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PROJECT PRAKASH

PRogrammed Approach to Knowledge And Sensitization on Hepatitis

TRAINING MODULE FOR

LABORATORY TECHNICIAN ON VIRAL HEPATITIS

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FOR

LABORATORY TECHNICIANS

ON

VIRAL HEPATITIS

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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. Age is no barrier to liver disease and, as such, raising awareness of risk factors across the age spectrum is essential. Viral Hepatitis is a large problem and is on the rising trend. There is a great need to reverse this growing problem and the promotion of a healthy liver as a way of life to this generation and the next is a key concept.

'Lab Technicians' are integral in making an impact on this liver disease; they can make every contact count by identifying risk factors and by offering health promotion and education to help individuals to make informed choices.

This module on viral hepatitis for lab technicians will describe the knowledge, skills and attitudes that are required to deliver patient-centered liver care. The module focuses on patient centered outcomes and will be an indispensable tool for those commissioning, managing and developing the workforce. The module is intended to be used with other local policies and pathways around the scope of practice undertaken.

This module may also be useful for those health care professionals (HCPs) i.e. GPs, Nurses, social workers, dieticians and drug and alcohol workers who are also working with patients with or at risk of liver disease in primary or secondary care 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 of caring for people with or at risk of liver disease.

MESSAGE FROM THE DIRECTOR

It gives me immense pleasure to know that the lab technicians from across the country would come to the Institute of Liver and Biliary Sciences (ILBS) to be trained in Laboratory Diagnostic Viral Hepatitis. 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 in Hepatology.

Institute of Liver and Biliary Sciences is pleased to welcome each and every one of you to this 'Training Program on Viral Hepatitis'. We intend to share with you standard protocols as well as new developments in screening, diagnosis and prevention of Viral Hepatitis. We hope that through this course we can help build a cadre of Lab Technicians in India who are trained in Viral Hepatitis and may lead the way in the National Program for Control of Viral Hepatitis which is recently launched by the Government of India.

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 Virology for putting up a wonderful course material which would serve as a ready reckoner for the learner.

I do hope that you will enjoy the learning process through Project PRAKASH; and in 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
Director, ILBS

ILBS - AS AN INSTITUTION

The Institute of Liver & Biliary Sciences (ILBS) has been established by the Government of the National Capital Territory of Delhi as an autonomous Super Specialty Institute, under the Societies Registration Act – 1860, New Delhi. ILBS has been granted deemed to be University status by the University Grant Commission under Section 3 of UGC Act, 1956 under de-novo category through the Ministry of Human Resource Development, GOI.

The mission of ILBS is to become a dedicated international Center of Excellence for the diagnosis, management, advanced training and research in the field of liver and biliary diseases. ILBS offers training programs for super-specialties related to Hepato-Biliary and Pancreatic sciences through the Post-Doctoral Courses: DM in Hepatology, Pediatric Hepatology, Organ Transplant Anesthesia and Critical Care; M.Ch. in Hepato-Pancreato-Biliary Surgery. PDCC courses in Virology, Microbiology, Biochemistry, Clinical Nutrition, Renal Replacement Therapy, Radiology, Intervention Radiology and Oncology for a duration of 1 year are also available. There is also a provision of short term courses and observership program for training the faculty and students of other institutes. The institute has also started M.Sc. in Nursing Program.

The mission of the Institute is to develop a facility with international standards, which could provide a comprehensive and a 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.

ILBS aims 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.

INTRODUCTION

The Institute of Liver and Biliary Sciences (ILBS) is an autonomous deemed-to- be University under the Government of National Capital Territory (NCT) of Delhi. It is India's first and the only medical institute dedicated exclusively to the treatment, advanced training and research in Liver, Biliary and Allied Sciences.

Our mission is 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.

There are many challenges in prevention and eradication of viral hepatitis in India. Health professionals in the country need to join hands to deliver best services for viral hepatitis, so that we could achieve the target of viral hepatitis elimination by 2030 which is also the global call for action by WHO.

Project PRAKASH aims to build capacity of primary care physicians and paramedical professionals for the management of viral hepatitis in the country. This training will build capacities in the existing health care delivery system through comprehensive knowledge sharing among technical experts from ILBS and health professionals in India using a common platform.

❖ Hepatitis Induction Program (HIP):

- A one-day training program on laboratory diagnostics of viral hepatitis at ILBS.
- Didactic lectures by the faculty, Live Demonstration and Workshops for the participants
- Some of the topics covered during the day are Overview and burden of Viral Hepatitis, Laboratory diagnosis of Viral Hepatitis A, B, C and E, Universal work precautions and good laboratory practices etc.
- Live Demonstration and hands-on training on serological Techniques of ELISA, CLIA, Rapid card tests etc.

