Hepatitis Serology and Background Notes

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NSW GOVERNMENT Health Pathology

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Hepatitis Viruses

- Virus
- Disease
- Transmission
- Prevalence
- Vaccines
- Serology and NAT diagnostic tests.

Hepatitis viruses

- Early 1960's Saul Krugman differentiated short incubation hepatitis (Infectious Hepatitis - Hepatitis A) from long incubation hepatitis (Serum Hepatitis - Hepatitis B)
- Hepatitis A - identified by Feinstone 1973
- Hepatitis B - Australia antigen identified by Blumberg 1965
- Hepatitis C - cloned by Houghton 1989
- Hepatitis D - identified by Rizzetto 1977
- Hepatitis E - identified by Reyes 1990

Hepatitis A Virus

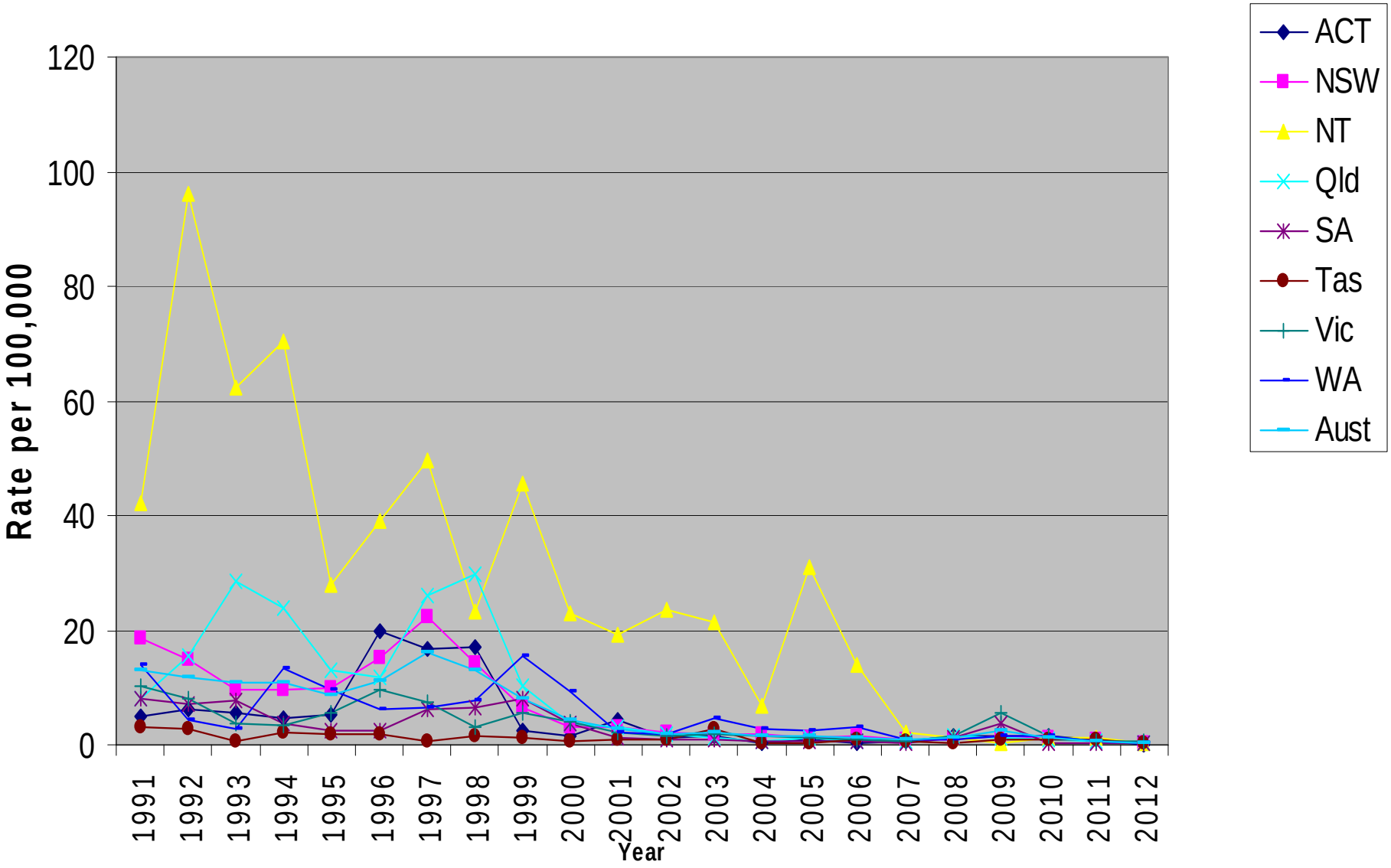
- ss RNA virus of the Heparnavirus genus, Picornaviridae family
- Incubation period 2-6 weeks (usually 4 weeks)
- Endemic throughout the developing world - most cases in Australia are seen in travellers returning from these areas
- Faecal/ oral transmission – not normally sexually transmission but may occur with anal intercourse
- Outbreaks have occurred in the male homosexual population in Sydney
- Vaccine preventable since 1992.

Prevalence

Acute Hepatitis A in Australia is predominantly seen in:

- travelers returning from an endemic area.
- Homosexual males
- IVDU
- the developmentally disabled & associated carers
- child care (both children & carers)
- indigenous population
- occasional food outbreaks
- 0.6 per 100,000 or 144 cases in Australia 2011

Hepatitis A Australia



http://www.health.gov.au/cda/Source/CDA-index.cfm

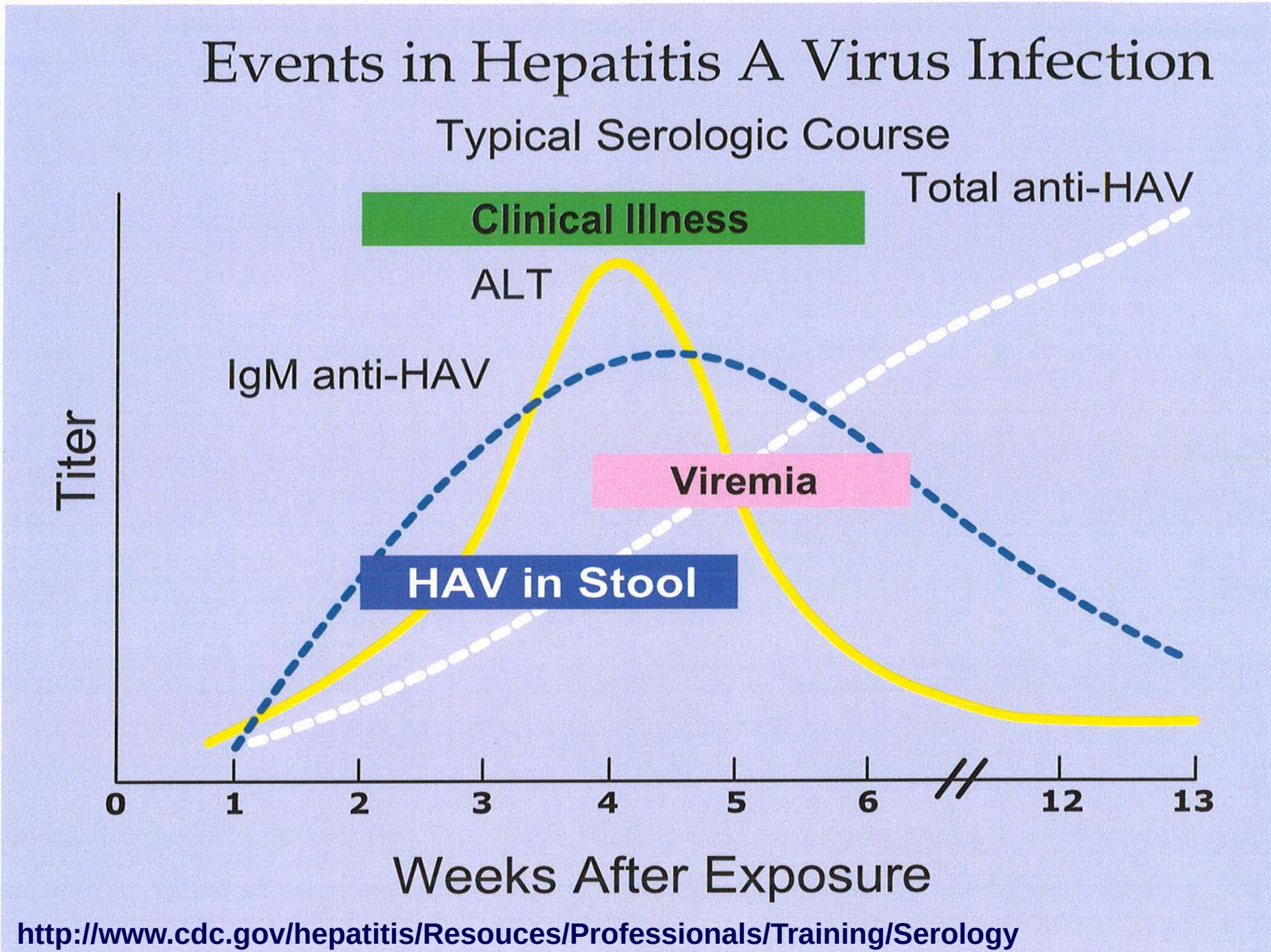
Hepatitis A Diagnostics

Serology Markers:

- Anti-HAV or HAV total antibody (competitive EIA) >= 20 mIU/ml = immune
- HAV IgG S/CO > 1 = immune
- HAV IgM S/CO > 1.2 = acute illness

