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HBV and HCV Serological and Molecular diagnoses

Introduction

- Principal viral diagnostic laboratory methods:
  - Culture
  - Antigen detection
  - Nucleic acid amplification test (NAAT)
  - Serology

Introduction

- NAAT
  - Highest sensitivity
  - Greater latitude in specimen collection, window for collections of specimen, and transport
  - Throughput time: One day or less

Introduction

- NAAT
  - Viral nucleic acid fragments may persist into recovery phase of illness (limiting their use as tests of cure)
  - Mutations in DNA or RNA target sequences can reduce sensitivity

Introduction

- NAAT
  - PCR
  - Real time PCR
  - SDA (standard displacement amplification)
  - NASBA (nucleic acid sequence based amplification)
  - TMA (transcription mediated amplification)
  - LAMP(loop mediated isothermal amplification)
  - INAAT(isothermal nucleic acid amplification technology)

Introduction

Nucleic Acid Amplified Test (NAAT)		
NAAT (PCR, Isothermal Amplification (IAP, LAMP)	• Most sensitive • Define genotype sensitivities and prognosis • Moderate to very easy to perform • Low contamination risk if closed configuration used	• May be more expensive than other options • Only detects pathogens defined by primers and probes • Limited multiplex options
NAAT Panels	• Comprehensive group of targets, not restricted to viruses • Highly sensitive • Same-day results	• Expensive • Only detects pathogens defined by primers and probes • Does not differentiate between carrier vs. pathogenic viruses
Next-Generation Sequencing	• Emerging technique that can be used for viral detection and "de novo" characterization of unknown viruses • Assessment of antiviral susceptibility • Very high sensitivity compared to Sanger Sequencing • Exploration of viral outbreaks • Detection of viral variants	• Very expensive • Not currently available to most clinical virology laboratories • Bioinformatics required
Serology		
IgM	• May be present during primary infections	• Not useful for infections with short prodromal incubation times • Cross-reactivity with closely related viruses • Can also be detected during secondary infections
IgG	• Presence indicates immunity to certain viruses	• Presence of viral-specific IgG does not always indicate immunity • Detection of rising IgG titers from acute and convalescent sera can take several weeks

Introduction

- Quantitative real time PCR
  - Standard of care in assessing viral loads and monitoring response to therapy

Introduction

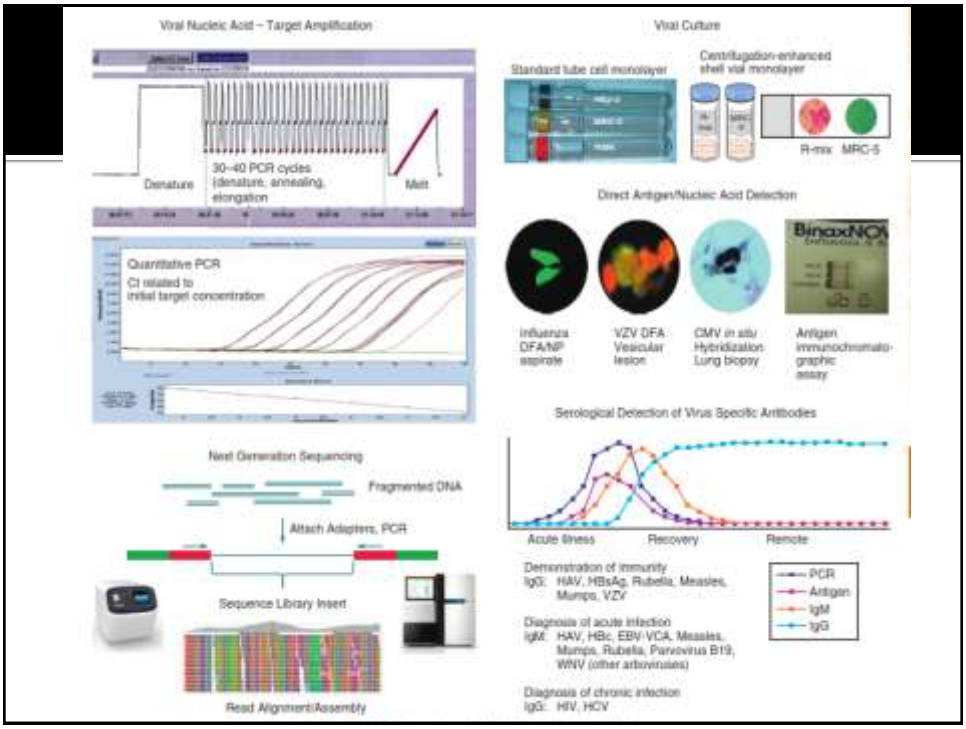
- Viral Serology
  - Serum specimen are easy to obtain, transport and store
  - Two major clinical application:
    - Diagnosis of recent infection
    - Determination of immunity

