Laboratory Diagnostics of Viral Hepatitis B: Testing Challenges and Case Scenarios

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Perinatal Hepatitis B Prevention Program Summit

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Content

- Hepatitis B viral structure and role of various components in the diagnostics of HBV infection.
- HBV genotype distribution
- Challenges in the diagnostic of HBV infection
- Using testing algorithms to facilitate accurate HBV diagnostics
- Diagnostic quandaries and potential resolution

Viral Hepatitis

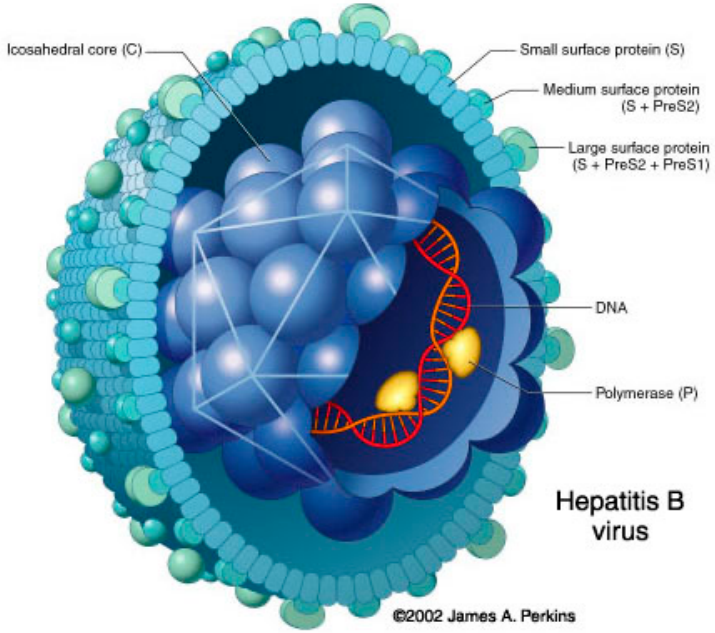
One Clinical Case Definition:

- **Acute illness**
- **Discrete onset of symptoms and jaundice**
- **Or elevated serum aminotransferases**

Five Causative Agents:

- **Hepatitis Viruses A – E**
- **13 serodiagnostic markers**
  - HAV, HBV and HCV tests approved by FDA
- **At least one molecular test for each virus**
  - FDA-approved for HBV, HCV NAT
- **No FDA-approved Tests for HDV and HEV diagnostic**
  - CLIA validated at CDC

Hepatitis B Virus (HBV)