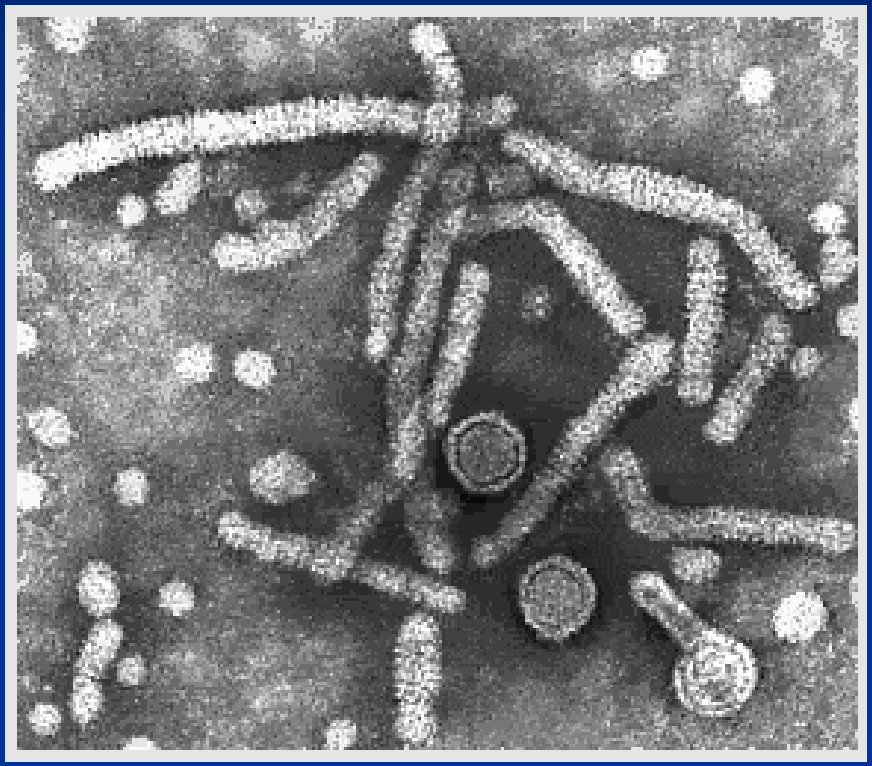
NAT:

- HAV genotype for research or epidemiology only (VIDRL)



http://www.cdc.gov/hepatitis/Resouces/Professionals/Training/Serology

HBV Structure



Hepatitis B virus (HBV) is a spherical particle 47nm in diameter containing circular, partially double-stranded DNA. There are 2 protein shells –

1. surface antigen (HBsAg), which is produced in excess & forms long filamentous aggregates and small round aggregates - 20nm in diameter

2. core antigen (HBcAg) – 27nm in diameter.

The HBcAg is not found without the HBsAg outer shell outside the hepatocyte.

In addition to these 2 antigens, a soluble protein (HBeAg) is associated only with the whole viral particle and therefore is usually associated with viraemia & infectivity

HBV Transmission & Prevalence

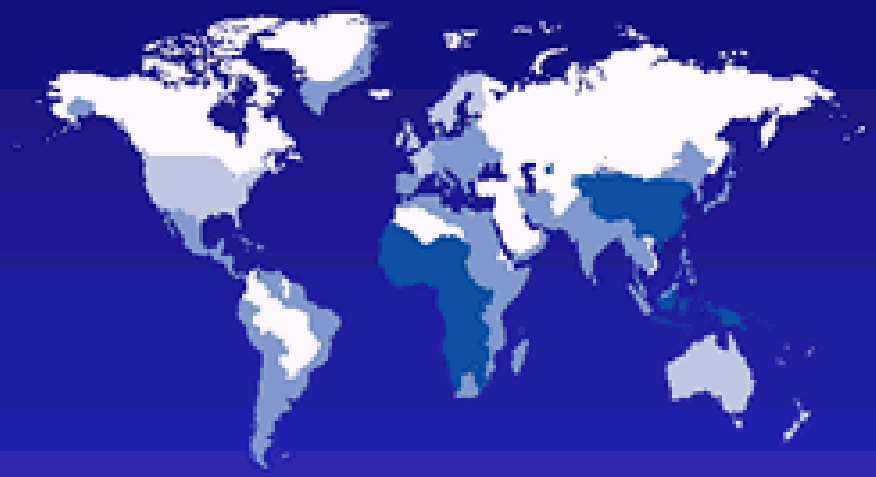
TRANSMISSION

- Vertical
- Blood & blood products - IVDU, tattoos, sharps injuries
- Sexual transmission

PREVALENCE

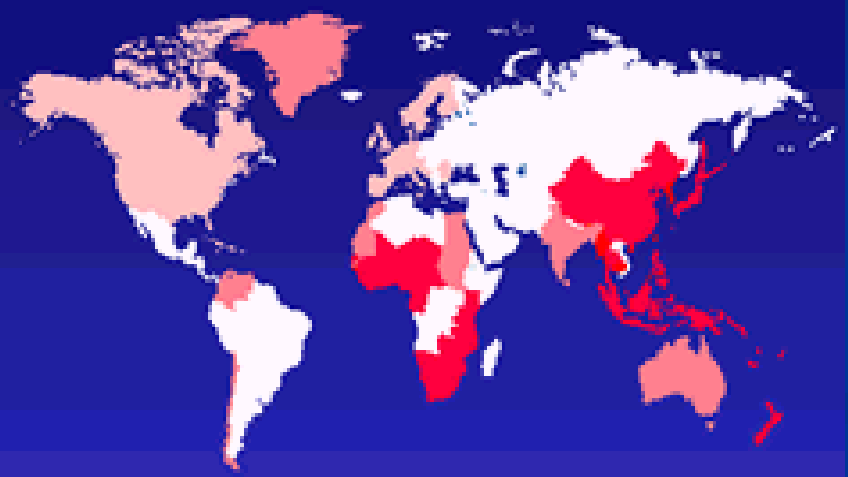
- 29.8 per 100,000 in Australia 2011 = 6648 in 2011
- Endemic in different areas of the world

Worldwide Prevalence of Hepatitis B and Incidence of HCC



World prevalence of HBV carriers

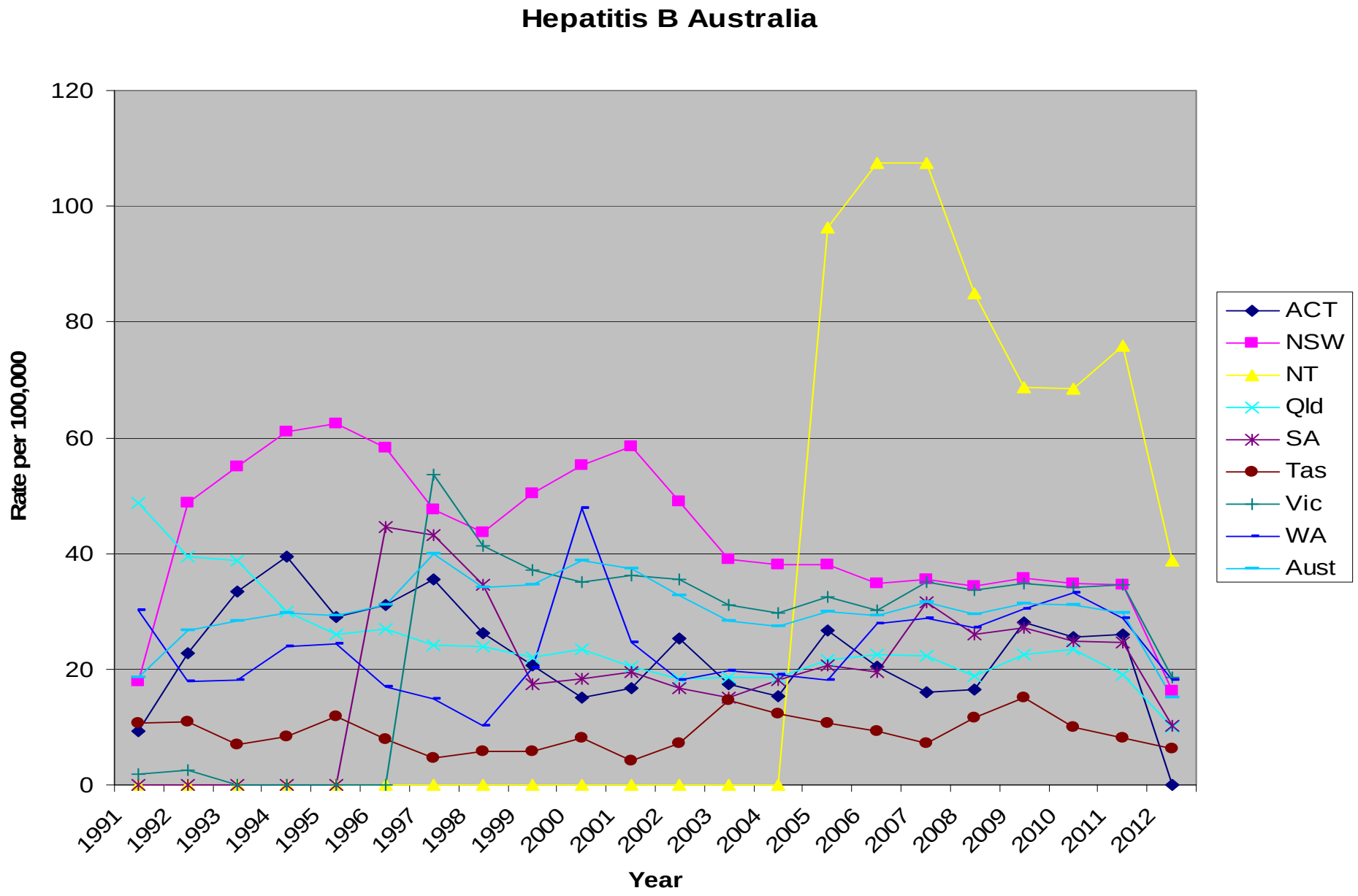
HBs Ag carriers prevalence
- <1%
- 1-10%
- >10%
- poorly documented



