

Basics of hepatitis B diagnostics

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Basics of hepatitis B diagnostics

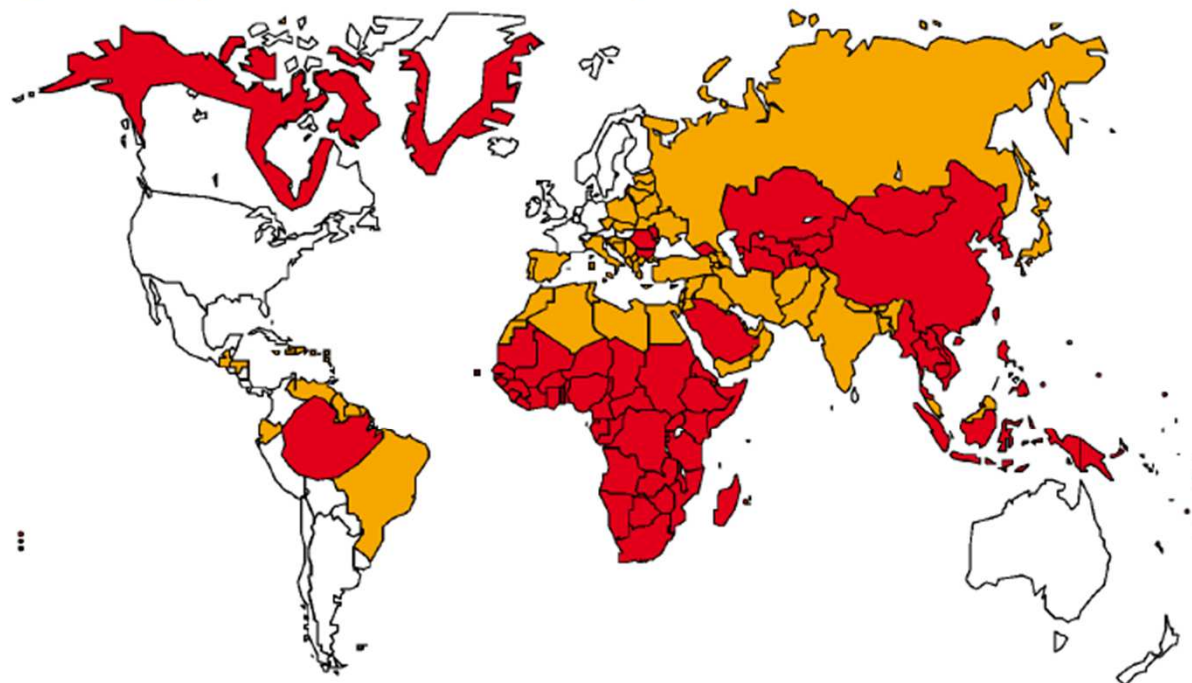
- Background
- Epidemiology
- Morphology
- Life-cycle
- Diagnostic markers
- Mutations

Hepatitis B – background

- Previously ‘serum hepatitis’
 - 1883 Bremen, German shipyard workers vaccinated against smallpox
 - 191/1289 developed jaundice ‘serum hepatitis’
 - Those unaffected vaccinated with different batches
- Blumberg ID ‘Australia antigen’ / HBsAg
 - 1963 Ab from haemophiliacs reacted with Ag from sera from an Aborigine
 - 1967 Australia Ag found to be causative agent of hepatitis B
 - Screening assay developed
 - 1969 developed vaccine
- 1970 Dane discovered HBV by electron microscopy

Hepatitis B - Epidemiology

Figure 1. Geographical distribution of HBV endemicity



Red: High (HBsAg prevalence $\geq 8\%$)

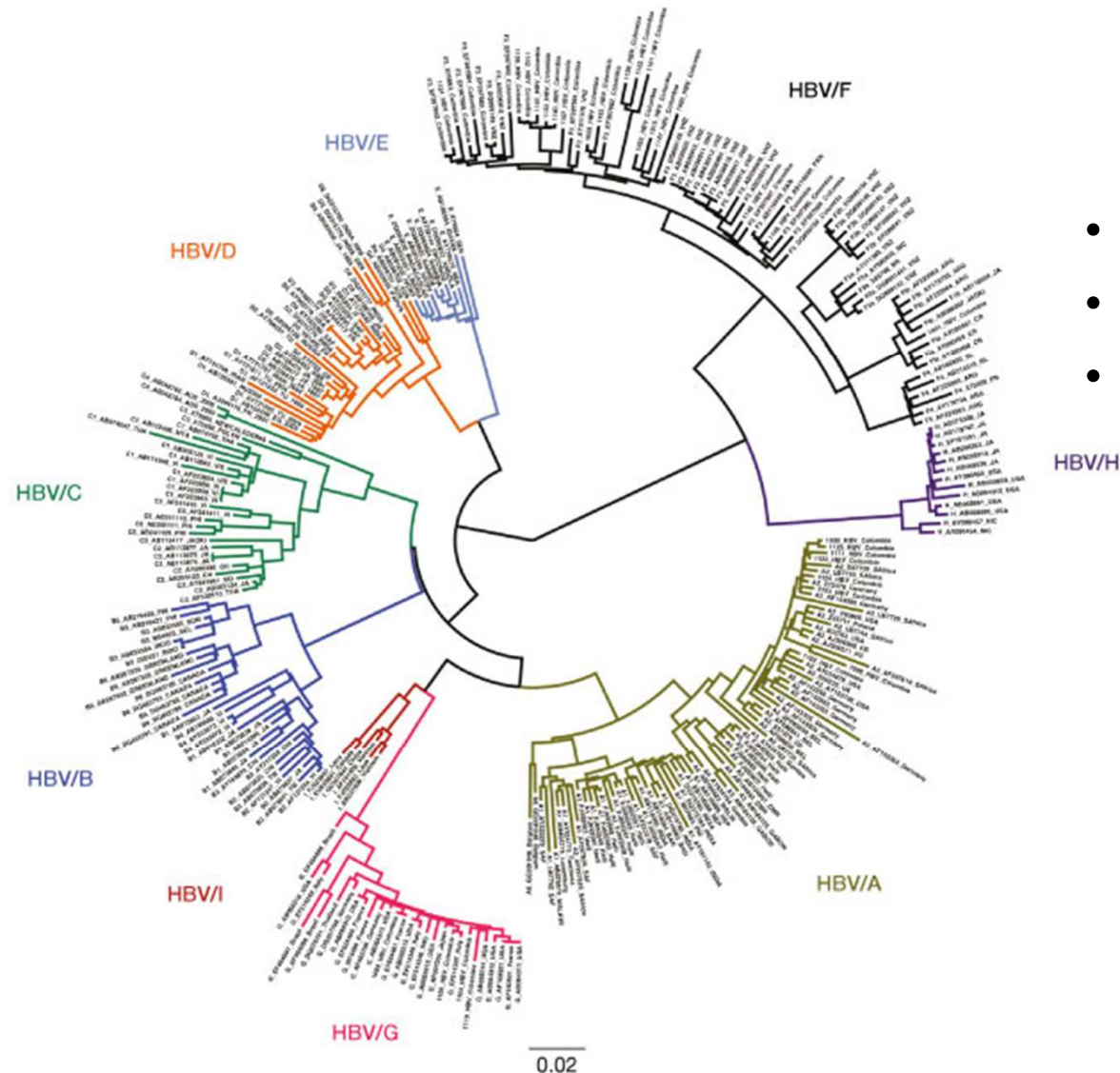
Orange: Intermediate (HBsAg prevalence 2%–7%)

White: Low (HBsAg prevalence $< 2\%$)

Source: World Health Organization. Introduction of hepatitis B vaccine into childhood immunization services. Management guidelines, including information for health workers and parents. Geneva: WHO; 2001.

