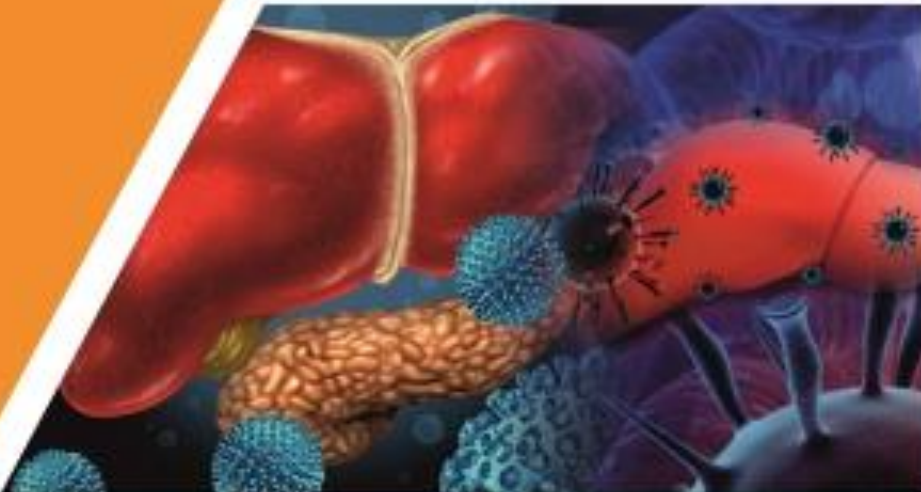




PROJECT PRAKASH

Programmed Approach to Knowledge and Sensitization on Hepatitis



HEPATITIS INDUCTION PROGRAM FOR DOCTORS

LABORATORY DIAGNOSIS OF VIRAL HEPATITIS

Dr. Ekta Gupta,
Additional Professor,
Dept of Hepatology, ILBS

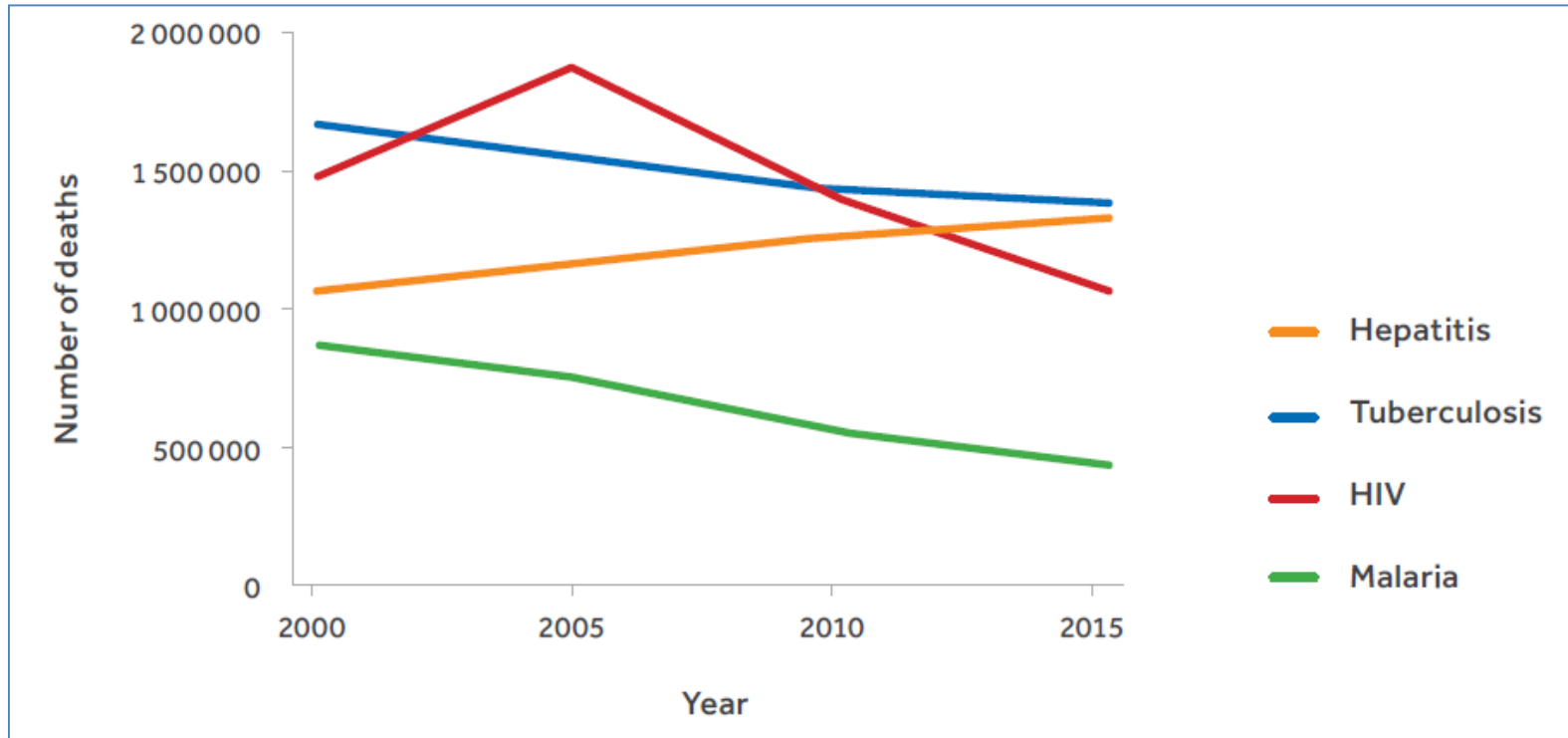
INSTITUTE OF LIVER & BILIARY SCIENCES, NEW DELHI

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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%.

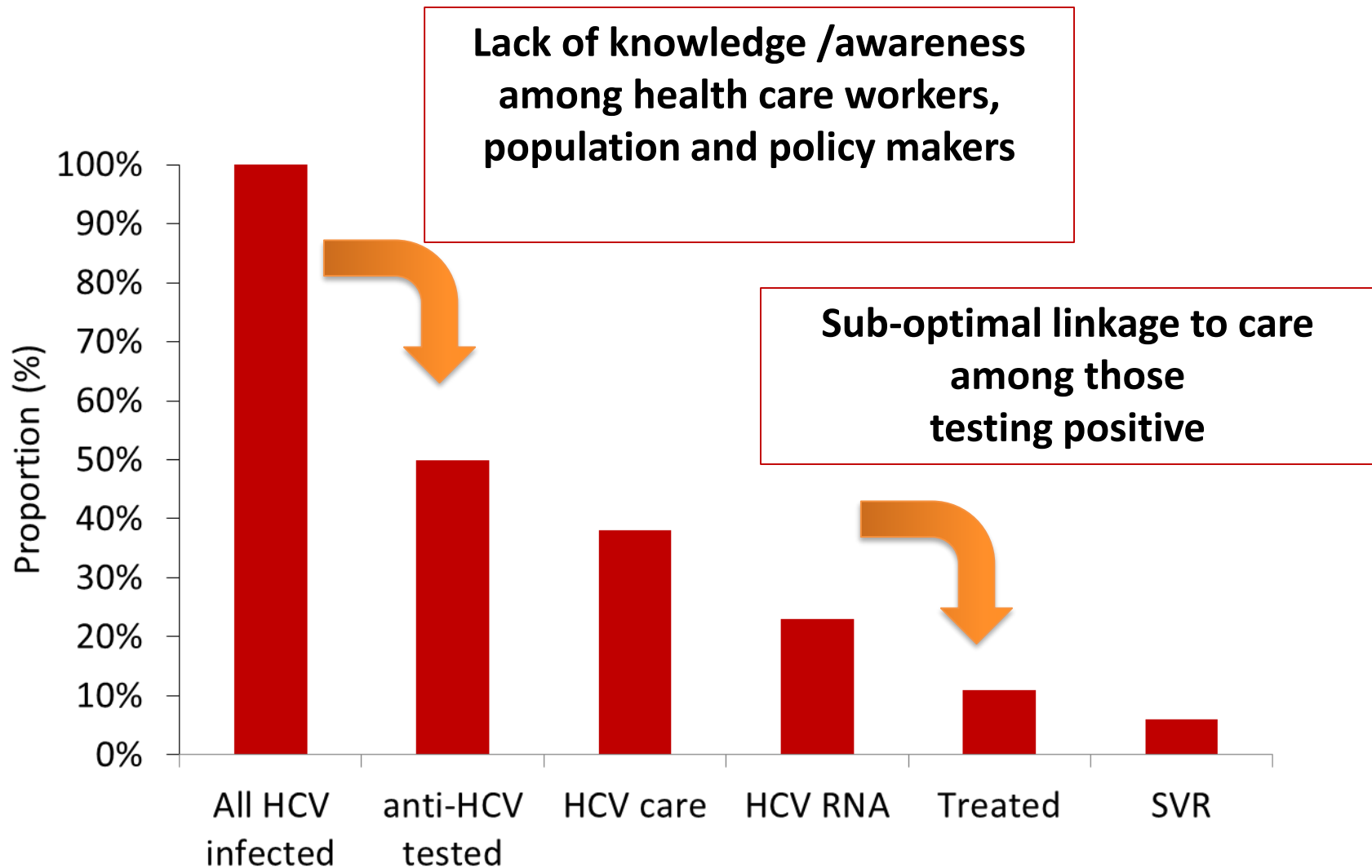
India : Viral Hepatitis Burden



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	Fecal-oral	Per-cutaneous, Per-mucosal	Per-cutaneous Per-mucosal	Per-cutaneous Per-mucosal	Fecal-oral
Chronic infection	No	Yes	Yes	Yes	No/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

Large number remains undiagnosed



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??*

Challenges to scale-up of Testing

**Many markers : confusing
Lack of Diagnostic strategy/algorithm**

**Lack of simple, reliable, low
cost diagnostic tests, Poor quality kits, No quality
assurance.**

**Laboratory capacity, infrastructure, trained
manpower
lack of uniformity in reporting.**

**Lack of uniform practices
and linkage to care facility.**

Importance of screening

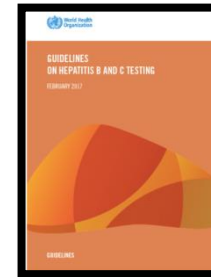
- Early identification, prevent development of serious liver damage.
- Refer to treatment, cure.
- Appropriate education : risk behavior modification.
- Prevention of onward transmission.
- Immunization to other infections: improves health outcome.
- Development of evidence based public health interventions.

