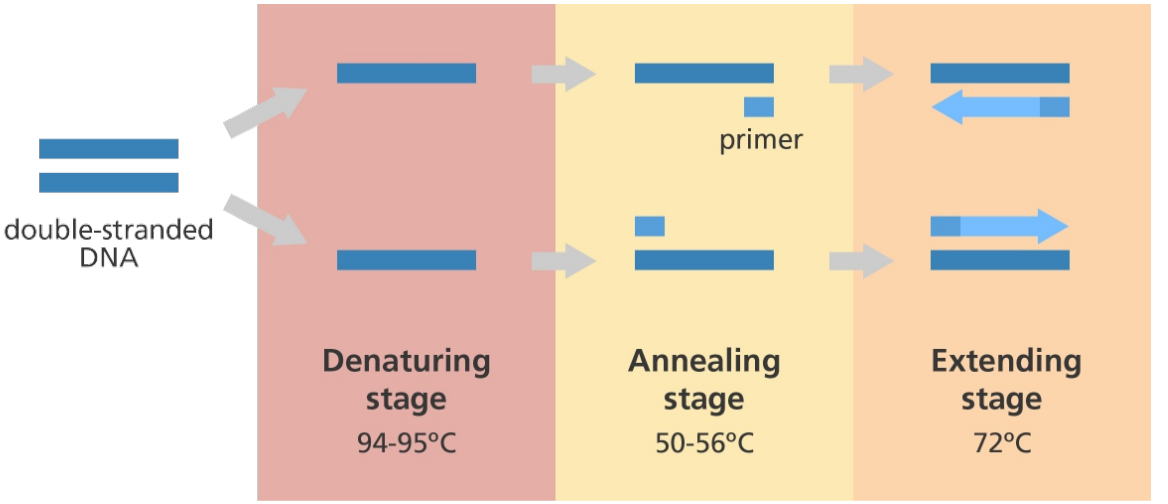
A. Denaturation
The DNA template is heated to 94° C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA.

B. Annealing
The mixture is cooled to anywhere from 50-70° C. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

C. Extension
The reaction is then heated to 72° C, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template.

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially.

Figure 16: Illustration showing the main steps in the polymerase chain reaction (PCR)



There are different types of PCR

- Real-time PCR
- Quantitative real time PCR (q-RT PCR)
- Reverse Transcriptase PCR (RT-PCR)
- Multiplex PCR
- Nested PCR

GOOD LAB PRACTICES & QUALITY CONTROL

GOOD LABORATORY PRACTICES

Biosafety guidelines are provided for the protection of workers in diagnostic laboratories handling infectious materials and agents of blood borne diseases such as HIV, HBV and HCV etc. The risk of laboratory-acquired infection with blood borne diseases are primarily from contamination of hands, mucous membranes of eyes, nose and mouth by infectious blood and other body fluids. Though occupational risk is low, the consequences of infection of blood borne diseases are dire.

Good laboratory practices include various aspects that are mentioned below.

Universal precautions for laboratory workers

- Wear gloves when handling infectious material.
- Do not touch eyes, nose or skin with gloved hands.
- Do not leave the work place or walk around wearing gloves, remove gloves after completion of work.
- Wash hands with soap and water immediately after any contamination.
- Wear laboratory apron only when working in laboratory.
- Entry to laboratory should be restricted, doors should have a 'Biohazard' sign and 'Restricted Entry' labels.
- Keep laboratory clean, neat and free from extraneous materials and equipment's.
- Disinfect work surfaces at the end of procedures and each working day with 1% hypochlorite solution.
- Avoid usage of needles and other sharp instruments and if in use place them in puncture-resistant container.
- Do not recap used needles and do not remove needles from syringes.
- Never pipette by mouth, use pipetting aid.
- Do not eat, drink, smoke, apply cosmetics or apply contact lens in the laboratory.
- Remove gloves before attending the telephone while working in the laboratory.
- Use separate markers, pens and other stationary for laboratory work.

