PROJECT PRAKASH
PRogrammed Approach to Knowledge And Sensitization on Hepatitis

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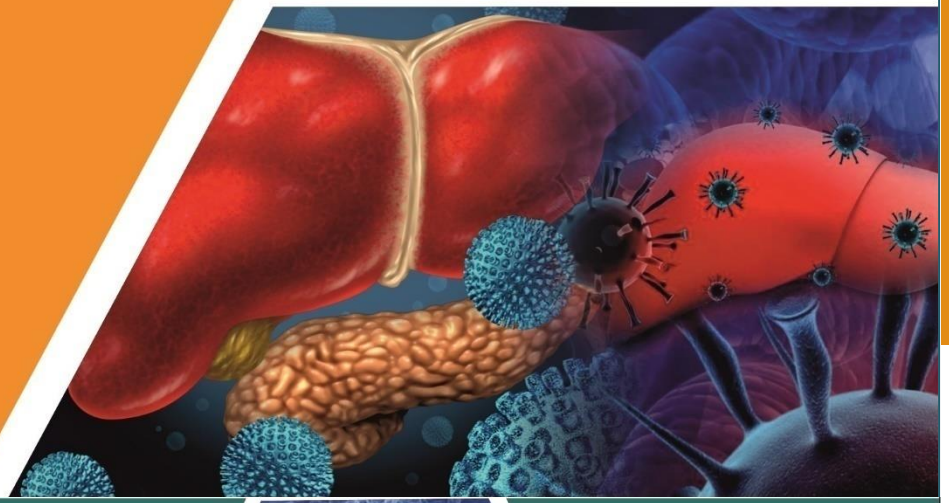

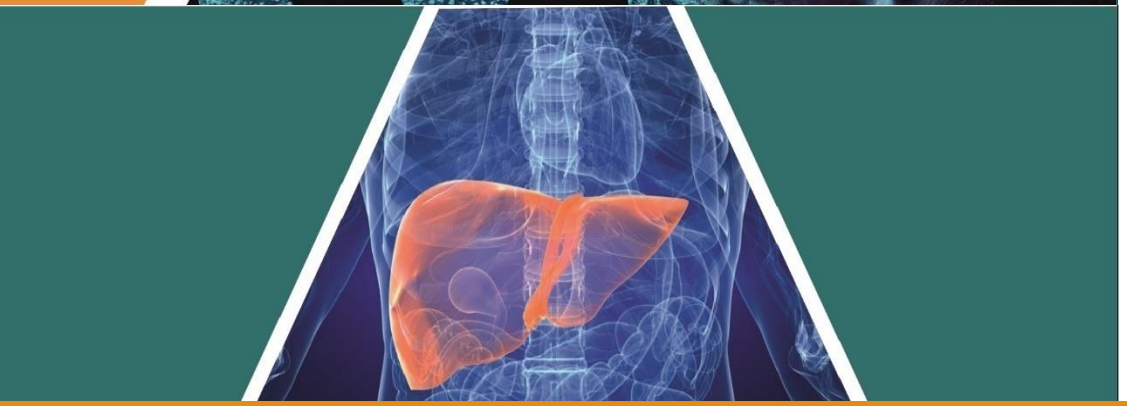

HEPATITIS INDUCTION PROGRAM

FOR LAB TECNICIANS

OVERVIEW OF VIRAL HEPATITIS A to E

Dr. Ekta Gupta,
Additional Professor & In charge
Dept of Clinical Virology, ILBS

INSTITUTE OF LIVER & BILIARY SCIENCES, NEW DELHI
www.ilbs.in

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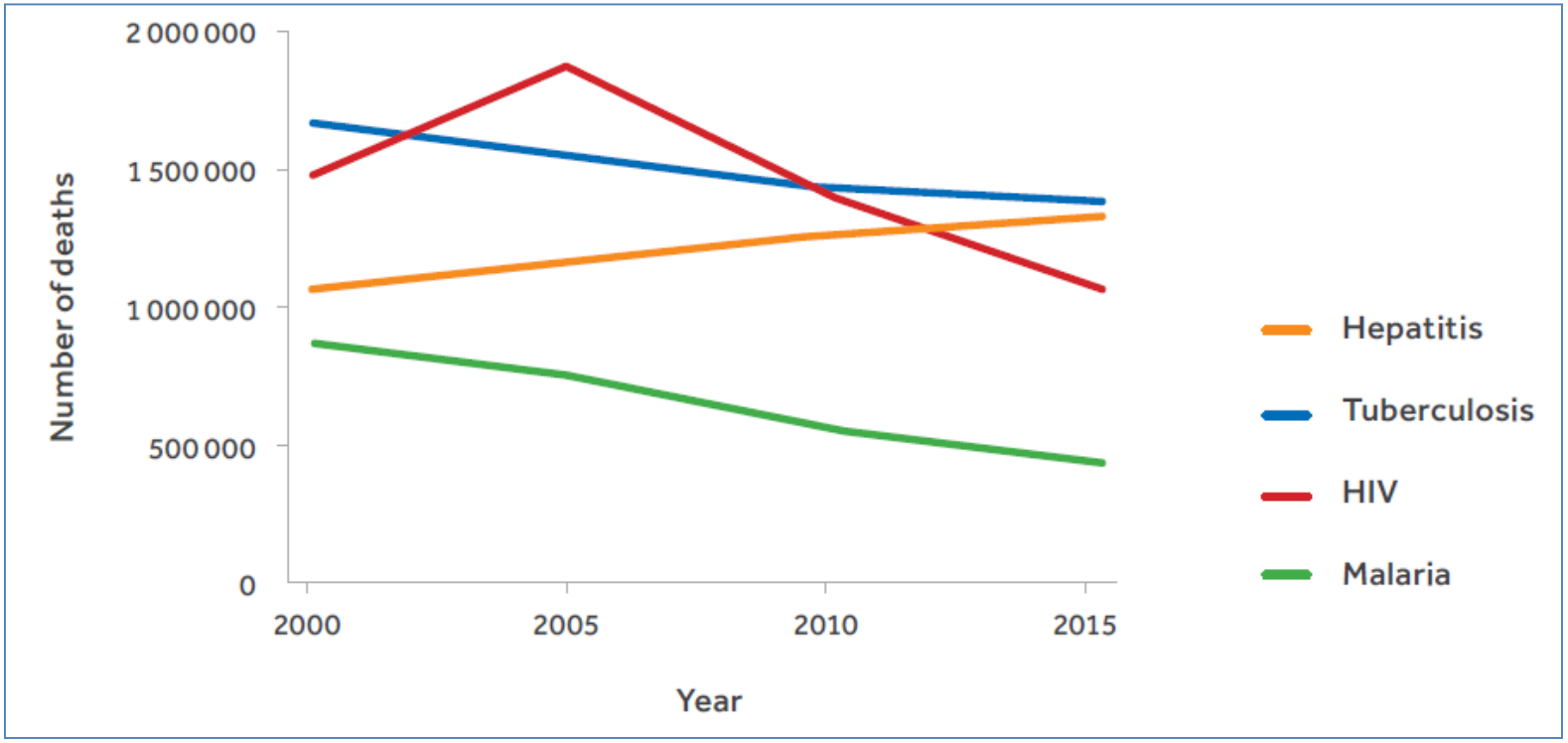
INSTITUTE OF LIVER & BILIARY SCIENCES, NEW DELHI

Viral Hepatitis : a major public health problem



" Elimination of viral hepatitis as a major public health threat by 2030"

Reducing new infections by 90% and mortality by 65%.

GHSS strategy on VH, Global Hepatitis Report WHO,2017

India : Viral Hepatitis Burden

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40 million
11% of world
24% High viremic

12 Million
Prevalence:
0.094% to
15.0%.

Hyperendemic

Hyperendemic

- 3% die per year from B and C
- Varied prevalence

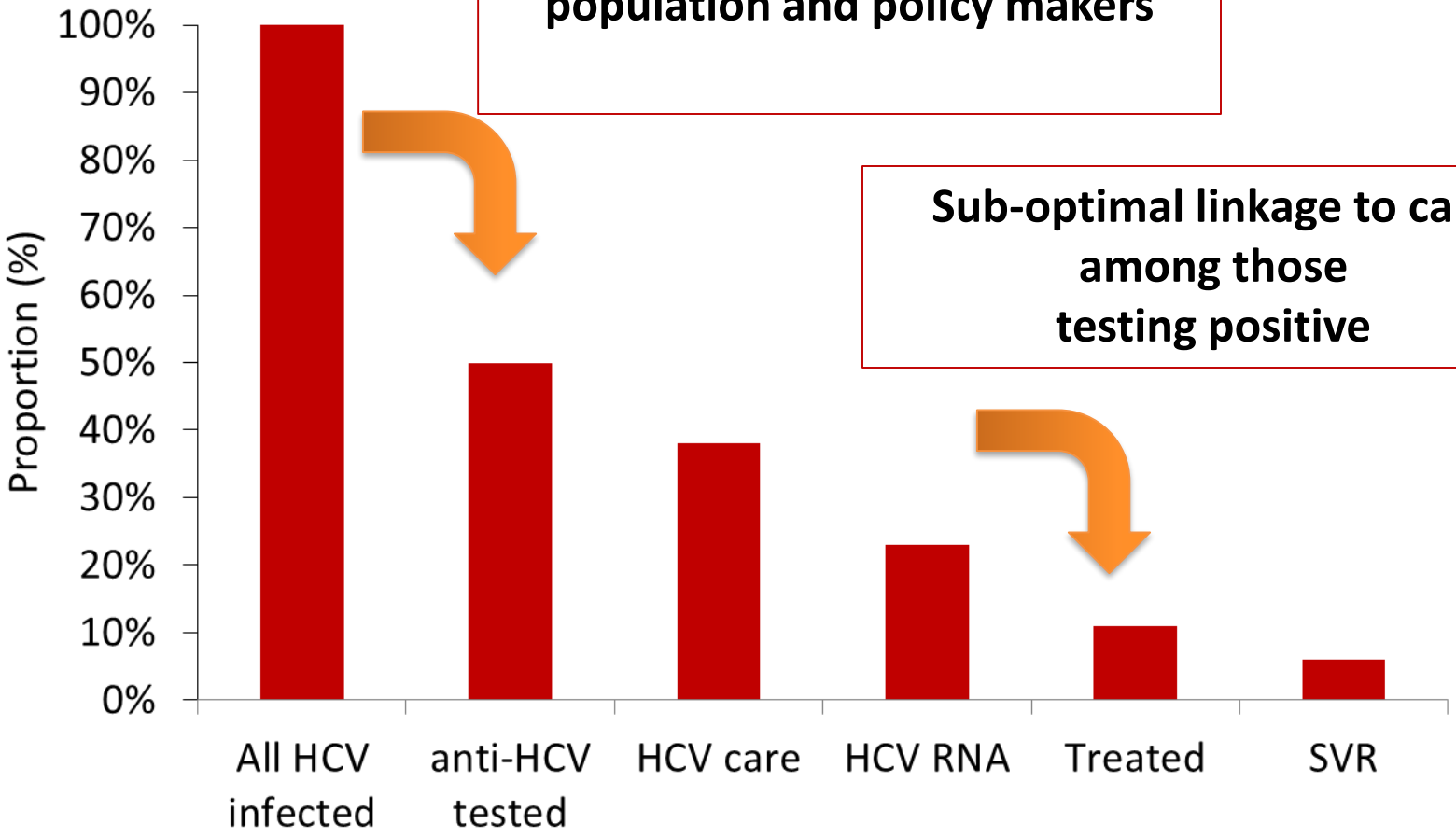
Hepatitis Viruses-an overview

	HAV	HBV	HCV	HDV	HEV
Source	Feces	Blood/blood-derived body fluids	Blood/blood-derived body fluids	Blood/blood-derived body fluids	Feces
Transmission	Food/water	Mother to child,Blood borne,injections	Blood borne Unsafe injections	Blood borne Mother to child	Food/water
Chronic infection	No	Yes	Yes	Yes	**Yes?**
Prevention	Vaccine Sanitation Ensure safe drinking water	Pre/post exposure immunization	Blood donor screening, Risk behaviour modification	Pre/post exposure immunization	Sanitation Ensure safe drinking water
Treatment	Nothing	Yes, lifelong	Yes, DAA Limited course	Yes	Nothing

Large number remains undiagnosed

Lack of knowledge /awareness among health care workers, population and policy makers

Sub-optimal linkage to care among those testing positive



Holmberg et al, *NEJM* 2013; 368:1859-62

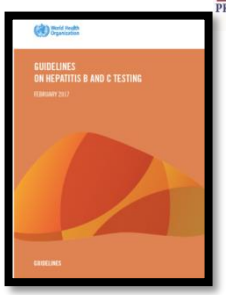
VH Testing in India : current practices

- Blood/organ donors : HBsAg, anti HCV, HBcTotal?
- **Patients with abnormal LFT:** HBV,HCV,HAV? HEV?
- HCW with NSI: HBV,HCV
- Newborns born to HBs Ag positive mothers
- *Pregnant females*
- *Prior to surgery??*

Importance of screening

- Early identification, prevent development of serious liver damage.
- Refer to treatment, cure.
- Appropriate education : risk behavior modification.
- Prevention of onward transmission.
- Immunization to other infections: improves health outcome.
- Development of evidence based public health interventions.