- 2 billion past or current infection
- 240 million chronic infection
- 30% of liver cirrhosis
- 50% HCC
- 780,000 deaths / year
- Despite HBV vaccine since 1982

Hepatitis B – Genotypes



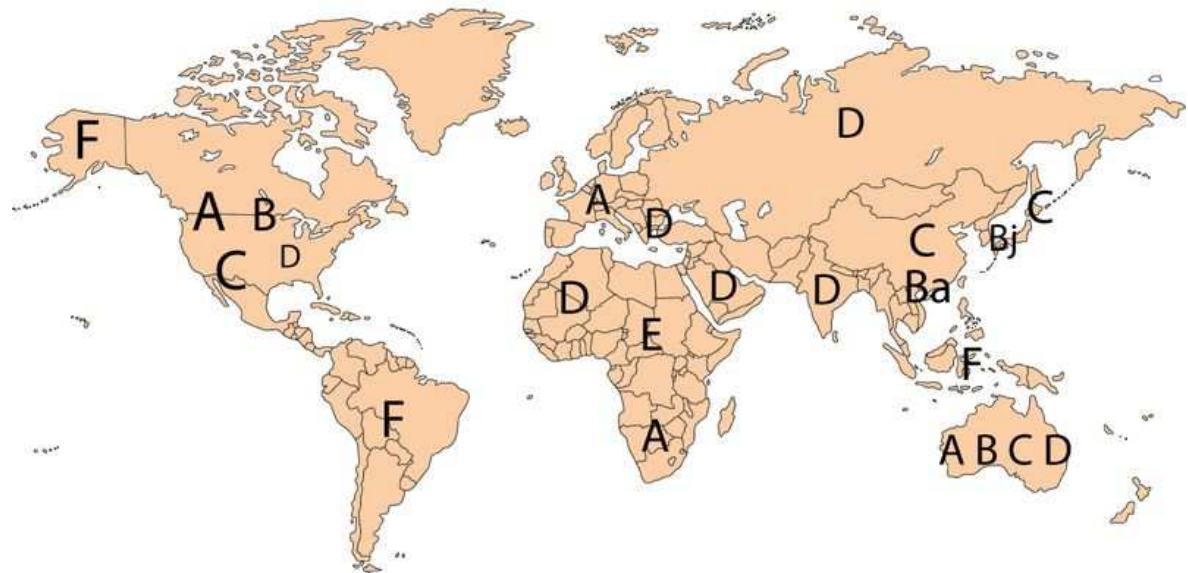
- 8 Genotypes
- A-H
- > 8% divergence in complete genome sequence

Hepatitis B – Genotypes

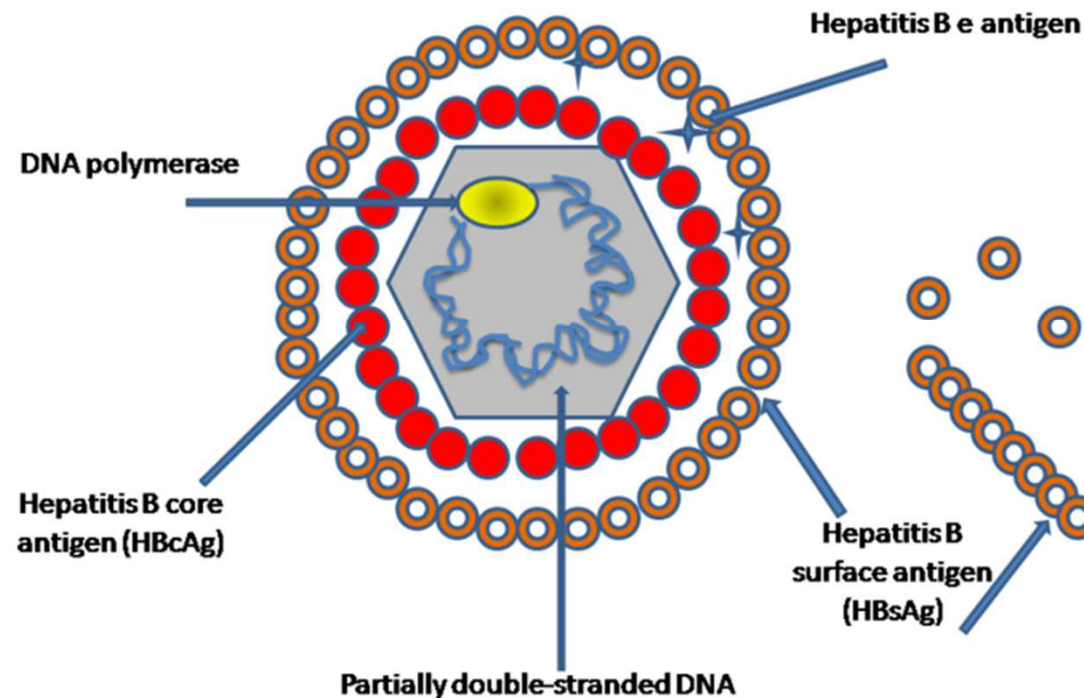
Table 1. Geographic Distribution of HBV Genotypes

HBV genotypes	Geographic distribution
A	Northwest Europe, North America, Africa
B	Asia
C	Asia
D	Worldwide distribution
E	Africa
F	South America, Central America (Hispanic origin)
G	France, UK, Italy, Germany, USA
H	Central America (Amerindian populations)

Geographic Distribution of Hepatitis B Virus Genotypes

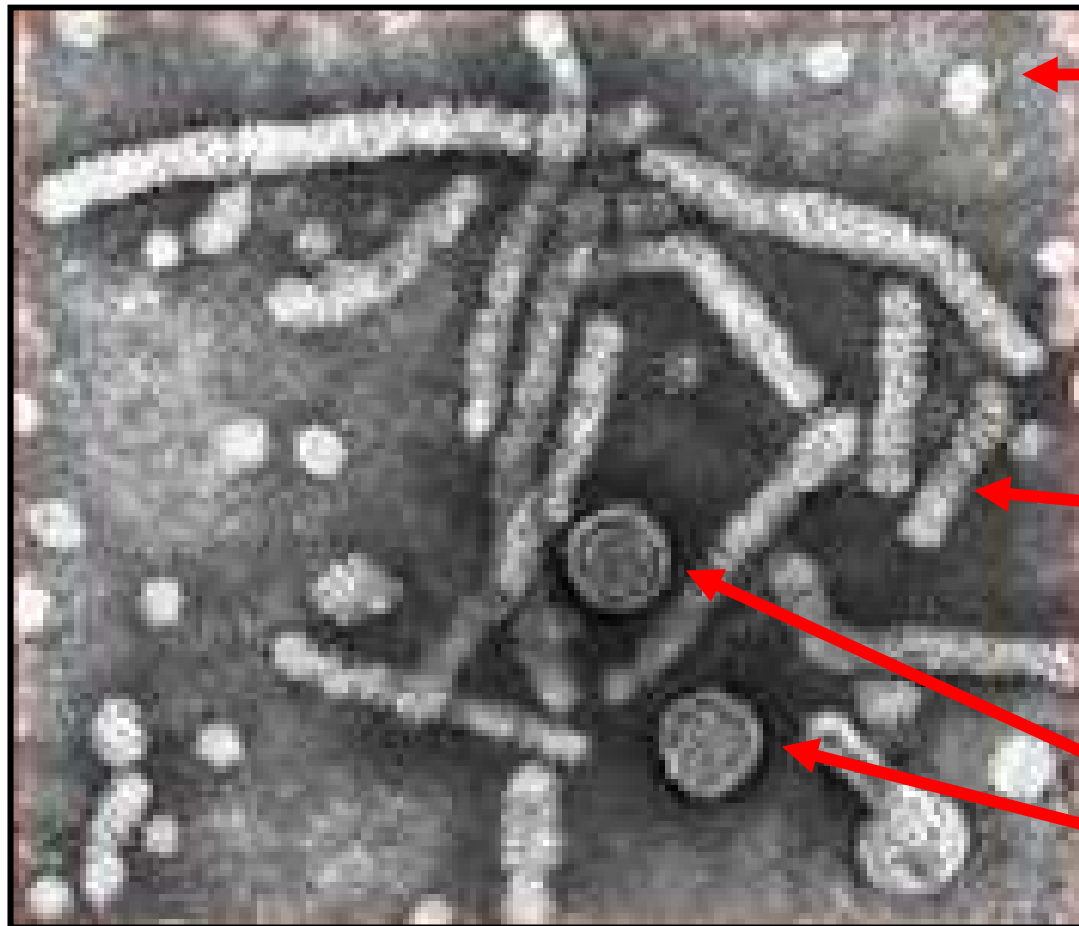


Hepatitis B – Viron



- Hepadnaviridae family
- Small – 42nm
- Lipid envelope with embedded proteins (L, M, S)
- Nucleocapsid core
- HBeAg is secreted in blood
- Partially double stranded DNA