CORE TEAM

Dr. S.K.Sarin,
Director

Dr. Anil Agarwal,
Head, Admin

Dr. Ekta Gupta,
Additional Professor,
Dept. of Clinical Virology

Dr. Reshu Agarwal,
Assistant Professor,
Dept. of Clinical Virology

Arun Prakash,
Assistant Manager,
Admin

Ms. Akanksha Bansal,
Manager (Projects)

Dr. Vikas Bansal,
Program Director

Dr. Sapna Chauhan,
Program Coordinator

Saurabh Gupta,
IT Engineer

Mr. Vinay Kumar,
Assistant Admin

Ashish Kumar,
Assistant Admin

COURSE CURRICULUM

VIRAL HEPATITIS TRAINING PROGRAM
Training Agenda for Lab Technicians

SESSION	DETAILS	SPEAKER	DURATION	TIME
Opening	Participant Registration and Module Distribution	TEAM	75 MINS	8:00 AM - 9:30 AM
	KAP STUDY/PRE -TEST			
TEA BREAK 9:30 AM - 10:00 AM				
Introduction and Overview	Introduction	Akanksha Bansal	15 MINS	10:00 AM - 10:15 AM
	Overview of Viral Hepatitis (A -E)	Dr. Ekta Gupta	45 MINS	10:15 AM - 11:00 AM
Lab Diagnosis	Serological methods in viral hepatitis testing	Dr. Krithiga Ramachandran	30 MINS	11:00 AM - 11:30 PM
	Molecular methods in viral hepatitis testing	Dr. Nitin Kumbhar	30 MINS	11:30 AM - 12:00 NN
Bio - Safety	Good lab practices and quality control	Dr. Kritiga Ramachandran	30 MINS	12:00 NN - 12:30 PM
	Needle Stick Injury and Post exposure prophylaxis	Dr. Reshu Agarwal	30 MINS	12:30 PM - 01:00 PM
LUNCH BREAK 1:00 PM - 2:00 PM				
Hands-on & Demonstration	Rapid card tests	SR/Technician	30 MINS	2:00 PM - 4:00 PM
	ELISA	SR/Technician	30 MINS	
	CLIA	SR/Technician	30 MINS	
	Micro pipetting techniques and calibration	SR/Technician	30 MINS	
Closing	1. Feedback About Training	TEAM	60 MINS	4:00 PM - 5:00 PM
	2. Post - Test			
	3. Valedictory and Certificate Distribution			
HIGH TEA 5:00 PM - 5:30 PM				

Virology Department Message

Department of Clinical Virology Laboratory at the Institute provides a full range of diagnostic services for the detection and monitoring of infection caused by all the Hepatitis viruses (Hepatitis A, B, C, D and E). The laboratory takes pride in performing several diagnostic assays for the first time in the country, like testing for drug resistant substitutions in HCV (RAS), antiviral drug resistance testing in HBV; HBs Ag quantification in clinical samples; testing for Occult HBV infection and cccDNA in biopsy samples; screening for various mutations in HBV like Pre-core, Basal core promoter mutations; 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 Hepatitis 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 Virology 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 lab in India. The Virology Lab has made an exhaustive protocol for Needle Stick Injuries, post exposure prophylaxis for healthcare professionals at ILBS and also keeps a track of the HBV immunization status of the entire ILBS staff.

This training program will capacitate laboratory staff in Viral Hepatitis testing and will be an important link in the National Hepatitis Control Program recently launched by the Government of India.

Dr. Ekta Gupta,
Additional Professor & In-charge
Dept of Clinical Virology
Member Secretary IEC/IRB
Nodal Officer,WHO CC
Institute of Liver & Biliary Sciences

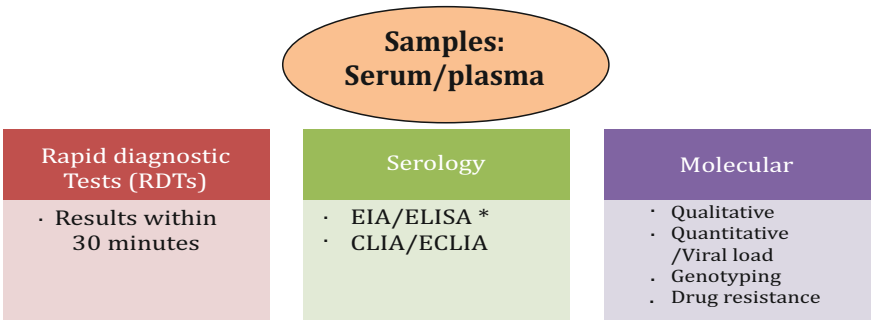
OVERVIEW OF VIRAL HEPATITIS

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, E, and G. 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, D and C may cause acute or chronic infection.