Annual incidence of primary HCC

Cases/100,000 population
- 1-3
- 3-10
- 10-150
- poorly documented

CDC, 2007

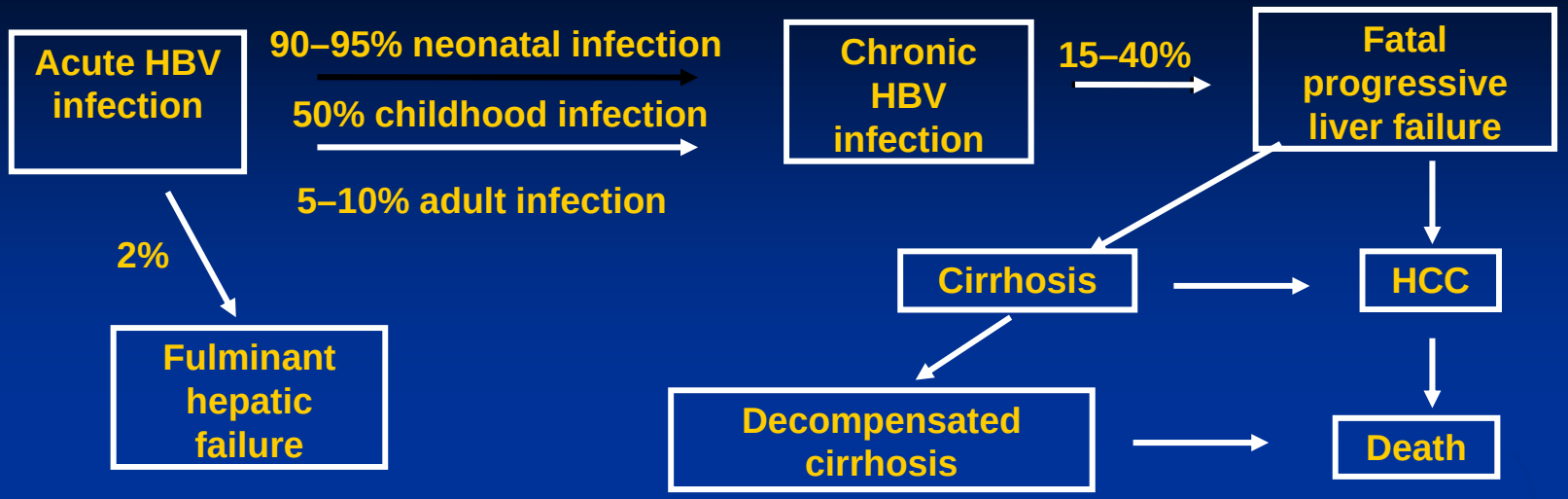


http://www.health.gov.au/cda/Source/CDA-index.cfm

HBV Genotypes and Subtypes

Genotype	Subtype	Areas of prominence
A	adw2, ayw1	NW Europe, USA, Central Africa
B	adw2, ayw1	Taiwan, Japan, Indonesia, China, Vietnam
C	adw2, adrq+, adrq-, ayr	E Asia, Taiwan, Korea, China, Japan, Vietnam
D	ayw2, ayw3	Mediterranean area, India
E	ayw4	W Africa
F	adw4q, adw2, ayw4	Central and S America
G	adw2	France, USA
H		**Central and S America**

Course of HBV Infection



Acute HBV infection

HBV infection may be successfully cleared by the immune system during acute phase.

It begins with an immune tolerance phase and typically lasts for 45–160 days.The next phase is immune clearance. Between 90–95% of infected adults make a full recovery without medical intervention.

Chronic HBV infection

Acute infection progresses to chronic infection if the immune system fails to clear HBV within 6 months. Between 5–10% of adults and 90–95% neonates with acute infection develop persistent chronic infection.

Between 25–40% of all individuals chronically-infected with HBV develop progressive liver disease.

HBV chronic infections can be treated with anti-virals. Patients are more difficult to treat if they have

- High pretreatment HBV DNA
- Low baseline ALT levels
- HBV Genotype C (Patients with HBV Genotype C are less responsive to IFNa therapy than patients with HBV Genotype B)

Hepatitis B Diagnostics

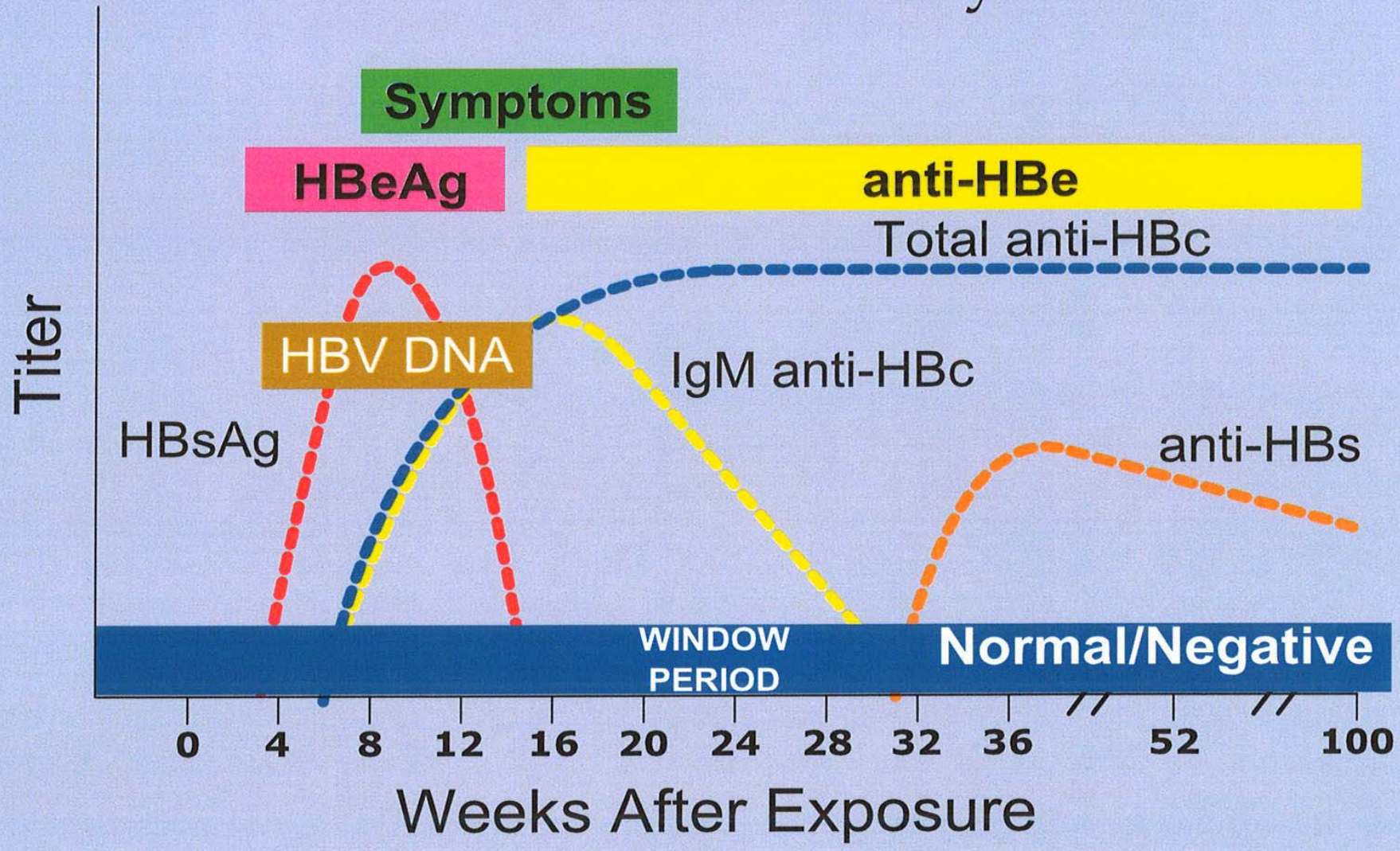
Serology Markers:

- HBsAg and neutralising antibody to confirm [quantitative?]
- Anti-HBs [HBsAb] > 10 mIU/ml protective
- Total anti-HBc, Anti-HBc IgM
- HBeAg, anti-HBe

NAT:

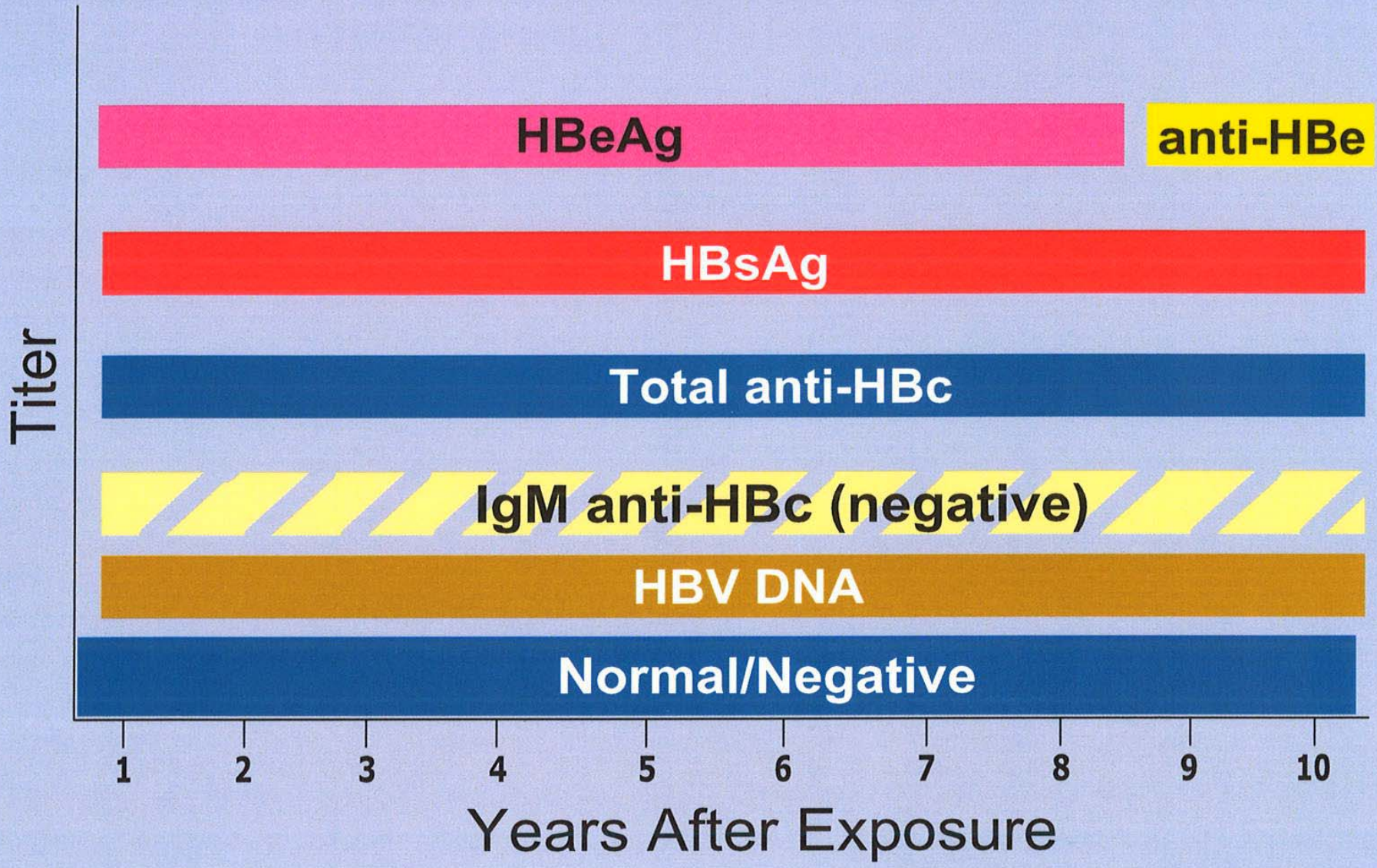
- HBV DNA qualitative / quantitative
- HBV genotype for research or epidemiology only (VIDRL)

