The ABC's (and D & E's) of the Viral Hepatitides
Part 2

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Objectives 2

1. Explain the various modes of transmission of each virus discussed.
2. Describe the mortality and morbidity associated with the viral hepatitides.
3. Give a basic description of the makeup and structure of each virus.
4. Be able to select the correct lab diagnostics for each virus discussed.
5. Discuss current treatments and efficacy.

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Disclosures 3

- I have nothing relevant to this topic to disclose.
- All tables and graphs have been drawn from public domain websites.

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DIAGNOSTIC TESTS

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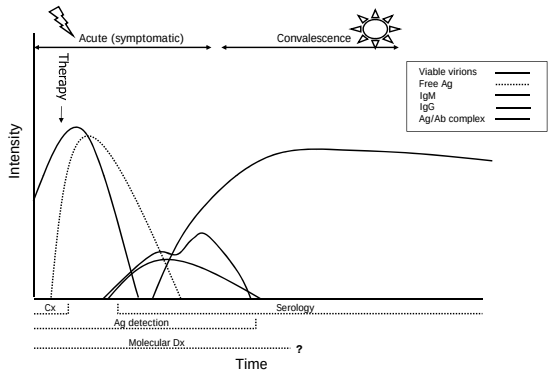
Review of Acute Antigen (Ag)/Antibody (Ab) Responses 5



Ag/Ab Relationships in Chronic Infection 6

- These patterns apply to acute viral hepatitis (VH) infections, but change in chronic VH:
  - Viral antigen and virions (i.e. viral particles) usually remain detectable in blood and other fluids.
  - IgG class Abs directed against the virus do not appear as expected.
- **These differences present many diagnostic opportunities.**

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Blood Studies for Suspected VH 7

- Alanine and aspartate amino transferases
- Total and Direct bilirubin
- Albumin and total protein
- CBC
- aPTT, PT/INR
- Alpha fetoprotein
- HAV,HBV,HCV Ab/Ags (more in a moment…)

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HAV & HEV 8

- Diagnosis of HAV and HEV is less complex than the others, due to no chronic phase.
- The primary markers measured are virus-specific antibodies.
- However, the lack of an IVD assay for HEV antibody hampers testing in the USA.

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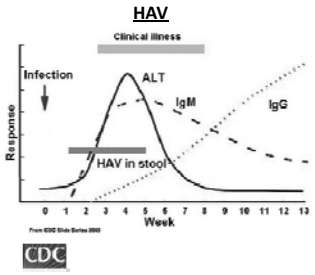
HAV & HEV Abs 9



HEV similar, EXCEPT that IgG anti-HEV falls off rapidly after 6-12 months, and may not confer immunity for life.

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HAV Diagnosis 10

- IgM detection is required for diagnosis; many commercial assays are available.
- Total/IgG anti-HAV assays assess immune status only, since IgG anti-HAV persists for decades.
  - While a positive result indicates immunity, commercial assays don't reliably detect vaccine-induced immunity.
- Antigen detection and nucleic acid amplification tests (NAAT) are not commercially available, and HAV is not cultivable.

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HEV Diagnosis 11

- IVD assays not available. At least one commercial reference lab and the CDC offers anti-HEV antibody testing: IgM detection is the key to diagnosis.
- CDC also performs RT-PCR and genotyping.
- Virus is not cultivable.
- Best approach? Contact the WI DPH or WSLH if HEV is suspected.

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HBV/HDV Diagnosis 12

- The diagnosis of HBV & HDV is more complex due to acute and chronic phases of infection.
  - Diagnosis initially made by detection of viral Ag & Ab.
  - Staging and monitoring therapy also uses quantitative nucleic acid amplification testing (NAAT; i.e. 'viral load').
- In contrast to HCV, HBV genotyping is not routinely done to begin therapy.

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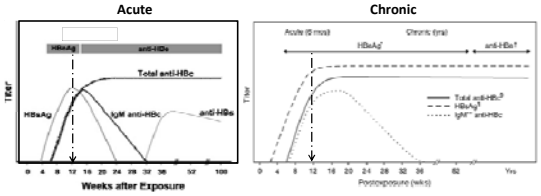
HBV Ag/Ab Detection: The Players 13

Ag/Ab	Abbreviation
Hepatitis B surface antigen	HBsAg
Hepatitis B core antigen	Not detectable
Hepatitis B e antigen	HBeAg
Anti-HBsAg antibody (Total)	Anti-HBs
Anti-HBcAg antibody (IgM &Total)	Anti-HBc
Anti-HBeAg antibody (Total)	Anti-HBe

Serology of HBV Acute and Chronic Infection 14



http://publichealthlab.ca/wp-content/uploads/2012/10/GTS-Anti-HBs-Chart-1.jpg
http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/viral-hepatitis-figure4.html
(accessed 02/27/2013)

HBV Serological Tests 15

Primary Markers

❖HBsAg Indicates ongoing infection, acute or chronic.

OR

❖Anti-HBs Indicates resolved infection. Alone, a marker of vaccination (≥ 10mIU/mL considered protective).

❖IgM anti-HBc Indicates acute infection.