Hepatitis B – 3 morphological forms

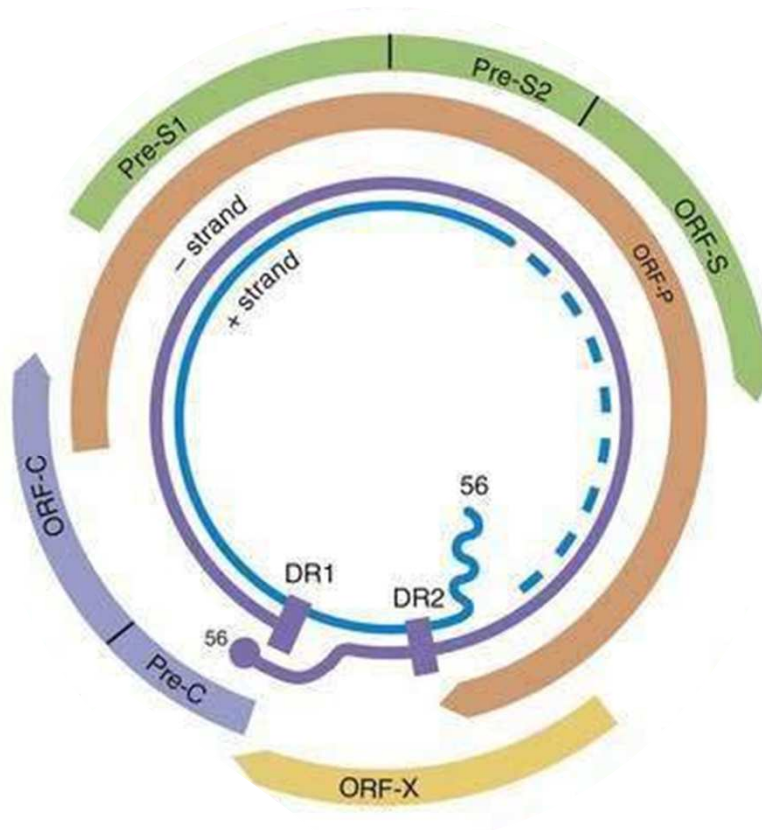


20nm non
infectious particles
– HBsAg

Tubular, filamentous
forms made of
aggregated HBsAg

43nm complete
HBV virions –
enriched with
surface proteins

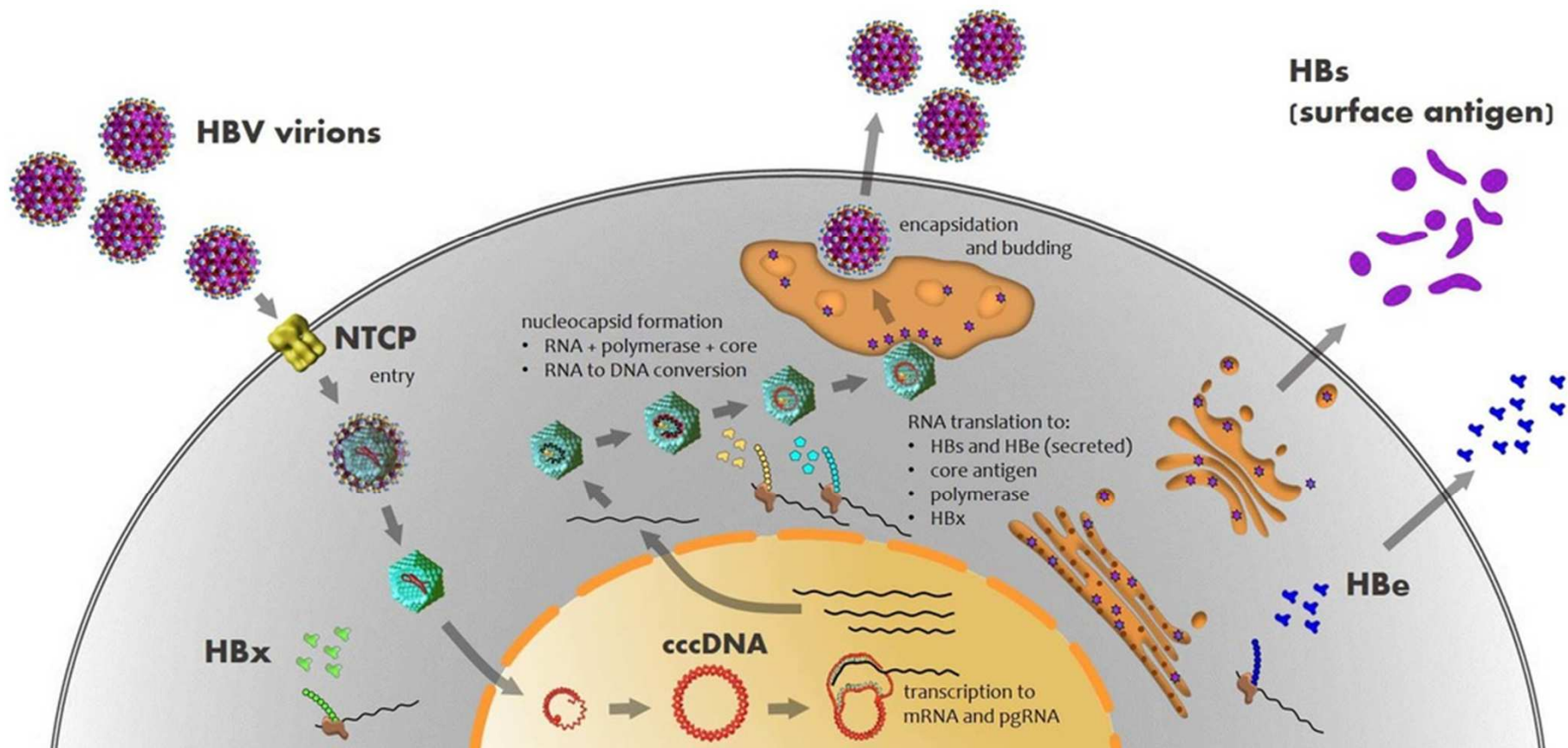
Hepatitis B – Genome



4 overlapping open reading frames

- **S gene**: 3 start codons at pre-S1 (L), pre-S2 (M), S (S) region
- **C gene**: 2 start codons at pre-core (HBeAg) and core (HBcAg)
- **P gene**: HBV polymerase
- **X-gene**: HBV X protein: role unclear