Acute Hepatitis B Virus Infection with Recovery



http://www.cdc.gov/hepatitis/Resouces/Professionals/Training/Serology

Chronic Hepatitis B Virus Infection



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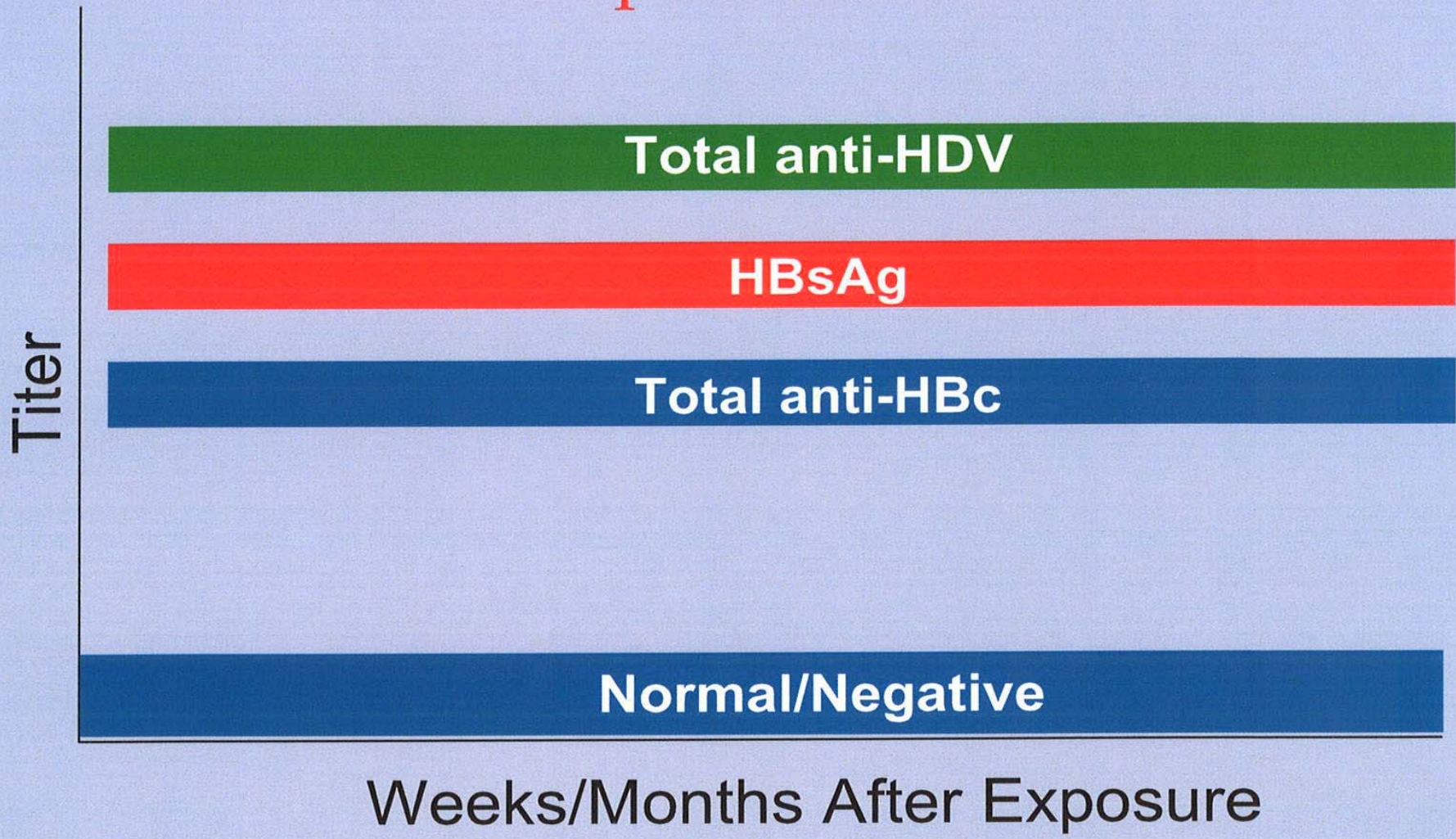
HBV-HDV Infection

Super Infection



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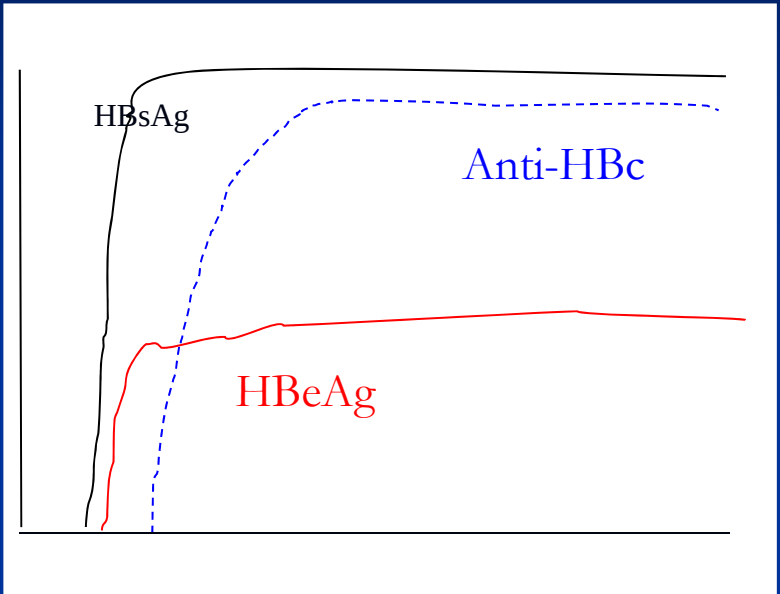
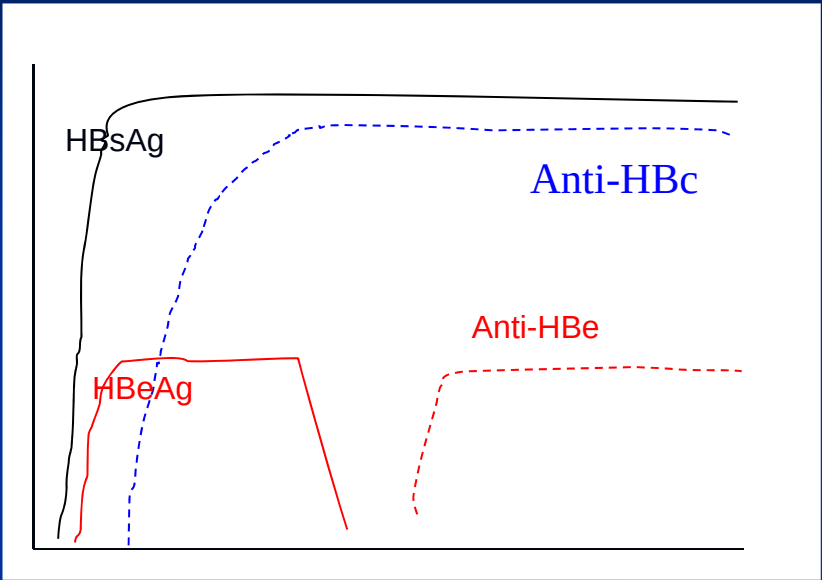
HBV carriers



Supercarrier - ~20% progress to HCC - ~90% chance of vertical transmission without vaccination



Healthy carrier – usually no chronic liver disease – ~5-10% chance of vertical transmission without vaccination

Assessing stage of HBV infection

- Ever infected – anti-HBc
- Active infection – HBsAg (confirmed by neutralisation), HBeAg
  - HBVDNA > 20 IU/ml (≈100 copies/ml)
- Resolution – anti-HBs

HBV Mutants

- **Vaccine escape mutant:**
  - HBsAg aa 145 (Gly → Arg) - changes 3-dimensional conformation (Arg much larger & charged) → current vaccine non-protective
  - binding with monoclonal antibodies much reduced (most current assays) - if HBsAg positive usually at a low level
- **Pre-core mutant:**
  - nucleotide 1896 in pre-core region - TGG (tryptophan) → TAG (stop codon) → HBeAg not replicated
  - high LFT's, aggressive liver disease
  - higher fatality rate than wild-type acute HBV (fulminant hepatitis)
  - can be responsible for fatal exacerbations of chronic HBV

Hepatitis C Virus

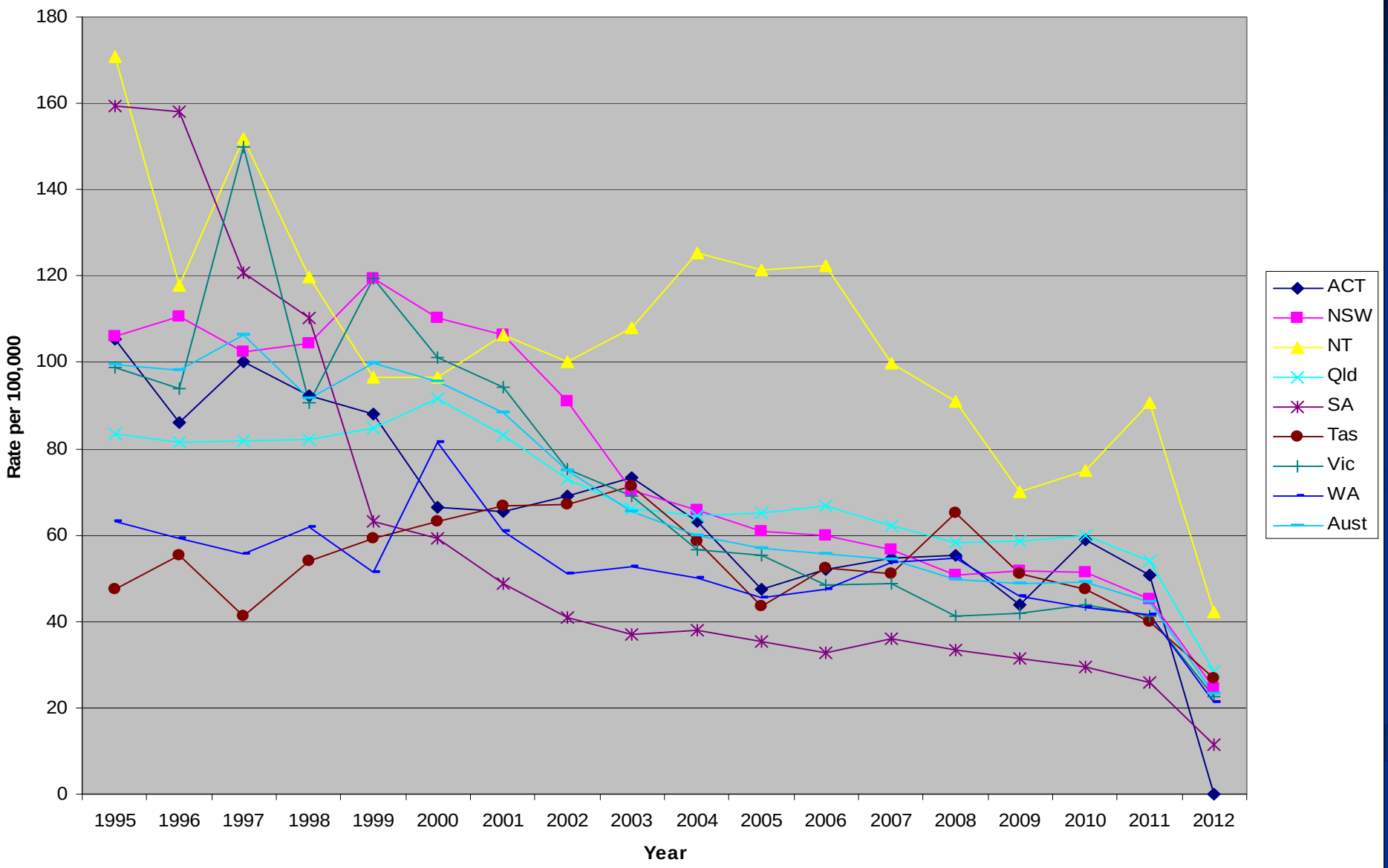
NANB hepatitis. ssRNA. Family Flaviviridae.