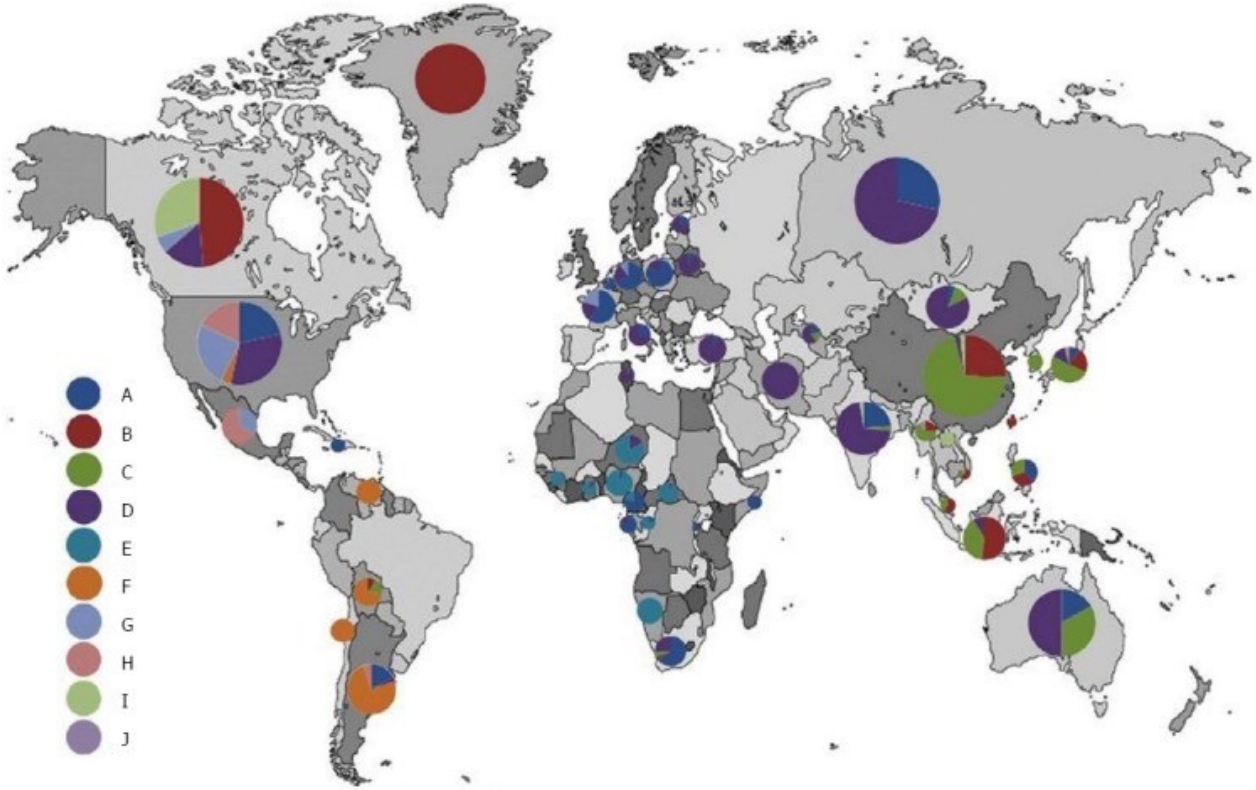


- **Hepadnaviridae family**
- **Virion (40-42nm)**
  - Surface antigen (HBsAg) part of the envelope
- **Core particle (28nm)**
  - Core antigen (HBcAg)
  - Extracellular form (HBeAg)
- **Partially double-stranded DNA (3.2 kb)**
- **Nine serologic subtypes of HBsAg:**
  - adrq+, adrq–, ayr, ayw1, ayw2, ayw3, ayw4, adw2, adw4

HBV Genotype Distribution

10 HBV genotypes (A-J)

- North America: A, D, G, F, H, I
- South America: F
- Mexico & Central America: H
- Greenland: B
- NW Europe, Central Africa: A, D
- Eastern Europe, Mediterranean region, Middle East, South Asia: A, D
- West Africa: E
- Asia: B, C
- Japan: J
- Vietnam, Laos: I
- Australia: A, D, C



Routes of Transmission

- **Percutaneous, mucosal, or nonintact skin exposure to infectious blood, semen, and other body fluids.**
  - HBV is concentrated most highly in blood, and percutaneous exposure is an efficient mode of transmission.
  - Infected hepatocytes secrete very high levels of virus particles resulting in viremia of up to 10^8-10^{10} particles per ml
  - HBV is most infective of all blood borne viruses- 100x more than HIV
- **HBV is transmitted primarily through:**
  - Birth to an infected mother
  - Sexual contact with an infected person
  - Sharing contaminated needles, syringes, or other injection-drug equipment
- **Less commonly through:**
  - Needle-sticks or other sharp instrument injuries
  - Organ transplantation and dialysis
  - Interpersonal contact through sharing items such as razors or toothbrushes or contact with open sores of an infected person
  - However, with successful prevention of perinatal transmission, these routes may take centerstage.

Newly Acquired HBV Infection

Incubation Period: From exposure to onset of jaundice From exposure to onset of high ALT	 Mean : 90 days (60 – 150 days) Mean : 60 days (40 - 90 days)
Symptomatic: anorexia, malaise, nausea, vomiting, abdominal pain, and jaundice	Age up to 12 months: <1% 1-5 years old: 5 - 15% >5 years old: 30 - 50%
Fulminant liver failure	1-2% of symptomatic cases
Case fatality rate	Overall: 0.5 - 1%

http://www.cdc.gov/hepatitis/resources/professionals/pdfs/abctable.pdf

Potential for Chronic Infection after Acute Infection

Chronic HBV infection develops in:

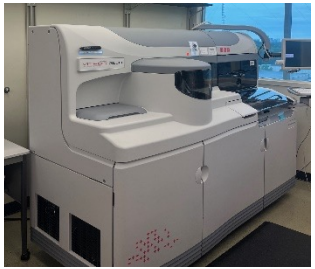
- 90% of infants after acute infection at birth
- 25%–50% of children newly infected at ages 1–5 years
- 5% of people newly infected as adult