WHO 2017 testing guidelines



	Who to test?	How to test?	How to confirm current/viraemic infection?	Monitoring treatment response	Promoting uptake of testing and linkage to care
HBV	Who to test	How to test (HBsAg)	Confirmation of viraemic HBV infection (HBV DNA)	Monitoring of HBV treatment response and disease progression	Dried blood spots for HBV serological and virological testing
HCV	Who to test	How to test (anti-HCV antibody)	Confirmation of viraemic HCV infection (HCV RNA or cAg)	Assessment of HCV treatment response (test of cure)	Dried blood spots for HCV serological and virological testing

Pre- and post-test counselling

Other interventions to promote uptake of testing and linkage to care

Only for HBV & HCV

Focus on LMIC ,Help in development of National Guidelines

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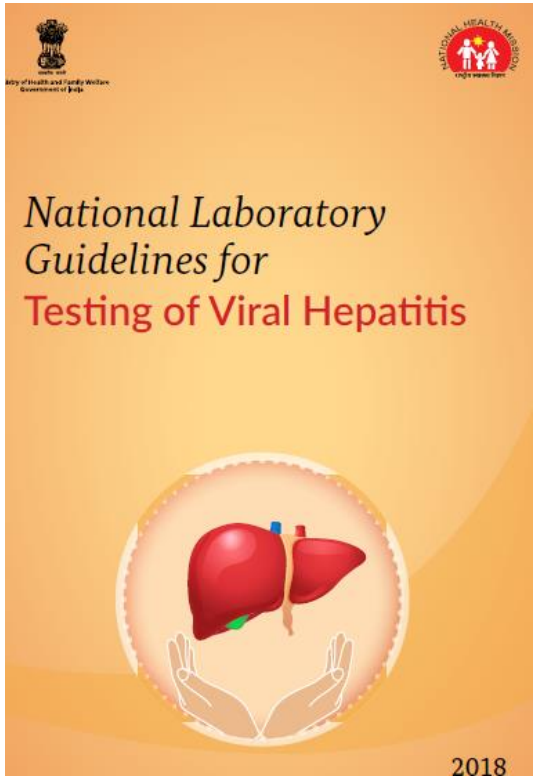
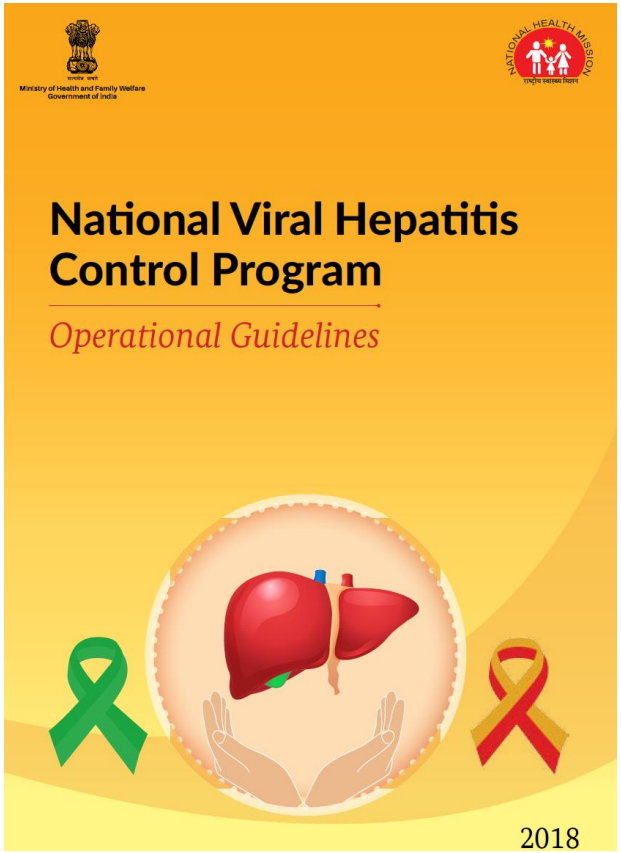
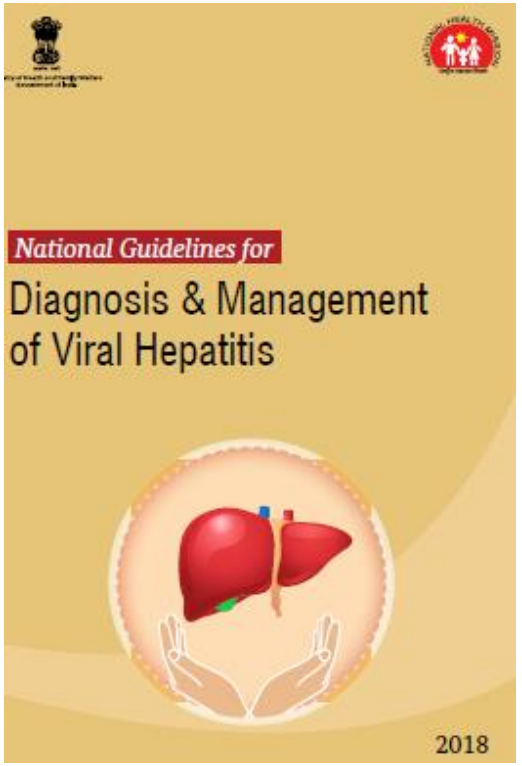
National Viral Hepatitis Control Program India



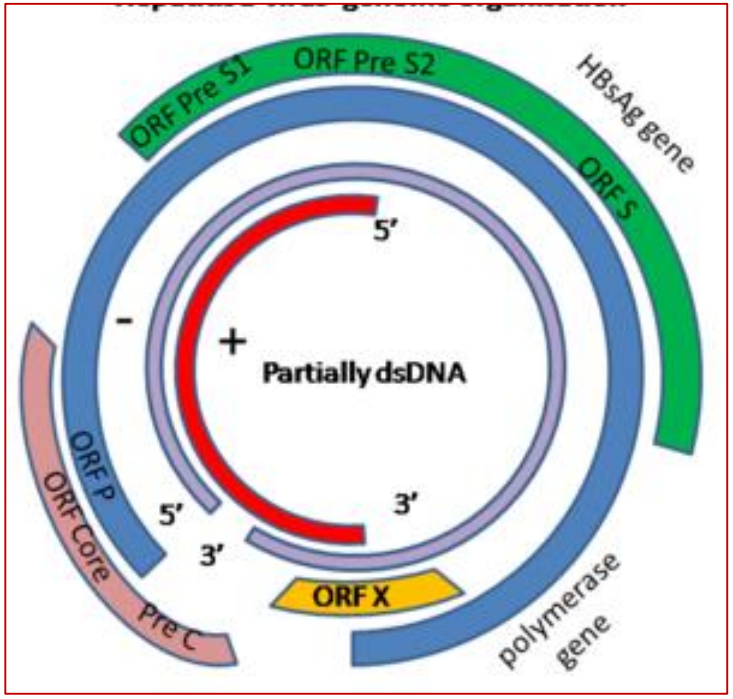




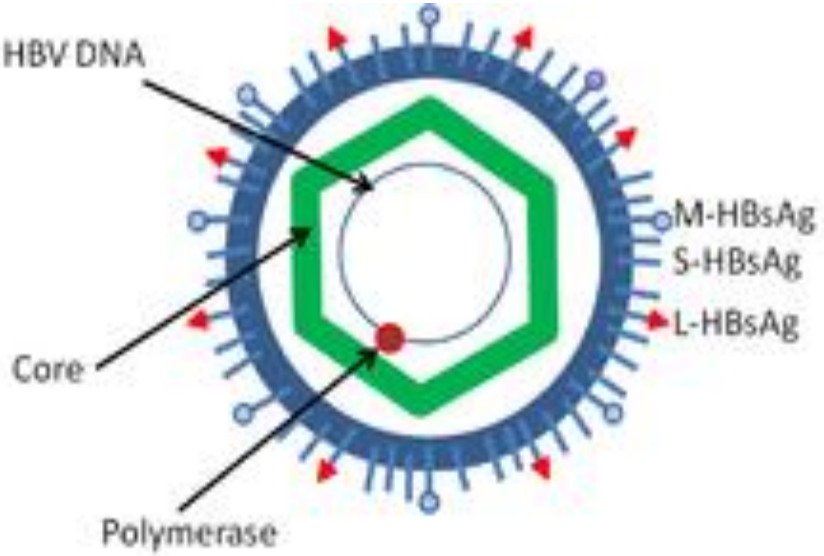
NVHCP launched by GoI on 28th July 2018

Hepatitis B virus infection

HBV Virus

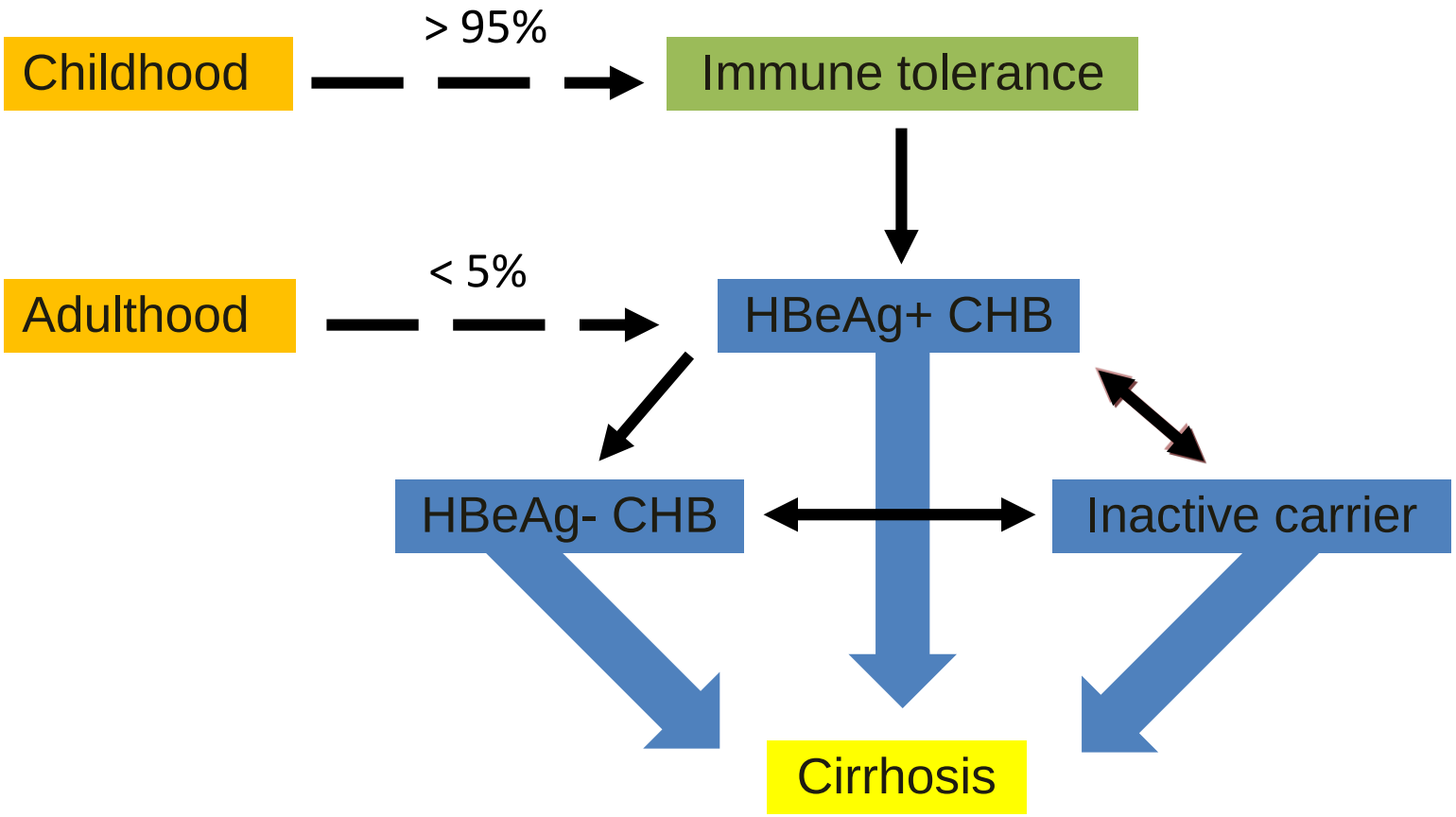


- Partially double stranded DNA virus
- 3.2 kb, *Hepadnaviridae*
- 4 ORF's
- **Surface** (PreS1/S2/S)
- **Core (precore /core) –** core & e antige
- **Polymerase –** DNA Polymerase
- **X -** a transactivator of viral transcriptior