WHO envisions

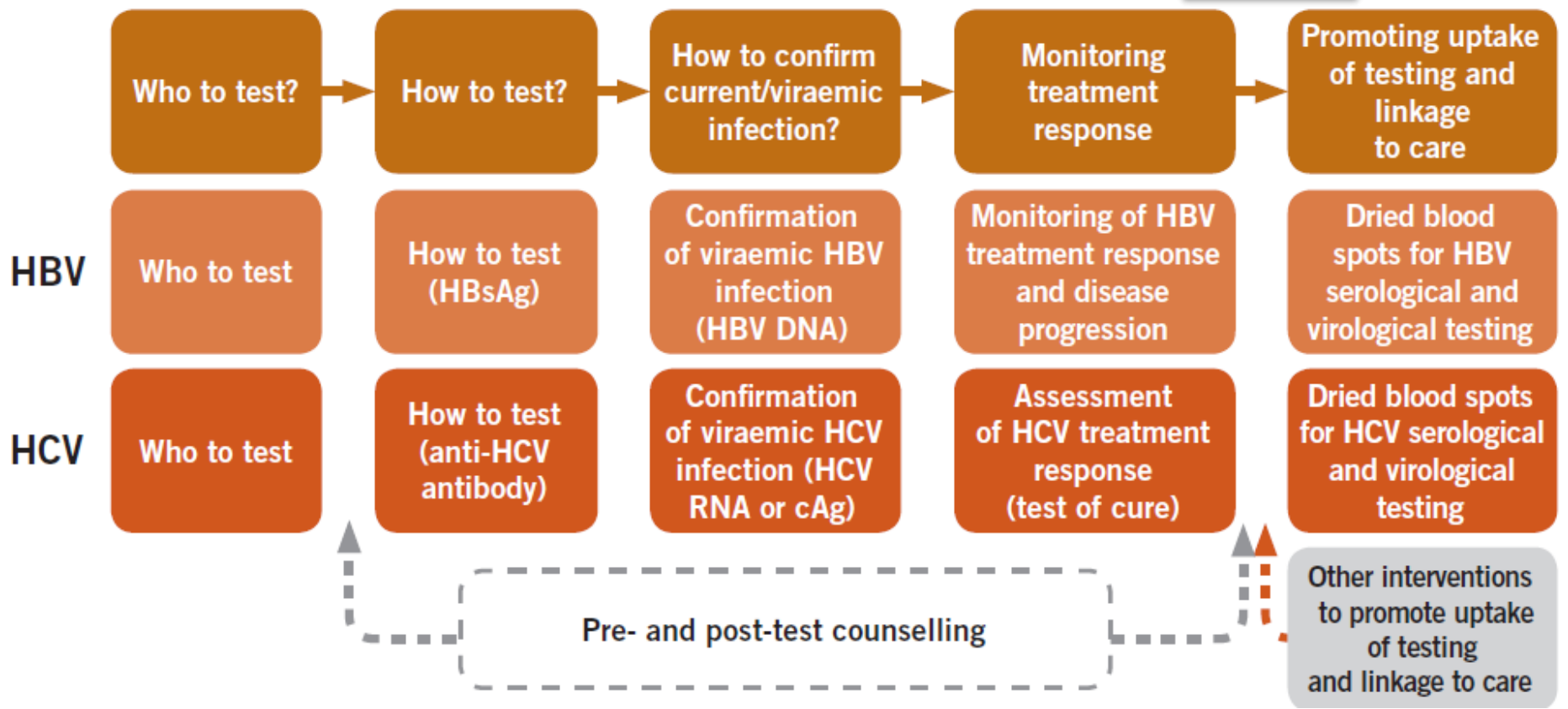
“ Elimination of viral hepatitis as a major public health threat by 2030 ”

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

Major bottle neck is laboratory diagnosis of VH.



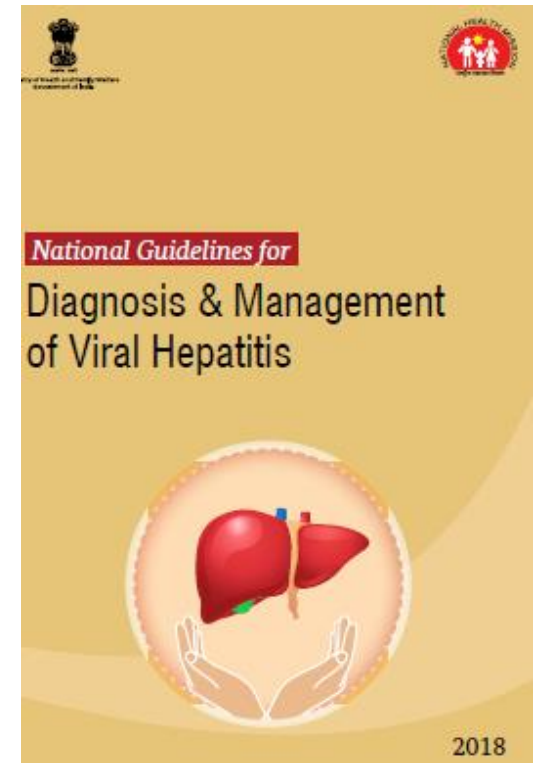
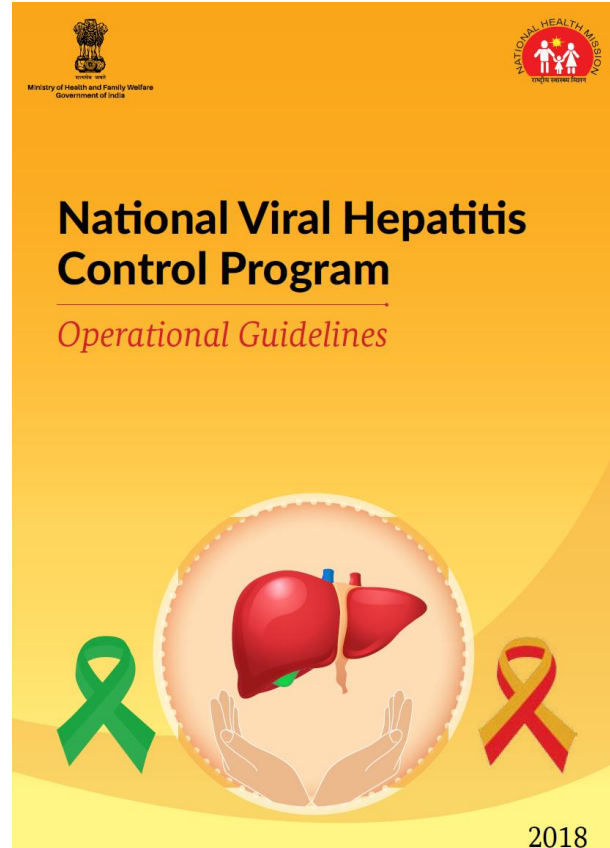
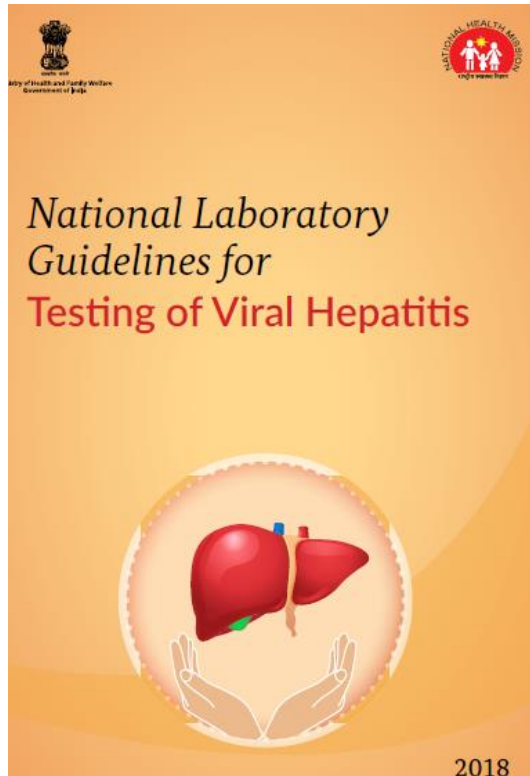
WHO 2017 testing guidelines



Only for HBV & HCV

Focus on LMIC ,Help in development of National Guidelines

National Viral Hepatitis Control Program India



NVHCP launched by GoI on 28th July 2018

Current Testing Methods for VH

Samples: Blood plasma/serum



Rapid Diagnostic Tests (RDTs)

Serological result within 30 minutes

Serological Tests

- EIA/ELISA
- CLIA/ECLIA
- Confirmatory
Neutralization
Immunoblot

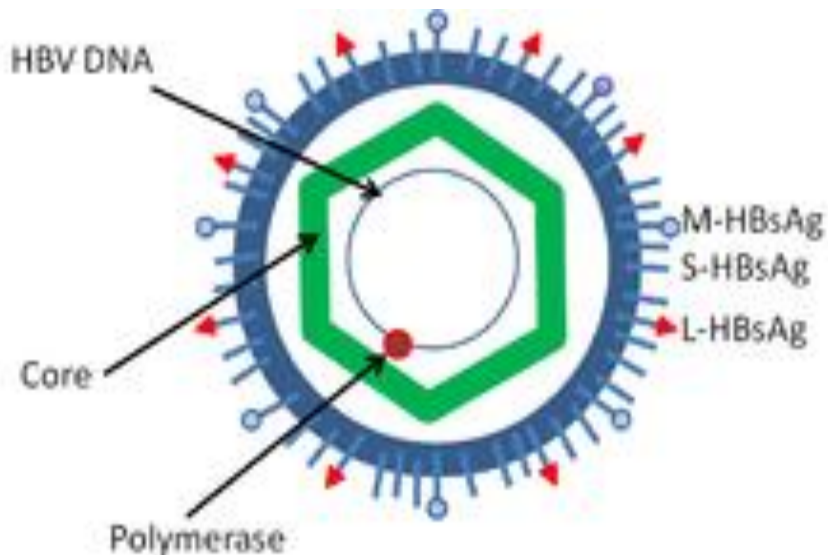
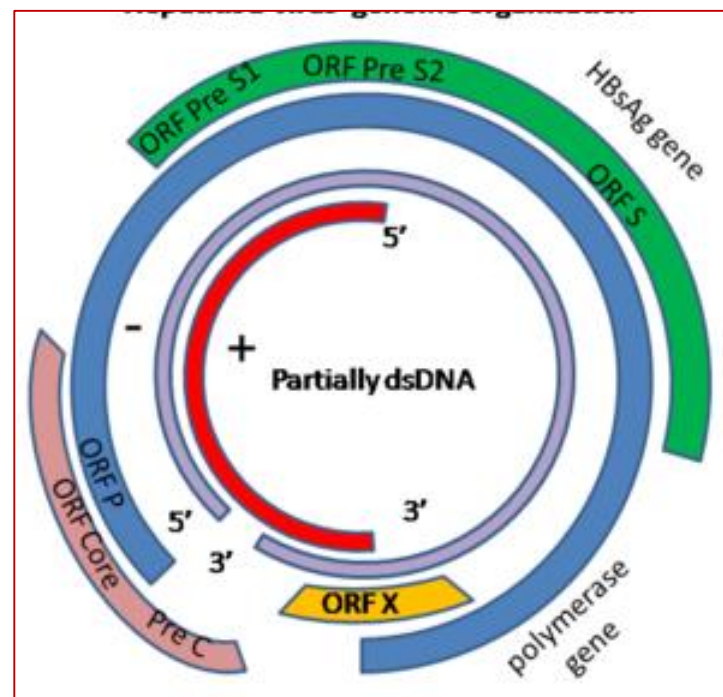
Molecular Tests(NAT)