- 3-4 million infections worldwide yearly
- 150 million chronic HCV infections
- 350,000 HCV related deaths
- 44.6 per 100,000 in Australia 2011
- 9974 with HCV of some form in Australia 2011.

http://www.who.int/topics/hepatitis/factsheets/en/index.html

http://www.health.gov.au/cda/Source/CDA-index.cfm

Hepatitis C Australia



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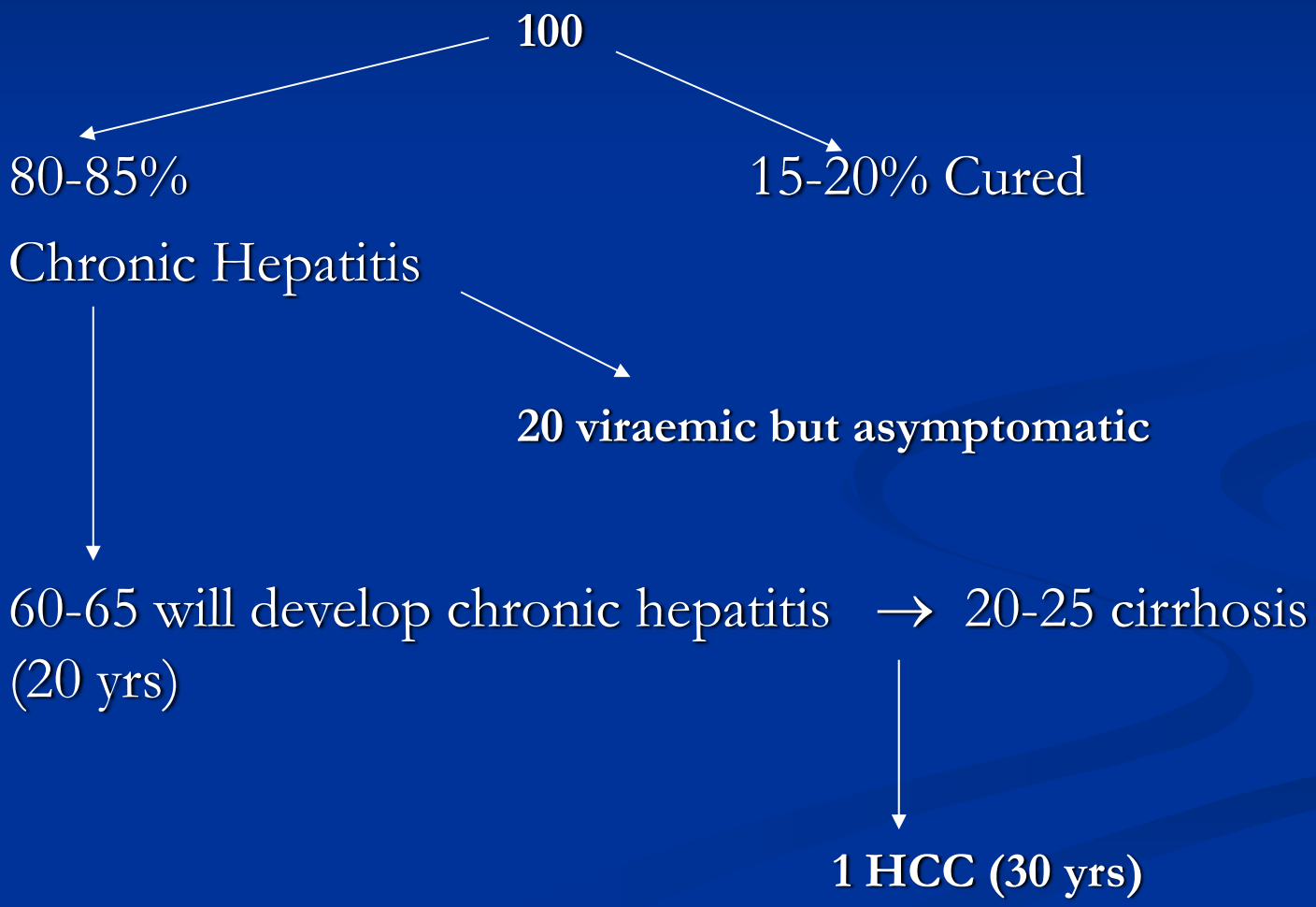
HCV Natural History



HCV Routes of Transmission

- IVDU
- Recipients of blood, blood products prior to Feb. 1990 **especially pts. receiving pooled products haemophilia, agammaglobulinaemia, receiving i.v. gammaglobulin (NZ)**
- Patients with tattoos
- Other parenteral exposure **e.g. dentists, ear piercing, other body piercing, hairdresser, barber**
- Nosocomial inf. **e.g. dialysis patients, haematology pts., endoscopy**
- Occupational exposure
- Sexual (minimal)
- Vertical (minimal)

Hepatitis C Diagnostics

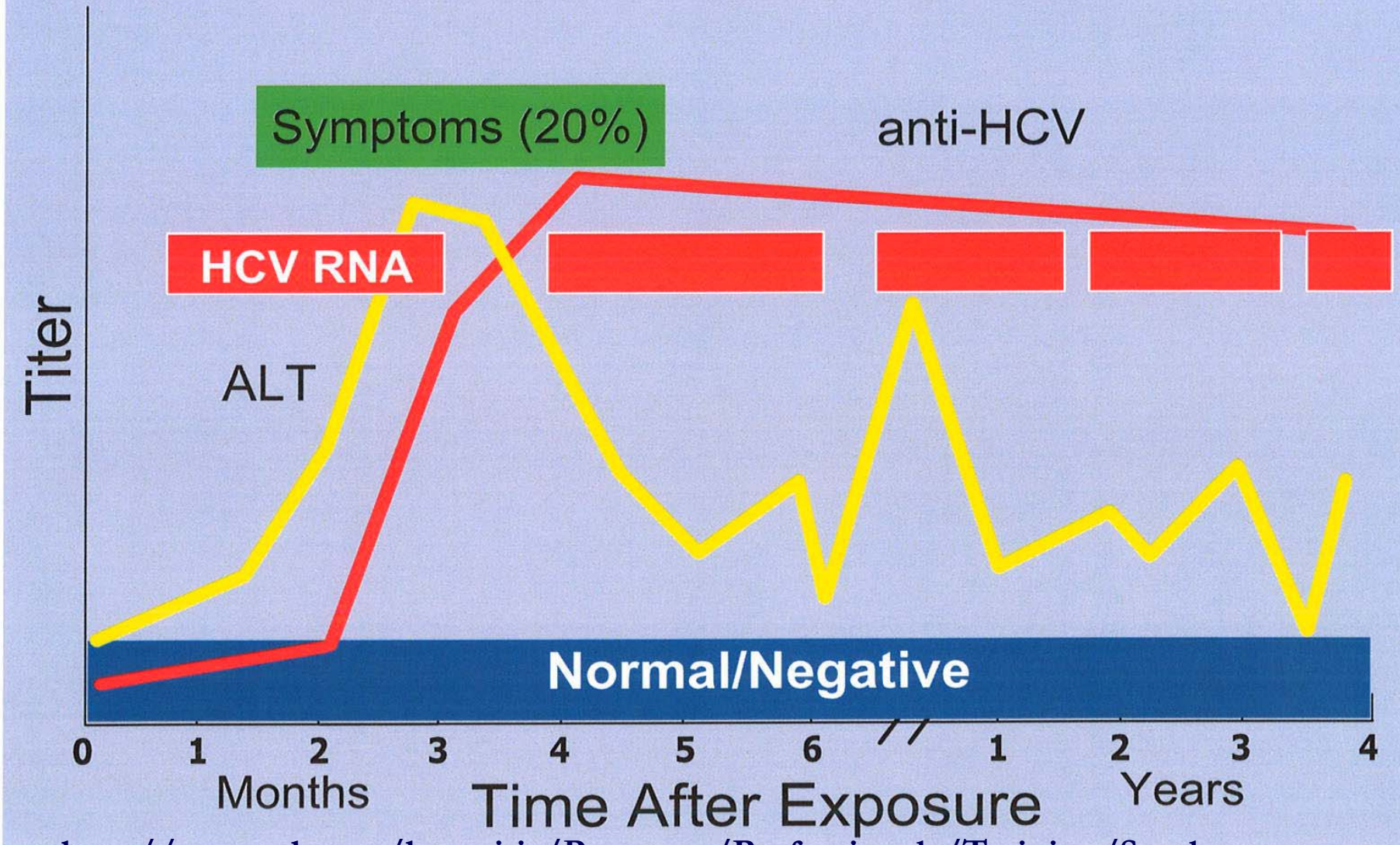
Serology markers:

- HCV combined antibody/antigen[after 2 weeks]
- HCV antibody [after 6-8 weeks]
- HCV antigen [after 1-2 weeks]

NAT:

- HCV RNA qualitative [Detected / Not detected]
- HCV RNA quantitative [15 to 6.9 x 10^7 IU/ml]
- HCV RNA genotyping [1a,1b,2a,2b,2c,3a,3k,4,5a,6a,6b]

Acute HCV Progressing to Chronic HCV Infection



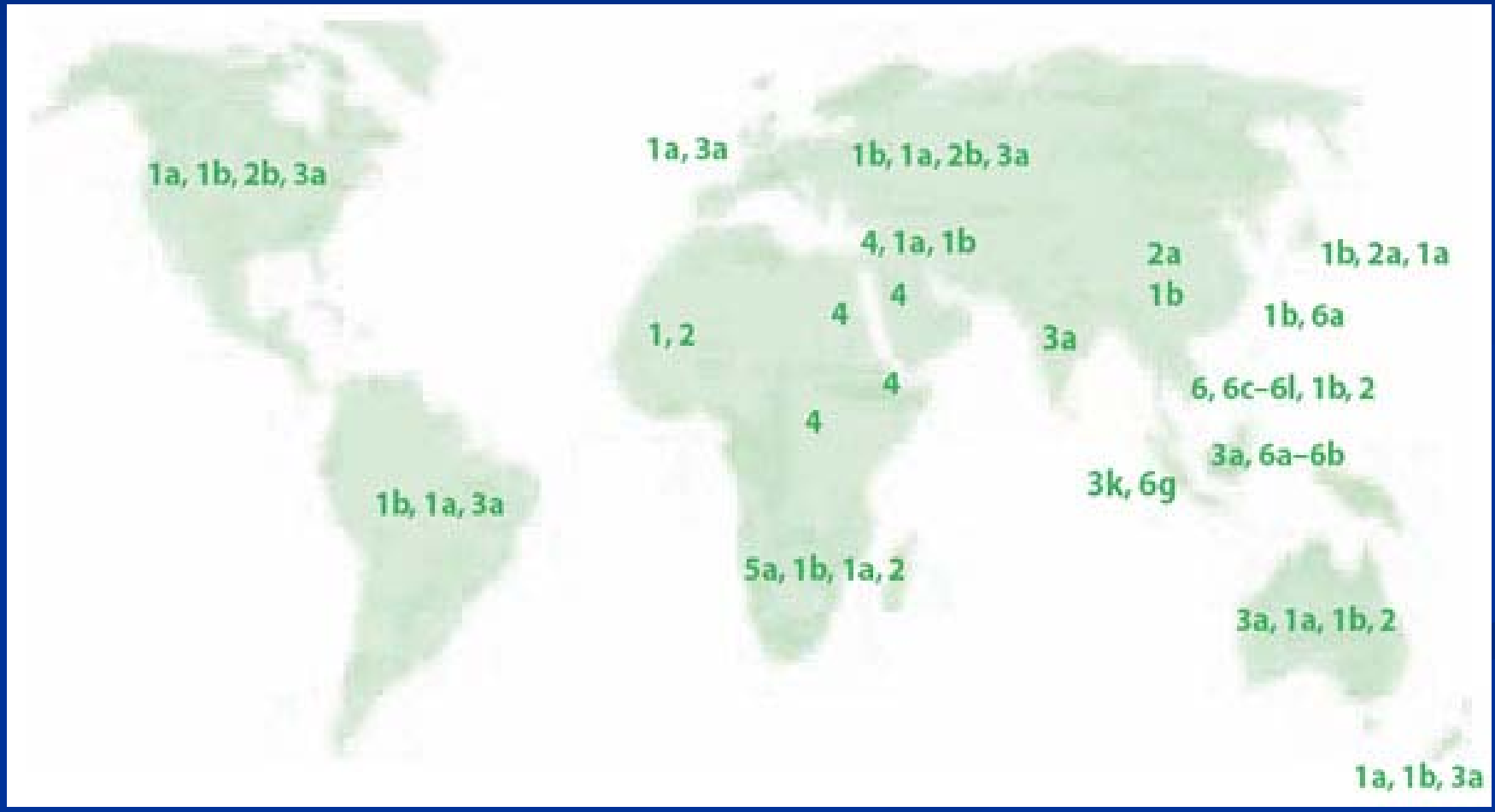
http://www.cdc.gov/hepatitis/Resouces/Professionals/Training/Serology

HCV genotypes

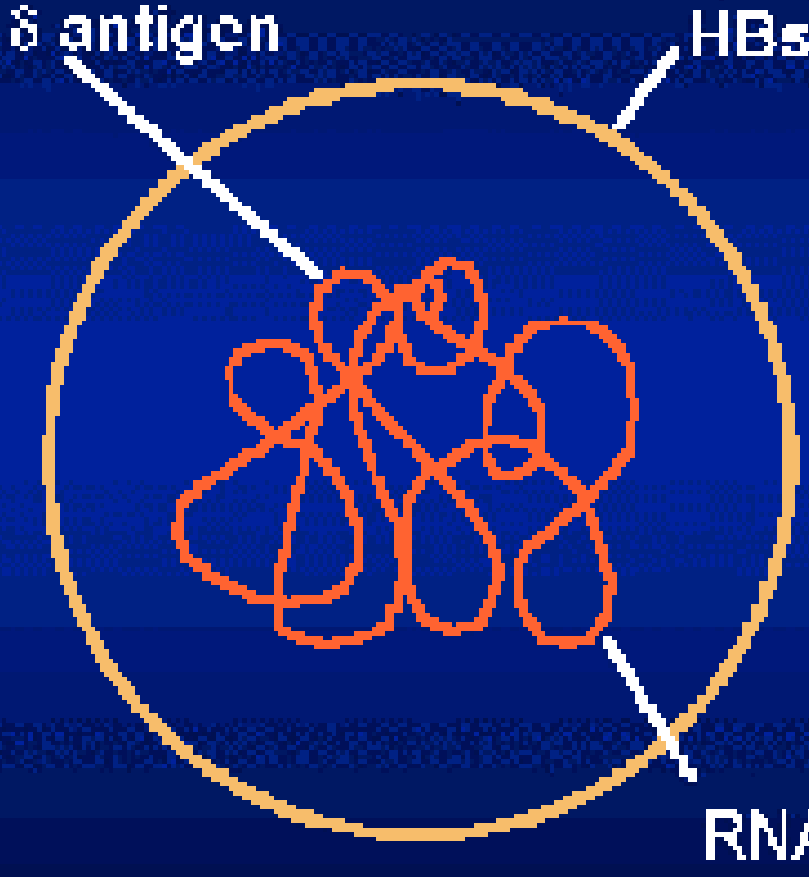
		Prevalence	% at Westmead
1a	Americas, Europe	15	52%
1b	Europe & Asia	23	
2a	Japan & China	0.2	
2b	US & northern Europe	2.9	9.3%
2c	Western & southern Europe	5.7	
3a	Worldwide	26	32%
3b	Nepal, India, Thailand		
4	Egypt, Central Africa, Middle East		5.5%
5a	South Africa		0%
6a	SE Asia (Hong Kong, Vietnam)		1.7%
7-9*	Vietnam, Thailand		0%
10a**	Indonesia		0%

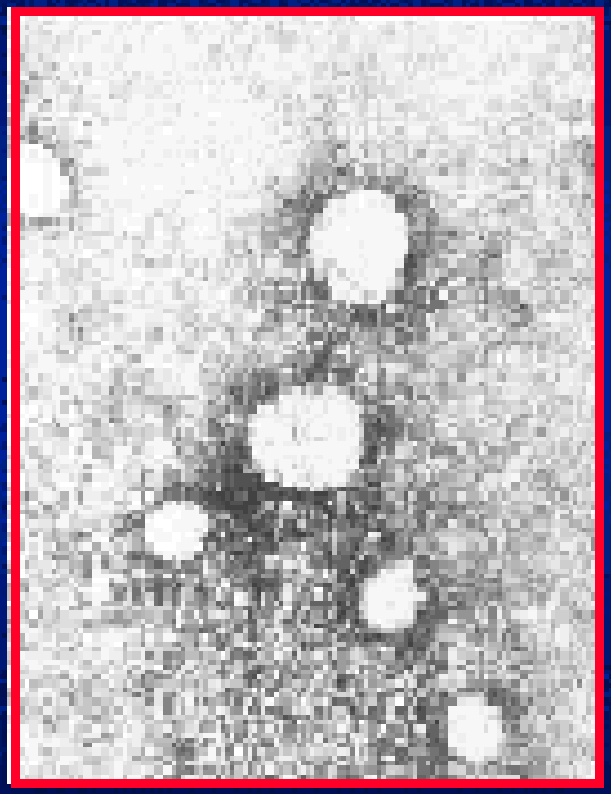
- * now classified as Clade 6
  ** now classified as 3k

HCV genotype distribution



Hepatitis D (Delta) Virus





Delta Hepatitis (HDV)

Delta antibody test is performed in the following circumstances:

The patient is HBsAg POSITIVE (or in rare cases where anti-HBc is the only positive marker)

AND one of the following

1. patient has biphasic transaminases
2. patient has fulminant hepatitis
3. patient is a Hepatitis B carrier and has a sudden flare up of symptoms
4. patient has liver failure
5. prior to interferon therapy

Delta Hepatitis (HDV)

- HDV can be transmitted via blood exchange (especially needle sharing), sexual contact and from mother-to-child.
- Majority of HDV in Australia is in IVDU but we are seeing an increasing amount in Somalian/ Sudanese refugees
- Anti-HDV antibodies are found in 20-40% of HBsAg carriers in Africa, the Middle East, and Southern Italy
- By vaccinating against HBV you prevent HDV transmission
- 0.2 per 100,000 in Australia 2011
- 36 notifications in Australia 2011 (CDI, 2012)