Standard precautions

- Hand hygiene, respiratory hygiene and cough etiquettes
- Personal protective equipment (PPE)
- Safe patient placement and transport
- Cleaning and disinfection of devices and surfaces
- Biomedical waste management
- Safe handling of sharps , linen

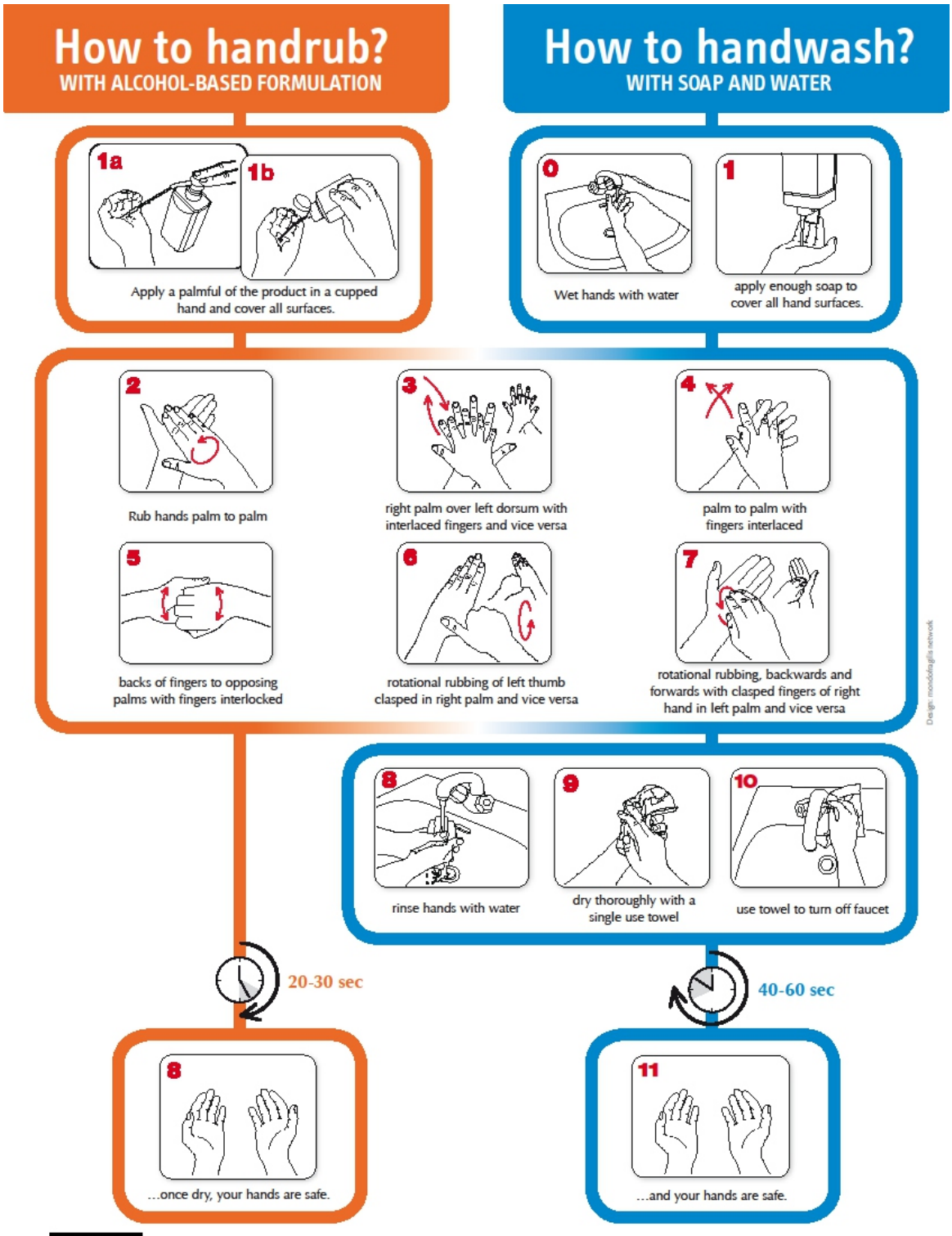


Figure 17: WHO recommended steps of handrub & handwash

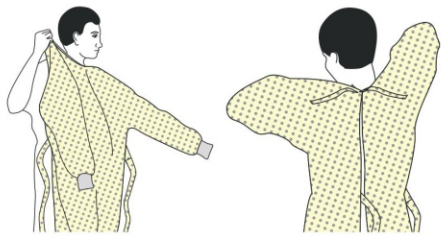
Figure 18: Sequence for donning of PPE

SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

The type of PPE used will vary based on the level of precautions required, such as standard and contact, droplet or airborne infection isolation precautions. The procedure for putting on and removing PPE should be tailored to the specific type of PPE.