REPLACED BY

❖Total anti-HBc Denotes evolved infection; does not differentiate chronic vs. resolved infection.

Secondary Markers

❖HBeAg Indicates rapid viral replication, high infectivity.

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❖Anti-Hbe Seen in resolved acute & inactive chronic infection.

Chronic HBV Markers: A Closer Look 16

Phase (Stage)	Positive Lab Findings
Immune Tolerance	HBsAg, Total anti-HBc, HBeAg, ⇑ HBV DNA
Immune Clearance	HBsAg, Total anti-HBc, HBeAg, ⇑ HBV DNA, ⇑ transaminases, abnormal liver histology
Inactive CS	HBsAg, Total anti-HBc, anti-HBe, (Transaminases may flare)
(Occult hepatitis B)	± findings for Total anti-HBc, ⇑ transaminases, abnormal liver histology. HBV DNA detectable only by sensitive research assays
Isolated Anti-HBc – Positive	Negative for both HBsAg & anti-HBs is not unusual (0.5-20%); meaning unclear. Needs follow-up; may be infectious.

CS, HBsAg Carrier State; ⇑ HBV DNA, ≥ 20,000IU/mL

Horvat, RT and GE Tegtmeier 2011. Hepatitis B and D Viruses, p 1670-71. *In* J. Versalovic *et al* (eds), Manual of Clinical Microbiology, 10th ed. ASM Press, Washington DC & A Valsamakis 2007 Clin Microbiol Rev 20:426

HBV NAAT 17

❖ Quantitative NAAT

- ❖Several commercially available assays
- ❖A WHO HBV international standard exists. However, quantitation still varies among assays.
- ❖Best practice is to consistently use the same assay.
  - ❖If changing a viral load assay, perform and report both assays (only charging for one) for 3-6mos to 'reset' all patient's viral loads as a service to your providers.

HBV NAAT 18

Some HBV Viral Load Interpretive Guidelines

- ❖>1log$_{10}$ (i.e. >10-fold) **reduction** is predictive of therapeutic success, while >1log$_{10}$ **increase** during treatment suggests development of antiviral resistance.
- ❖Increasing levels correlate histologically with cirrhosis.
- ❖Sustained suppression of viral DNA is the best measure of long term therapeutic success.

Horvat, RT and GE Tegtmeier 2011. Hepatitis B and D Viruses, p 1671. *In* J. Versalovic *et al* (eds), Manual of Clinical Microbiology, 10th ed. ASM Press, Washington DC

HDV Diagnosis 19

- HBV co-infection required-also test for HBV.
- Anti-HDV Ab detection
  - Screen with Total anti-HDV
  - Reflex to IgM anti-HDV to assess for acute state
- Viral load testing for disease activity
- But, there are no FDA cleared IVD assays.

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HCV Diagnosis-Some Differences… 20

- Acute infection usually asymptomatic, so most cases appear after years of silent chronic infection.
- Unlike HBV, HCV genotype or level of viremia do not correlate with severity or likelihood of chronic disease progression. **However** genotype and viral loads are critical in making therapeutic decisions.
- Only Total anti-HCV Ab is measured.
- Recombinant immunoblot assay (RIBA) still used.
- But like HBV, HCV diagnostics are not simple.

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HCV Diagnosis 21

- Gen 3.0 HCV Ab assays predominate in the USA, but one 2.0 assay still marketed.
  - 3.0 has better sensitivity and equal specificity, shortening seroconversion window by ~2 weeks.
- Several 3.0 assays may report positive results when S/CO ratio is above a threshold set by manufacturer.
  - If below threshold, **OR** any positive value in the other assays, requires confirmation.

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HCV NAAT 22

- Both qualitative and quantitative HCV RNA assays available as FDA-IVD, ASR or RUO.
- Qualitative assays traditionally considered most sensitive, but several current quantitative assays are almost as sensitive.
- HCV NAAT can assist in diagnosing acute infection before seroconversion (e.g. after exposure), but is most often used in Tx of chronic HCV.

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HCV NAAT 23

- HCV NAAT in chronic infection
  - Plays an important part in the confirmation of anti-HCV Ab screen results.
  - HCV viral loads, along with HCV genotype, are critical in monitoring and tailoring therapy.
- Most labs now only use highly sensitive quantitative NAATs in all phases of diagnosis and treatment.

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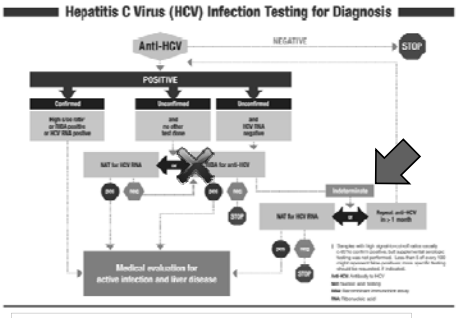
HCV Diagnosis 24



http://www.cdc.gov/hepatitis/hcv/labtesting.htm (accessed 03/04/12)

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PREVENTION & TREATMENT

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HV Prevention 26

- Basic Public Health Efforts
  - Clean water
  - Sanitation systems
  - Screening of blood/blood products supply
  - Promotion of safe sex practices
  - IVDU → Reduce/educate/needle exchange
  - Vaccination

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VH Vaccines 27

- HAV Yes.
  - Used in low-moderate incidence countries where symptomatic disease in adults occurs.
  - Not necessary in highly endemic countries where all are asymptomatically infected as infants, and where there is no chronic stage.
- HBV Yes. Used worldwide. (179 countries in 2010)
  - Universally attractive since it prevents advancement to chronicity, cirrhosis and HCC.
- HCV Many attempts, none successful.