Table 1: Comparative features of Viral Hepatitis:

Feature	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	Acute: good Chronic: poor	Good
Prophylaxis	Immunoglobulin, Inactivated vaccine	HBIG, Recombinant vaccine	None	HBV vaccine	Unknown

Table 2: Currently available testing methods for viral hepatitis



* **EIA** - Enzyme immunoassays
ELISA - Enzyme-linked immunosorbent assay
CLIA - Chemiluminescent immunoassay
ECLIA - Electrochemiluminescence immunoassay

LABORATORY DIAGNOSIS OF VIRAL HEPATITIS

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 3: Serological viral markers for Hepatitis A

Serological viral markers	Importance
a) Anti -HAV IgM antibodies	Confirms diagnosis of acute Hepatitis A Rapidly increases in titre over 4 -6 weeks Declines to non -detectable within 3 -6months
b) 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.

LABORATORY DIAGNOSIS OF HEPATITIS B VIRUS (HBV)

Hepatitis B, a DNA virus belonging to *Hepadnaviridae 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 chronic infection. Diagnosis of HBV infection is based on two categories of laboratory tests, serological and molecular.

1. Serological tests

Table 4: Serological viral markers for the 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 5: 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

2. Molecular tests:

a) Viral load testing: 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.

b) Genotyping: 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).

c) Drug resistance: Direct sequencing and reverse hybridization are currently the main methods for detecting drug-resistance mutations of hepatitis B virus (HBV).

LABORATORY DIAGNOSIS OF HEPATITIS C VIRUS (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.

❖ **Initial testing for hepatitis C has three parts:**

1. Serological test: for anti- HCV antibody
2. Molecular test: for HCV RNA
3. HCV genotyping

1. Screening tests for anti-HCV antibody detection are:

a. Enzyme Linked Immunosorbent Assay (ELISA):

b. Chemiluminescent microparticle immunoassay (CMA):

c. 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%.

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

2. Molecular test: for HCV RNA

a. HCV RNA is a confirmatory test for hepatitis C virus infection.

b. 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.

c. 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.

3. HCV Genotyping Testing:

a. 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.

b. 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.

LABORATORY DIAGNOSIS OF HEPATITIS D VIRUS (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.

HDV Lab diagnosis:

- Serology (antibody detection)- anti-HDV-total and IgM anti-HDV
- Antigen detection- HDAg- ELISA, Western blotting
- NAAT –RT-PCR (Nucleic Acid Amplification Test)

LABORATORY DIAGNOSIS OF HEPATITIS E VIRUS (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.

Serological tests

The methods available of detection of antibodies are ELISA

Table 7: Serological viral markers for Hepatitis E

Serological viral markers	Importance
a) 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 mths in 50% patients
b) 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.

BIOSAFETY AND UNIVERSAL WORK PRECAUTIONS

Introduction:

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.

Important Terms:

- Hazard- Capable of causing harm to humans & environment.
- Risk- Probability of causing harm to humans and environment.
- Control-Measures to minimize exposure to hazard.

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 equipments.
- 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.

Different Levels:

I. Work that involves agents of no known or minimal potential hazard to laboratory personnel and environment

II. Work that involves agents of moderate potential hazard to personnel & environment

III. Work that involves indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation.

Procedures done in the laboratory require Biosafety level II precautions

Biosafety Level II:

All blood samples in the laboratory should be handled as if it is infectious.

Precautions:

- Bench tops of impervious material decontaminated daily with hypochlorite.
- Lab rooms with closable doors and 'One pass in flow' air system.
- Sinks, waste decontamination facility available within the work area.
- Gowns & gloves used routinely.
- No mouth pipetting allowed.
- No eating, drinking, smoking etc in the work area.
- High risk activities clearly separated from low risk activities.
- Extreme caution in handling needles.
- Any accidental exposure immediately reported to the laboratory director.

Causes of Occupational Injury:

- Wrong work practices (e.g. mouth pipetting).
- Ignorance, inexperience.
- Failure to follow established procedures.
- Overcrowding.
- Non-availability of biosafety devices.

Managing Spills of Blood and Plasma:

- 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 mins.
- 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.