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

Hepatitis B virus infection

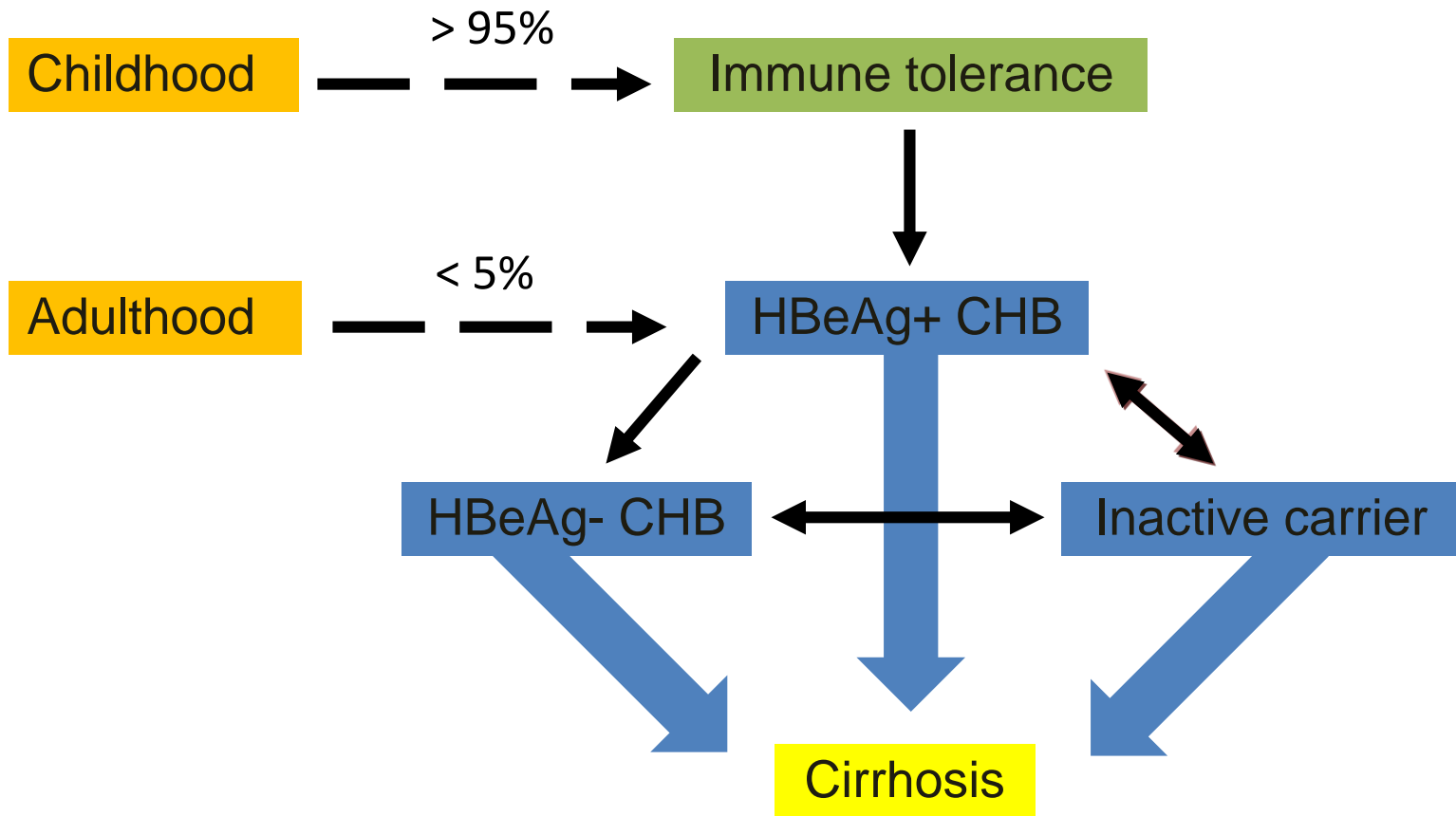
HBV Virus

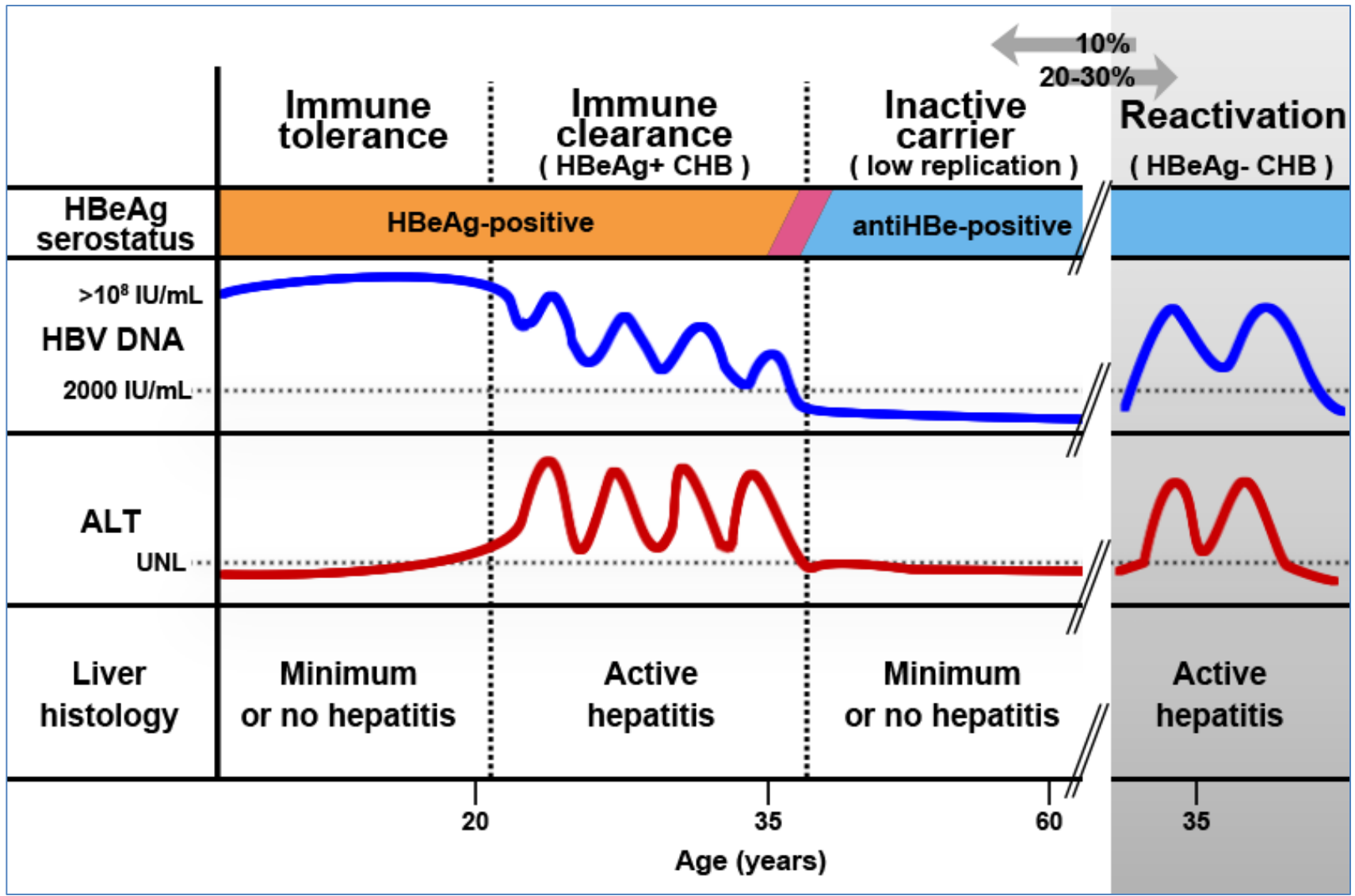
- Partially double stranded DNA virus
- 3.2 kb, *Hepadnaviridae*
- 4 ORF's
- **Surface** (PreS1/S2/S)
- **Core (precore /core)** – core & e antigen
- **Polymerase** – DNA Polymerase
- **X** - a transactivator of viral transcriptior



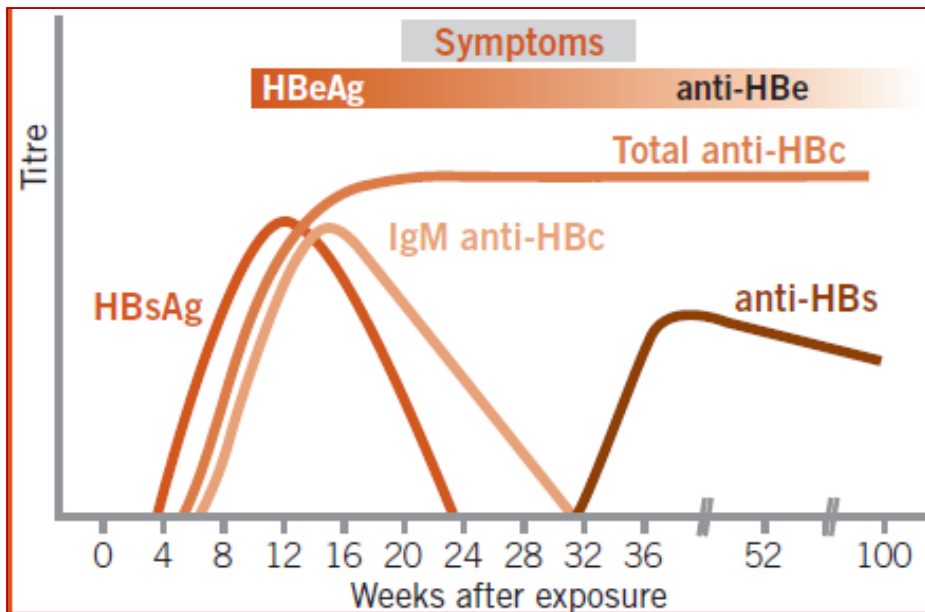
Natural History of HBV Infection

Most of the CHB seen are mother to child transmission

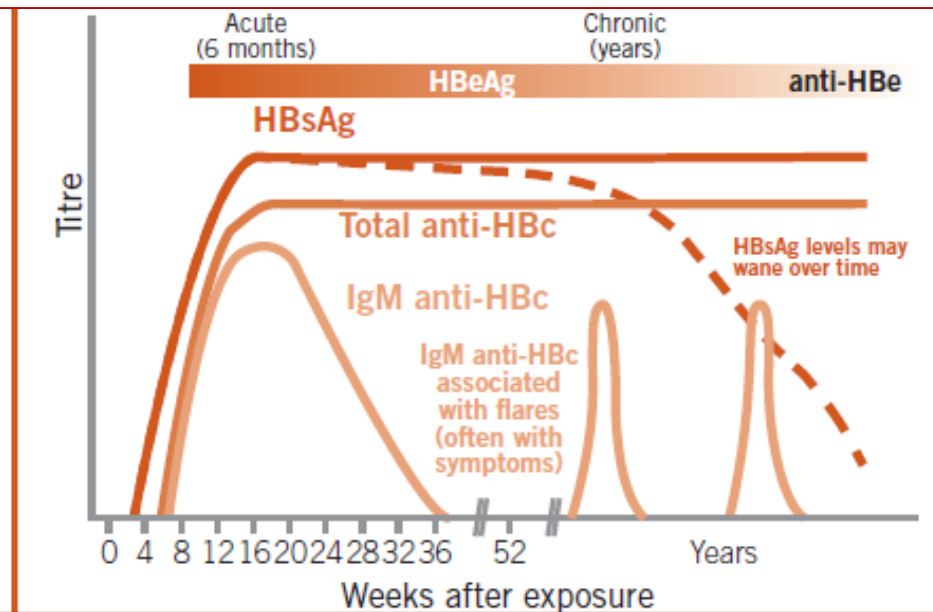




Diagnostic markers in HBV infection



Acute HBV infection



Chronic HBV infection

Who to test for Chronic HBV Infection

- **General population** : Intermediate > 2% or high prevalence >5%
- **Routine testing in pregnant women:** $\geq 2\%$ or $\geq 5\%$ 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