Introduction

- Current/recent infection:
  - Virus specefic IgM during the acute stage
  - Significant rise in virus specefic IgG titre between acute and convalescent sera

Introduction

- General response patterns
  - Ig M appears in first week, dissappears within 1 to 3 months
  - EIA methods are more sensitive
  - Ig G is produced 1-2 weeks following infection, peaks at 4-8 weeks and then declines…
  - Reactivation: IgM may reappear transiently, IgG rapidly increase in titre.



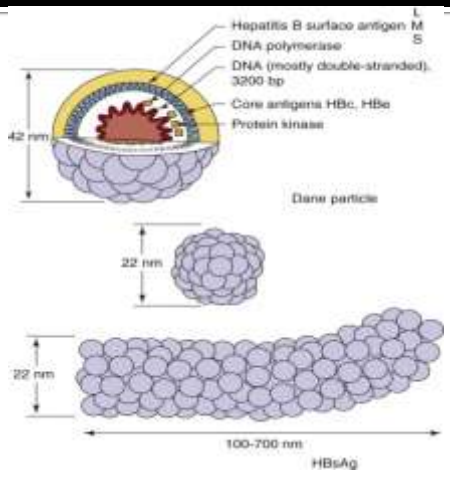
HBV

- Laboratory tests
  - assessment of liver disease
  - serologic markers of HBV replication
  - tests for coinfection with HCV, HDV, or HIV for those at risk.
  - The presence of HBsAg and HBeAg in addition to HBV DNA viral load quantitation are crucial components in the evaluation of patients with chronic HBV infection and in assessment of efficacy of anti viral treatment
  - Determination of the HBV genotype may be used to assess treatment efficacy and possible emergence of drug resistance

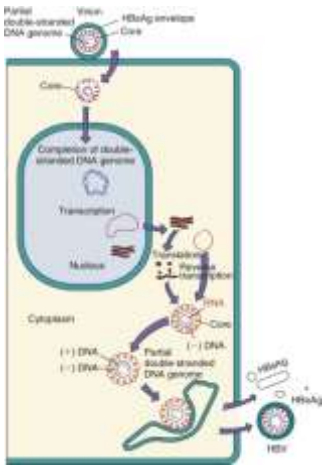
HDV?

- A defective RNA viroid that requires HBV surface antigen for full expression, replication, and transmission.
- HBV coinfection/superinfection with hepatitis D virus (HDV) is associated with a more severe course of hepatitis that frequently leads to rapid progressive fibrosis, hepatic decompensation, and the development of hepatocellular carcinoma.
- HDV is a blood-associated virus that may be transmitted from person to person in household situations in endemic areas (Middle East, South America, Central Africa, Mediterranean countries).
- HDV is rare in the United States; needle sharing among intravenous drug abusers is the principal risk factor.
- Anti-HDV IgG and IgM testing is available when HDV coinfection is suspected in HBsAg-positive individuals

HBV



HBV



HBV

- Serlogic markers:
  - HBS Ag:
    - A protein on the surface of hepatitis B virus;
    - during acute or chronic hepatitis B virus infection
    - the person is infectious
    - The body normally produces antibodies to HBsAg as part of the normal immune response to infection.
    - HBsAg is the antigen used to make hepatitis B vaccine.

HBV

Serologic markers:

- Anti-HBS:
  - recovery and immunity from hepatitis B virus infection.
  - Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B.