1. GOWN

- Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back
- Fasten in back of neck and waist



2. MASK OR RESPIRATOR

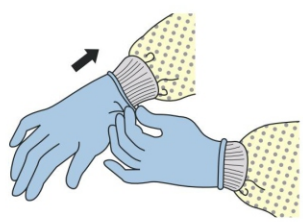
- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator

3. GOGGLES OR FACE SHIELD

- Place over face and eyes and adjust to fit

4. GLOVES

- Extend to cover wrist of isolation gown



USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION

- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene

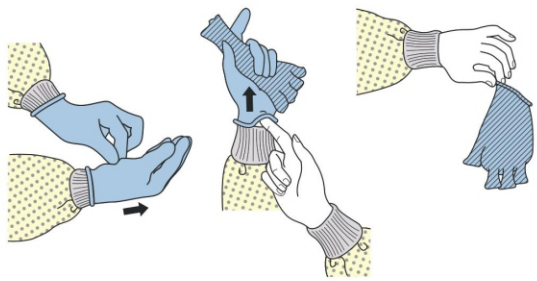
Figure 19: Sequence for doffing of PPE

HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 1

There are a variety of ways to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. Here is one example. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

1. GLOVES

- Outside of gloves are contaminated!
- If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove
- Hold removed glove in gloved hand
- Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove
- Discard gloves in a waste container

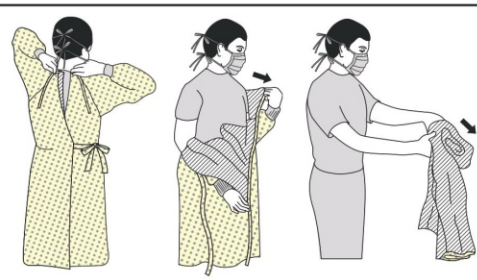


2. GOGGLES OR FACE SHIELD

- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band or ear pieces
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container

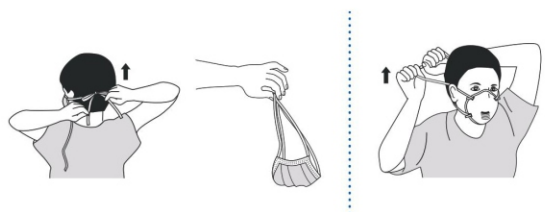
3. GOWN

- Gown front and sleeves are contaminated!
- If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties
- Pull gown away from neck and shoulders, touching inside of gown only
- Turn gown inside out
- Fold or roll into a bundle and discard in a waste container

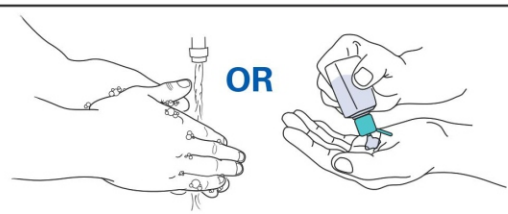


4. MASK OR RESPIRATOR

- Front of mask/respirator is contaminated — DO NOT TOUCH!
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container



5. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE



PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS BECOME CONTAMINATED AND IMMEDIATELY AFTER REMOVING ALL PPE

Different biosafety levels for infectious agents

Table 11: Biosafety levels for infectious agents

BSL	Agents	Practices
1	Not Known to consistently cause diseases in healthy adults	Standard microbiological practices
2	• Agents associated with human disease. • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.	**BSL-1 practice plus** Limited access Biohazard warning signs "Sharps" Precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	**BSL -2 practice plus** Controlled access Decontamination of all waste Decontamination of laboratory clothing before laundering
4	• Dangerous/exotic agents which post high individual risk of aerosol-trans-mitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments • Agents with a close or identical anti-genic relationship to an agent requiring BSL-4 until data are available to redesignate the level • Related agents with unknown risk of transmission	**BSL-3 practices plus** Clothing change before entering Shower on exit All material decontaminated on exit from facility

Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
No primary barriers required. PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
Primary barriers: BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPE: Laboratory coats, gloves, face and eye protection, as needed	**BSL-1 plus:** Autoclave available
Primary barriers: BSCs or other physical containment devices used for all open manipulations of agents PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	**BSL-2 plus:** Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory Entry through airlock or anteroom Hand washing sink near laboratory exit
Primary barriers: All procedures conducted in Class lll BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	**BSL-3 plus:** Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decontamination systems Other requirements outlined in the text