How to test for HBV

HBsAg \geq 0.4%

HBsAg < 0.4%

Assay 1

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

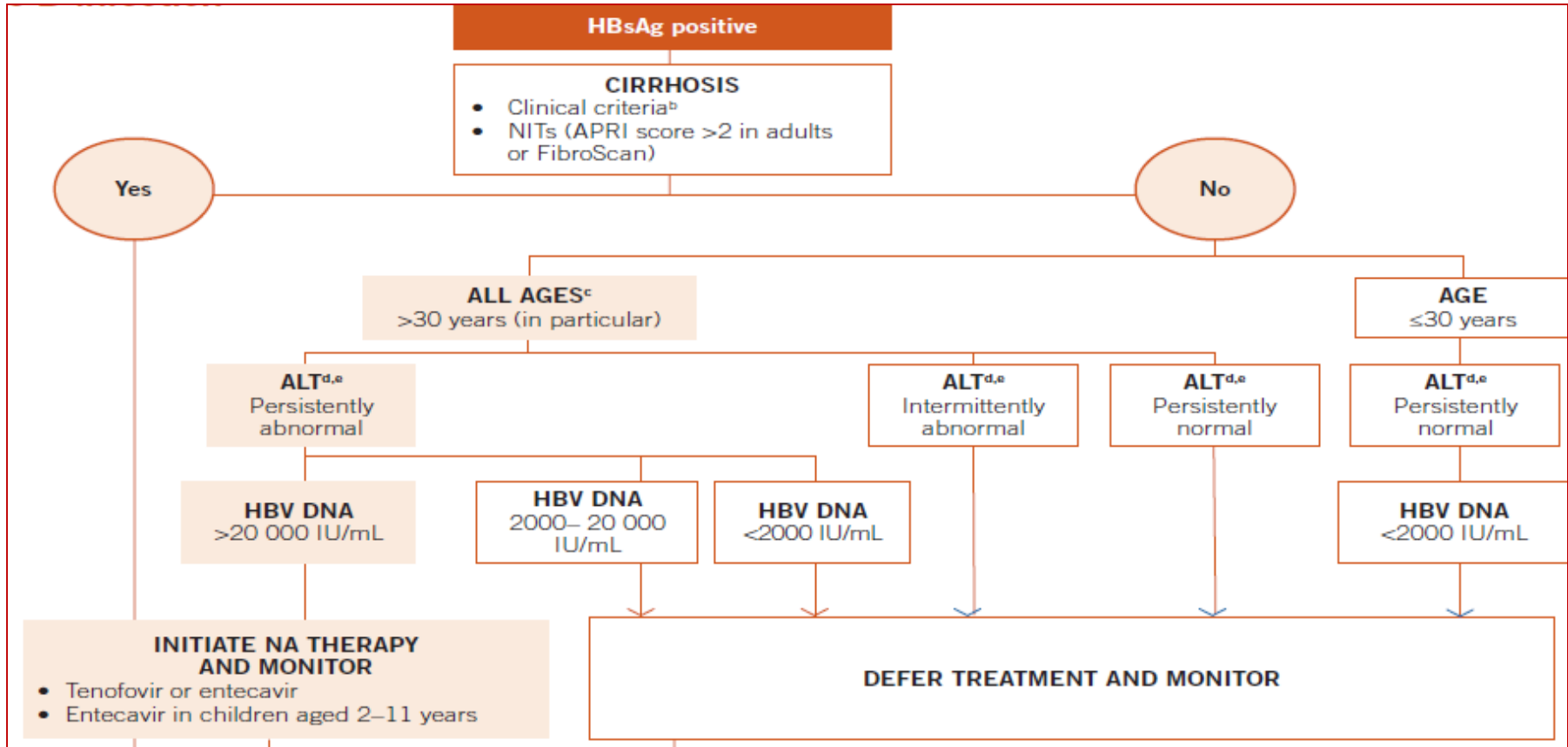
Further testing for viraemic infection

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**

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

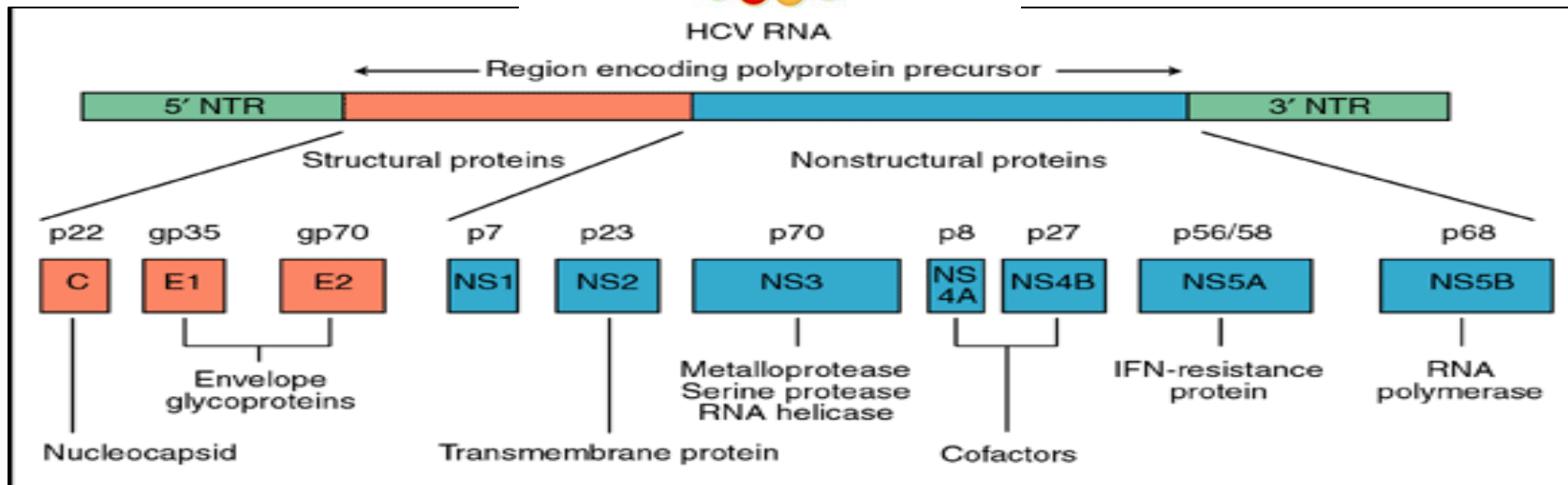
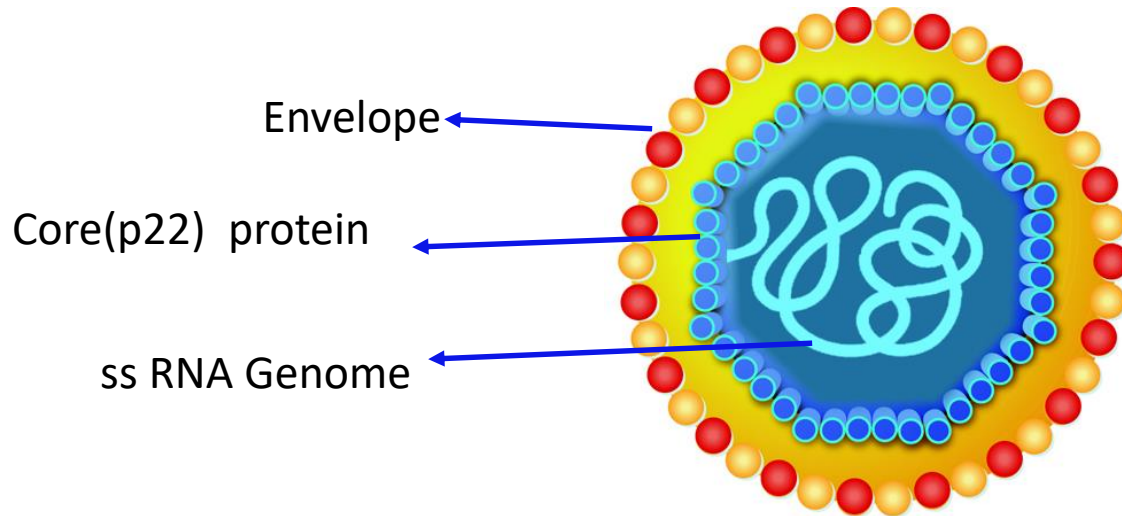
- Occult HBV diagnosis