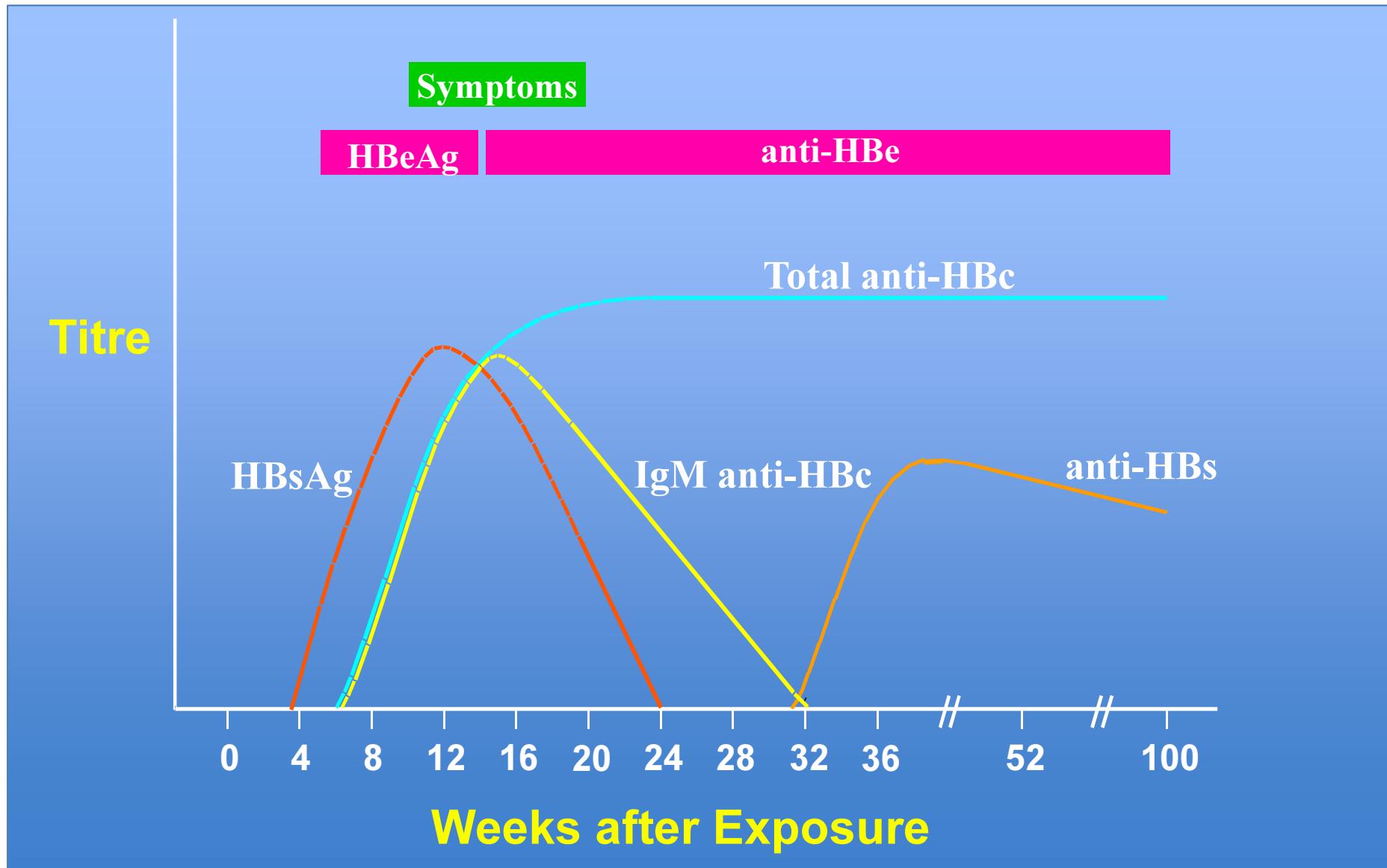
Hepatitis B – Life cycle



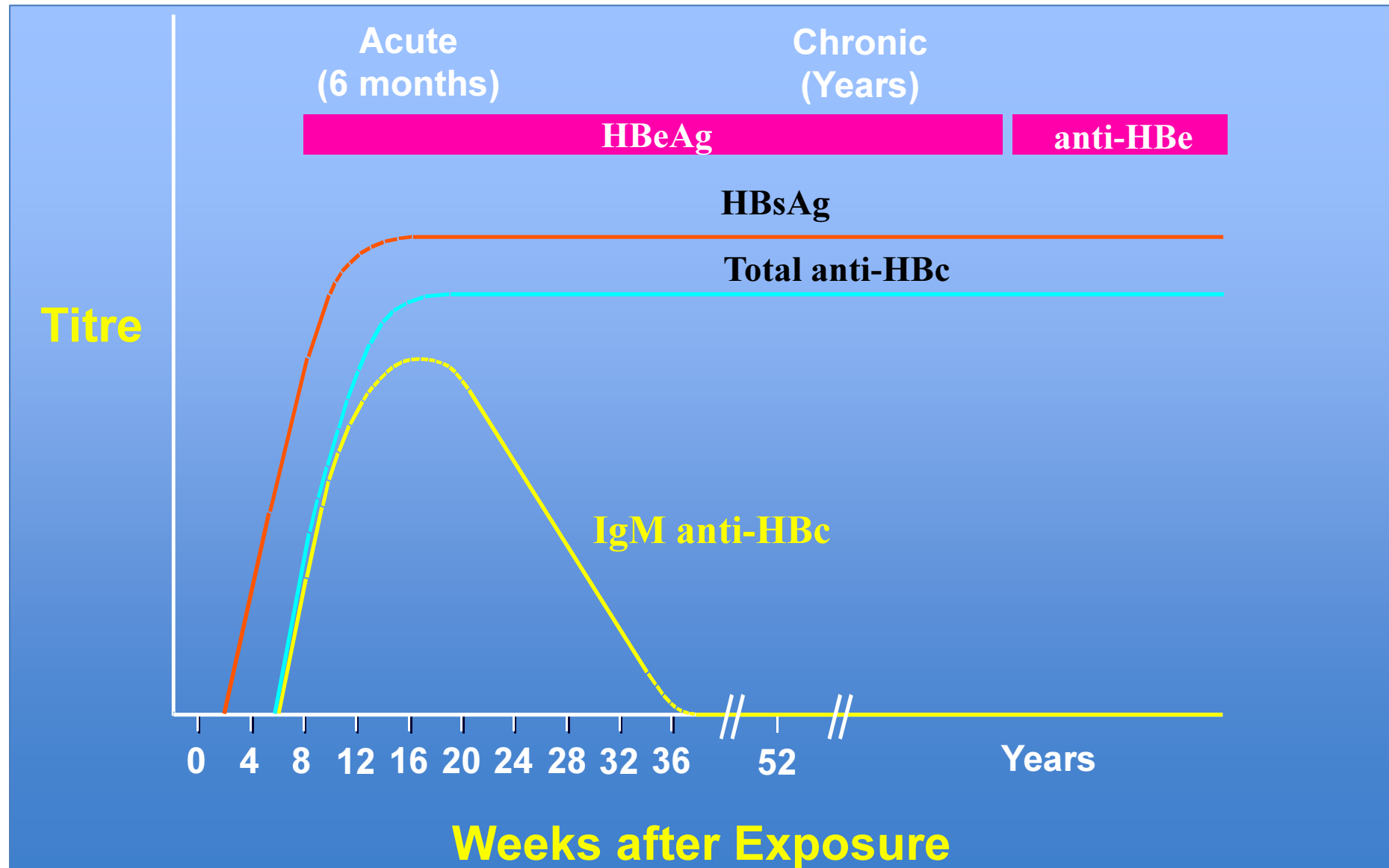
Hepatitis B – diagnostics

HBsAg	Hepatitis B surface antigen (envelope antigen)	Protein shell - complex of antigenic determinants found on the surface of HBV and of 22nm particles and tubular forms. Formerly designated Australia (Au) antigen
HBcAg	Hepatitis B core antigen	Antigenic specificity associated with the 27nm core of HBV. Not present in blood in free form
HBeAg	Hepatitis B e antigen	Antigenic determinant that is closely associated with the nucleocapsid of HBV. It circulates as a soluble protein in serum.
HBV DNA	Hepatitis B virus DNA	Presence indicates active infection
Anti-HBs	Antibody to HBsAg	Marker of past infection or vaccination
Anti-HBc	Total antibodies to HBcAg	Determines HBV infection at some time
Anti-HBc IgM	IgM antibodies to HBcAg	Determines acute HBV infection
Anti-HBe	Antibody to HBeAg	Determines HBeAg seroconversion

Hepatitis B – acute course



Hepatitis B – chronic course



Hepatitis B – diagnostics 1)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	< 10 IU/ML
Comment:	

Hepatitis B – diagnostics 1)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	< 10 IU/ML
Comment:	Consistent with current HBV infection

Hepatitis B – diagnostics 2)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	NEGATIVE
Anti-HBc	POSITIVE
Anti-HBs	35 IU/ML
Comment:	

Hepatitis B – diagnostics 2)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	NEGATIVE
Anti-HBc	POSITIVE
Anti-HBs	35 IU/ML
Comment:	Consistent with PAST HBV infection

Hepatitis B – diagnostics 3)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	NEGATIVE
Anti-HBc	NEGATIVE
Anti-HBs	> 100 IU/ML
Comment:	

Hepatitis B – diagnostics 3)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	NEGATIVE
Anti-HBc	NEGATIVE
Anti-HBs	> 100 IU/ML
Comment:	Consistent with previous HBV vaccination

Hepatitis B – diagnostics 4)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

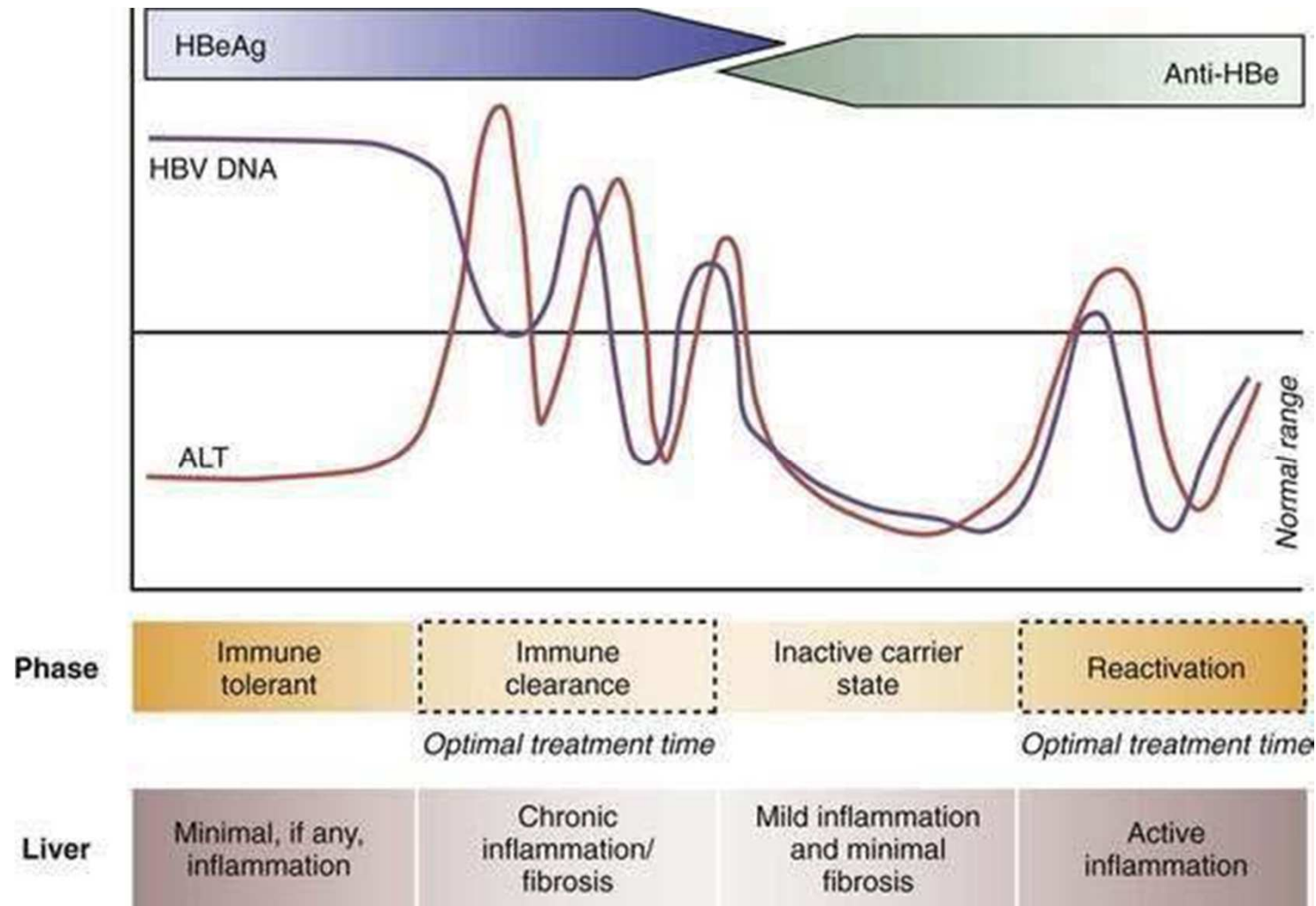
Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	POSITIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
Comment:	