HBV

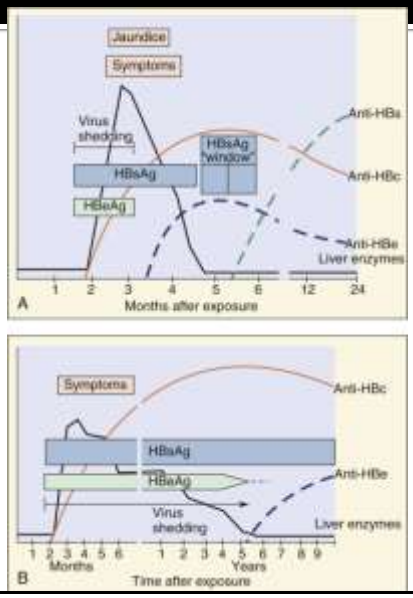
Serolgic markers:

- 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 hepatitis B virus in an undefined time frame.

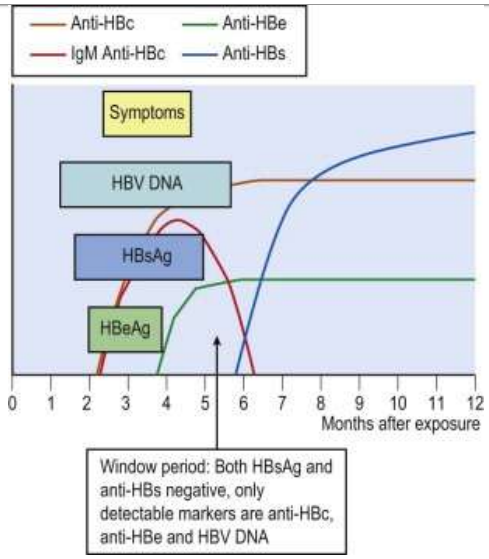
HBV

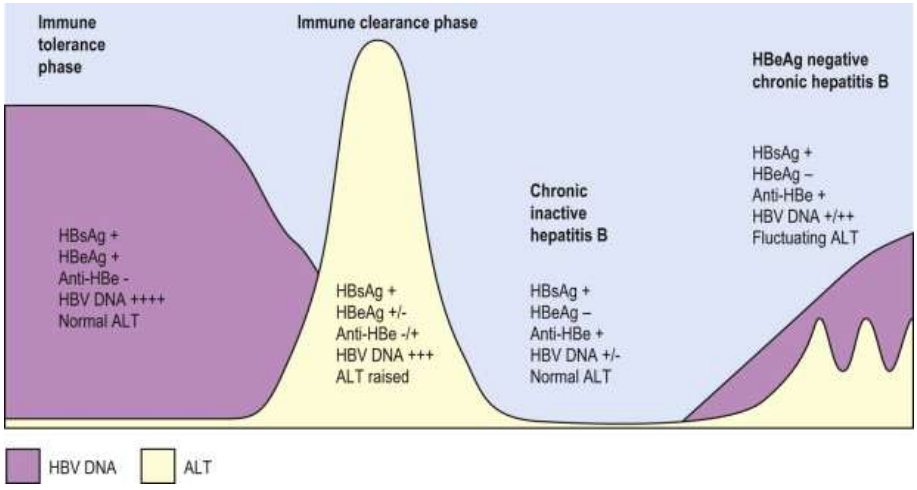
- Serologic markers:
  - IgM anti-HBc:
    - Positivity indicates recent infection with hepatitis B virus (< 6 months).
    - Its presence indicates acute infection.

HBV



HBV


Anti-HBc
Anti-HBe
IgM Anti-HBc
Anti-HBs
Symptoms
HBV DNA
HBsAg
HBeAg
0 1 2 3 4 5 6 7 8 9 10 11 12
Months after exposure
Window period: Both HBsAg and anti-HBs negative, only detectable markers are anti-HBc, anti-HBe and HBV DNA


Immune tolerance phase
Immune clearance phase
HBeAg negative chronic hepatitis B
HBsAg +
HBeAg +
Anti-HBe -
HBV DNA ++++
Normal ALT
HBsAg +
HBeAg +/-
Anti-HBe -/+
HBV DNA +++
ALT raised
Chronic inactive hepatitis B
HBsAg +
HBeAg –
Anti-HBe +
HBV DNA +/-
Normal ALT
HBsAg +
HBeAg –
Anti-HBe +
HBV DNA +/++
Fluctuating ALT
HBV DNA
ALT

?