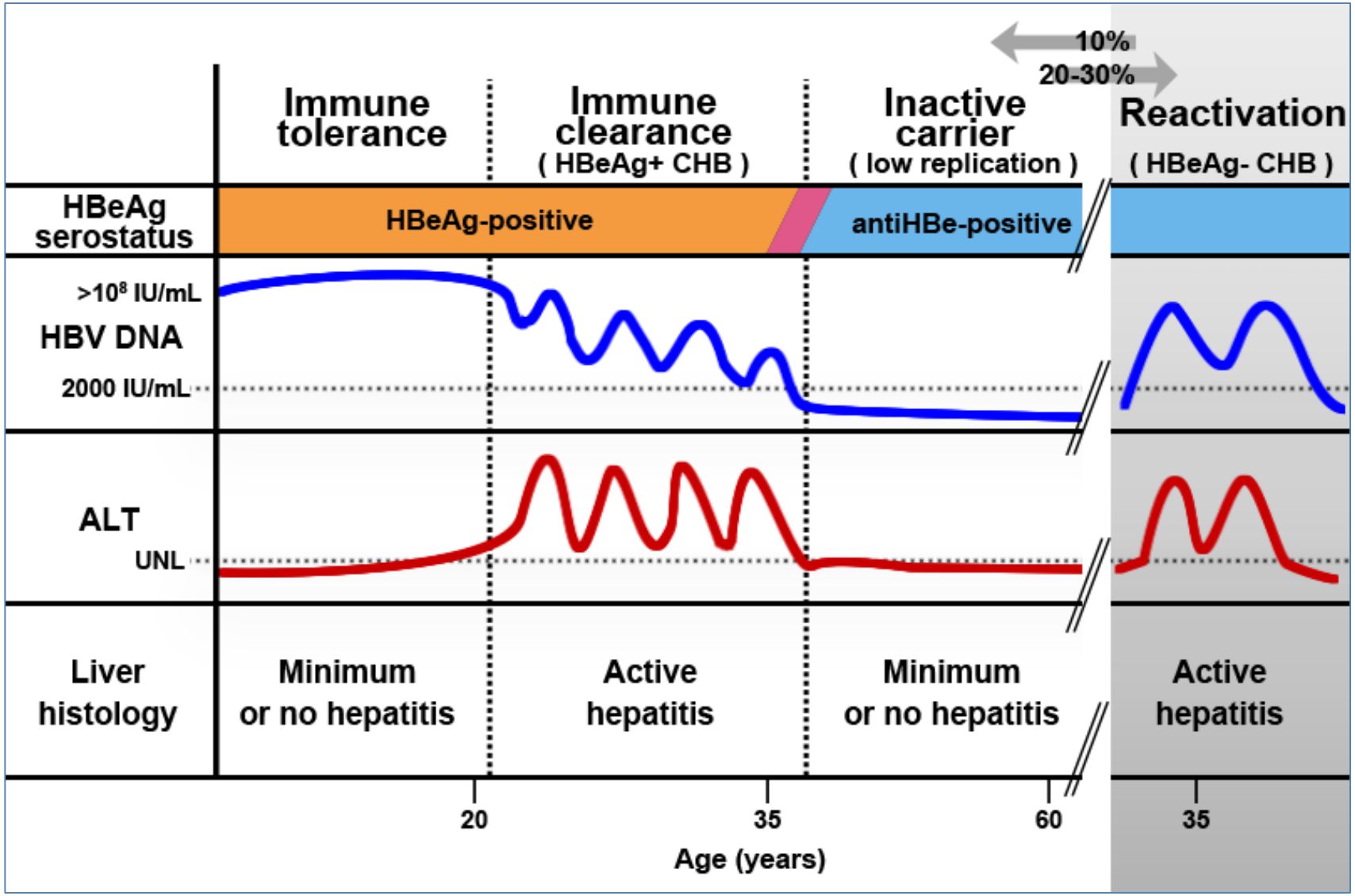


Natural History of HBV Infection

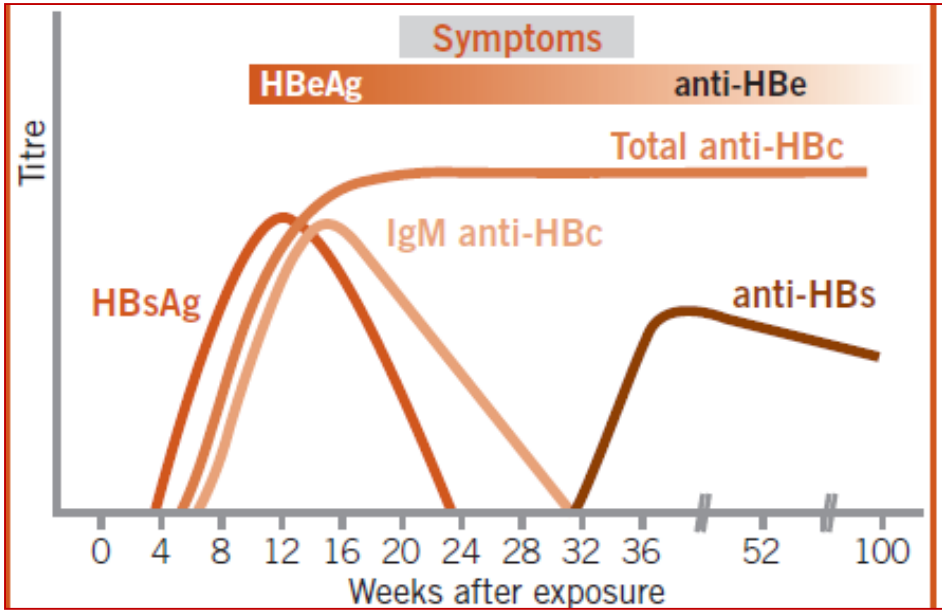
Most of the CHB seen are mother to child transmission

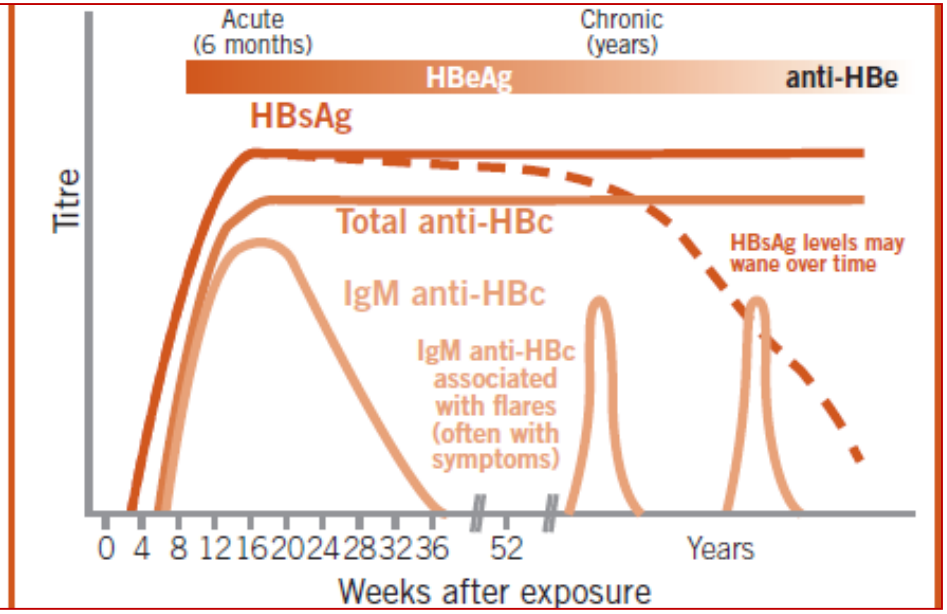


Seeff L, et al. N Engl J Med. 1987;316:965-970.


10%
20-30%
Immune tolerance
Immune clearance
(HBeAg+ CHB)
Inactive carrier
(low replication)
Reactivation
(HBeAg- CHB)
HBeAg serostatus
HBeAg-positive
antiHBe-positive
>10⁸ IU/mL
HBV DNA
2000 IU/mL
ALT
UNL
Liver histology
Minimum or no hepatitis
Active hepatitis
Minimum or no hepatitis
Active hepatitis
20
35
60
35
Age (years)

Diagnostic markers in HBV infection



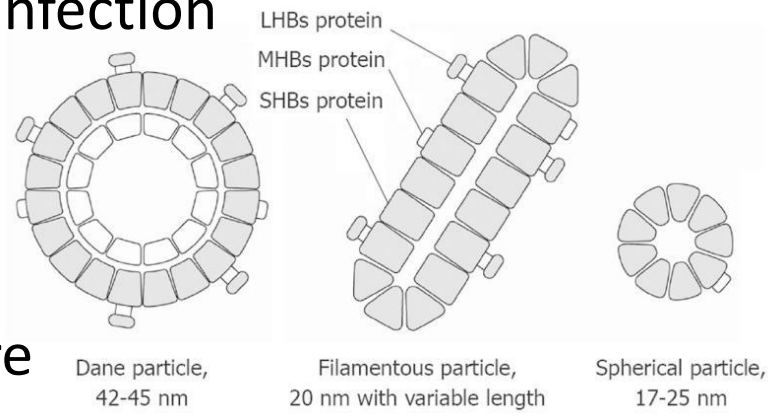


Acute HBV infection

Chronic HBV infection

HBsAg : Hallmark of HBV infection

- Marker of HBV infection
- Seroprevalence marker
- Helps in the viral entry
- Triggers immunity
- Levels reflects the stage of viral infection
- Transcriptional activity of cccDNA
- Seroconversion is functional cure



Antibody to S Ag :Anti HBs

- Immunity to HBV infection.
- Is measured as m IU/ml.
- Response to HBV vaccination (2-3 months after).
- ≥ 10 m IU/ml is protective.
- Titer weans off with time.
- Vaccine escape mutant: G 145 R. (break through infection: rare)

Antibody to Core Ag

- **IgM HBc:** marker of acute infection.
- Also seen during CHB reactivation.
- Can be semi quantified by CLIA: S/Co
- Levels of HBc IgM differs: Acute > 10 S/Co

- **Total HBc** : IgM + IgG
- Marker of exposure,occult HBV
- Quantitation: qHBc directly proportional to Liver inflammation and hepatitis.

Hepatitis B e Ag

- It is secreted from the infected cell but is not part of the virion structure and does not have any known role in replication.

- In the neonate born to an HBV-infected mother, it has been suggested that HBeAg crosses the placenta and induces tolerance.

- CHB e Ag positive : seroconversion Antibody to HB e

  (Anti HBe) :treatment response.

- Can be quantitated : PE IU/ml.or IU/ml.

Who to test for Chronic HBV Infection

- **General population :** Intermediate > 2% or high prevalence >5%

- **Routine testing in pregnant women:** ≥2% or ≥5%% HBsAg seroprevalence.

- **Focused testing in most affected populations**: in all settings

  High HBV seroprevalence or history of exposure /high-risk behaviours.

  Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis

  Sexual partners, children and other family members, and close household contacts

  HCWs

- **Blood donors**: in all settings

WHO guidelines ,2017

How to test for HBV

HBsAg ≥0.4%

Assay 1

HBsAg < 0.4%

Assay 1

Assays should meet minimum acceptance criteria of either WHO PQ or a stringent regulatory review for IVDs

Sensitivity : 90-98%,Specificity : 98-99%

2nd assay : PPV of >97%

Assay 2 : Confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT/EIA assay

Quantitative HBV DNA

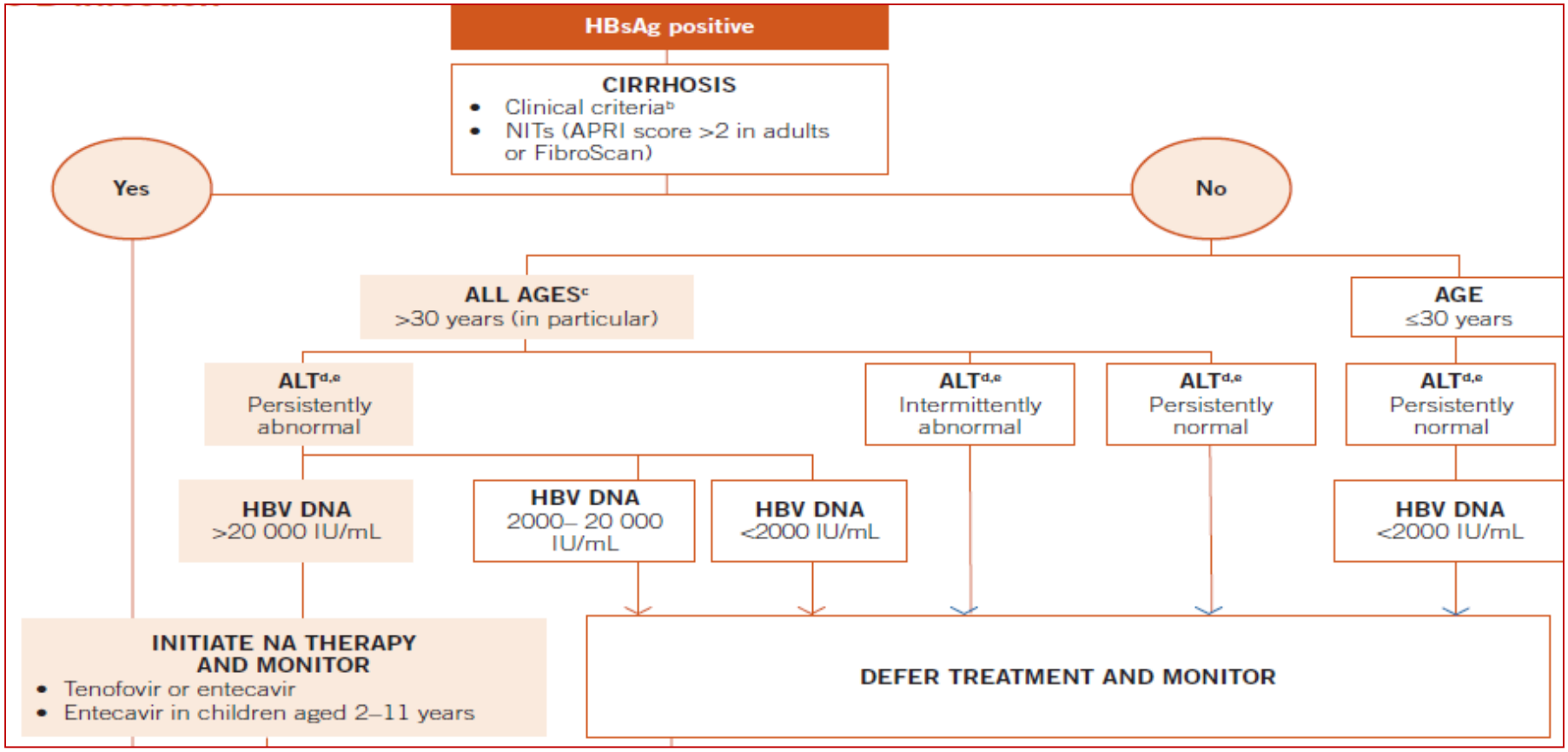
Further testing for viraemic infection

Further testing as appropriate

Repeat HBsAg after 6 months

WHO guidelines ,2017

Assessment for Treatment



Monitoring at least annually for treatment : **ALT, DNA**
On T/t every 3 monthly by **HBV DNA ± HBeAg**