Hepatitis B – diagnostics 4)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	POSITIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
Comment:	Consistent with current ACUTE HBV infection **** THIS IS A NOTIFIABLE DISEASE****

Hepatitis B – phases of infection



Hepatitis B – diagnostics 5)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
HBV DNA	5.6 X 10 ⁵ IU/ML
ALT	28 IU/L (<30)
Comment:	

Hepatitis B – diagnostics 5)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
HBV DNA	5.6 X 10 ⁵ IU/ML
ALT	28 IU/L (<30)
Comment:	Consistent with chronic HBV infection. Immune tolerant phase.

Hepatitis B – diagnostics 6)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
HBV DNA	4.2 X 10 ⁴ IU/ML
ALT	96 IU/L (<30)
Comment:	

Hepatitis B – diagnostics 6)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	POSITIVE
Anti-HBe	NEGATIVE
HBV DNA	4.2 X 10 ⁴ IU/ML
ALT	96 IU/L (<30)
Comment:	Consistent with chronic HBV infection. Immune clearance phase.

Hepatitis B – diagnostics 7)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	POSITIVE
HBV DNA	8.6 X 10 ² IU/ML
ALT	25 IU/L (<30)
Comment:	

Hepatitis B – diagnostics 7)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	POSITIVE
HBV DNA	8.6 X 10 ² IU/ML
ALT	25 IU/L (<30)
Comment:	Consistent with chronic HBV infection. Inactive carrier phase.

Hepatitis B – diagnostics 8)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

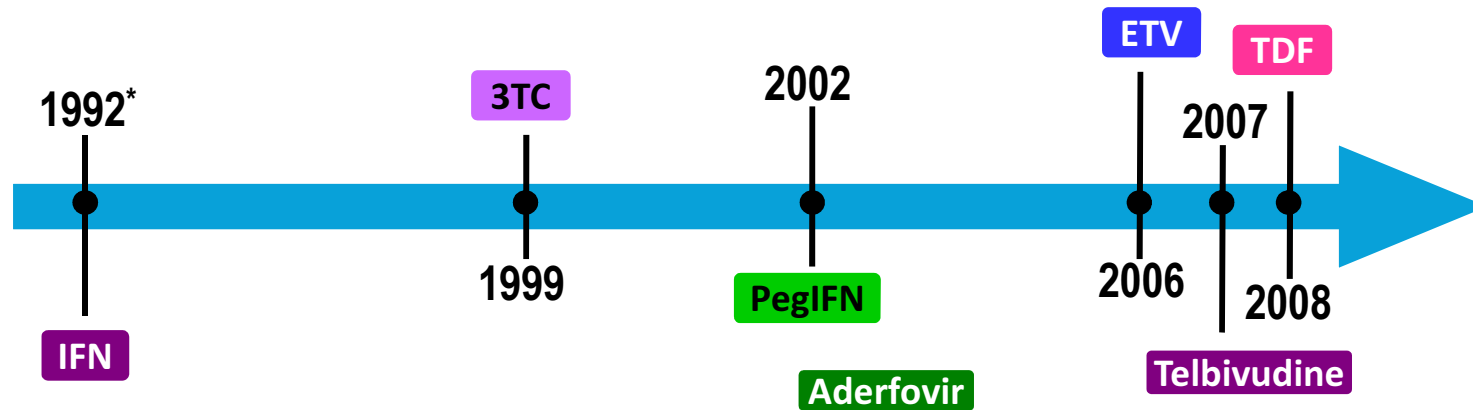
Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	POSITIVE
HBV DNA	5.6 X 10 ³ IU/ML
ALT	72 IU/L (<30)
Comment:	

Hepatitis B – diagnostics 8)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	POSITIVE
HBV DNA	5.6 X 10 ³ IU/ML
ALT	72 IU/L (<30)
Comment:	Consistent with chronic HBV infection. Reactivation phase.

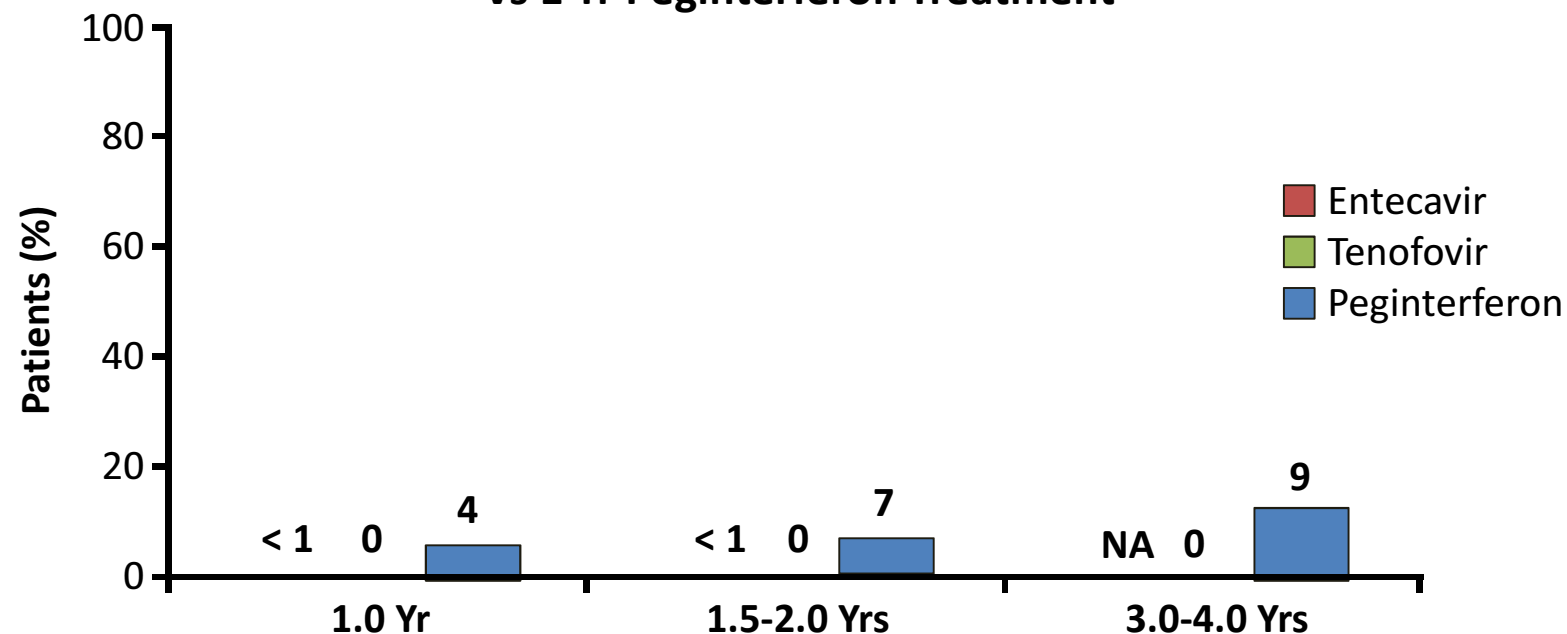
Hepatitis B – approved treatments



Hepatitis B – HBsAg loss

Not head-to-head trials; different patient populations and trial designs

Extended Treatment With Nucleos(t)ide Analogues* Vs 1 Yr Peginterferon Treatment



*With sustained undetectable HBV DNA.