HBV DNA/ ccc DNA PCR from tissue

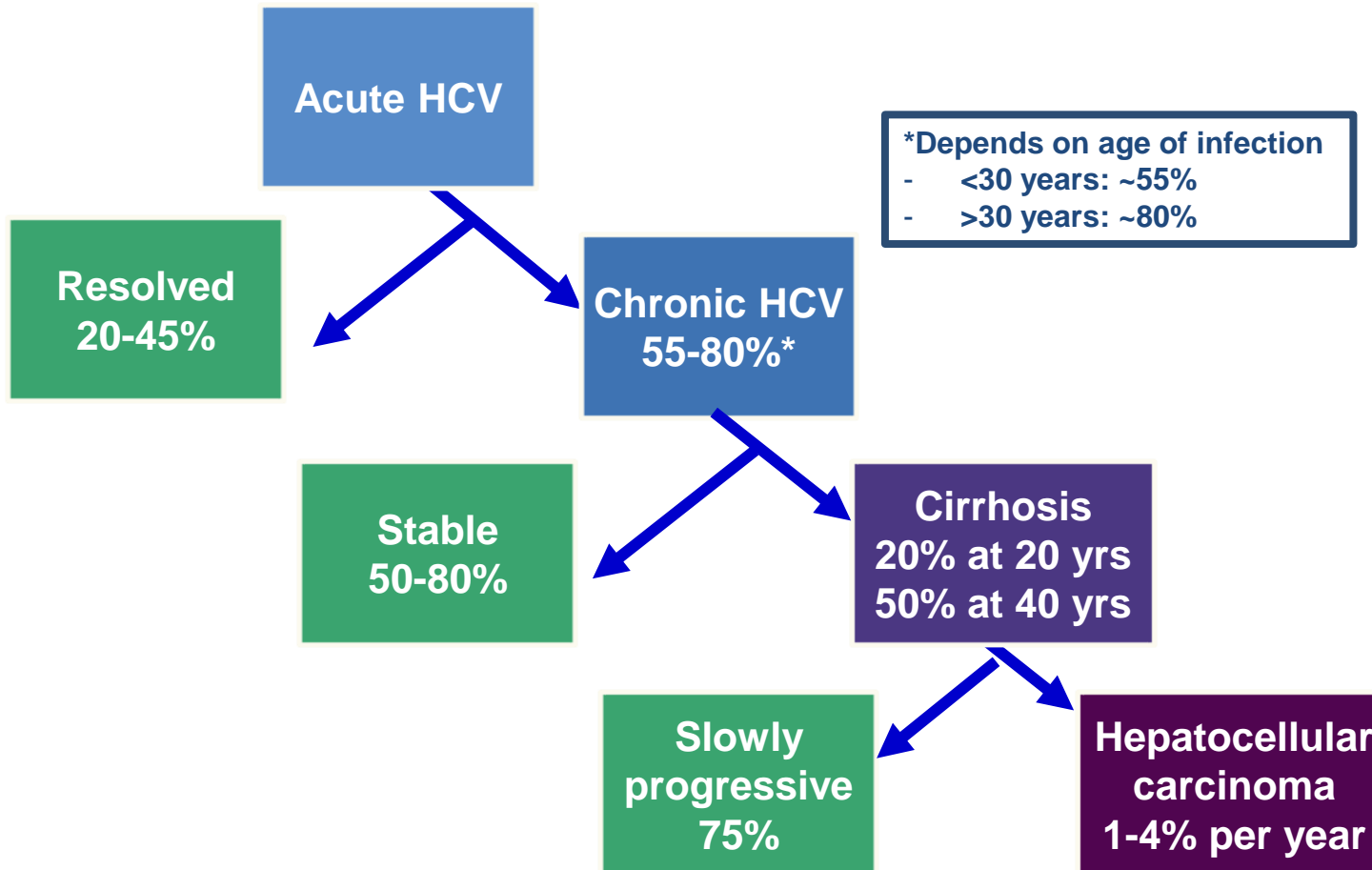
Hepatitis C Virus Infection

Hepatitis C virus

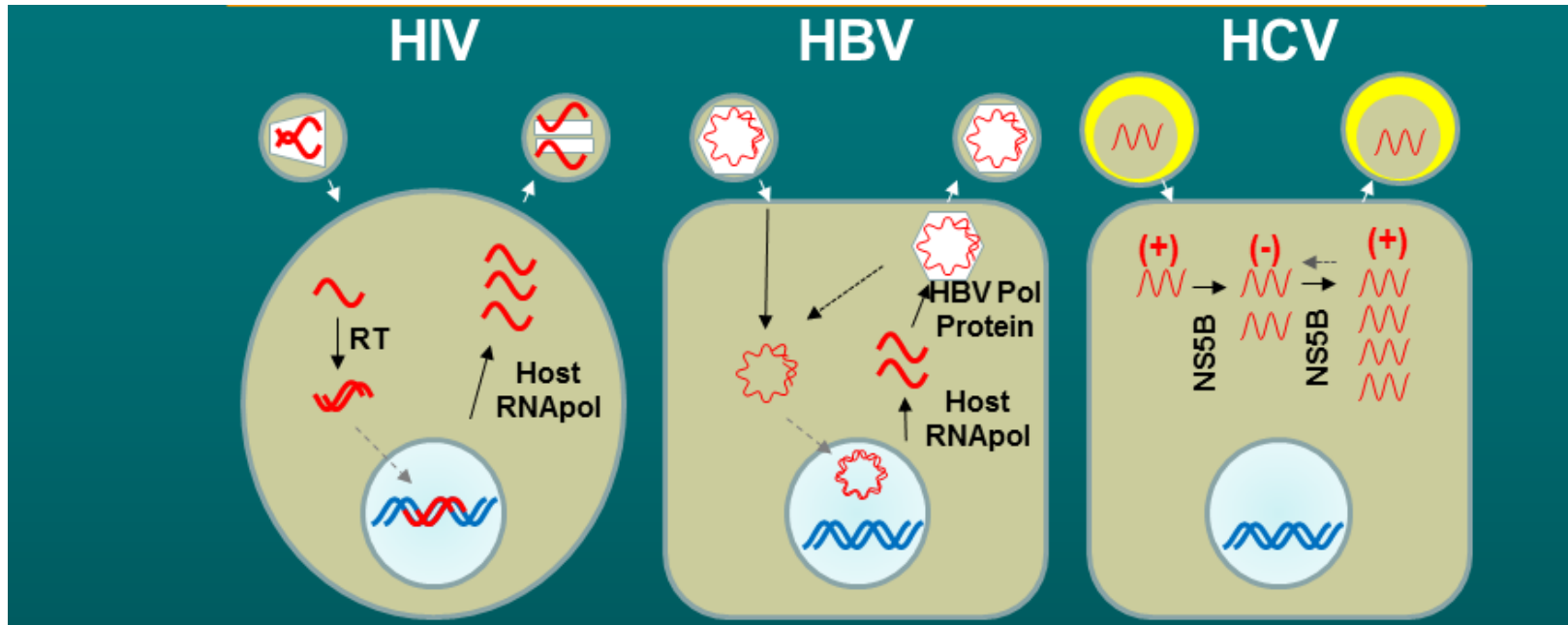
HCV is an enveloped, 9.6-kb, positive-sense, ss RNA virus family *Flaviviridae*



Hepatitis C Virus Infection: Natural History



Differences with HBV & HIV

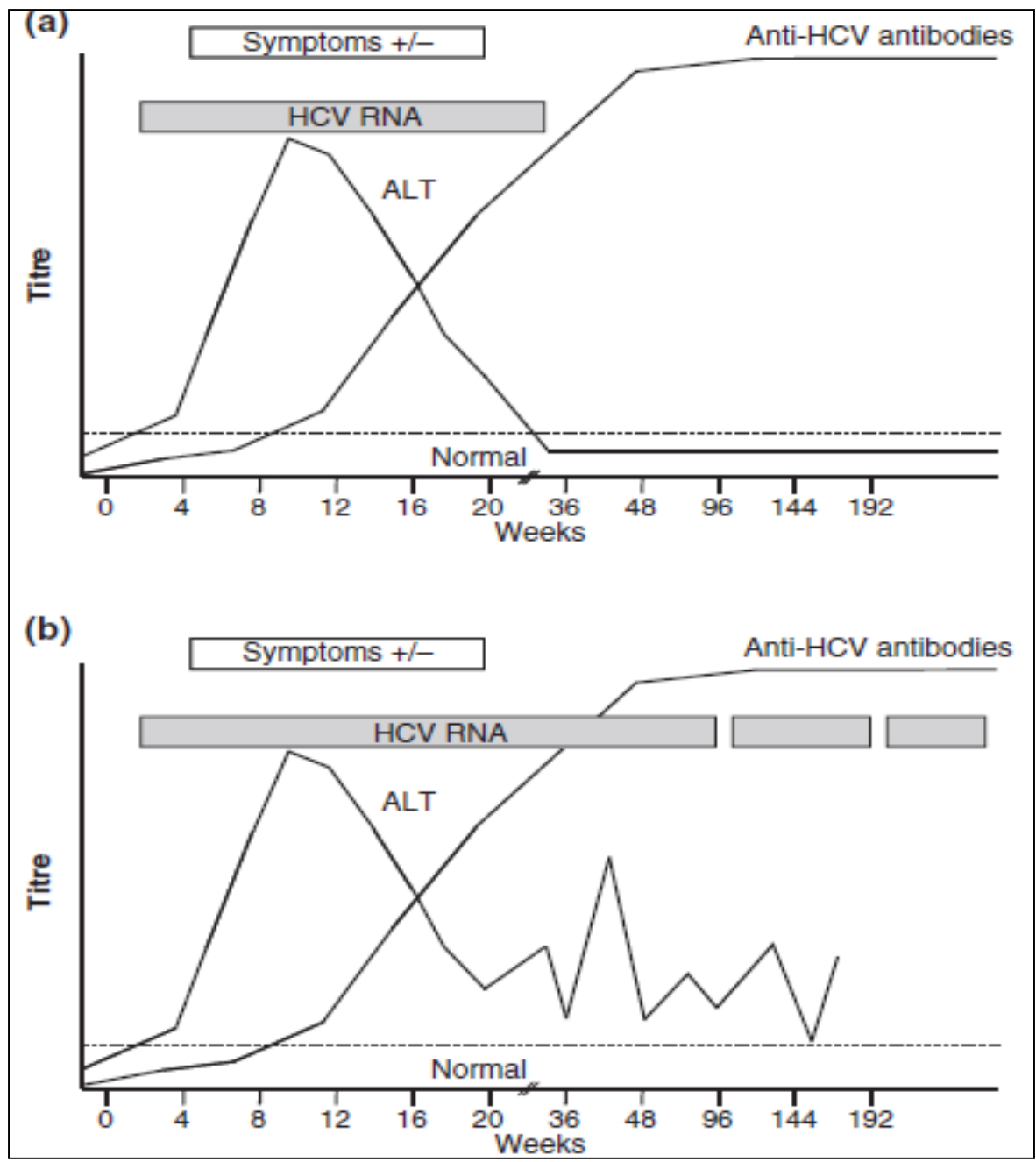


	HIV	HBV	HCV
Stable genome	Provirus	cccDNA	None
Virion RNA polymerase	Host RNA pol	Host RNA pol then HBV pol protein	HCV NS5B
Error-prone replications	One by HIV RT, host factors	HBV pol protein, host factors	HCV pol protein, host factors

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 !!