Overall, approximately 25% of persons who become chronically infected during childhood and 15% of those who become chronically infected after childhood die prematurely from cirrhosis or liver cancer

Serologic Tests for the Diagnostic of HBV Infection

Chemi-Immunoluminescent and Enzyme Linked Immunosorbent Assays (CIA and ELISA) for antigens and antibodies

- **HBsAg**
  - Detects at least 97% of HBV infections
  - High analytical sensitivity (0.1ng)
  - Confirmation test of an HBsAg screening-positive results is required if NAT not feasible
- **Total and IgM anti-HBc; HBeAg/anti-HBe**
- **Qualitative or Quantitative anti-HBs**



FDA approved IVD tests for HBsAg

- QuidelOrtho **VITROS**
- Abbott **ARCHITECT**
- Abbott **Alinity**
- Siemens **Advia Centaur**
- Roche Cobas **Elecsys**

Nucleic Acid Tests for the Diagnostic of HBV Infection

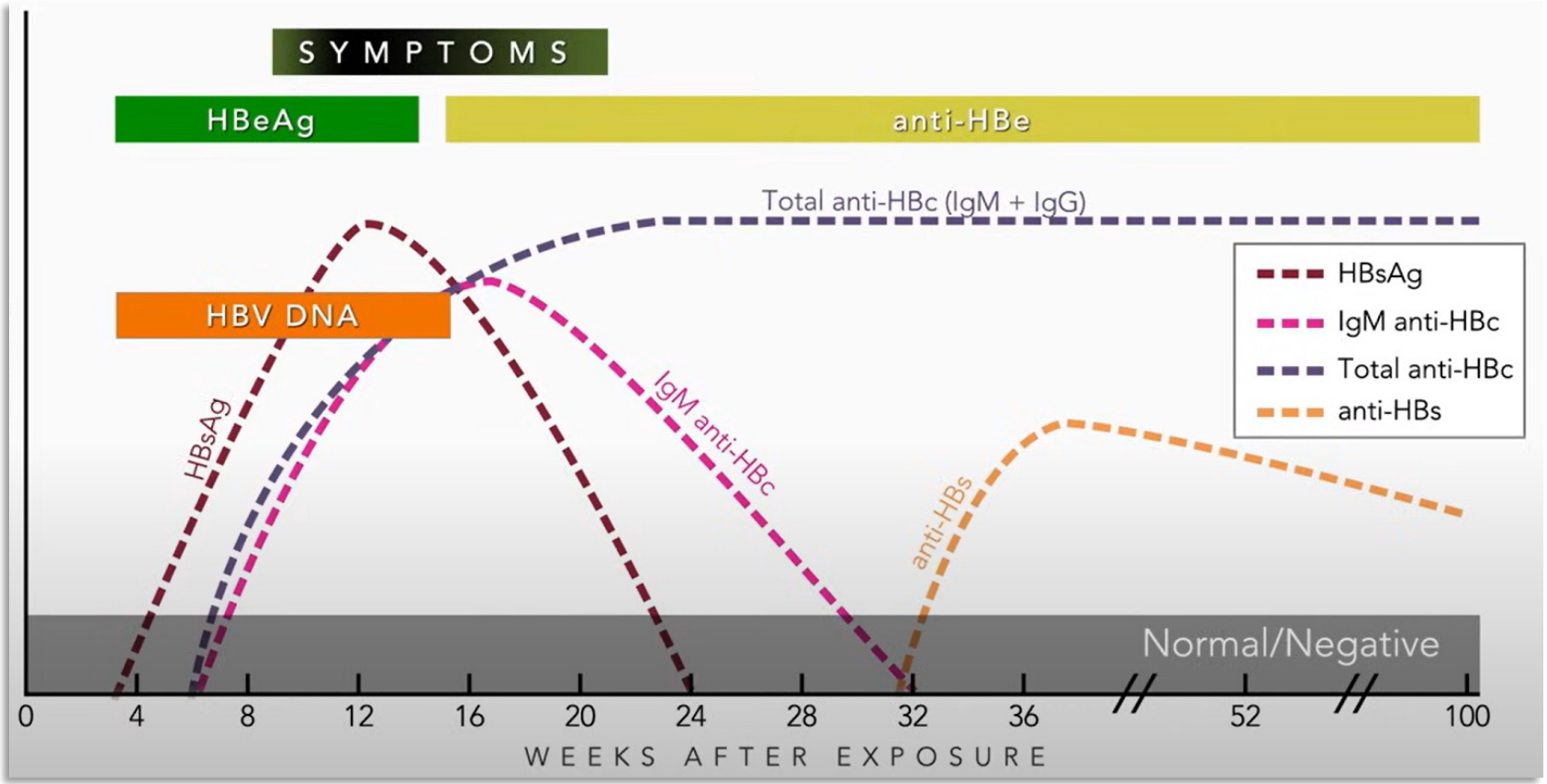
HBV DNA detection and quantification

- FDA Approved High performing platforms:
  - ROCHE COBAS
    - Various generations and throughput (e.g. 5800, 6800, 8800)
  - ABBOTT M2000 and Alinity M
  - Panther Hologic

Genotyping and Molecular Epidemiology

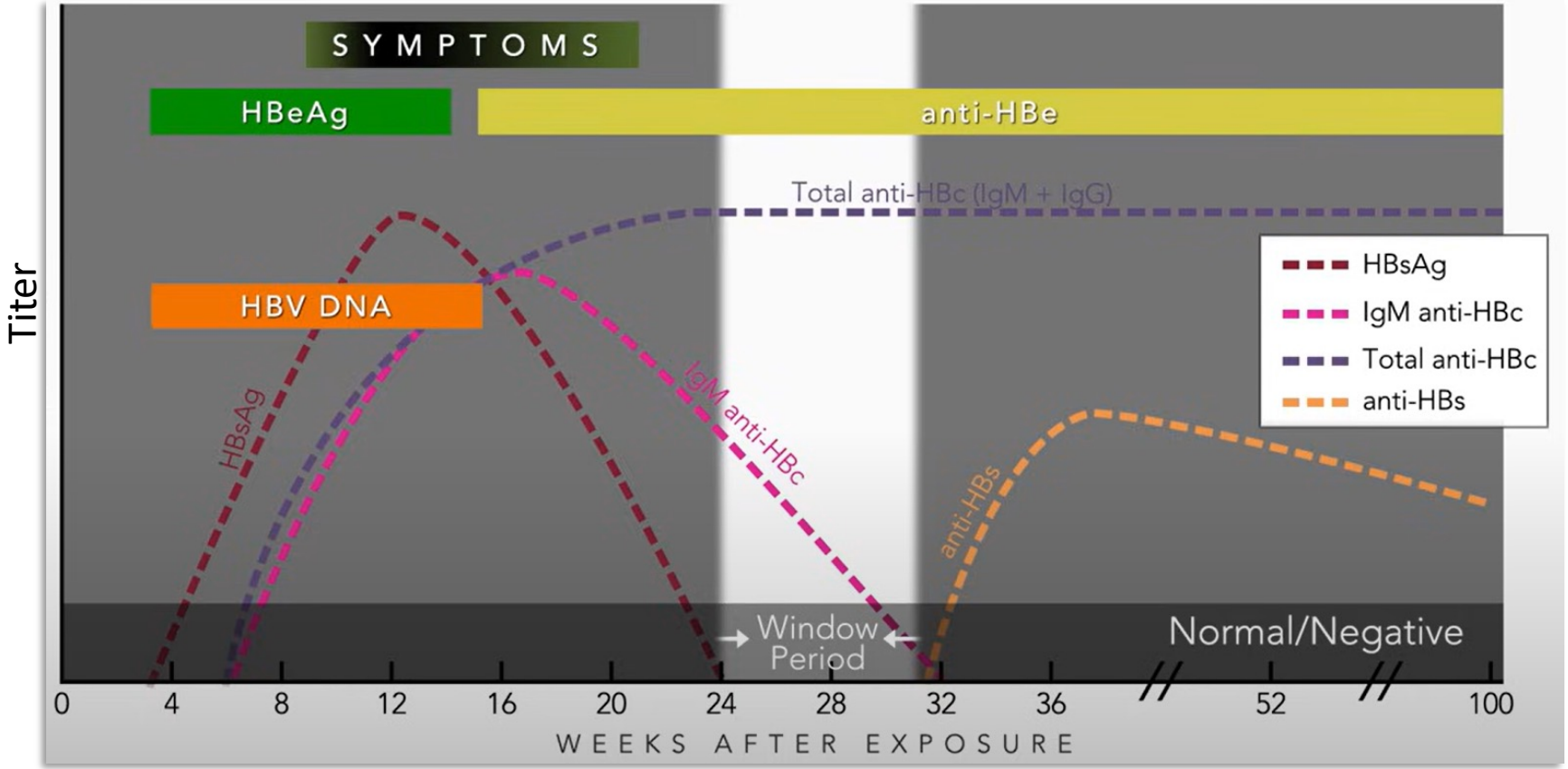
- S-gene or whole genome sequencing can provide evidence of genomic mutations or transmission linkages
  - Not FDA-approved and can be used for research only (RUO)