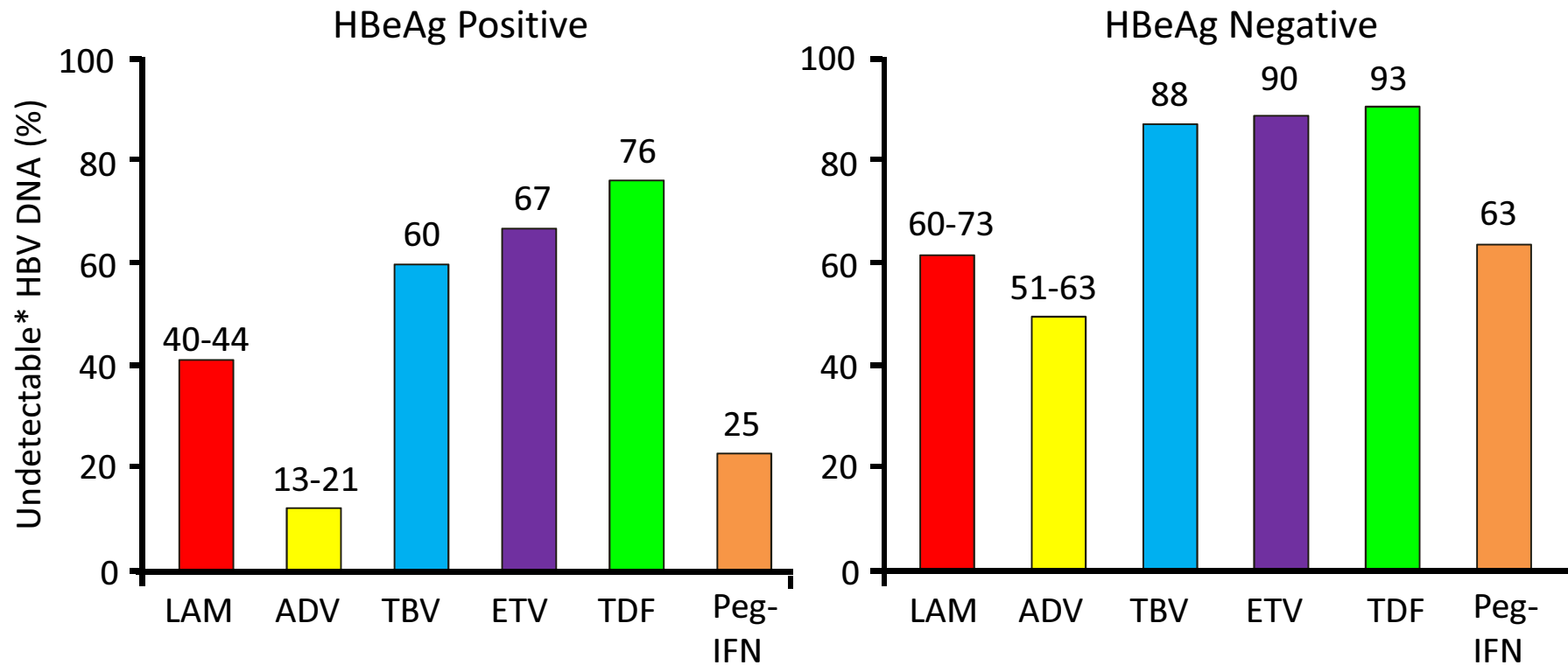
Lai CL, et al. N Engl J Med. 2006;354:1011-1020. Marcellin P, et al. N Engl J Med. 2008;359:2442-2455. Marcellin P, et al. AASLD 2008. Abstract 146. Shouval D, et al. J Hepatol. 2009;50:289-295.

Marcellin P, et al. AASLD 2009. Abstract 481. Brunetto M, et al. EASL 2008. Abstract 683. CCO Hepatitis

Hepatitis B – approved treatments

Undetectable* HBV DNA After 1 Year of Treatment

Not head-to-head trials; different patient populations and trial designs

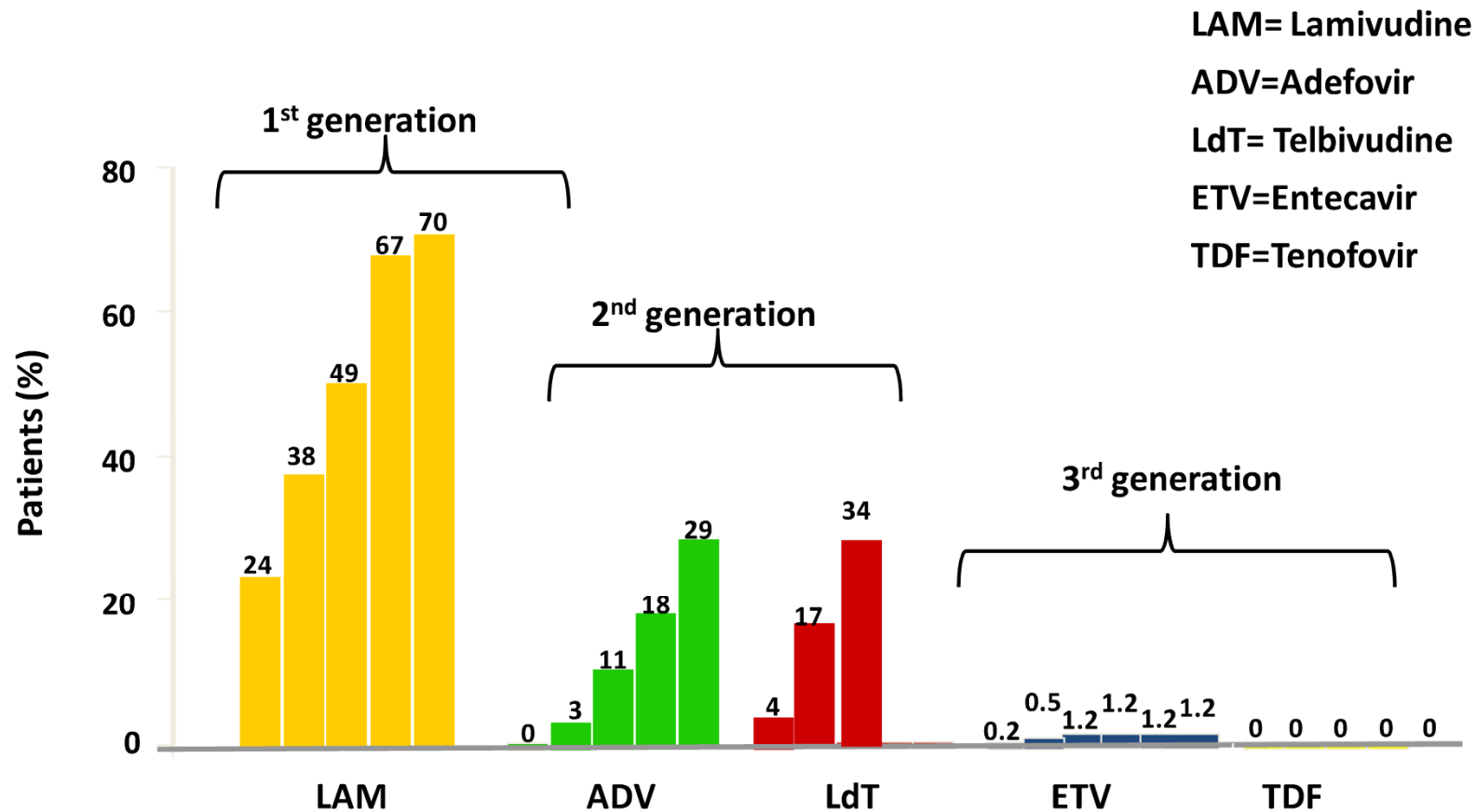


*By PCR-based assay (LLD ~ 50 IU/mL) except for some LAM studies.

Lok AS, et al. Hepatology. 2007;45:507-539. Lok AS, et al. Hepatology. 2009;50:661-662.