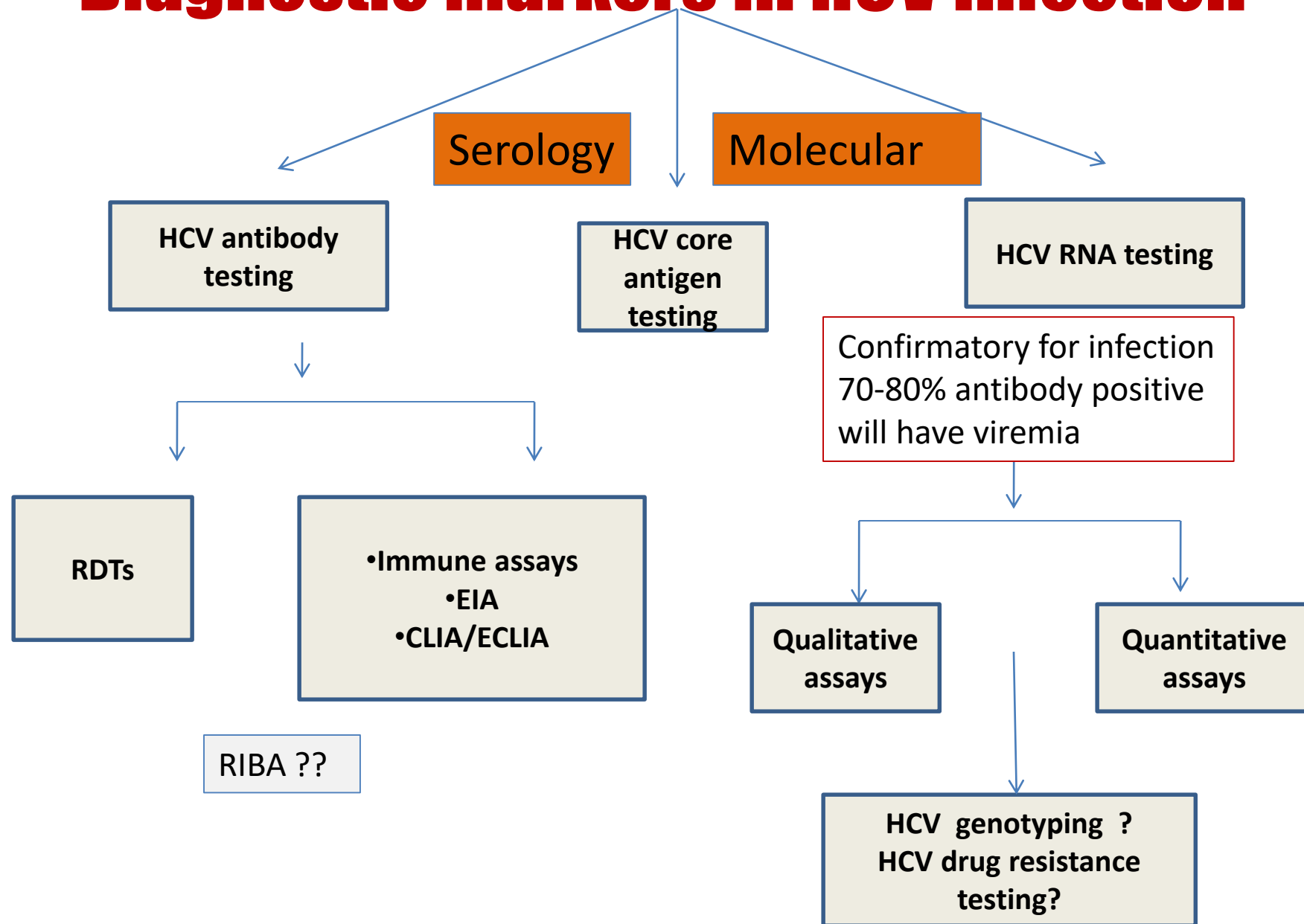
All oral DAA, Pangenotypic drugs : SVR > 95%



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

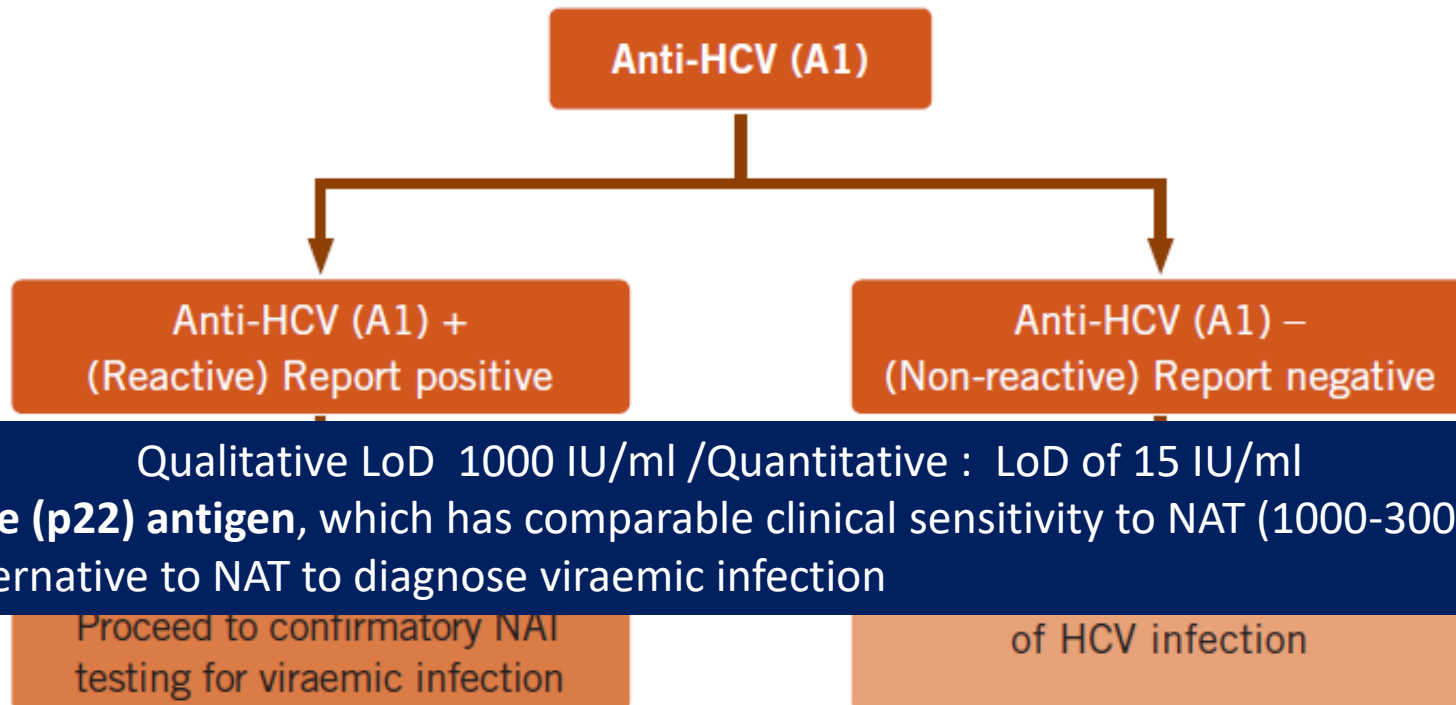
- **Identifying specific cohort for testing**

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.

How to test for HCV

Single-assay testing strategy irrespective of prevalence



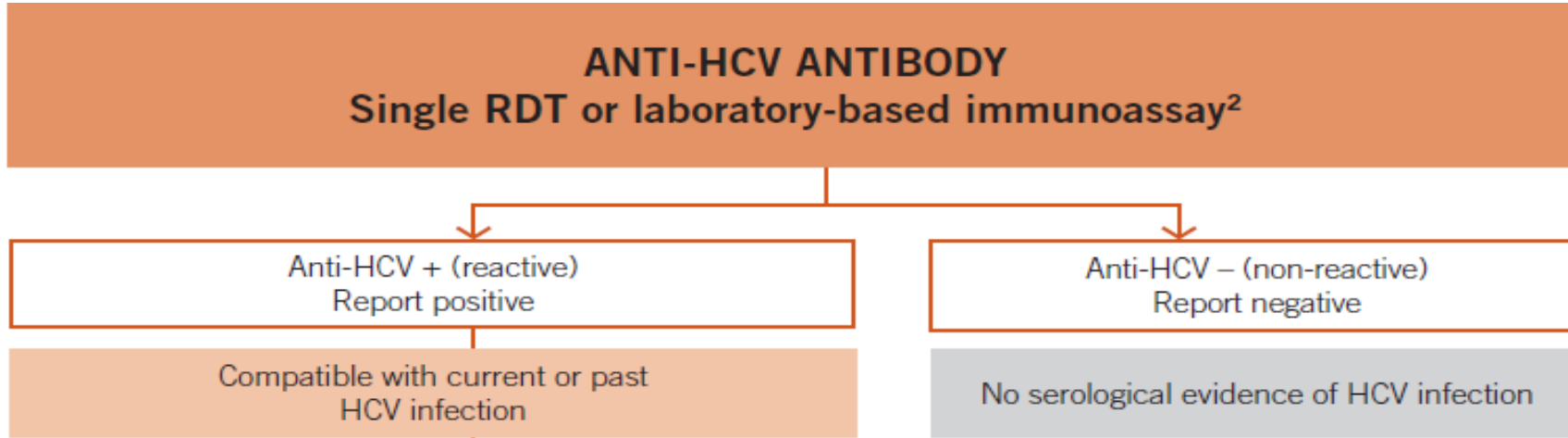
All assays used should meet WHO prequalification of IVDs

Stringent regulatory review for IVDs.

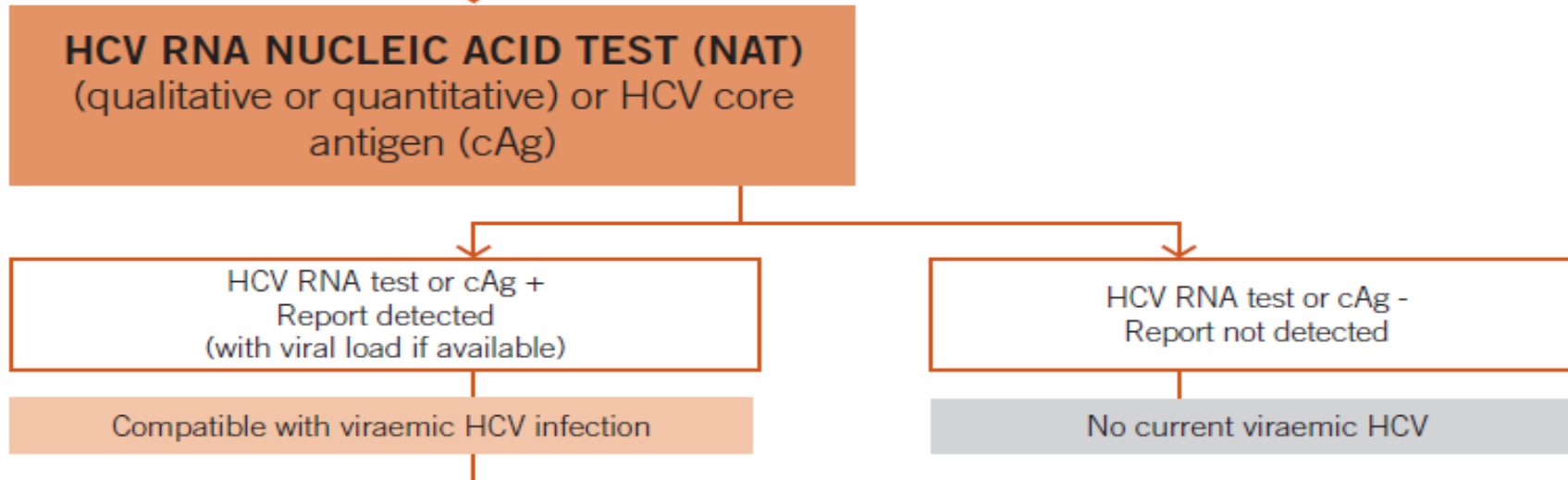
All IVDs should be used in accordance with manufacturers' instructions for use.