WHO guidelines ,2017

Interpretation of Screening Tests

HBsAg	Anti-HBc	Anti-HBs	Interpretation	Management	Vaccinate?
+	+	–	Chronic hepatitis B	Additional testing and management needed	No
–	+	+	Past HBV infection, resolved	No further management unless immunocompromised or undergoing chemotherapy or immunosuppressive therapy	No
–	+	–	Past HBV infection, resolved or false-positive	HBV DNA testing if immunocompromised patient	Yes, if not from area of intermediate or high endemicity
–	–	+	Immune	No further testing	No
–	–	–	Uninfected and not immune	No further testing	Yes

Other HBV markers for individual assessment of patient

Test Result	Interpretation
HBsAg (—) Total anti-HBc (—) anti-HBs (—)	Susceptible
HBsAg (—) Total anti-HBc (+) anti-HBs (+)	Immune due to natural infection
HBsAg (—) Total anti-HBc (—) anti-HBs (+)	Immune due to hepatitis B vaccination
HBsAg (+) Total anti-HBc (+) IgM anti-HBc (+) anti-HBs (—)	Acutely infected
HBsAg (+) Total anti-HBc (+) IgM anti-HBc (—) anti-HBs (—)	Chronically infected
HBsAg (—) Total anti-HBc (+) anti-HBs (—)	Four interpretations possible 1. Recovering from acute HBV infection 2. Distantly immune and test not sensitive enough to detect very low level of serum anti-HBs 3. Susceptible with a false positive anti-HBc 4. Chronic HBV infection with rare circumstance where HBV does not produce detectable HBsAg

Molecular tests for HBV

- **HBV DNA PCR : ** Qualitative

  (newborn/dialysis/immunosuppressed)

  Quantitative: treatment initiation/monitoring

- **HBV genotyping/ HBV drug resistance testing/HBV variants**

  screen: surface gene, PC/BCP

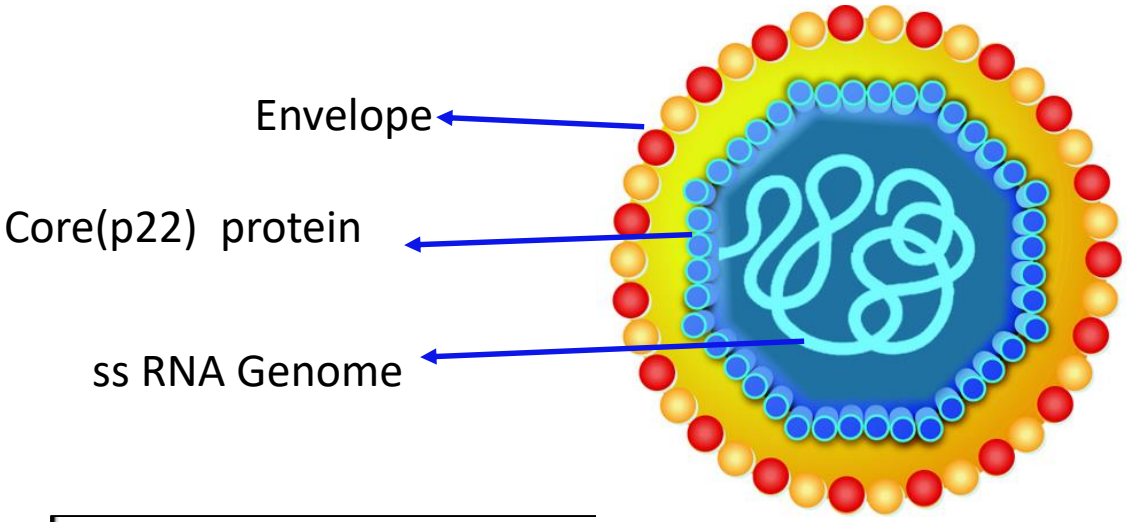
- Occult HBV diagnosis

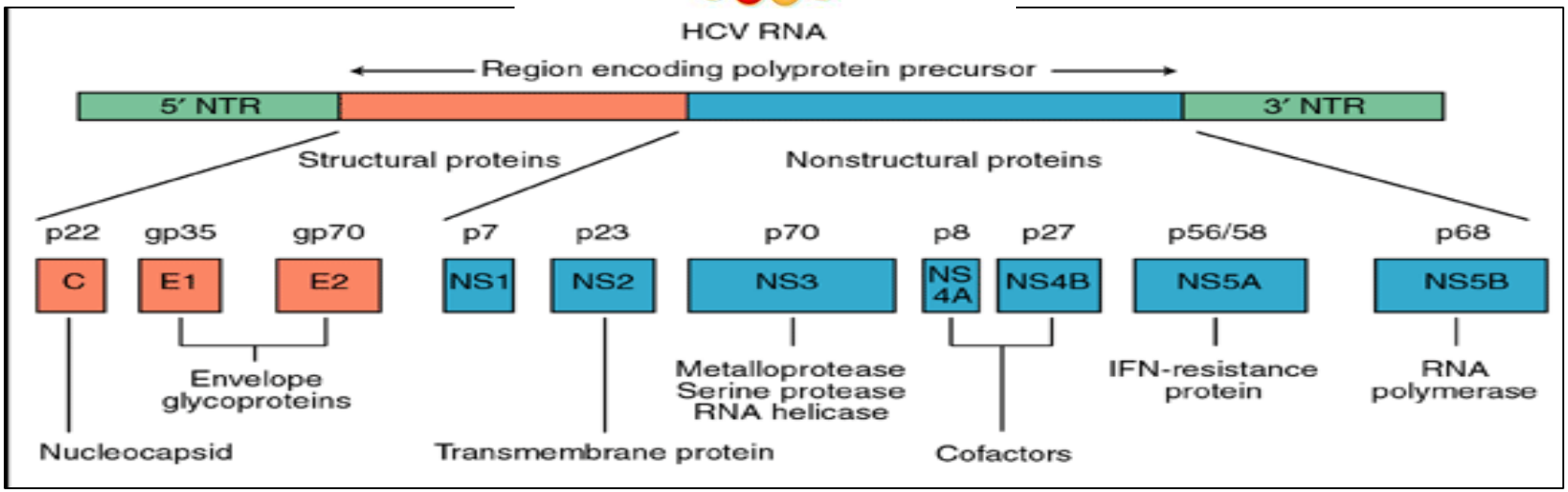
  HBV DNA/ ccc DNA PCR from tissue

Hepatitis C Virus Infection

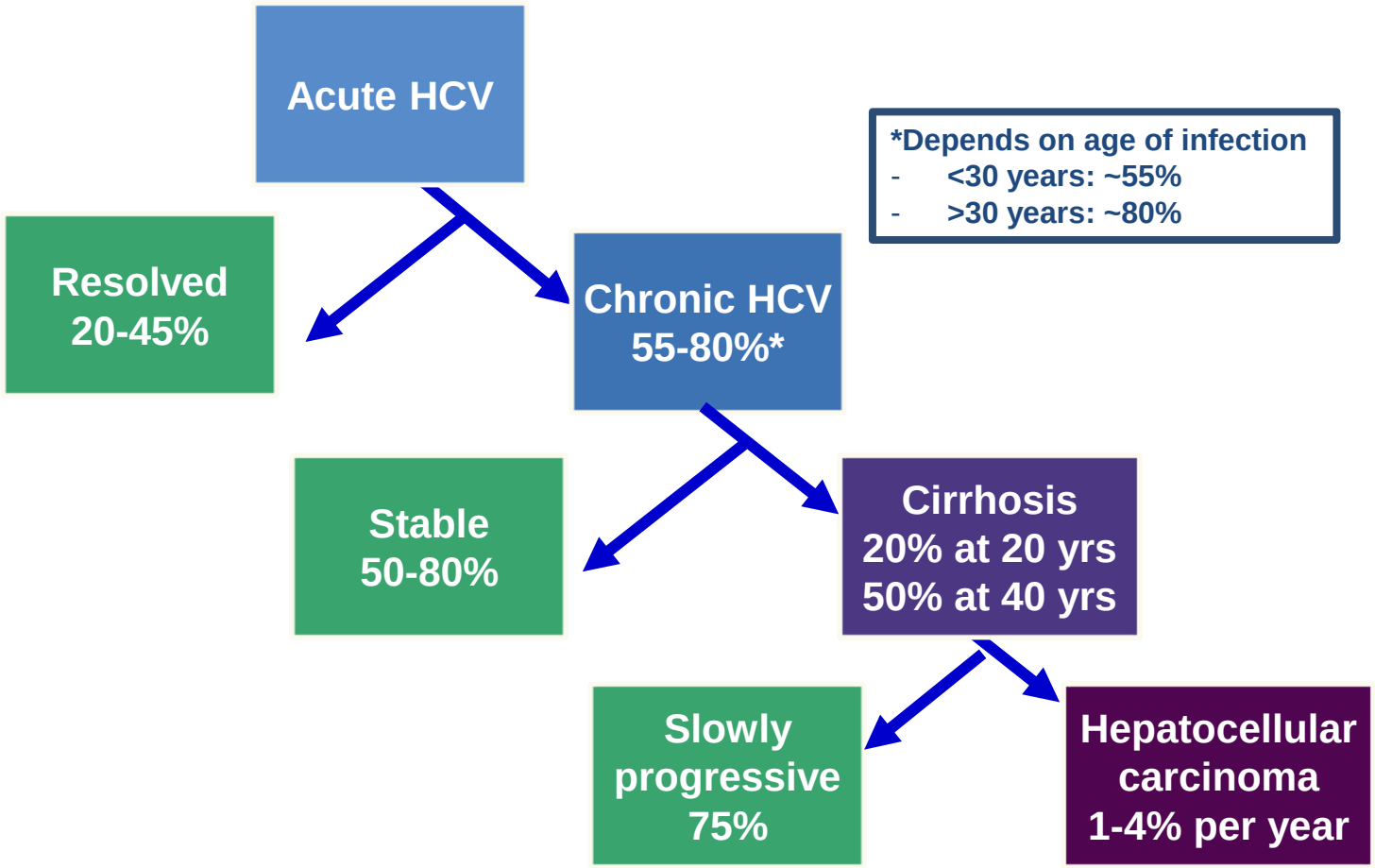
Hepatitis C virus

HCV is an enveloped, 9.6-kb, positive-sense, ss RNA virus family *Flaviviridae*



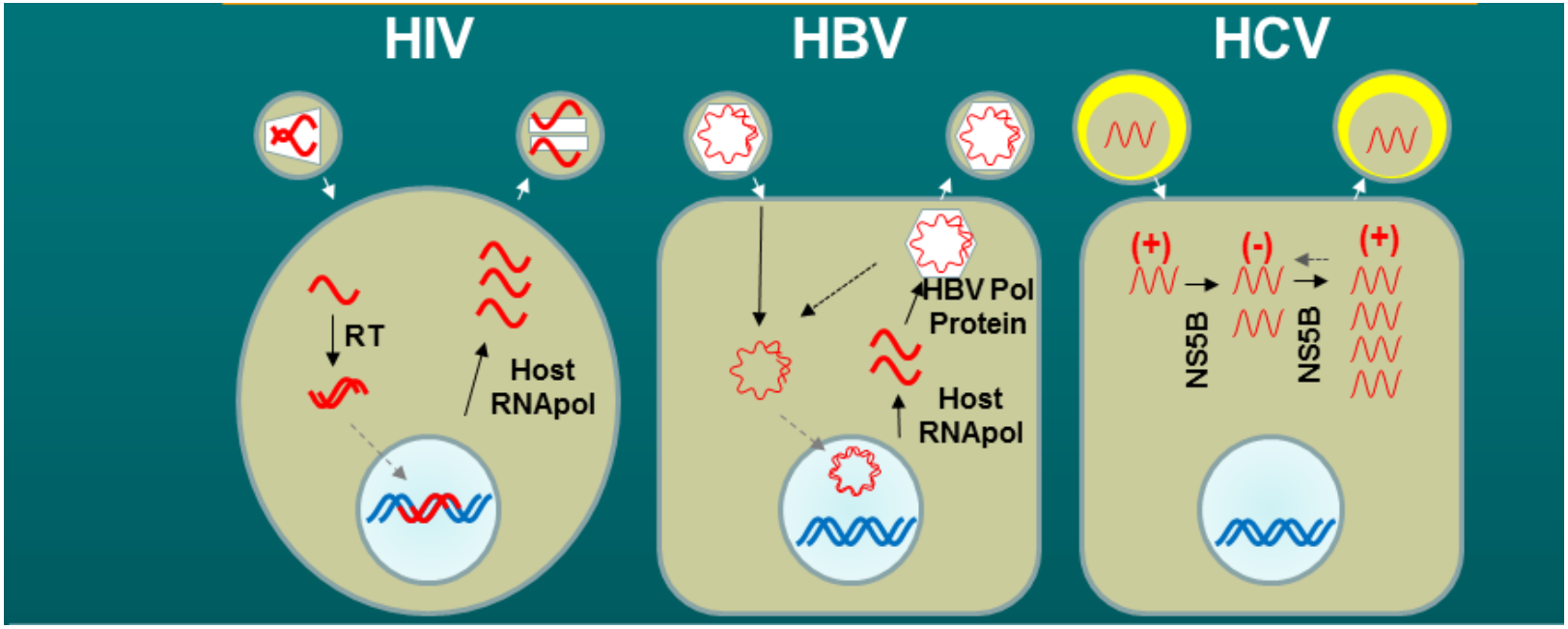


Hepatitis C Virus Infection: Natural History



Hepatology. 2000;31(4):1014-1018.