Hepatitis B – resistance

Resistance rates among nucleos(t)ide-naïve patients



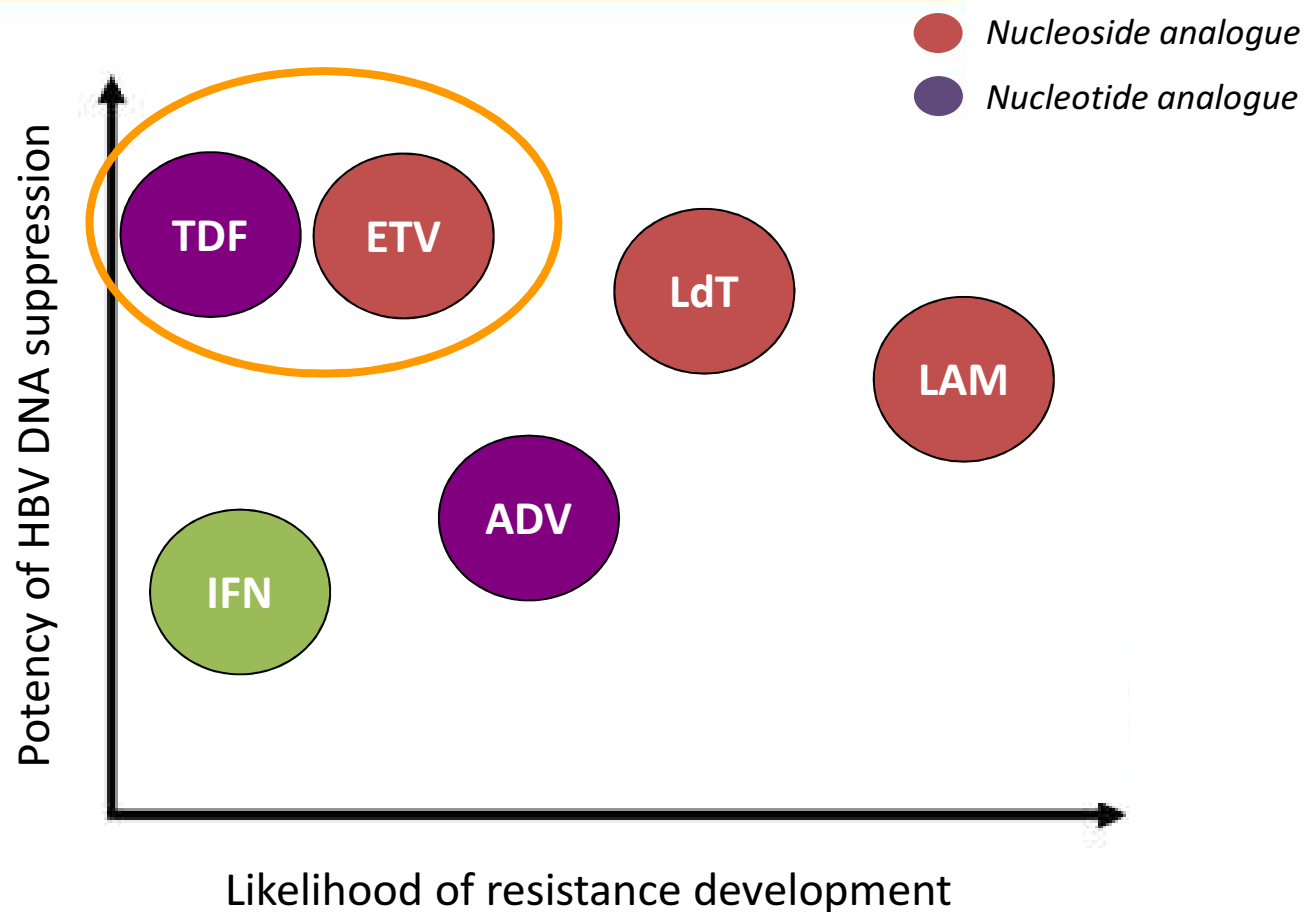
**Collation of currently available data – not from head-to-head studies*

Hepatitis B – viral kinetics

- Daily production of HBV virions: 1-10 trillion/day
- HBV polymerase enzyme: multifunctional enzyme – current target of nucleos(t)ide analogues
- Intrinsically error prone and lacks proof-reading mechanism – frequent replication errors
- It is predicted that every nucleoside of the 3.2kb HBV genome can be substituted every day within a given infected patient

Hepatitis B – resistance




Potency versus resistance



Hepatitis B – resistance

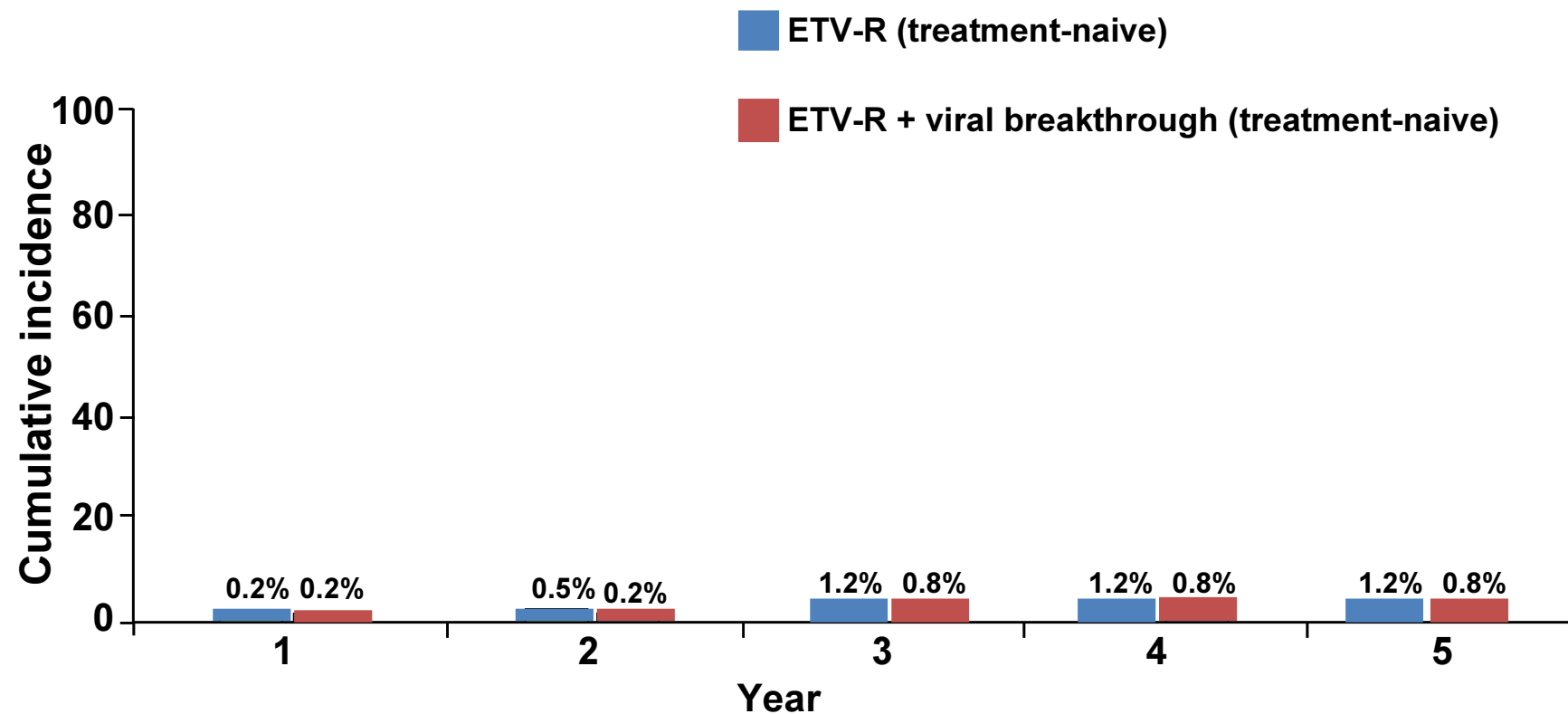
In vitro cross-resistance among oral anti-HBV drugs

	LAM	TBV	ETV	ADV	TDF
M204I	Resistant	Resistant	Intermediate	Susceptible	Susceptible
L180M+M204V	Resistant	Resistant	Intermediate	Susceptible	Susceptible
A181T/V	Intermediate	Susceptible	Susceptible	Resistant	Susceptible
N236T	Susceptible	Susceptible	Susceptible	Resistant	Intermediate
I169T+V173L+M250V	Resistant	Resistant	Resistant	Susceptible	Susceptible
T184G+S202I/G	Resistant	Resistant	Resistant	Susceptible	Susceptible