WHO guidelines 2017

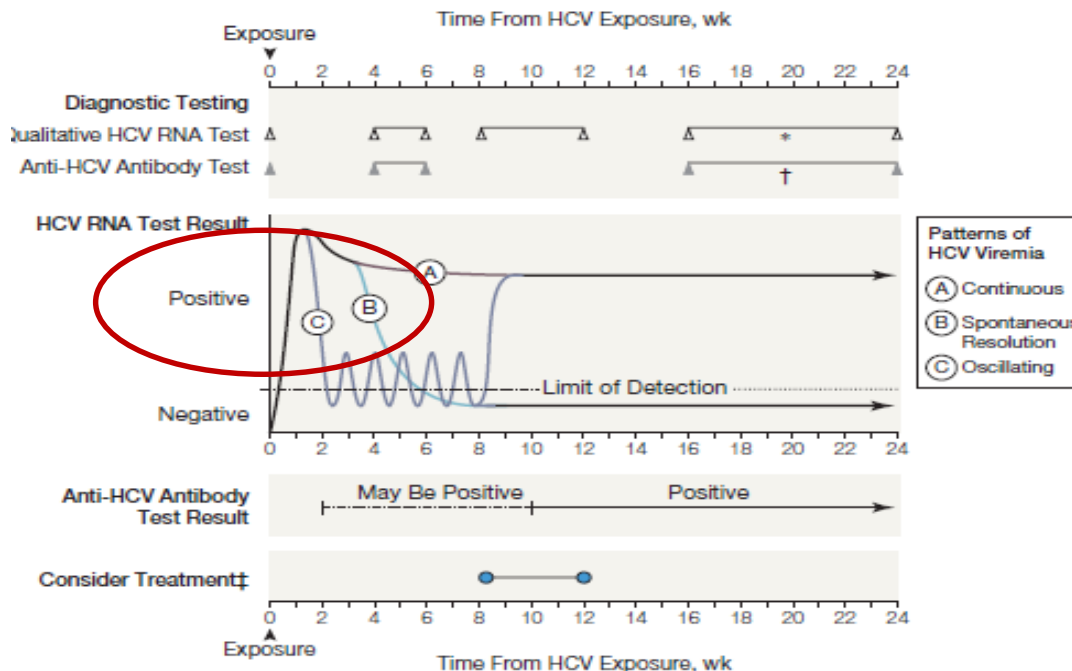
SEROLOGICAL TESTING



CONFIRMATION OF VIRAEMIC INFECTION



Acute HCV infection

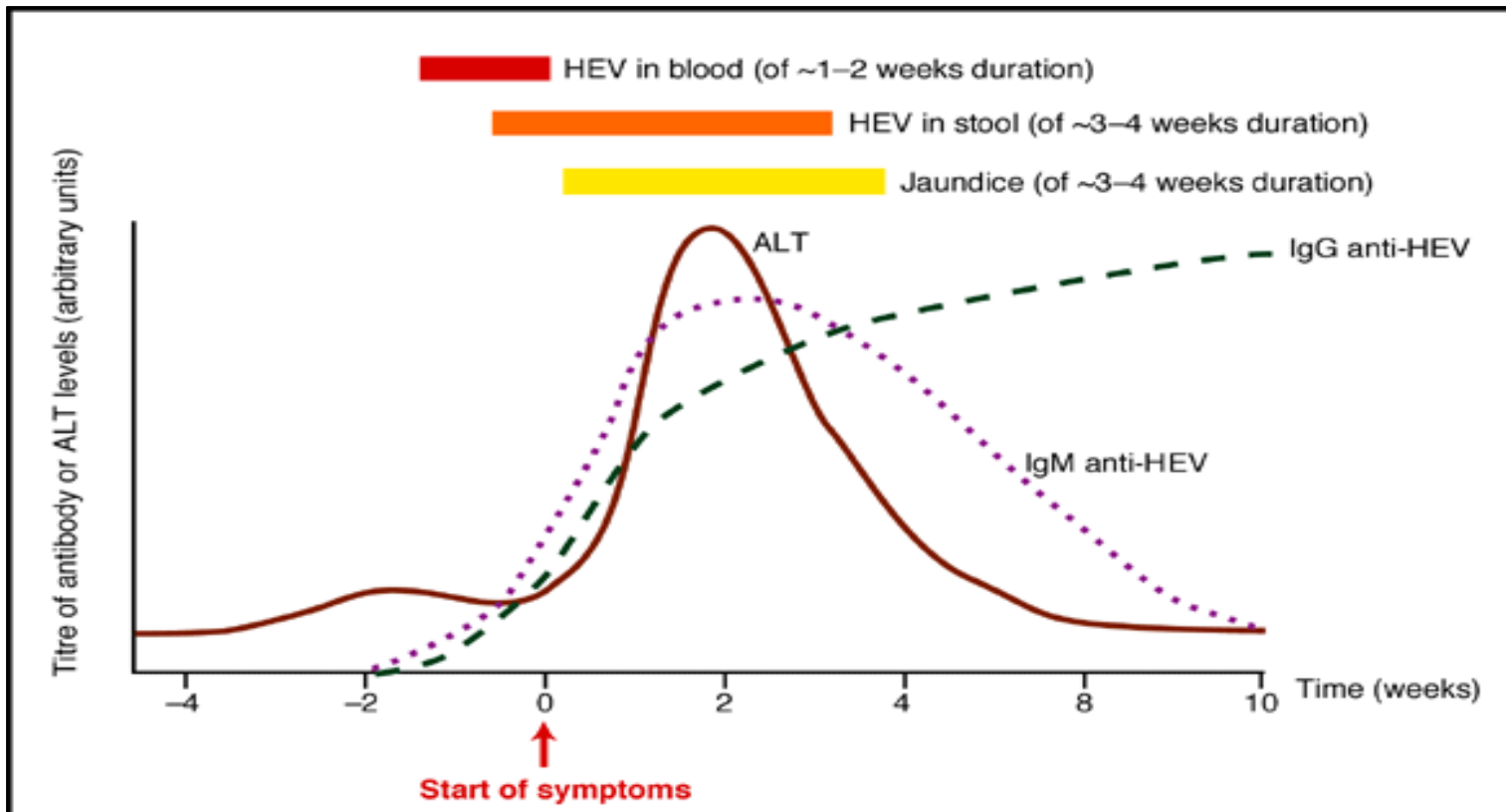


- | | |
|---------------------|---|
| HCV antibody | <ul style="list-style-type: none"> ■ May be negative in the first 6 weeks after exposure ■ May be delayed or absent when the individual is immunosuppressed ■ Presence alone does not distinguish between acute and chronic infection ■ Low signal-to-cutoff ratio may be present during acute HCV infection or represent a false-positive result |
| HCV RNA | <ul style="list-style-type: none"> ■ 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 |

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.

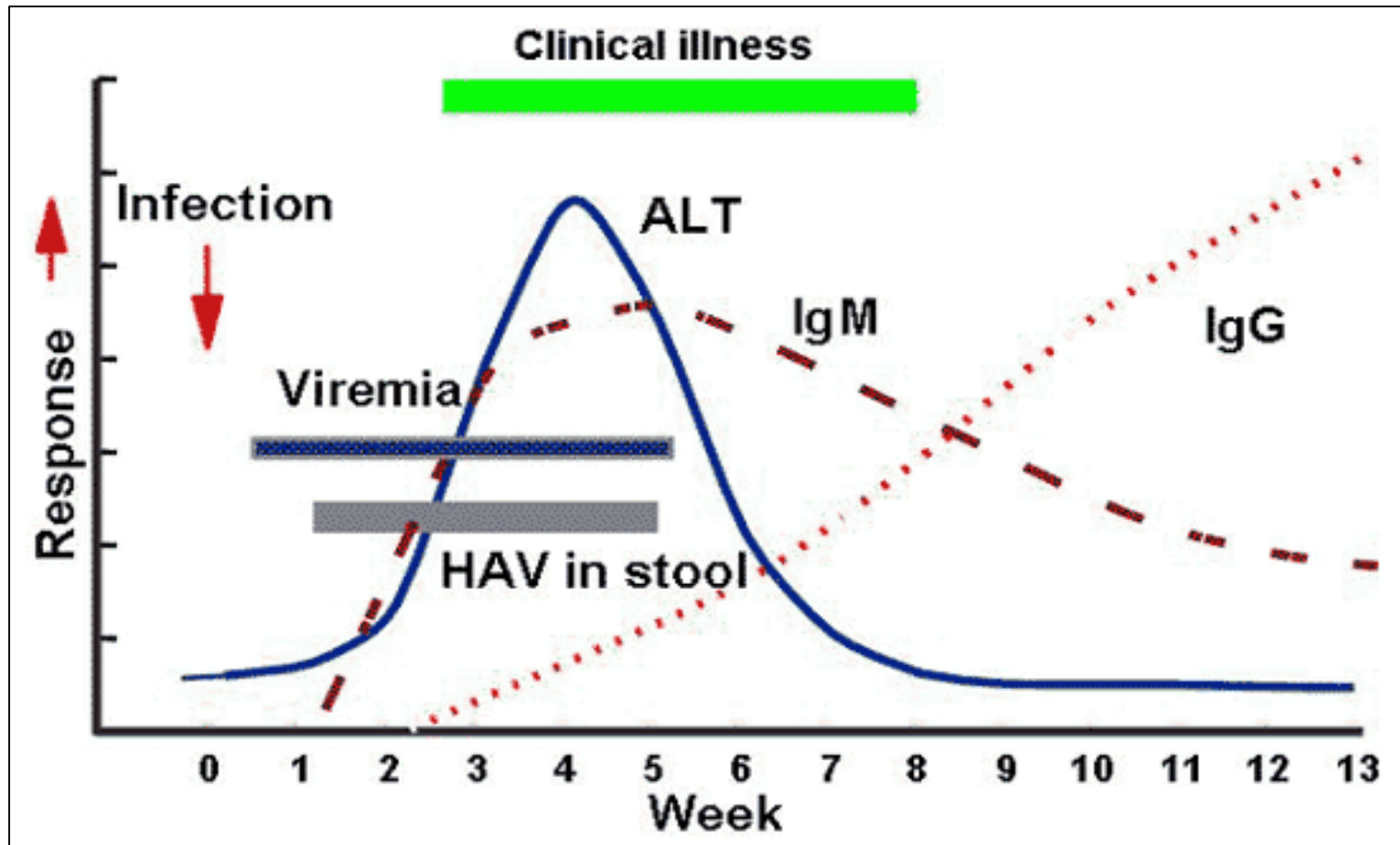
HEV Diagnostics

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, <u>cirrhosis</u>
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