Differences with HBV & HIV

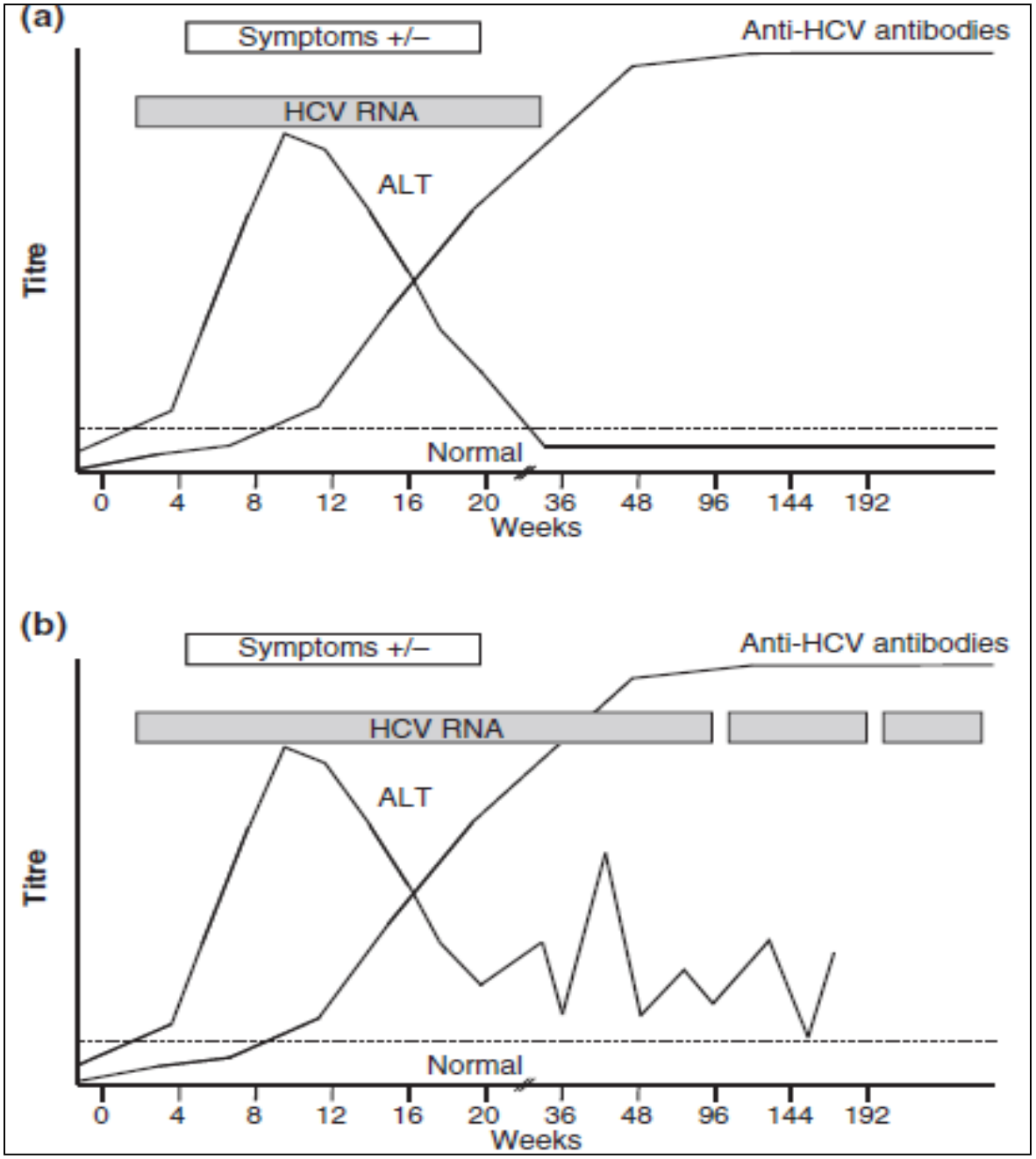


	HIV	HBV	HCV
Stable genome	Provirus	cccDNA	**None**
Virion RNA polymerase	Host RNA pol	Host RNA pol then HBV pol protein	HCV NS5B
Error-prone replications	One by HIV RT, host factors	HBV pol protein, host factors	HCV pol protein, host factors

Unlike HBV & HIV ,HCV is curable !

Virus	HIV	HBV	HCV
Genome	RNA	DNA	RNA
Mutation Rates	Very High	High	Very High
Virions produced Daily	10^{10}	10^{13}	10^{12}
Long-lived proviral reservoir	YES (Integrated viral DNA)	YES (cccDNA)	**NO**
Viral Targets of Therapy	Multiple	One	Multiple
Current Therapeutic Goal	Lifelong suppression	Lifelong suppression	**cure !!**

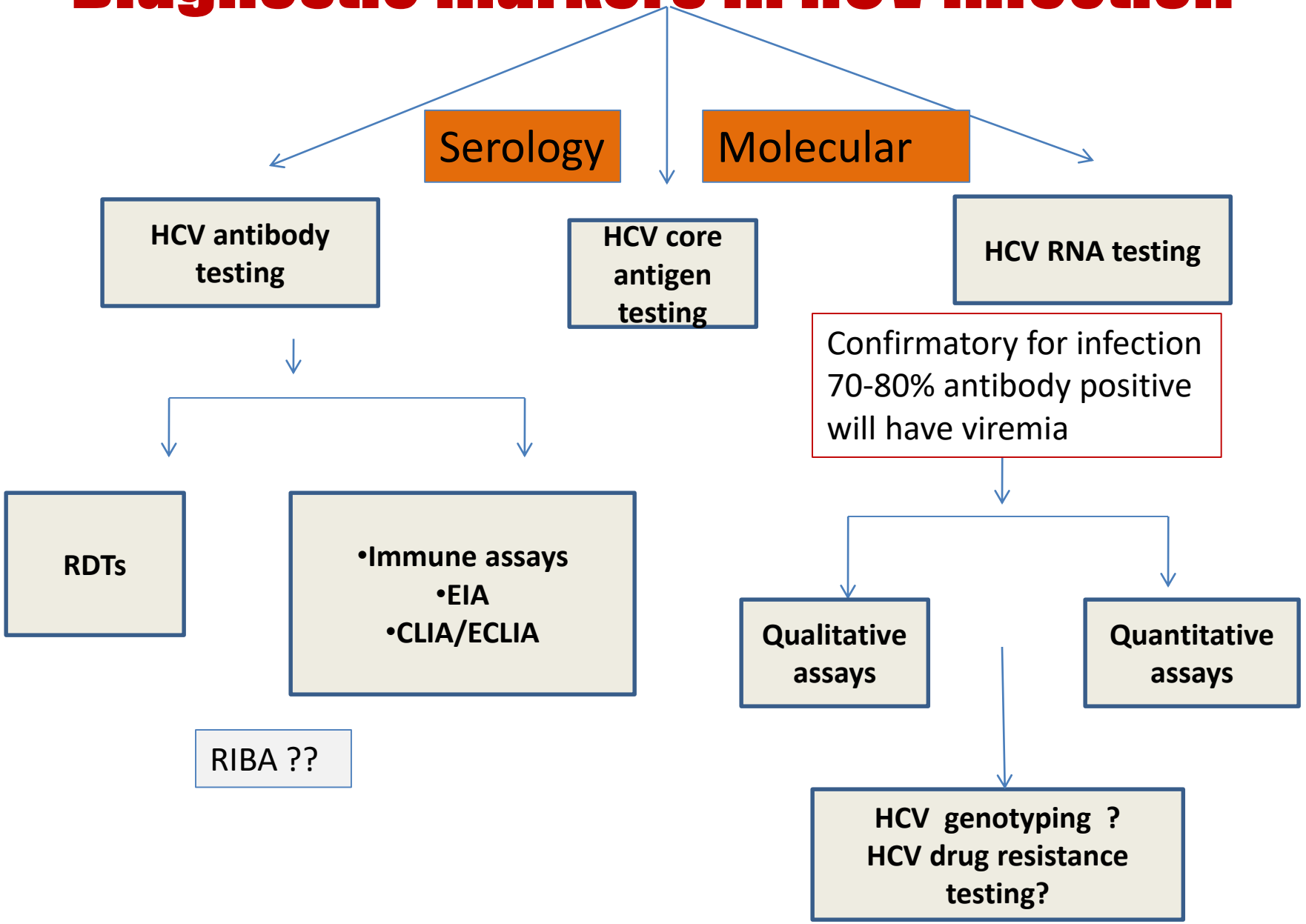
All oral DAA, Pangenotypic drugs : SVR > 95%



Acute HCV

Chronic HCV

Diagnostic markers in HCV infection



Whom to test for CHC

- **Focused testing in most affected populations**

  Who have a history of exposure and/or high-risk behaviours

  Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis (i.e. symptoms, signs, laboratory markers)

- **General population testing**

  ≥2% or ≥5% HCV antibody seroprevalence

- **Identifying specific cohort for testing**

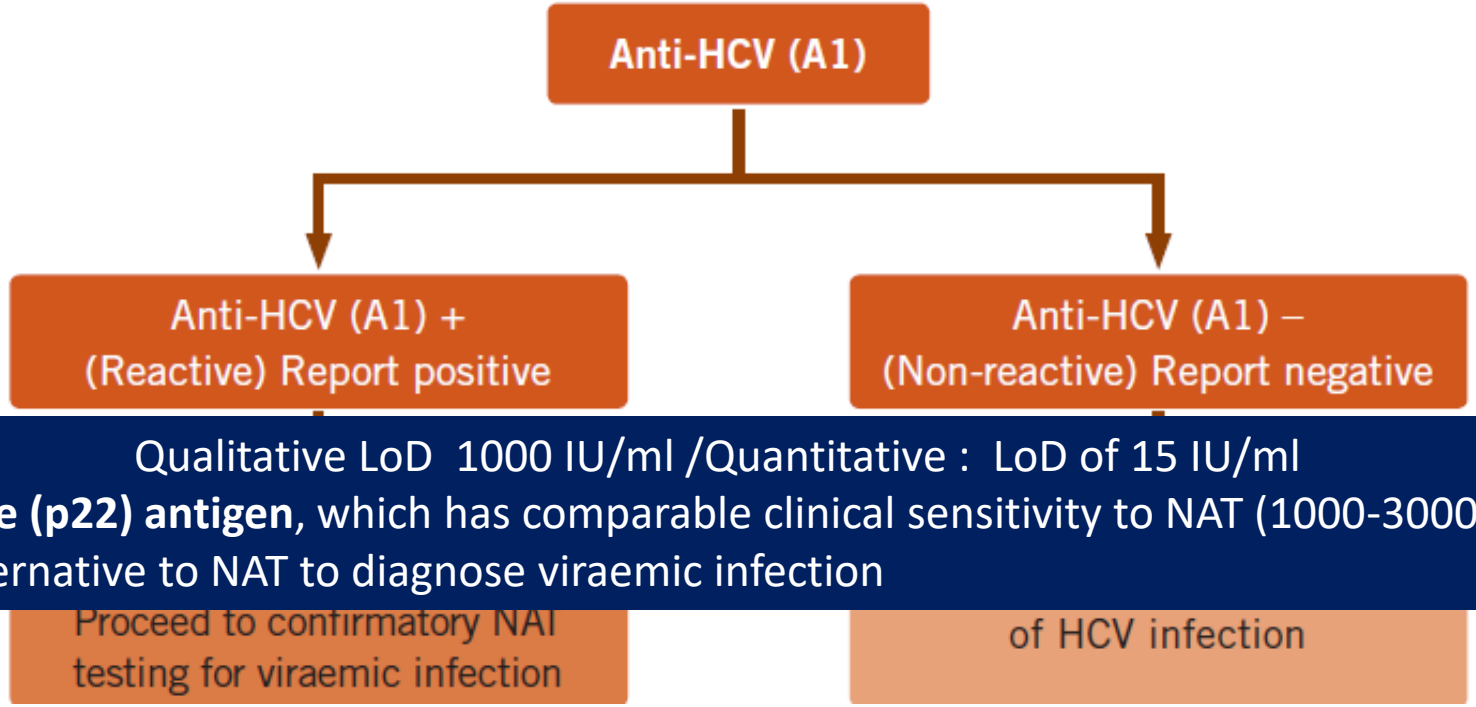
  Specific identified birth cohorts of older persons at higher risk of infection

“ Baby boomer testing” All adults born during 1945–1965 receive one-time testing for HCV.