Different types of biosafety cabinets

Table 12: Different types of Biosafety Cabinets

Type	Face Velocity (ipfm)*	Airflow Pattern	Radionuclides Toxic Chemicals	Biosafety Levels	Product Protection
Class I**	75	In at front, rear and top through HEPA filter	No	2 or 3	No
Class II Type a1	75	70% recirculated through HEPA filter, exhaust through HEPA filter	No	2 or 3	Yes
Class II Type A2	100	Same as Class II, Type A, but piena is under negative pressure to room and exhaust air is ducted	Yes	2 or 3	Yes
Class II Type B1	100	30% recirculated through HEPA filter, exhaust via HEPA filter and hard ducted	Yes	2 or 3	Yes
Class II Type B2	100	No recirculation through HEPA filter and hard ducted	Yes	2 or 3	Yes
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	Yes	3 or 4	Yes

Infrastructure of a good laboratory

- Lab should be clean and tidy.
- It should be free of smoke, smell, dust and pests.
- Ensure good ventilation, proper illumination and prefer natural light.
- Air condition the laboratory with humidity control.
- Enough space for the instruments & laboratory personnel.
- Take care of all the safety points including proper earthing as well as fire safety.
- Establish proper areas for storage of incoming samples as well as test completed samples.
- Establish proper sample collection place as well as packing and disposal of tested samples.
- Separate facilities/area for staff for hand washing, eating and storing food.
- Quality water supply for analytical purpose.
- Uninterrupted power supply.

Personnel, Training & Development

It is important to ensure appropriate staff strength and regular activities should be undertaken for training and development of the technical staff.

- Head & Quality Manager.
- Ensure appropriate staff strength with necessary qualification & experience.
- Roles & responsibilities of each staff member should be clearly defined.
- Conduct programmes and trainings for regular update of skills.
- Ensure periodic competence evaluation of each staff.
- A personal data file has to be maintained.

Equipment's

- All items should be suitably located
- Always ensure good capacity & working condition in a laboratory
- Conduct periodic inspections of equipment's and ensure timely cleaning of the devices and maintenance of records in a log book.
- Ensure standard SOPs for operation.
- Calibration & annual maintenance needs to be ensured.
- Performance checks of the equipment's must be done by using quality control materials.

Reagents & materials

- Appropriate storage conditions
- New reagents – check controls first
- Opening date should be written
- Reagents should be brought to room temperature before performing any test

Standard operating procedure

- Maintain document, which contains detailed, written instructions describing the stepwise process and technique of performing a test or procedure in the laboratory.
- Helps to ensure uniformity, consistency and control over the processes carried out. It ensures that the procedures are done in exactly the same way each time irrespective of the change in operator.

Testing & reporting of test results

- Maintain record of all specimens that are received in the laboratory.
- Patient's ID with requested tests, date & time of receipt of specimen all must be maintain. All patients must be provided with a unique lab ID.
- Controls should be checked before running samples.
- All the tests should be performed as per SOPs.
- Reports are to be clinically interpreted and must be duly signed by an authorized signatory.

Data management

- Always archive data for future reference including patient details, findings of analysis, reported results, internal & external quality control.
- Confidentiality of patient records must be ensured at all times with adequate usage of data protection & security systems.
- Standard SOPs have to be maintained.

Figure 20: Bio-medical waste as per BMWM rules

BIO-MEDICAL WASTE MANAGEMENT

Table 13: Bio-medical waste: its categories, segregation, treatment & disposal[351]

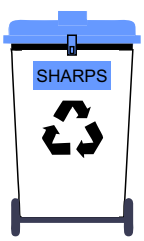
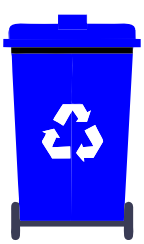
Bin category	Type of waste
 **Yellow (a-e)** coloured & coded, non-chlorinated plastic bags or containers.	a. Human Anatomical Waste b. Animal Anatomical Waste c. Soiled waste: dressings, plaster casts, cotton swabs and bags containing residual or discarded blood and blood components. d. Discarded or Expired Medicine e. Chemical Waste f. Chemical Liquid Waste g. Discarded Linen, Mattresses, beddings contaminated with blood, body fluids, routine mask and gown. h. Microbiology, Biotechnology and other clinical laboratory waste
 **Red** coloured non-chlorinated plastic bags (having thickness equal to more than 50 µ) and containers.	**Contaminated plastic waste (recyclable)**: Wastes generated from disposable items such as tubing, bottles, intravenous tubes and sets, catheters, urine bags, syringes without needles, fixed needle syringes with their needles cut, vacutainers and gloves.
 **White** coloured translucent, puncture proof, leak proof, temper proof containers.	**Waste Sharps** including metals Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded and contaminated metal sharps.
 **Blue (a-b)** Puncture proof and leak proof boxes or containers or card board boxes with blue colored marking	a. Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes. b. Metallic Body Implants.