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VH Vaccines 28

- HEV In development in China.
  - Desirable in high- endemicity countries where pregnancy-related fulminant hepatitis occurs.
- HDV Not needed - prevent HBV with vaccination.

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VH Treatment 29

- Acute HAV, HEV
  - No effective antiviral Tx
  - Supportive care as needed
- HDV
  - No effective antiviral Tx
  - Target efforts at the concomitant HBV infection

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Chronic HBV Treatment 30

- Due to expense and toxicities of Tx, not all chronic HBV cases are treated.
  - Tx reserved for those with high viral loads AND evidence of hepatic disease e.g. elevated transaminases (plus individual factors e.g. age, co-morbidities).
- Tx is of long duration: $\geq$ 48 weeks.
- Emergence of resistance is not uncommon.

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Chronic HBV Treatment **31**

- Two major drug classes
  - Pegylated interferon-alpha (PEG-INFα)
    - Stimulates immune response
    - No resistance noted
    - Cannot be given with severe liver disease
  - Nucleoside/nucleotide analogs
    - Similar to anti-HIV Rx; targets viral replication
    - Varying rates of resistance occur
    - Can be given when liver disease is severe
- May be combined, but which cocktail is best?

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Chronic HBV Treatment **32**

- Viral load is monitored during Tx
  - $\geq$ 1 $\log_{10}$ (> 10X) rise suggests a new resistance.
- Viral genotyping is of little benefit, but genotypic testing for antiviral resistance may be of value.
  - Lab-developed tests only-buyer beware.
  - Results are not straightforward.

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Chronic HBV Treatment **33**

- Determining treatment 'success' is difficult
  - Sustained suppression of viral replication?
  - Normal transaminases?
  - Anti-HBe seroconversion?
  - Anti-HBs seroconversion (rare i.e. HBsAg persists)?
  - Quantitation of HBsAg?
- Goal is to suppress virus enough to prevent sequalae, not to completely eradicate.

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Chronic HCV Treatment **34**

- Treatment is challenging due to the high mutability of HCV, leading to resistant strains in the background in an untreated patient.
  - Once Tx begins the resistant strain predominates.
- Combination therapy helps fight emergence of resistant strains.

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Chronic HCV Treatment **35**

- Principles of HCV treatment similar to that of chronic HBV, but are more developed.
- Standard treatment had been a cocktail of PEG-INFα and the antiviral ribavirin until the FDA approved boceprevir and telaprevir in 2011.
- boceprevir and telaprevir
  - Inhibits viral replication.
  - For use against chronic HCV due to genotype 1, the most common genotype in the USA, and most difficult to treat.
  - Used in combination with PEG-INFα and ribavirin.

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Chronic HCV Treatment - Definitions **36**

Treatment Response/Milestone	Definition
Rapid virologic response (RVR)	No detectable HCV RNA* at Tx wk 4
Early virologic response (EVR)	$\geq$ 2$\log_{10}$ drop in HCV RNA at Tx wk 12
End of treatment (EOT)	End of customized Tx period
End of follow-up (EFU)	Last time point for HCV RNA testing in VR+ patients, typically 24 wk EOT
Virologic response (VR)	No detectable HCV RNA* at EOT
Sustained VR (VR)	No detectable HCV RNA* at EFU

*HCV RNA limit of detection $\leq$50IU/mL; all HCV RNA measurements in plasma/serum

Forman, M and A Vasamakis 2011. Hepatitis C Virus, p 1439. *In* J. Versalovic *et al* (eds), Manual of Clinical Microbiology, 10th ed. ASM Press, Washington DC

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Chronic HCV Treatment **37**

- Length of treatment ranges between 24-72 wks.
- Is customized based on:
  - Baseline HCV RNA load (< 400,000 commonly)
  - Genotype (Gt1 requiring longer Tx)
  - Milestones (RVR and/or EVR shortens Tx period)
  - Co-morbidities.

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Chronic HCV Treatment **38**

- As with HBV, Tx is generally reserved for those with active disease.
- SVR ranges from ~40-80%.
  - Gt1, high initial viral load have lowest SVR rate.
- Relapse occurs; patients often respond to a second course of Tx.

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Summary **39**

1. The clinical laboratory plays a major part in both diagnosis and monitoring of treatment of HVs.
2. HAV and HBV are preventable with vaccination, while HCV is not.
3. While not fully curable, chronic HBV and HCV can now be successfully managed in the high resource setting of many developed countries.
4. In low-resource countries, HBV and in the future HEV vaccination offer the most effective approach.

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QUESTIONS?

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