NEEDLE STICK INJURY AND POST EXPOSURE PROPHYLAXIS

Definition

Needlestick 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.

First aid to be taken

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.

Do's	Don'ts
Remove gloves, if appropriate. Wash site thoroughly with running water. Irrigate thoroughly with running water or distilled 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.

PEP for HBV:

	Treatment when source is found to be		
Exposed person	**HBsAg Reactive**	**HBsAg Non reactive**	**Unknown/not tested**
Not Vaccinated	Hep B Immunoglobulin, initiate vaccination	Initiate vaccination	Initiate vaccination
Previously vaccinated, known responder	Test for anti -HBs If >10mIU/ml: nothing If < 10mIU/ml: booster dose vaccine	No treatment	No treatment
Previously vaccinated, known non responder	Hep B Immunoglobulin Vaccination	No treatment	If known high risk, may treat as if source were HbsAg positive
Response unknown	Test for anti -HBs If >10mIU/ml: nothing If < 10mIU/ml: Hep B immunoglobulin + booster dose vaccine	No treatment	Test for anti -HBs If >10mIU/ml: nothing If < 10mIU/ml: booster dose vaccine

***Responder-** Anti HBs titre > 10mlu/ml after completion of vaccination series.

***Non Responder-** Anti HBs titre < 10mlu/ml after completion of 2 series vaccination.

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 manangement.

QUALITY ASSURANCE IN CLINICAL VIROLOGY

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.

A. 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 assay/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 QC rules help analyze whether or not an analytical run in control or out of control. It uses a combination of decision criteria, usually six 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.

COEFFICIENT 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.

B. 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

COMMON SEROLOGICAL TECHNIQUES IN VIROLOGY

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.

1. 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

Well is precoated with antigen
↓
Test serum is added-antibodies bind
↓
Labeled antiglobulin is added
↓
Enzyme substrate is added
↓
Color change

Indirect ELISA

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

Sandwich ELISA

2. 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.

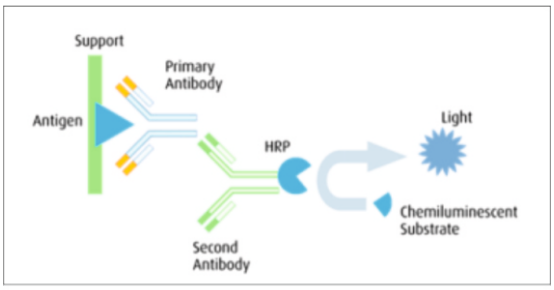


Fig: Principle of Chemiluminescence immunoassay

3. 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.

1. 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.

2. 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.

3. 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.

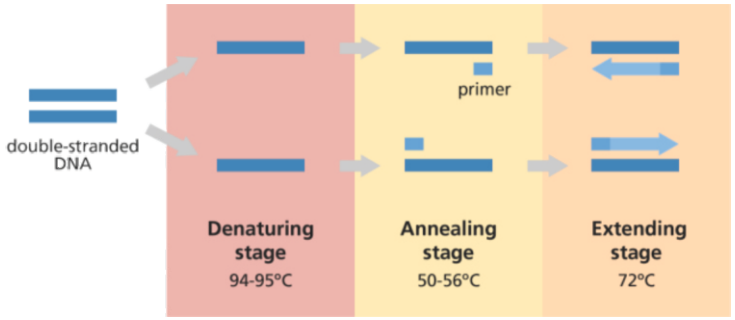


Fig: Steps of PCR

There are different types of PCR

1. Real-time PCR
2. Quantitative real time PCR (Q-RT PCR)
3. Reverse Transcriptase PCR (RT-PCR)
4. Multiplex PCR

PATIENT SECURITY AND COPYRIGHT ISSUES

Acknowledgement and Disclosure Statement

The authors sincerely appreciate the kind cooperation and expertise of all involved universities, institutions and organizations. We extend special thanks to Cipla Foundation who has provided financial grant through their charitable donation program. The grant is used only for the purpose of education and training and fulfillment of our research and education activities. There are no conflicts of interest or financial ties to disclose. We express our gratitude and appreciation to the Faculty Department of Virology ILBS who could only make this training program for lab technicians so successful.

Patient Security & Copyright Issues

The main subject of this project is to enhance medical education in the field of liver diseases. The contents of this project do 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. This being a learning program, ILBS or any of its faculty 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. It shall be the responsibility of the participating doctor/nursen/technician to utilize his/her skills and acumen for rendering most appropriate and ethical treatment to his/her patients.

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