Table 13: Bio-medical waste: its categories, segregation, treatment & disposal[351]

Treatment & disposal if HCF having tie-up with CBWTF	Treatment & disposal if HCF having its own treatment and disposal facility
Yellow category waste along with pre-treated waste (if treated) should be stored in central storage point and must be handed over to CBWTF.	Incineration or plasma pyrosis disposal in case of no linkage to CBWTF. Disposal of the waste in the deep burial pit should not be practiced unless the hospitals is located in rural or remote isolated place.
No onsite treatment of Red category waste is required. All such waste is needed to be sent to CBWTF for final treatment and disposal	All the recyclable waste generated from the HCF having its own treatment and disposal facility must be sterilised using autoclaving / microwaving / hydro-calving followed by shredding or mutilation or combination of sterilisation and shredding.
Handover the waste to CBWTF without any alteration or onsite treatment.	Sharps waste should be disinfected either with autoclaving or dry-heat sterilization or a combination of autoclaving cum shredding.
Dispose of the empty glass bottles by handing over to CBWTF without any onsite treatment. The residual chemicals in glass bottle should be collected as chemical waste in yellow coloured container / bags and over to CBWTF as yellow(e) waste.	The waste has to be sterilized or disinfected (either by autoclaving or microwaving or hydroclaving or by Sodium Hypochlorite Solution) followed by soaking & washing with detergent prior to sending it for recycling.

Figure 21: Storage & handling of COVID-19 waste

Bio-medical waste means any waste, which is generated during the diagnosis, treatment or immunisation of human beings or animals or research activities pertaining thereto or in the production or testing of biological or in health camps, including the categories mentioned in Schedule I appended to these rules BMW rules, 2016.

Isolation wards

- Use separate colour coded bins/bags/containers in wards.
- Use double, layered bags (using 2 bags) for collection of waste from COVID-19 isolation wards to ensure adequate strength and no-leaks;
- Use a dedicated collection bin labelled as "COVID-19" to store waste and keep separately in temporary storage room.
- Biomedical waste collected in isolation wards can also be lifted directly from ward into CBWTF collection van.
- Bags/containers used for collecting biomedical waste from COVID-19 wards, should be labelled as "COVID-19 waste".
- Maintain separate record of waste generated from COVID-19 isolation wards
- Use dedicated trolleys and collection bins in COVID-19 isolation wards.
- The (inner and outer) surface of containers/bins/trolleys used for storage of COVID-19 waste should be disinfected with 1% sodium hypochlorite solution daily.
- Depute dedicated sanitation workers separately for biomedical waste from isolation wards.
- Faeces from COVID-19 confirmed patient, if collected in diaper, must be treated as biomedical waste and should be placed in yellow bag/container.
- If a bedpan is used, then faeces to be washed into toilet and cleaned with a neutral detergent and water, disinfected with a 0.5% chlorine solution, then rinsed with clean water.
- Collect used masks (including triple layer mask, N95 mask, etc.), head cover/cap, shoe-cover, disposable linen gown, non-plastic or semi-plastic coverall, all types of used gloves except nitrile gloves and left-over food, disposable plates, glass, used masks, used tissues, used toiletries, etc. by COVID-19 patient in yellow bags.
- Collect used PPEs such as goggles, face-shield, splash proof apron, plastic coverall, hazmat suit, nitrile gloves, used gloves and plastic bottles from patients into red bag.

Sample collection centres & laboratories

- Report opening or operation of COVID-19 sample collection centres and laboratories to concerned SPCB.
- Guidelines given for isolation wards should be applied suitably in case of test

Quarantine camps/homes or home-care facilities

- BMW from quarantine centres/camps should be collected separately in yellow coloured bags provided by ULBs.
- Persons operating quarantine camps/centres should inform CBWTF operator to collect BMW as and when it gets generated.
- Hand over the yellow bags containing BMW to authorized waste collectors at door steps engaged by local bodies; or
- Deposit biomedical waste in yellow bags at designated deposition centres established by ULBs.; or
- Handover the biomedical waste to waste collector engaged by CBWTF operator at the doorstep.
- Persons operating quarantine camps/centres or quarantine-homes/home-care should report to ULBs in case of any difficulty in getting the services for disposal of solid waste or biomedical waste.