-  Susceptible
-  Intermediate
-  Resistant

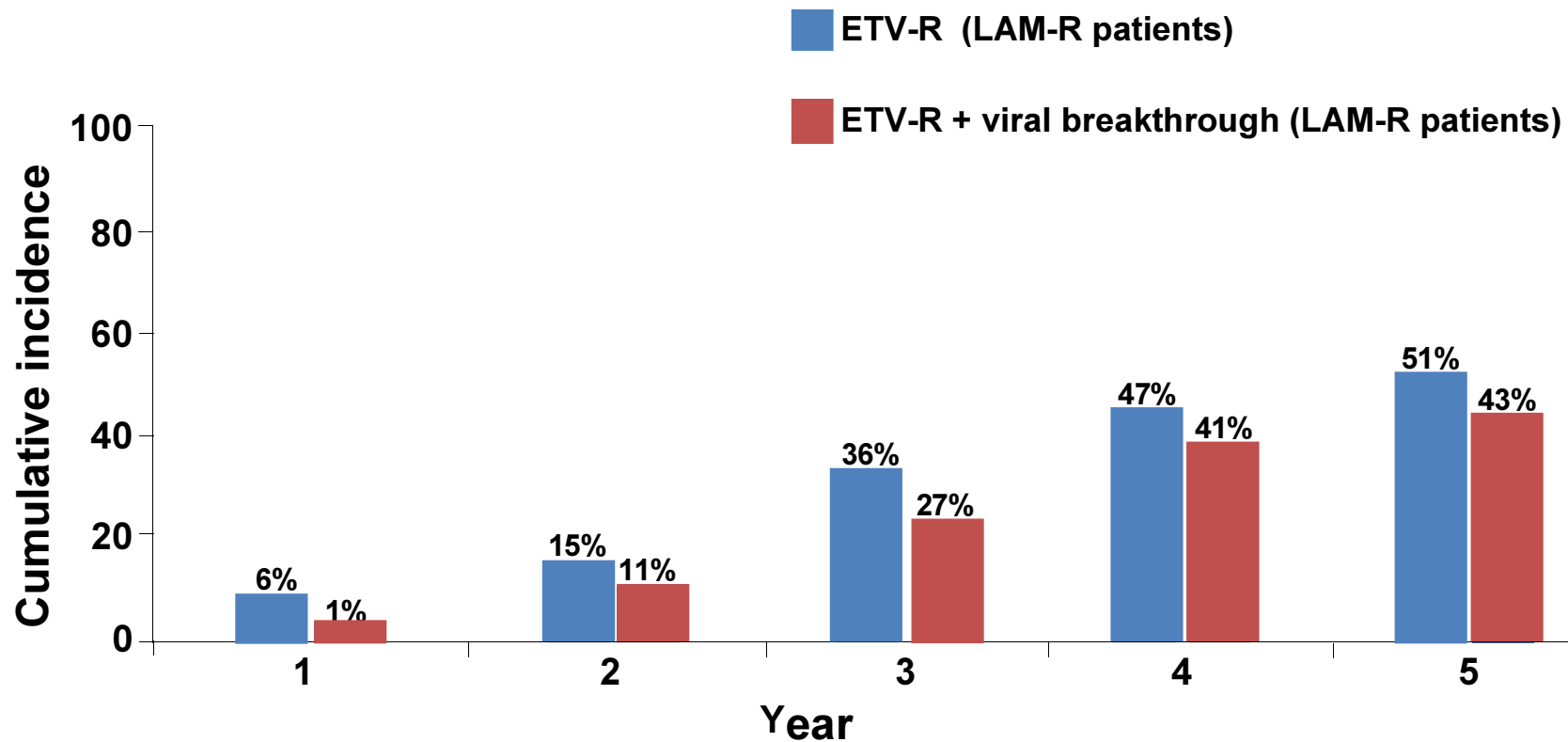
Hepatitis B – resistance

Entecavir Resistance Rates in Treatment-Naïve Patients



Hepatitis B – resistance

Entecavir Resistance Rates in LAM-R Patients



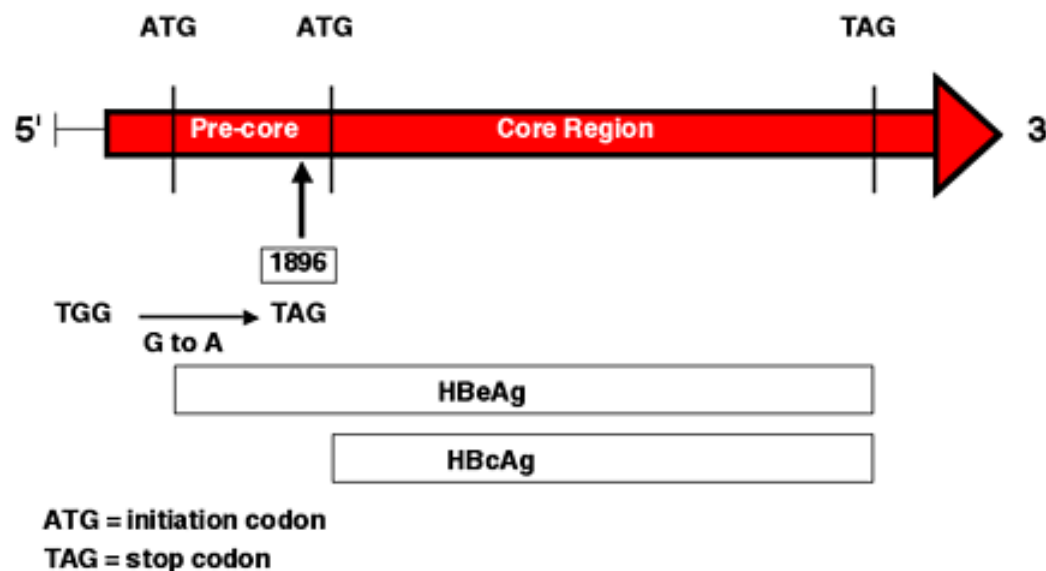
Hepatitis B – resistance

Monitoring for Resistance whilst on therapy

- HBV DNA levels every 3 months until HBV DNA levels become undetectable
- Longer intervals (approx 3-6 months) if entecavir or tenofovir are used
- Shorter intervals (approx 3 months) if lamivudine, telbivudine or adefovir are used
- If HBV DNA levels are rising, it is important to repeat the level and review compliance with the patient
- If confirmed, testing for antiviral resistance is advised.

Hepatitis B – pre-core mutant

Stop-codon Mutation in Precore Region of HBV Genome



- HBeAg coded for by Pre-core and Core
- HBcAg coded for by Core
- With HBeAg seroconversion precore mutations
- HBeAg negative
- HBeAb positive
- High HBV DNA
- Raised ALT

Hepatitis B – occult HBV

Nucleotide substitutions in the HBV S gene of OBI cohort with corresponding amino acid changes in the small S protein and polymerase.

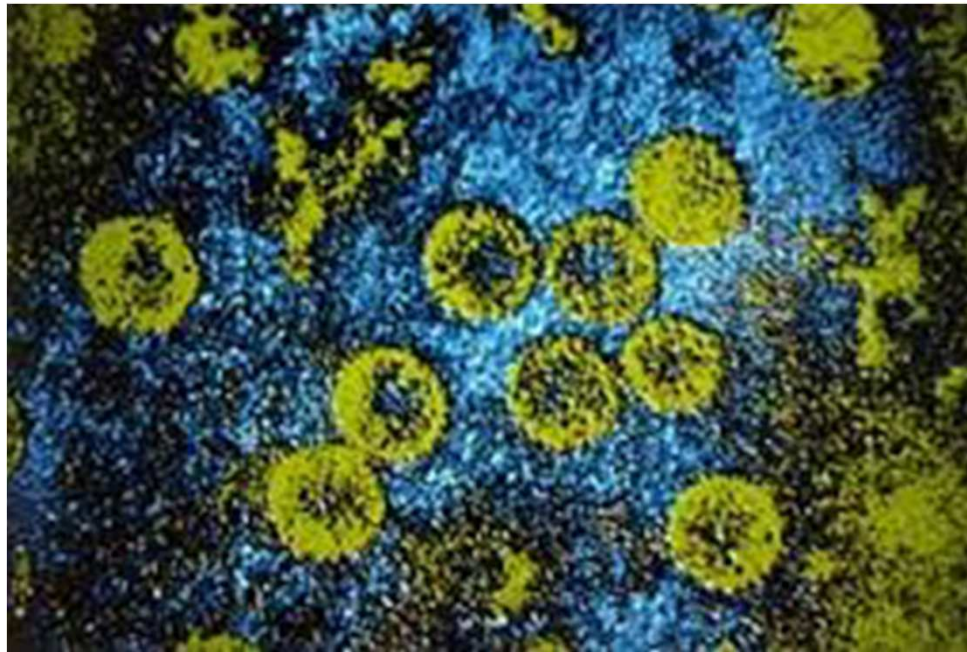
Nucleotide Substitution	Amino Acid Substitution (Small S Protein)	Amino Acid Substitution (Polymerase)
C11T	T4I	non-sense
G133T/A	A45S/T	rtS53N/I/V
A139G and C140T/G	T47V/G	rtH55R
C146T	P49L	non-sense
A337T	T113S	rtN121I
G479A	R160K	non-sense
A620G	N207S	non-sense

doi:10.1371/journal.pone.0117275.t002

**Martinez MC, Kok CC, Baleriola C, Robertson P, Rawlinson WD (2015) Investigation of Occult Hepatitis B Virus Infection in Anti-HBc Positive Patients from a Liver Clinic. PLoS ONE 10(3): e0117275.
doi:10.1371/journal.pone.0117275**

- Occult HBV: absence of HBsAg despite HBV DNA in liver, blood +/- PBMCS
- Prevalence not clearly defined
- Under lying mechanisms are not clear - ? Mutations in S & preS1/2 genes
- Above study identified 7 possible associated nucleotide substitutions

Basics of hepatitis B diagnostics



Hepatitis B – diagnostics 4)

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Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	NEGATIVE
Anti-HBc	POSITIVE
Anti-HBs	NEGATIVE
Comment:	

Hepatitis B – diagnostics 6)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
Anti-HBc	NEGATIVE
Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	NEGATIVE
Comment:	

Hepatitis B – diagnostics 6)

NHS No.	Use the NHS No.	Unit No.	M149970
Surname	149970	Forename	149970
DOB	14/02/1976	Sex	M
Report No.	L,15.2603869.Y	Collection Date	20/08/2015
Title	Hepatitis B Screen		
Clinical Details	Danger of infection		
Sample Type	Blood		
Sample Comment			

Result	Value
HBsAg	POSITIVE
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Anti-HBs	NEGATIVE
Anti-HBc IgM	NEGATIVE
HBeAg	NEGATIVE
Anti-HBe	NEGATIVE
Comment:	Consistent with current ACUTE HBV infection **** THIS IS A NOTIFIABLE DISEASE****