Acute Hepatitis B Virus Infection / With Recovery

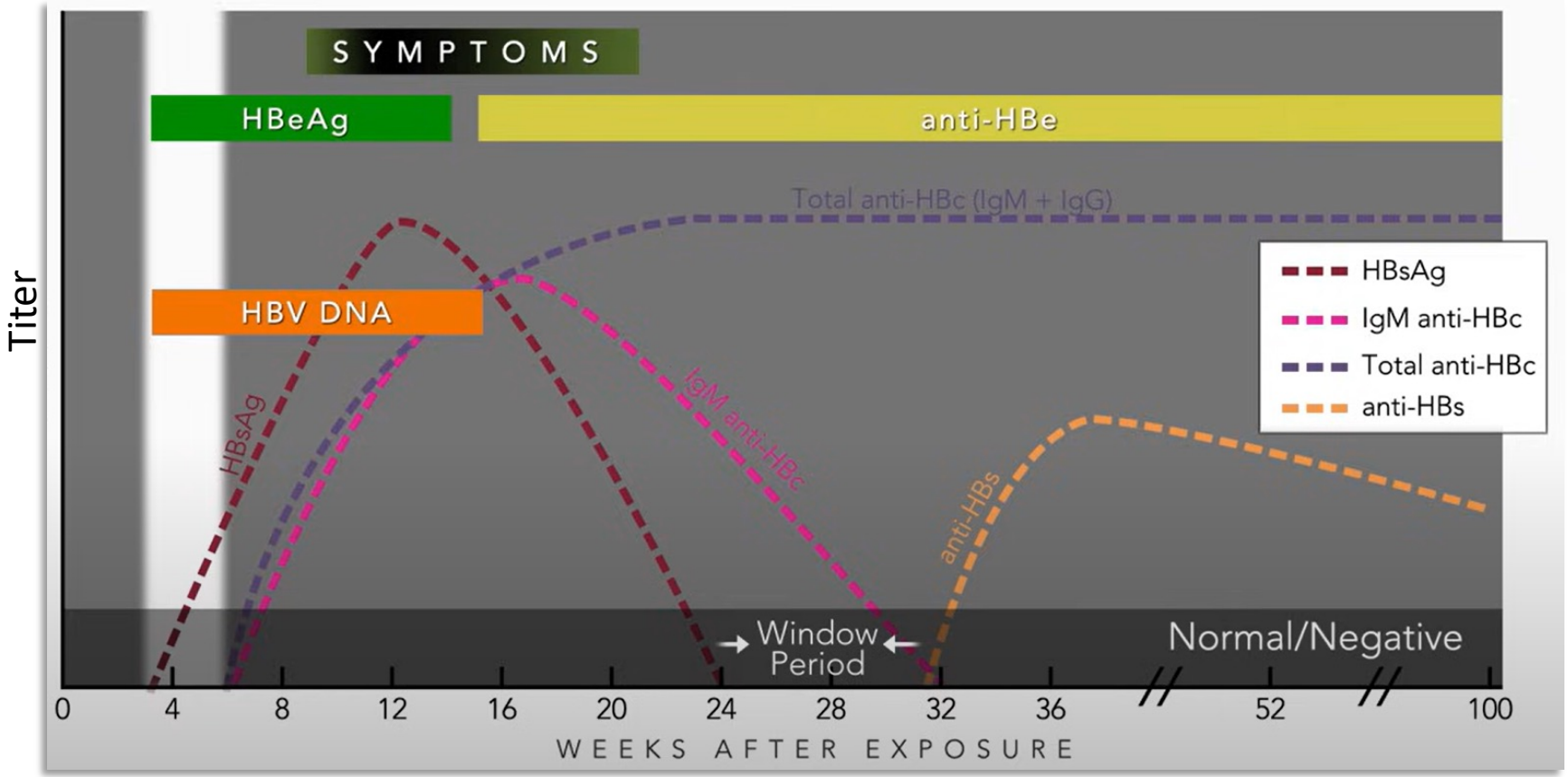