SPILL MANAGEMENT

Healthcare workers must follow proper guidelines in case of a blood, plasma or chemical spill in hospitals/laboratories.

Blood or plasma spill

- Clean the area taking all precautions (gloves, mask, protective gown etc.)
- Mark the area using demarcation tape.
- Cover the spillage with a filter paper sheet or absorbent material.
- After soaking of spillage discard the filter paper in yellow bin.
- Pour 1% sodium hypochlorite over the area and leave for 15-20 minutes.
- After this mop the area with water, then wipe it with 1% sodium hypochlorite solution (at least three times and finally dry the area).
- Broken glass/plastic should be swept with a brush and dustpan (Do not use hands).
- All spills and accidents are to be reported to the lab supervisor.

Mercurey spill

- Healthcare facilities have to ensure environmentally sound management of mercury or other chemical spills as per steps as given in CPCB guidelines explained below:
- Evacuate area: As far as possible, keep people who are not involved in the clean-up away from spill area to limit exposures and to prevent the spread of contamination.
- Put on face mask: In order to prevent breathing of mercury vapour, wear a protective face mask.
- Remove jewellery so that the mercury cannot combine (amalgamate) with the precious metals.
- Put on rubber or latex gloves. If there are any broken pieces of glass or sharp objects, pick them up with care using brush and a dustpan. Place all broken objects on a paper towel, fold the paper towel and place in a secure puncture proof yellow bag or container and label it as items contaminated with mercury.
- Check a wide area beyond the spill: Locate all mercury beads and look for mercury in any surface cracks or in hard-to reach areas of the floor using flashlights.
- A syringe (without a needle) shall be used to suck the beads of mercury. Collected mercury should be placed into an unbreakable plastic container/glass bottle with an airtight lid half filled with water. After removing larger beads, use sticky tape to collect smaller hard-to-see beads.
- Place all the materials (used & collected) from the spill area into a yellow plastic

bag or container with lid and seal properly and label it as mercury containing waste.

- Sprinkle sulphur or zinc powder over the area. Either powder will quickly bind any remaining mercury. Use the cardboard and then dampened paper towels to pick up the powder and bound mercury. Place all towels and cardboard in a yellow plastic bag and seal all the bags that were used and store in a designated area.
- All the mercury spill surfaces should be decontaminated with 10 % sodium thiosulfate solution. Keep a window open to ventilate after the clean-up.

Waste collected from chemical spills has to be categorized as yellow-e waste, which shall be collected in separate yellow bag and handed over to operator of CBWTF or Hazardous Waste TSDF.

Figure 22: Spill management guidelines

NEEDLE STICK INJURY & PEP

Needle stick injuries (NSIs) are puncture wounds, cuts, or scratches inflicted by medical instruments intended for cutting or puncturing (cannulae, lancets, scalpels, etc.) that may be contaminated with a patient's blood or other body fluids. Contact of blood with non intact skin and contact with mucous membranes (eye, mouth, nose) are also subsumed under the term "needlestick injury."

Post Exposure prophylaxis (PEP)

PEP refers to the comprehensive management given to minimize the risk of infection following potential exposure to blood-borne pathogens (HIV, HBV, HCV). The ultimate goal of PEP is to maximally suppress any limited viral replication that may occur, and to shift the biological advantage to the host cellular immune system to prevent or abort early infection.

Every HCW who sustains a NSI should have access to post-exposure prophylaxis (PEP), as appropriate, within hours of the injury, along with counseling, confidential testing, and follow-up.

Table 14: First aid to be taken in case of a needle stick injury

Contaminated Wound	Contaminated Intact Skin
Encourage bleeding from the skin wound and wash injured area with soap and water. DO NOT squeeze.	Wash the area with soap and water.
Contaminated Eyes	**Contaminated Mouth**
Gently rinse the eyes wide open with distilled water	Spit out any fluid - rinse the mouth with water and spit it out again.