WHO guidelines ,2017

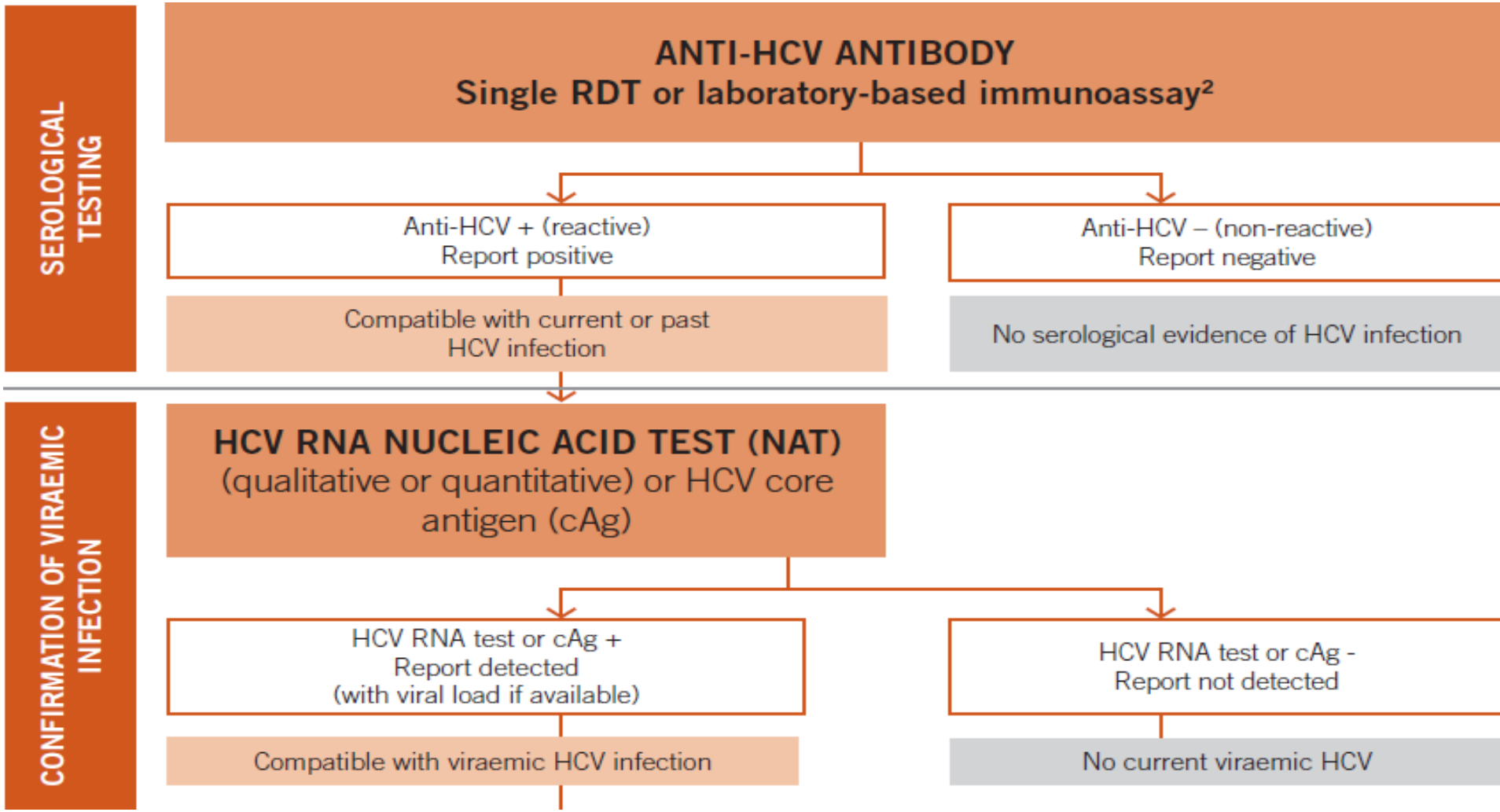
How to test for HCV

Single-assay testing strategy irrespective of prevalence



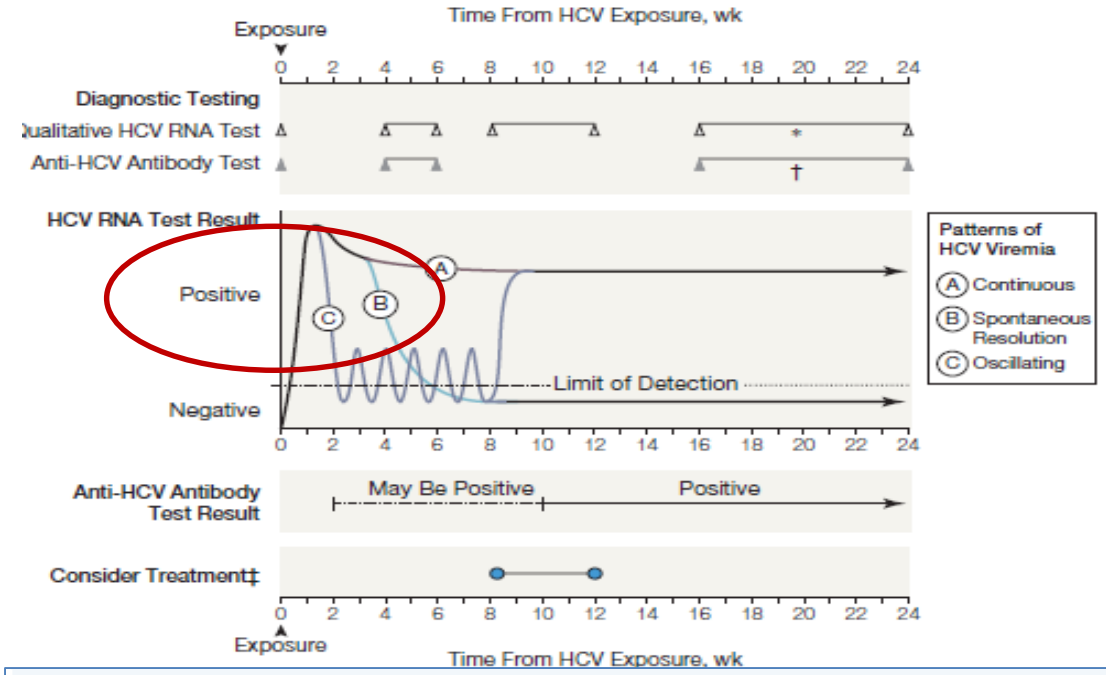
All assays used should meet WHO prequalification of IVDs
Stringent regulatory review for IVDs.
All IVDs should be used in accordance with manufacturers' instructions for use.

WHO guidelines 2017



WHO guidelines 2017

Acute HCV infection



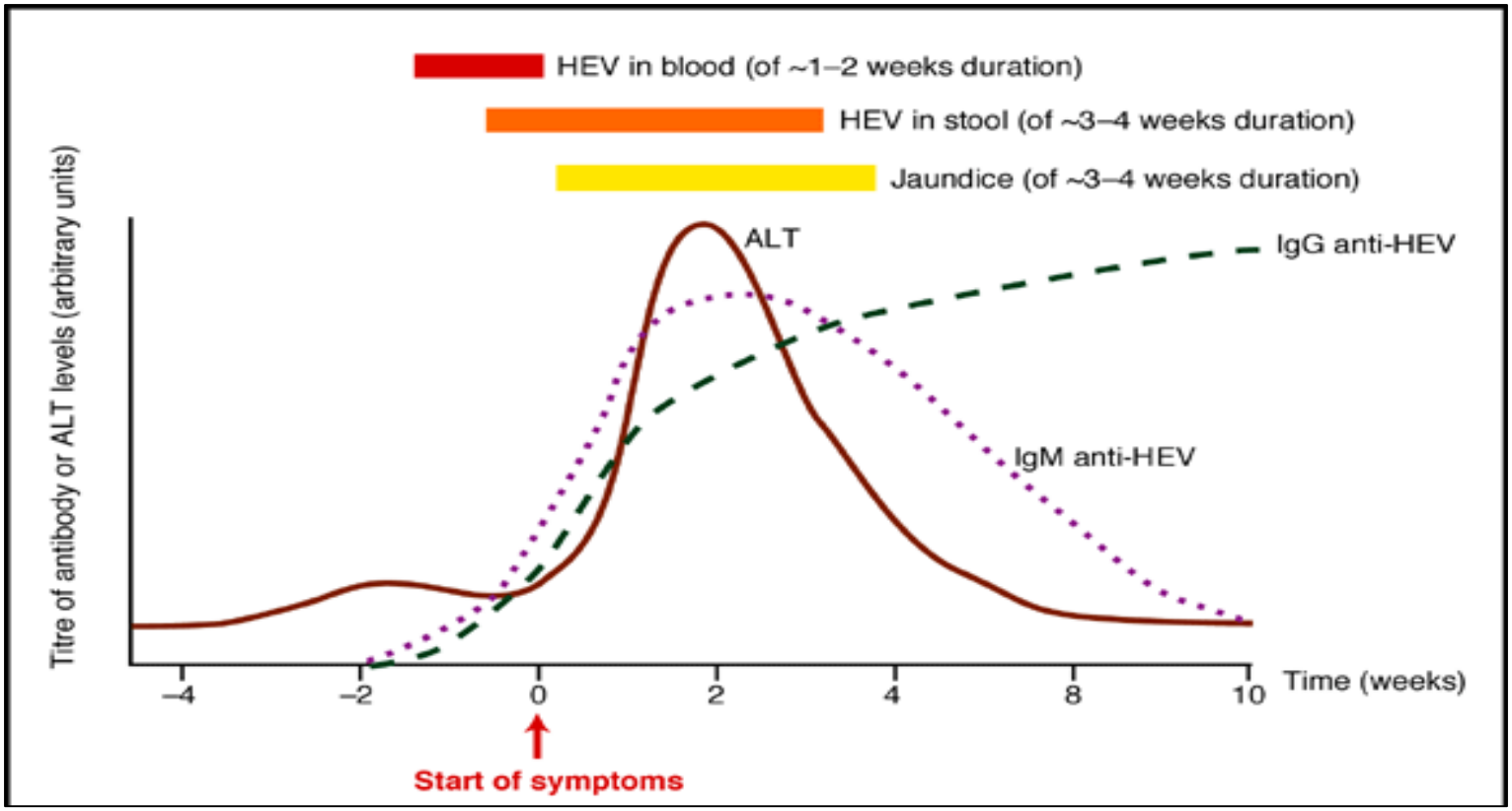
HCV antibody	▪ May be negative in the first 6 weeks after exposure ▪ May be delayed or absent when the individual is immunosuppressed ▪ Presence alone does not distinguish between acute and chronic infection ▪ Low signal-to-cutoff ratio may be present during acute HCV infection or represent a false-positive result
HCV RNA	▪ Viral fluctuations greater than 1 log10 IU/mL may indicate acute HCV infection ▪ May be transiently negative during acute HCV infection ▪ Alone does not distinguish between acute and chronic infection

AASLD,2015

Monitoring for HCV treatment response

- SVR 12 : NAT for qualitative or quantitative detection of HCV RNA after completion of antiviral treatment.
- No need for on-treatment laboratory monitoring.
- HCV c Ag as a test of cure : limited data, not recommended.

Hepatitis E virus



- Family *Hepeviridae,* 7 known genotypes.
- Genotypes 1 and 2 infect only humans.
- Genotype 3 and 4 strains have been isolated from various animals.

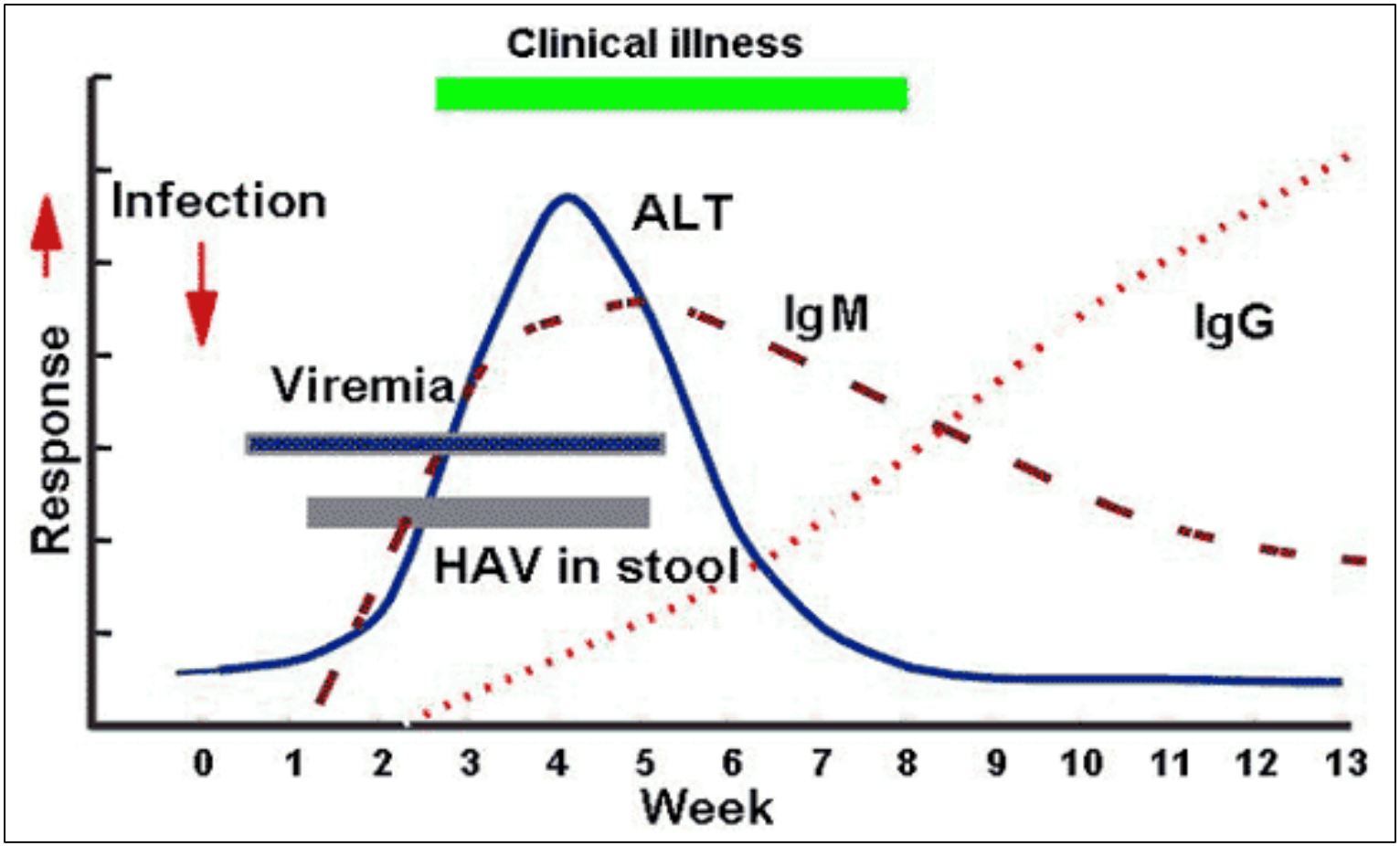
HEV Diagnostics

Test	Utility
IgM anti HEV	First line diagnostic assay in the immunocompetent
IgG anti HEV	Marker of past infection Seroprevalence estimation Vaccine efficacy
HEV RNA	First line diagnostic assay in the immunocompromised Establishing chronicity Antiviral treatment response
HEV Antigen	Diagnosis of early active infection Cost-effective alternative to HEV RNA
Genotyping	Distribution of viral strain