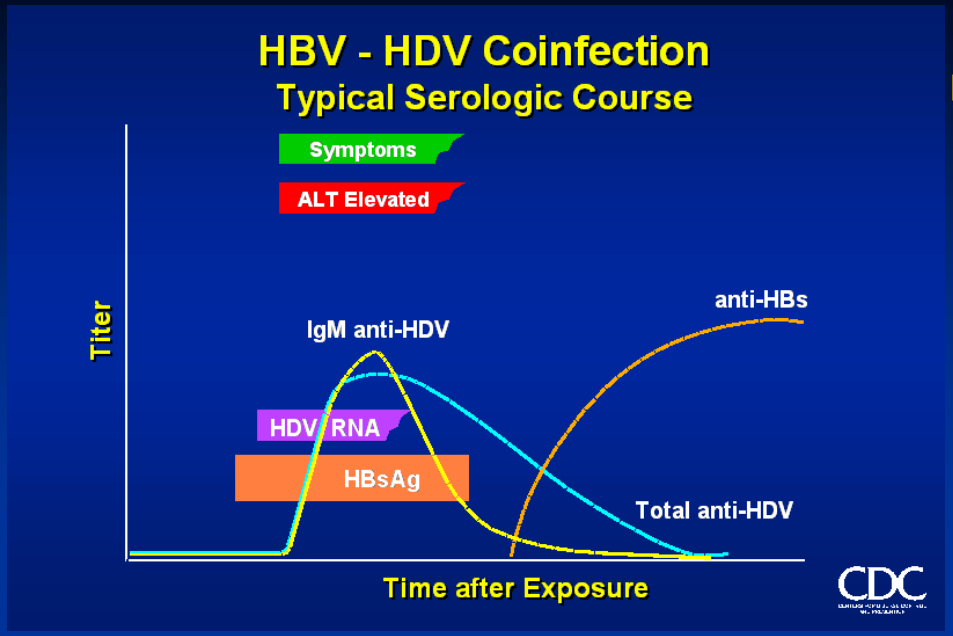
HDV Diagnostics

Serology markers:

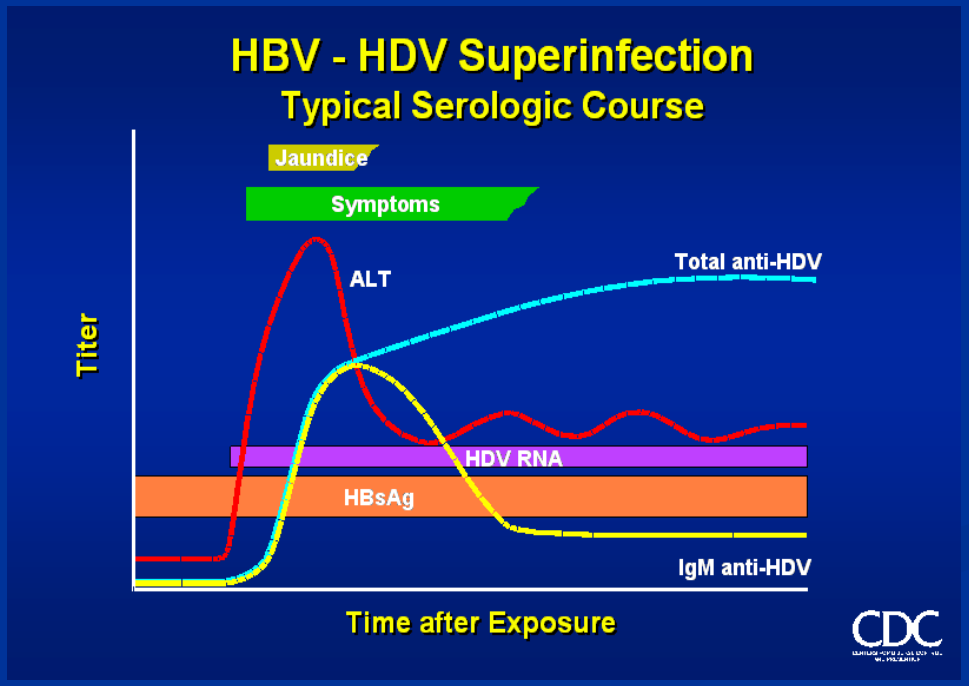
- Total anti-HDV (or HDV total Ig) competitive EIA
- HDV IgM EIA

NAT:

- HDV NAT – research only (VIDRL)



- **Co-infection**
  - More likely to have fulminant hepatitis than HBV alone but no more likely to become chronic carrier



- **Superinfection**
  - More likely to progress to cirrhosis than HBV alone

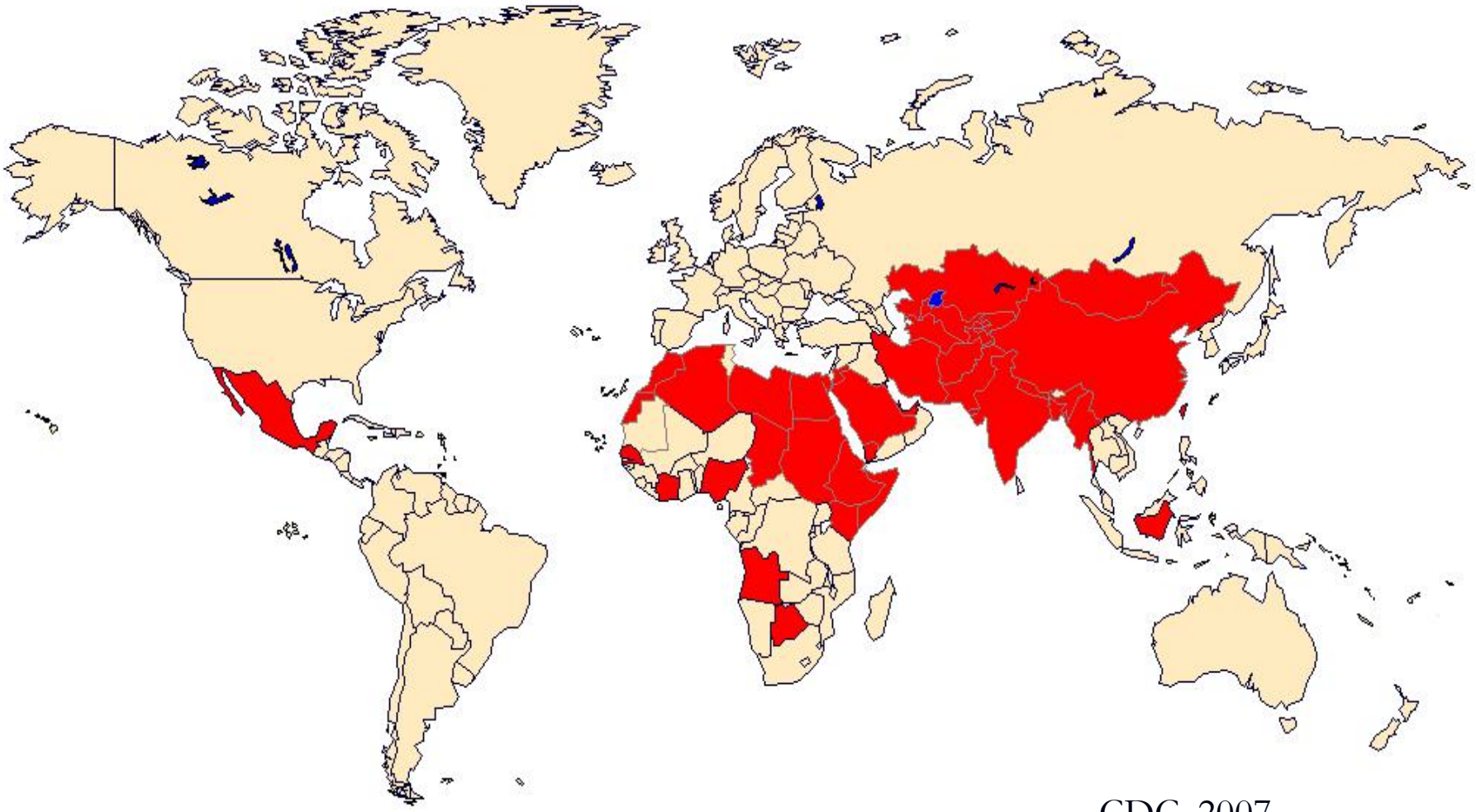
Hepatitis E Virus (HEV)

Calici-like ss RNA 27-34 nm, 3 serotypes

- Faecal/oral Tx - waterborne infection - little or no intra-familial spread
- incubation 2-9 weeks (commonly 26-42 days)
- no life-long immunity
- high mortality rate in pregnant women (especially in 3rd trimester); fulminant hepatic failure and death (of the mother) in up to 15%-20% of cases.
- 0.2 per 100,000 = 40 (20 in NSW) notifications in Australia 2011 (CDI, 2012)
- Possible zoonosis with connection to pigs and deer.

Geographic Distribution of Hepatitis E

Outbreaks or Confirmed Infection in >25% of Sporadic Non-ABC Hepatitis



CDC, 2007

HEV Diagnostics

Serology markers:

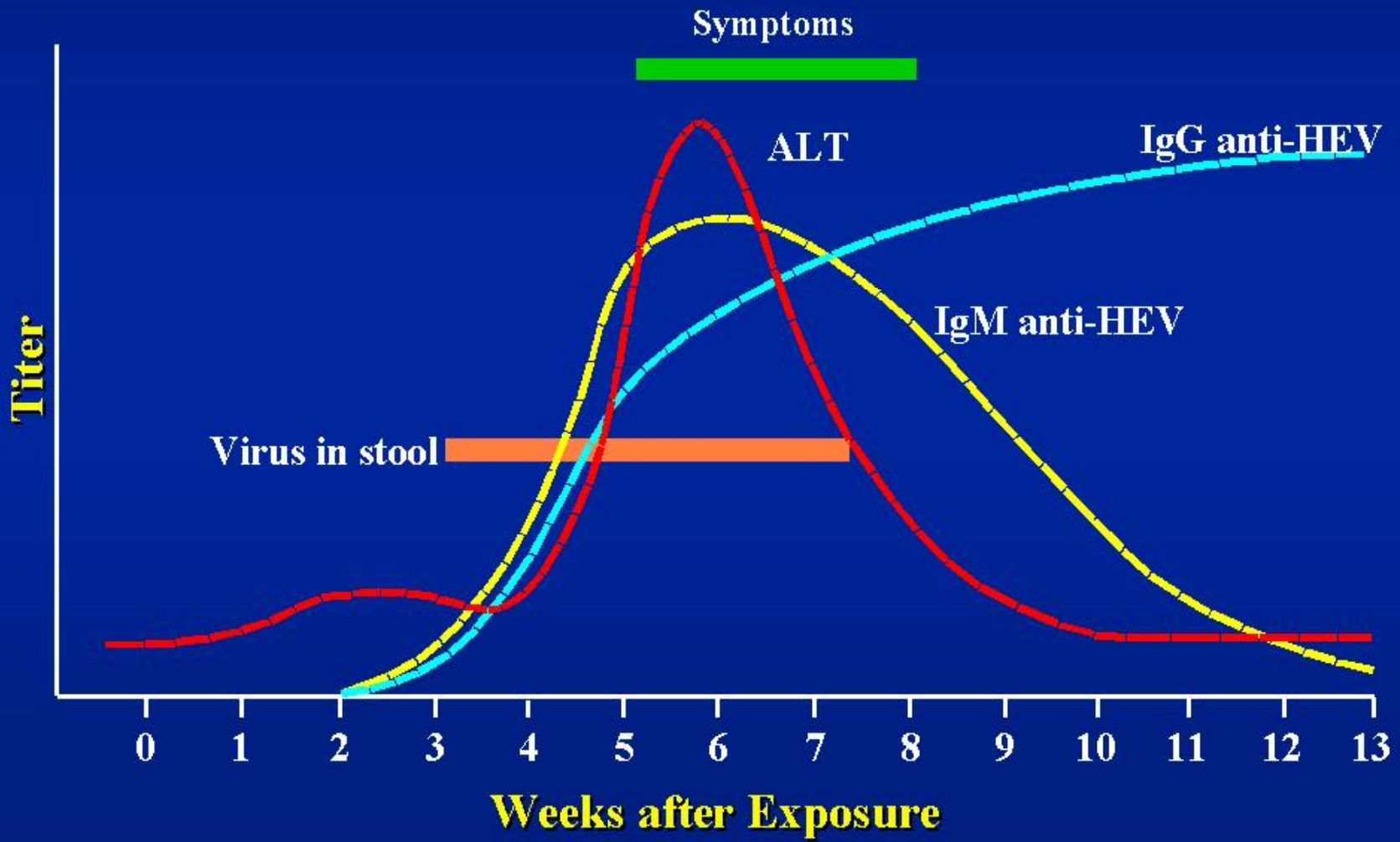
- HEV IgG EIA
- HEV IgM EIA

NAT:

- HEV RNA NAT – research only (VIDRL)
- Electron microscopy of faeces – not routine.

Hepatitis E Virus Infection

Typical Serologic Course



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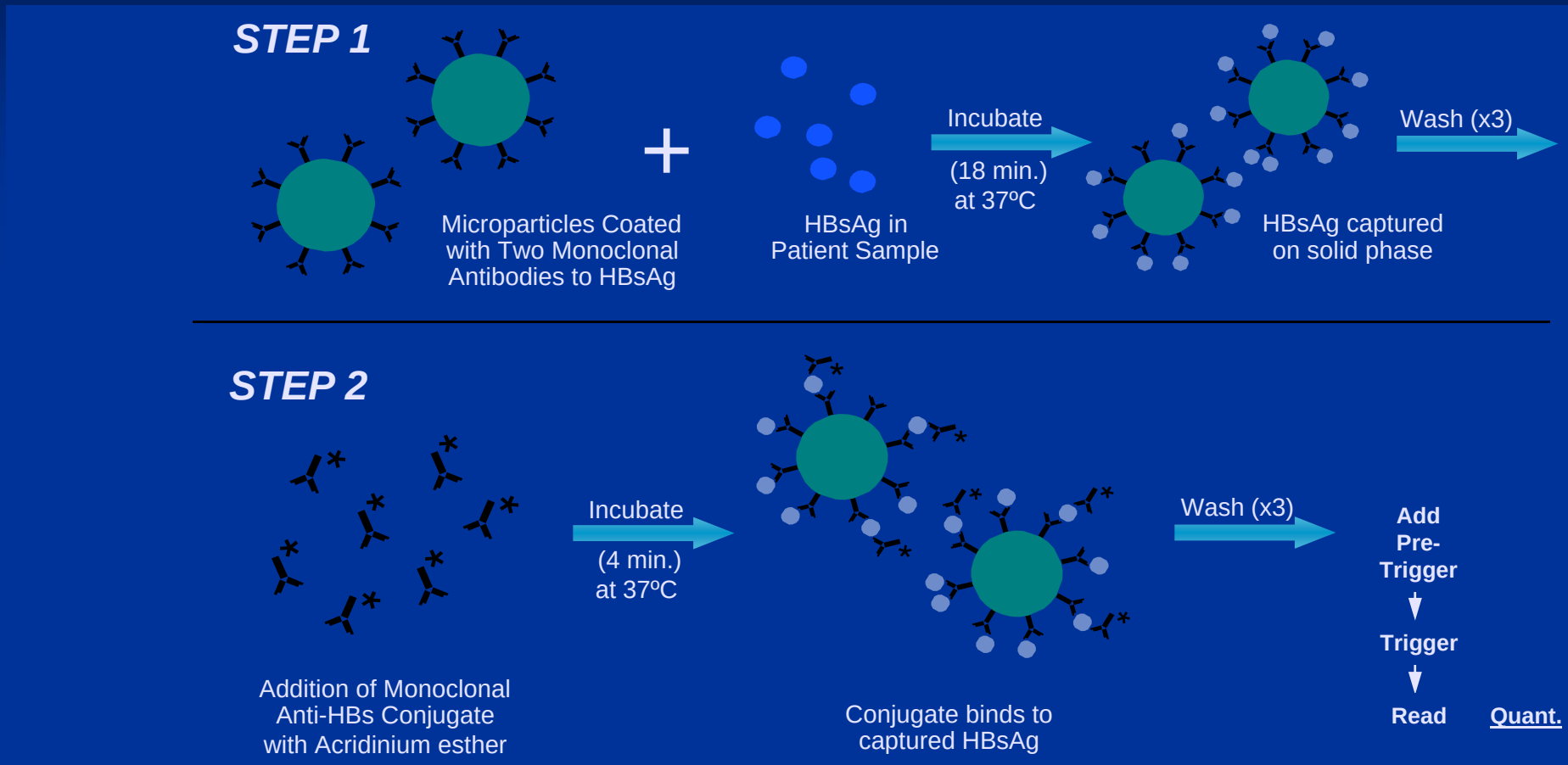
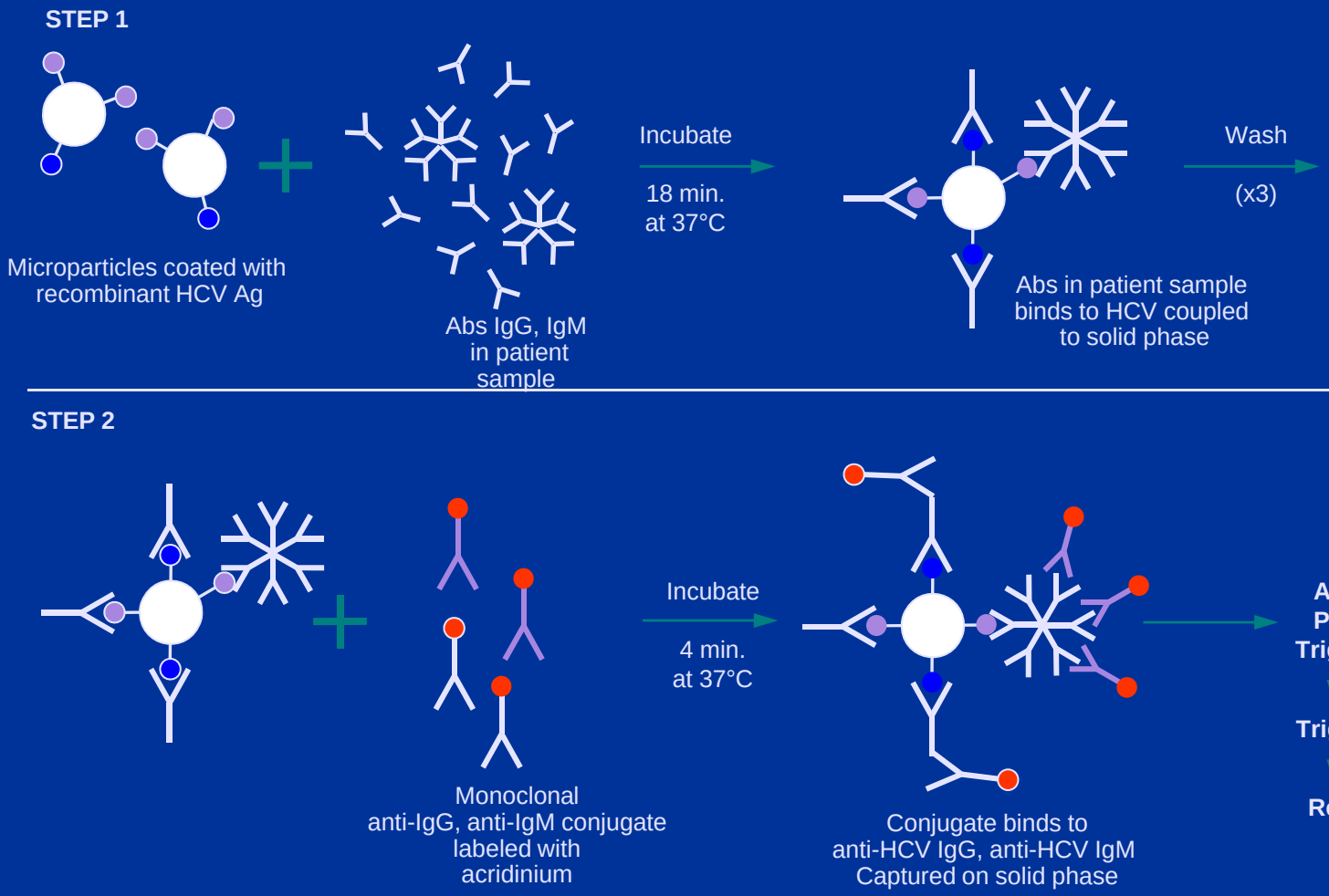
Abbott Architect i2000

HBsAg – direct sandwich



Abbott Diagnostics

Anti-HCV



Abbott Diagnostics

Roche Instrumentation

COBAS® AmpliPrep /
COBAS® TaqMan48® and
COBAS® AMPLICOR® System

COBAS® AmpliPrep /
COBAS® TaqMan® Docked System

cobas p 630,
COBAS® AmpliPrep /
COBAS® TaqMan® Docked System

Roche Instrumentation

COBAS® TaqMan® (CTM)

- Real-time amplification and detection using Taqman® technology
- On-board capacity of up to 96 samples
- Independent thermal cyclers – 4 different assays may be run simultaneously
- Ability to be docked to CAP for FULLY automated workflow from sample in to result out