Table 15: Do's & Don'ts in case of a needle stick injury

Do's	Don'ts
• Remove gloves, if appropriate. • Wash site thoroughly with running water. • Irrigate thoroughly with running water or distill water if splashes have gone into the eye or mouth.	• Do not panic! • Do not reflexively place pricked finger into mouth. • Do not squeeze blood from wound, this cause trauma and inflammation, increasing risk of infection transmission. • Do not use bleach, alcohol, betadine, or iodine, on the wound surface as this may further increase trauma.

Testing of the source patient for HBsAg, Anti-HCV and HIV1 and 2 (after consent) should be done. Depending on the source serostatus PEP may be started.

PEP for HIV: If the source is HIV positive or unknown source, antiretroviral prophylaxis should be started ideally within 2 hours of exposure, or within 72 hours. The health care worker should also be tested for HIV at baseline and then 6 to 8 weeks later.

Table 16: Post-exposure management of health care personnel after occupational percutaneous or mucosal exposure to blood or any fluids, by health care personnel Hep B vaccination and response status.

	Post exposure testing		Post exposure prophylaxis	
HCP status	**Source patient (HBsAg)**	**HCP testing (anti-HBs)**	**HBIG**	**Vaccination**
Documented responder after complete series			No action needed	-
Documented nonresponder after two complete series	Positive/Unknown Negative	-*	HBIG x2 separated by 1 month No action needed	
Response unknown after complete series	Positive/Unknown Negative Any result	<10 mIU/mL <10 mIU/mL ≥10 mIU/mL	HBIGx1 None No action needed	Innitiate revaccination Innitiate revaccination
Unvaccinated / incompletely vaccinated or vaccine refusers	Positive/Unknown Negative	- -	HBIGx1 None	Complete revaccination Complete revaccination

Abbreviations: anti HBs= antibody to hepatitis B surface antigen ; HBIG = hepatitis B immune globulin ; hepatitis B surface antigen ; HCP =health care personnel; *Not indicated

Source: MMWR recommendations and reports/vol.67/No.1 by CDCP.

PEP for HCV

In case source is anti HCV reactive, the HCW should be checked for baseline anti HCV and liver function test and followed up periodically with the same. If the HCW becomes anti HCV positive, he should be referred for appropriate management.

QUALITY ASSURANCE

Quality is the ability of a product or service to satisfy the needs of a specific customer. It may be achieved by conforming to established requirements and standards.

Quality assurance is the implementation of systematic coordinated activities in a system to ensure quality results and services.

All aspects of laboratory operation (organizational structure, processes and procedure) needs to be addressed to assure quality.

Internal quality control (IQC)

Internal quality control (IQC) is for detection and minimization of error, and also to check reproducibility in the Lab and is achieved by the following methods:

- Proper maintenance of equipment
- Monitoring of long term of precision and accuracy of results
- Usage of quality reagents and methods/techniques
- Reagent Calibration as recommended by the manufacturers
- Regular running of controls as per individual assay SOP
- Periodic training of technical staff
- Blanks
- Matrix spikes
- Duplicate samples
- Replicate testing
- Retesting of retained items
- Correlation (delta checking)

Levey–Jennings (L-J) charts

The L-J chart is a graph wherein quality control data is plotted to give visual indication whether an ssay/test result is reported correctly. It should be monitored on a monthly basis for all controls (negative as well as positive) for all assays performed.

Westgard rules (WR)

WR rules are multirule quality control rules helps to analyze whether or not an analytical run is in control or out of control. It uses a combination of decision criteria, usually five different consol rules to judge the acceptability of an analytical run. It is monitored for all controls (negative as well as positive) for all assays performed by the chemiluminescence (CLIA) technology.

Co-efficient of variation (CV %)

It is a statistical measure of the dispersal of data points in a data series around the mean. It represents the ratio of standard deviation to the mean. It is useful statistic for comparing the degree of variation from one data series to another. It is used in manual ELISAs to monitor their controls.

External quality control

This includes methods that allow comparison of laboratory's testing to a source outside of the laboratory. It can be achieved in the following ways.