HEV infection in Transplant recipients

	HEV in immunocompetent	HEV in immunocompromised
Clinical presentation	Acute self limiting hepatitis	Chronic HEV infection
Complications	none	Graft hepatitis, rejection, graft fibrosis, **cirrhosis**
Genotype	India: genotype 1	Genotype 3/4
HEV RNA	Spontaneously clears	Persistent >3 months
Treatment	None	Ribavarin Interferon α (?),DAA ?
Diagnosis	**HEV IgM**	**HEV RNA PCR**

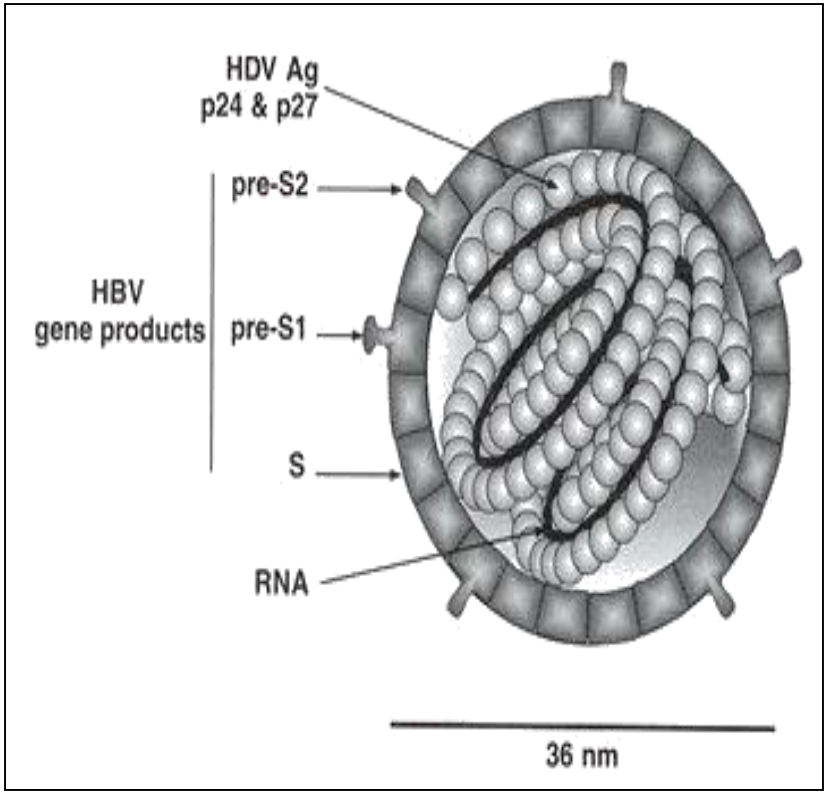
Hepatitis A virus Infection



HAV IgM : diagnosis
HAV IgG: exposure/protection.
HAV RNA PCR: epidemiological typing

Hepatitis D virus infection

- HDV is a defective RNA virus dependent on HBV for its replication.
- Two distinct patterns of HDV infection HDV-HBV co-infection and HDV superinfection.
- Anti HDV IgM: recent infection
- Anti HDV IgG: chronic existence
- **<u>HDV RNA PCR: confirmation of viremia</u>**
- Prevalence of HDV in India is < 5%



Innovations in VH testing

Area of Innovation	Technology or strategy	Primary testing target	Potential future testing target	Potential impact
1. Simplification of algorithms	• Elimination of need for genotyping with access to pan-genotypic DAAs, and only a single time point (SVR12) for assessment of cure	HCV		Reduce costs; Improve uptake
2. Sampling approaches	• Dried blood spots (DBS) • Oral fluid	• HCV • HCV		Increase access and coverage, reaching key and target populations
3. Innovative testing approaches	• Self-testing • Combo integrated multi-disease tests • Integrated multidisease testing platforms (centralised and decentralised)	• HCV • HCV • HCV	• HBV • HBV	Increase awareness, reduce stigma; Maximise programme synergies and reduce costs. Improve access to testing,
4. New technologies	• Point-of-care (POC) NAT • HCV core antigen test (as laboratory based assay and in future as POC)	• HCV • HCV	• HBV	Increase access to confirmation of viraemia
5. Health system improvements	• Integrated testing and service decentralization • Data connectivity • Sample chain and supply management	• HCV	• HBV	Improve timely receipt of results and linkage to care; improve supply chain management; Optimize use of current infrastructure sample workflow

BMC Infectious Diseases 2017, 17(Suppl 1):699

Current Testing Methods

Samples: Blood plasma/serum

Rapid Diagnostic Tests (RDTs)

Serological
result within 30 minutes

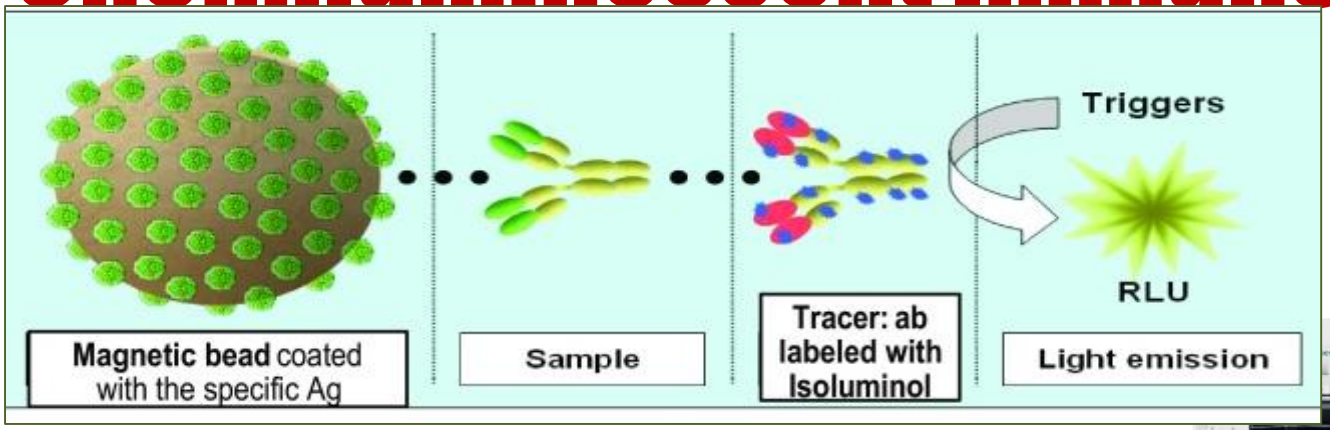
Serological Tests

- EIA/ELISA
- CLIA/ECLIA
- *Confirmatory*
  Neutralization
  Immoblot

Molecular Tests(NAT)

- Qualitative
- Quantitative /Viral load
- *Genotyping*
- *Drug Resistance*

Chemiluminescent Immunoassay



Measure light which is emitted which is directly related to concentration (Luminol, Acridium esters, Ruthenium derivatives, Nitrophenyl oxalates).

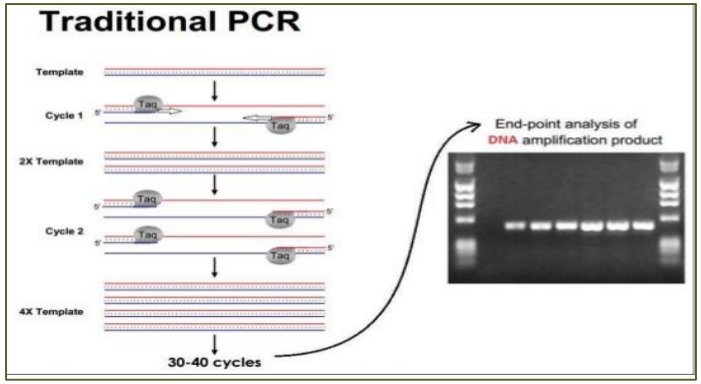
Advantages:

- More sensitive, specific.
- Easy to perform
- Fast TAT
- Semi quantitative results
- Quantitative serology !!

Disadvantages:

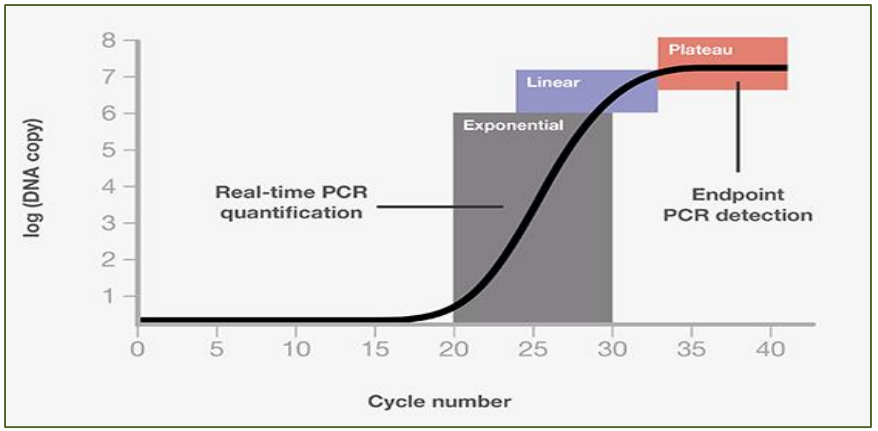
- Expensive
- Infrastructure requirement

End Point PCR



- Results are read at the end of amplification.
- Only qualitative.
- Lower limit of detection is much higher.
- Errors cannot be comprehended.

Real Time PCR



- Amplification occurs in Real Time
- Quantitative.
- Low LOD and wide dynamic range.
- Useful monitoring tool.

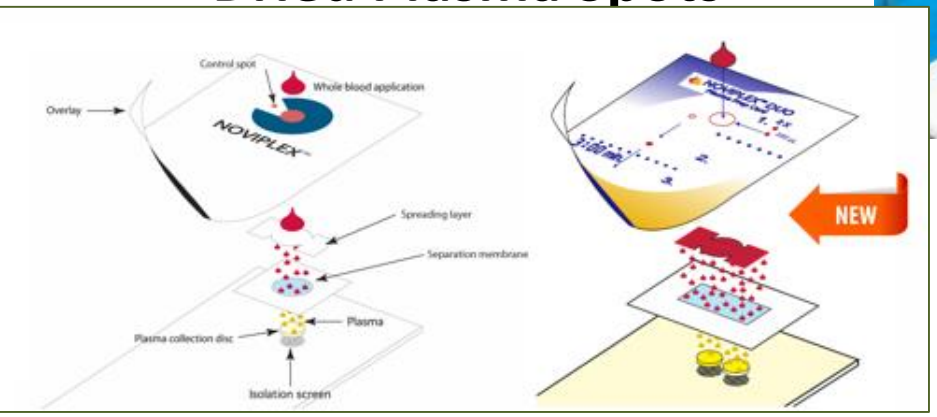
Sampling Innovations

Dried Blood spots (DBS)