- HBS Ag Negative
- Anti HBc Negative
- Anti HBS Negative

- Susceptible

?

- HBS Ag Negative
- Anti HBc Positive
- Anti HBS Positive

- Immune due to natural infection

?

- HBS Ag Negative
- Anti HBc Negative
- Anti HBS Poistive

- Immune due to vaccination

?

- HBS Ag Positive
- Anti HBc Positive
- Ig M anti HBc Positive
- Anti HBS Negative

- Acutely infected

?

- HBS Ag Positive
- Anti HBc Positive
- Ig M anti HBc Negative
- Anti HBS Negative

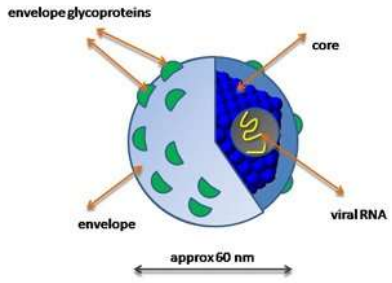
- Chronically infected

?

- HBS Ag Negative
- Anti HBc Positive
- Anti HBS Negative

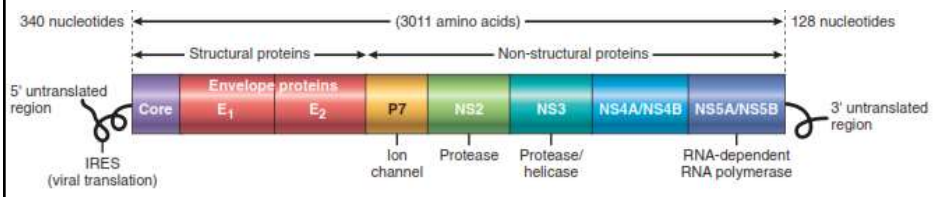
- 1. Resolved infection (most common)
- 2. False-positive anti-HBc, thus susceptible
- 3. "Low level" chronic infection
- 4. Resolving acute infection