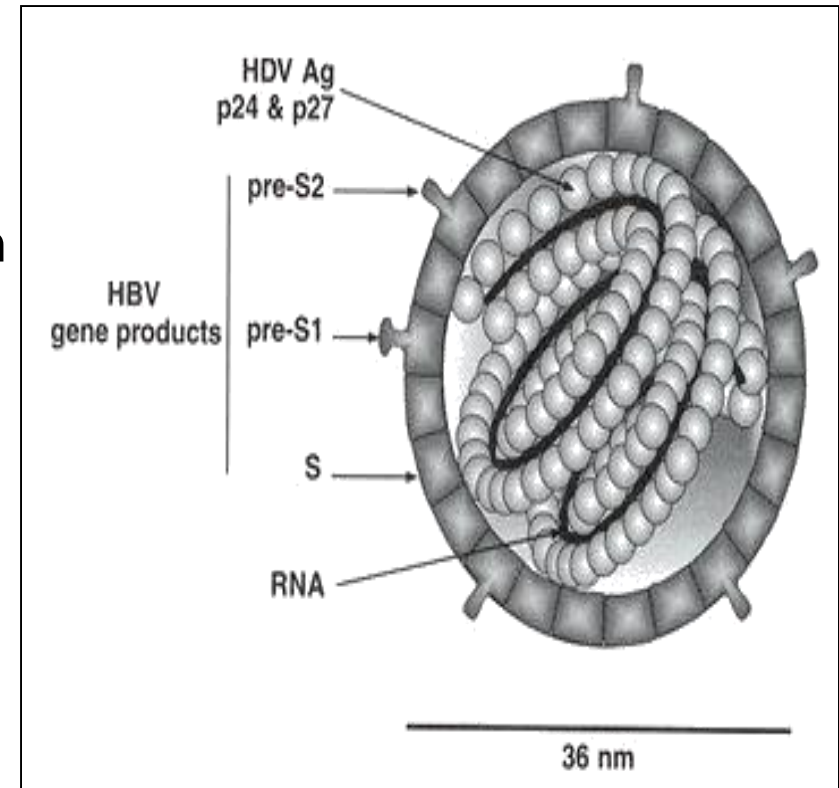
HAV IgM : diagnosis

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
- **HDV RNA PCR: confirmation of viremia**
- 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Dried blood spots (DBS) • Oral fluid 	<ul style="list-style-type: none"> • HCV • HCV 		Increase access and coverage, reaching key and target populations
3. Innovative testing approaches	<ul style="list-style-type: none"> • Self-testing • Combo integrated multi-disease tests • Integrated multidisease testing platforms (centralised and decentralised) 	<ul style="list-style-type: none"> • HCV • HCV • HCV 	<ul style="list-style-type: none"> • HBV • HBV 	Increase awareness, reduce stigma; Maximise programme synergies and reduce costs. Improve access to testing,
4. New technologies	<ul style="list-style-type: none"> • Point-of-care (POC) NAT • HCV core antigen test (as laboratory based assay and in future as POC) 	<ul style="list-style-type: none"> • HCV • HCV 	<ul style="list-style-type: none"> • HBV 	Increase access to confirmation of viraemia
5. Health system improvements	<ul style="list-style-type: none"> • Integrated testing and service decentralization • Data connectivity • Sample chain and supply management 	<ul style="list-style-type: none"> • HCV 	<ul style="list-style-type: none"> • HBV 	Improve timely receipt of results and linkage to care; improve supply chain management; Optimize use of current infrastructure sample workflow

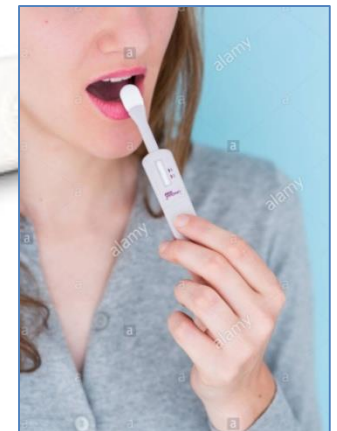
Innovations in VH testing

Dried Blood spots (DBS)



Alternate to plasma, easy to store, transport

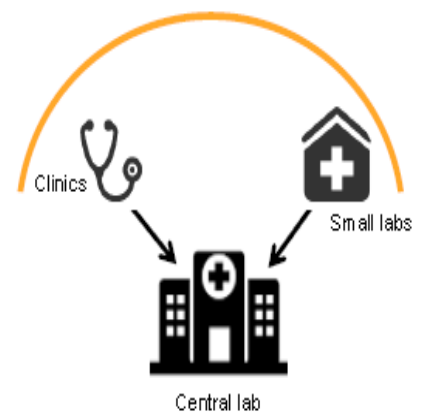
Oral Fluid testing



Self testing

The need for a decentralised molecular test

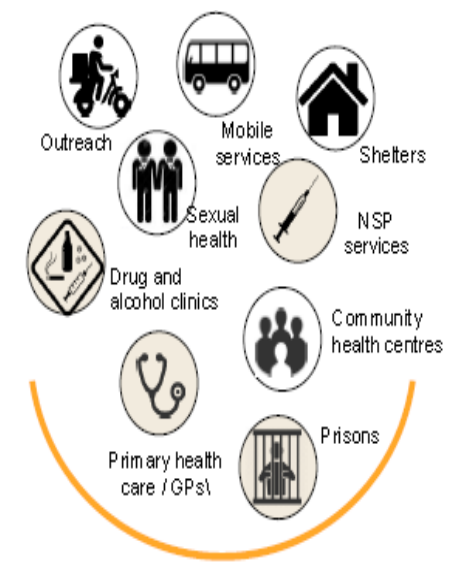
46% of patients never receive confirmatory RNA testing



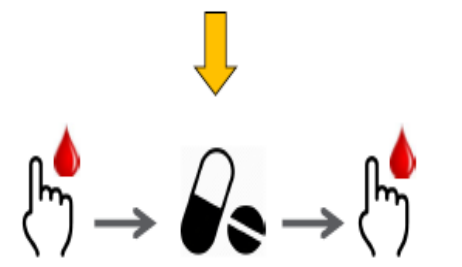
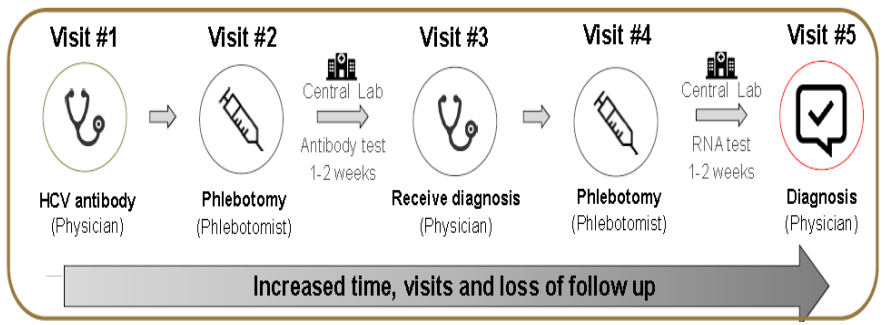
Centralized testing



Take the test to the patient




Decentralized services



Test → Treat → Confirm cure
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.

Innovations in Molecular Testing Platforms



Near patient Point of care of testing -PCR

dd PCR

Multiplex Real time PCR

Fully automated extraction/detection

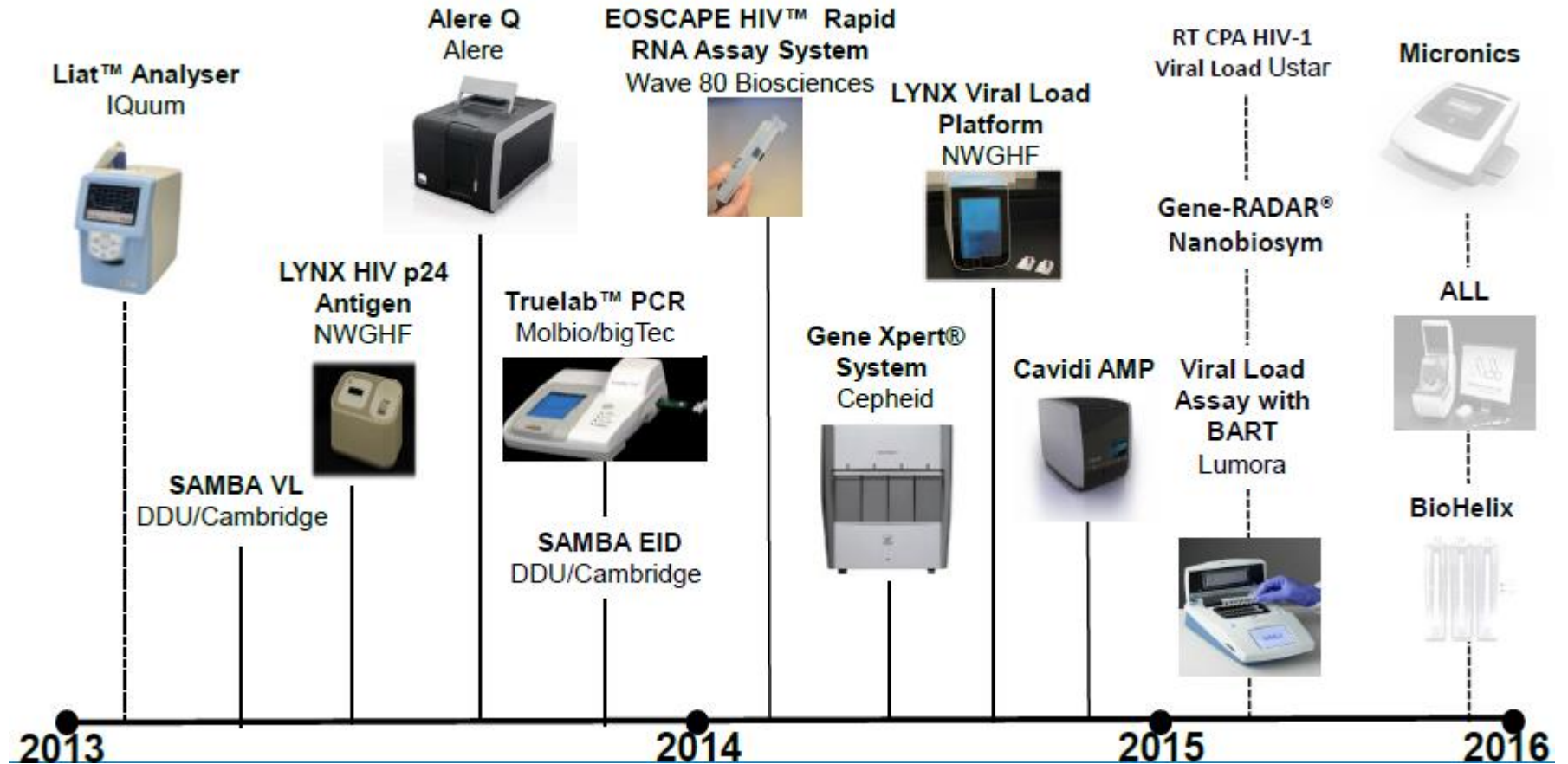
Real time PCR: manual/semi-automated

bDNA/Hybrid capture

LAMP
NASBA

Endpoint PCR

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 amplification 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

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 and very soon National policy and testing guidelines will be launched.

PDCC course in HepatoVirology at ILBS

Thank you!