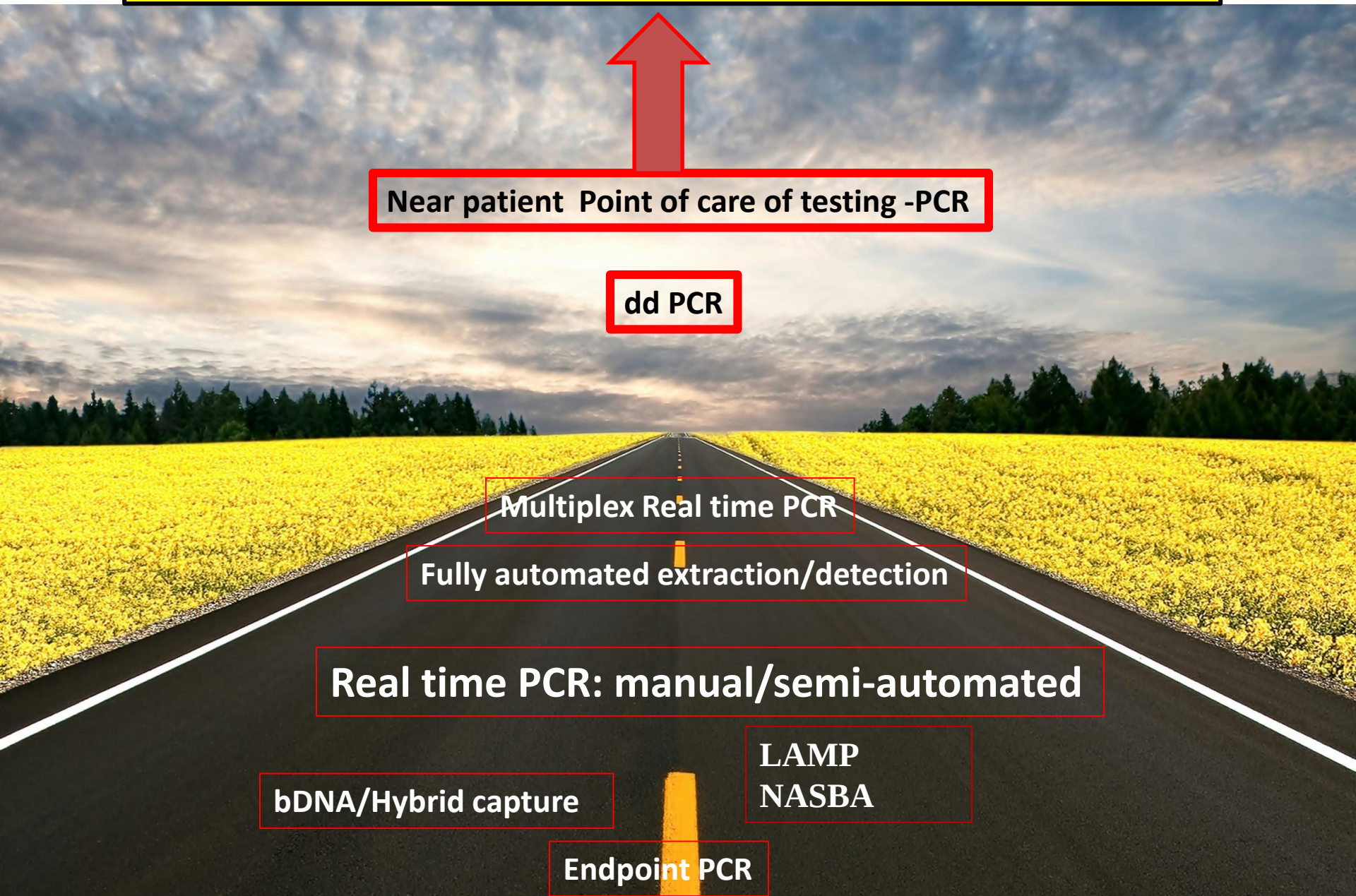
Oral Fluid testing

Self testing

Dried Plasma Spots

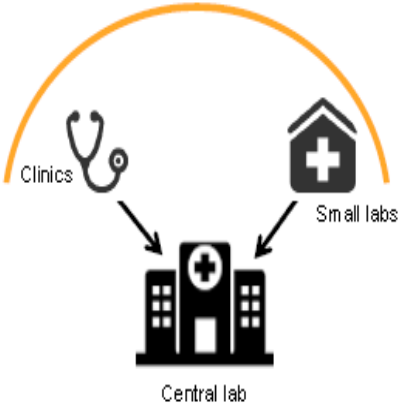


Innovations in Molecular Testing Platforms



The need for a decentralised molecular test

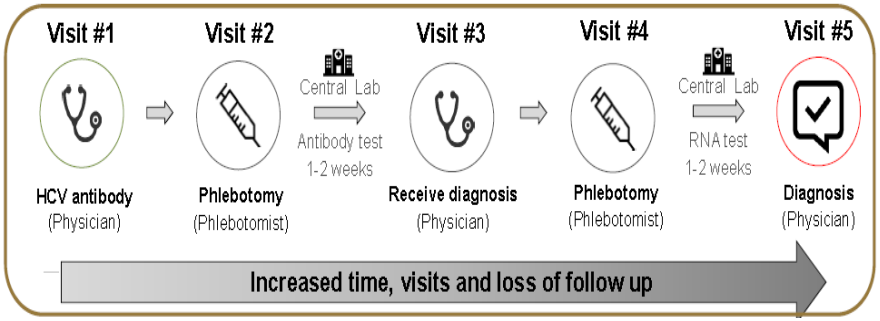
46% of patients never receive confirmatory RNA testing

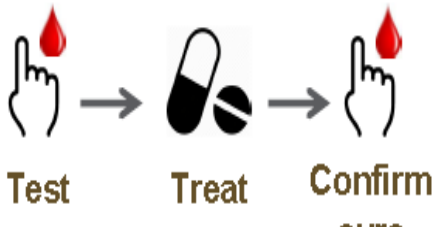


Centralized testing

Take the test to the patient

Decentralized services



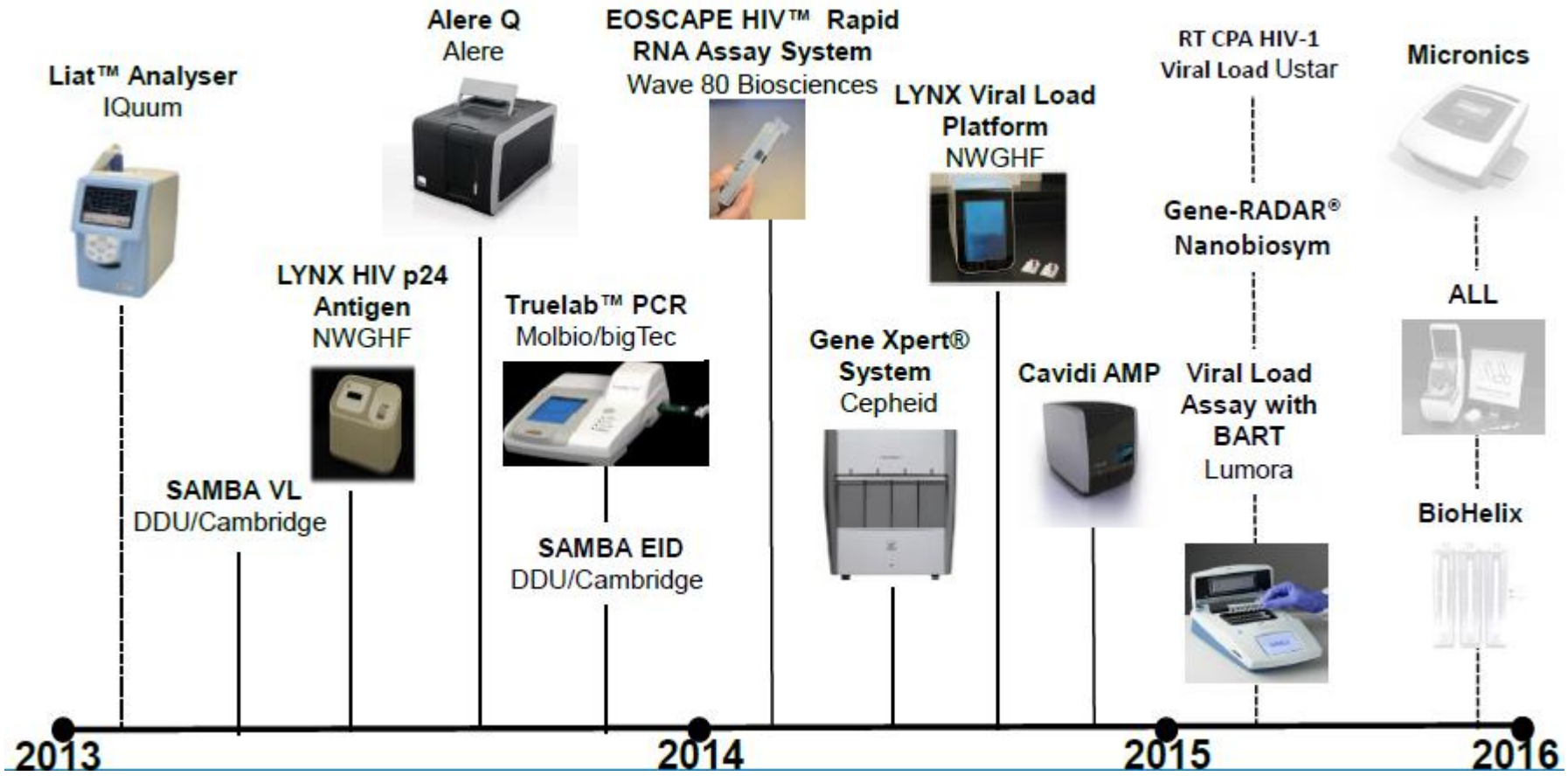


Point of care testing

Sources: Grebely J., Applegate T.L., Cunningham P. & Feld J.J. (2017): Hepatitis C point-of-care diagnostics: in search of a single visit diagnosis, Expert Review of Molecular Diagnostics, DOI: 10.1080/14737159.2017.1400385

T. Applegate, et al. Point of care and dried blood spot HCV testing – a practical introductory workshop. INHSU 2017.

Small integrated viral load testing assays



All steps of qPCR and analysis in one chamber ,TAT 30-50 minutes

Cartridge based nucleic acid testing (CBNAAT)

- Fully automated and integrated system with specimen purification, nucleic acid amplificatio and detection using real-time reverse transcriptase PCR (RT-PCR) which uses fluorescence to detect the RNA of interest.
- Rapid TAT.
- Minimal sample handling.
- Biosafety free.

Gene Xpert Instrument

True POC molecular tests

Daktari system

Genedrive system

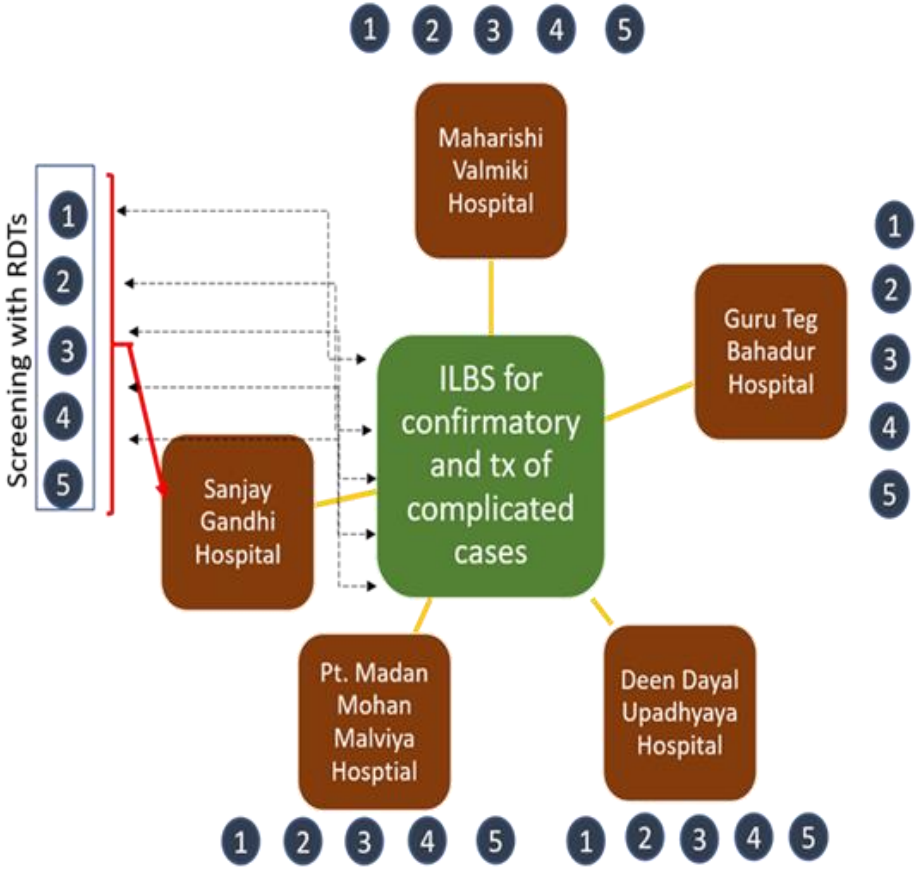
Fingerstick Capillary Blood

1 Collect 100µL capillary blood by finger-prick into a Minivette

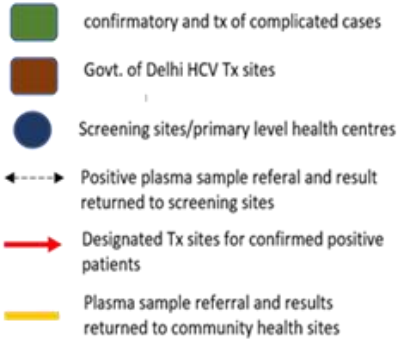
2 Load blood directly into Xpert HCV Viral Load cartridge

3 Result <60min

HCV : Project Head start in Delhi



- Screening at community sites using RDTs
- Treatment at corresponding hospital for non-complicated cases (~80%)
- Confirmatory testing at ILBS which will treat the complicated cases



HCV screening for 50,000 population and providing treatment free of cost to confirmed cases.

Role of Genotyping

- Limited to Epidemiology.
- Treatment failure. Drug resistance testing
- Prognosticate the patient.
- Unusual outbreak investigation.
- Sequencing based assays, Hybridisation assays, allele specific Real time PCR, Pyro sequencing.

Conclusions

- Laboratory diagnosis for Viral hepatitis is crucial in identification of infection and treatment monitoring.
- Need for simplified algorithms of testing.
- Lack of good quality kits.
- Quality control is very important especially in molecular tests.
- GOI is committed towards elimination of VH,National Viral Hepatitis Control Program is launched.

PDCC course in HepatoVirology at ILBS

ilbs

PROJECT PRAKASH
PRogrammed Approach to Knowledge And Sensitization on Hepatitis

Thank you!