HCV

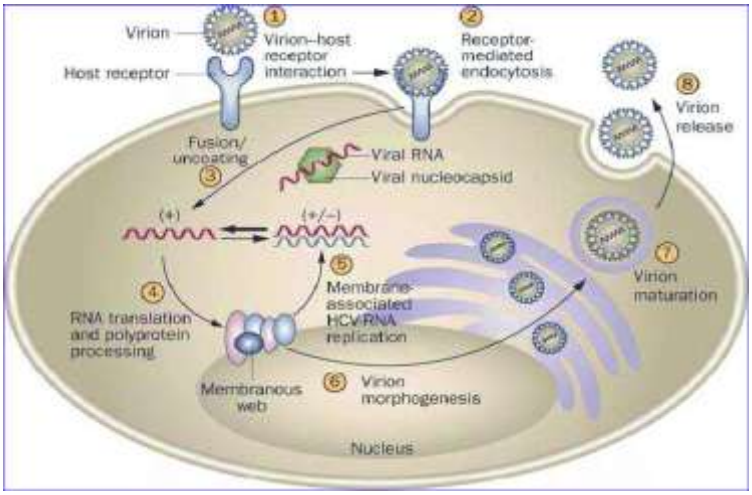


Structure of Hepatitis C Virus

HCV



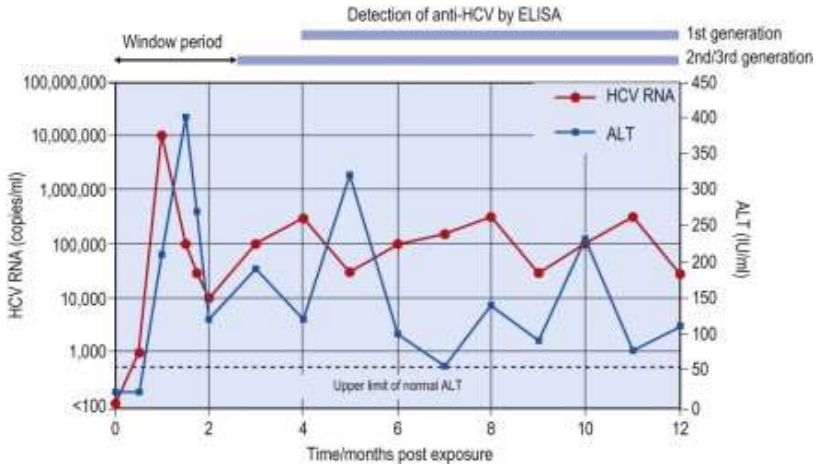
HCV



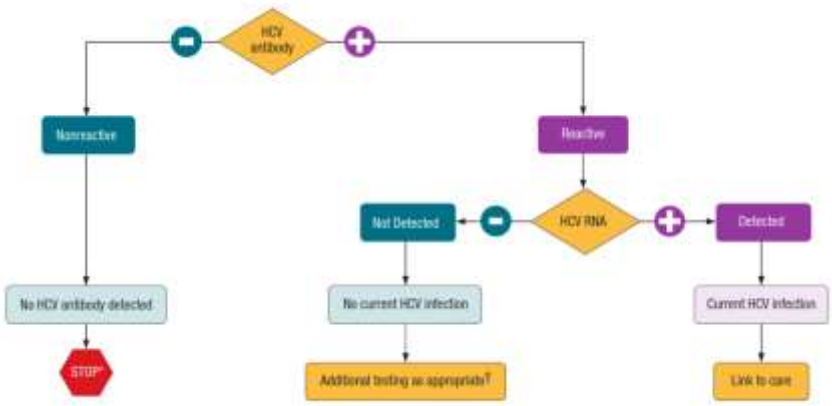
HCV

- Available third-generation serology assays : high sensitivity and specificity
- POC tests can increase HCV screening opportunities.
- Presence of HCV-RNA reflects viral replication; sensitive molecular assays are used to diagnose active HCV infection in patients with a positive antibody test.
- The HCV-RNA can be detected before specific antibodies become detectable (within 1 to 3 weeks post exposure).
- The diagnosis of a chronic HCV infection: the presence of both HCV antibodies and HCV-RNA over a period of 6 months
- Quantitative HCV RNA tests are used to follow antiviral therapy in order to minimize side-effects, monitor emergence of resistance, and minimize costs.

HCV


Detection of anti-HCV by ELISA
Window period
1st generation
2nd/3rd generation
HCV RNA
ALT
HCV RNA (copies/ml)
ALT (IU/ml)
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
<100
450
400
350
300
250
200
150
100
50
0
Upper limit of normal ALT
0
2
4
6
8
10
12
Time/months post exposure

HCV


HCV antibody
Nonreactive
Reactive
Not Detected
HCV RNA
Detected
No HCV antibody detected
STOP
No current HCV infection
Additional testing as appropriate†
Current HCV infection
Link to care

HCV

- HCV genotyping is helpful for selecting appropriate treatment.

Serologic and Virologic Tests for Hepatitis Viruses

	IgM Anti-HAV	IgG Anti-HAV	HBsAg	Anti-HBs	IgM Anti-HBVc	HBeAg	Anti-HBe	HBV DNA	Anti-HCV Screen	HCV RNA	Anti-HDV	Anti-HEV
HAV:												
Acute	+	+										
Remote	+	+										
HBV:												
Early			+	−	+	+	−	+				
Window			−	−	+	−	−	+				
Resolving			−/+	+	−	−/+	+	−/+				
Chronic			+	−/+	−	+	+/−	+				
TX monitoring								+				
Remote			−	+	−	−/+	+/−	−/+				
HCV:												
Screen									+	+		
Acute									+/−	+		
Chronic									+	+		
TX monitoring										+		
Remote									+	−/+		
HDV:												
Superinfection			+	−	+/−	+	−/+	+/−			+	
HEV												+

Data from CDC: Surveillance for acute hepatitis, United States, 2007, *MMWR* 58(SS-3), 2009.
HAV, hepatitis A virus; HBc, hepatitis C; HBeAg, hepatitis E antigen; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; IgM, immunoglobulin M; +, positive; −, negative.