- Proficiency testing
- Rechecking/retesting
- On-site evaluation
- Inter-laboratory comparison
- Participation in external quality assurance (EQAS) programmes

PATIENT SECURITY & DISCLOSURE STATEMENT

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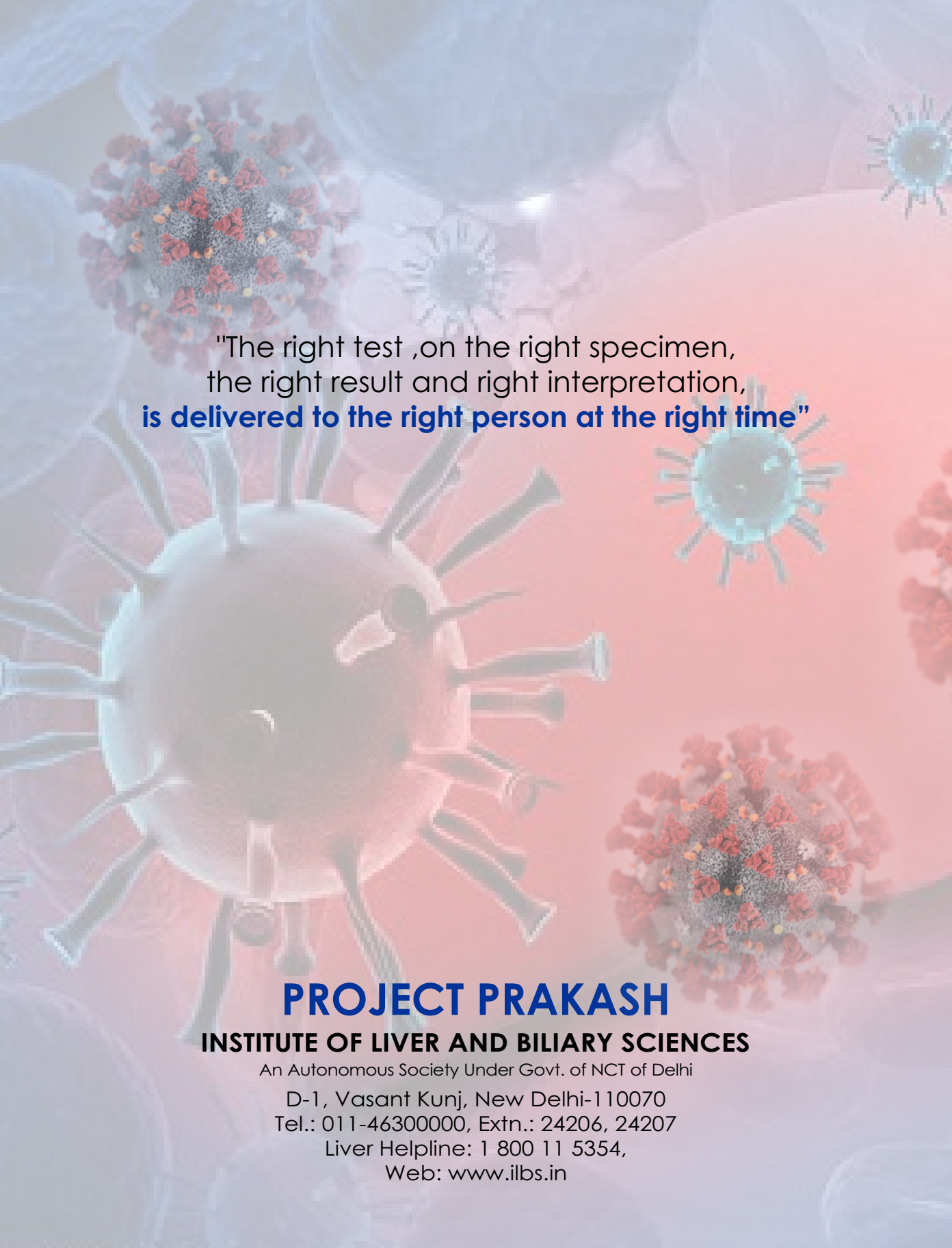
Patient Security & Copyright Issues

The main subject of this training program is to enhance medical education in the field of viral hepatitis and COVID-19. The content of this program does not include any patient related information, and we pay careful attention to protecting patient privacy by for example, not disclosing patient identity in our presentations and avoiding photographing patient faces in presentations. Therefore, our basic standpoint is that there is no need for further protection during transmission of the lectures during face to face conferences or over the internet/telemedicine sessions. This being a learning program, ILBS or any of its faculty/speakers or project PRAKASH staff will not be liable or legally accountable for any clinical or administrative, management complications and or any mishap that arise in any patient or hospital setting. It shall be the responsibility of the participating healthcare professional to utilize their skills and acumen for rendering most appropriate and ethical treatment to their patients.



"The right test ,on the right specimen,
the right result and right interpretation,
is delivered to the right person at the right time"

PROJECT PRAKASH

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