Acute Hepatitis B Virus Infection / With Recovery

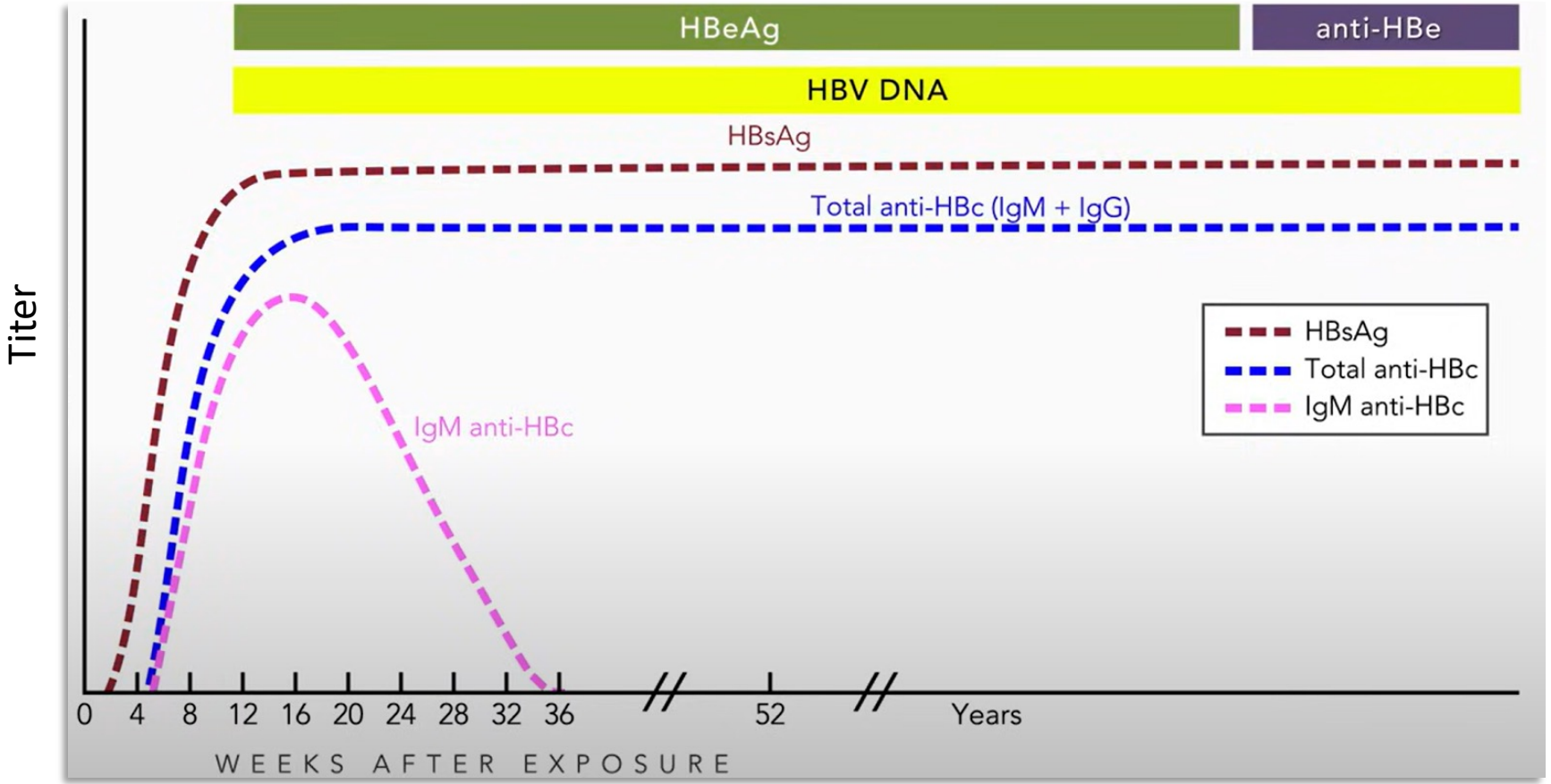
Window Period



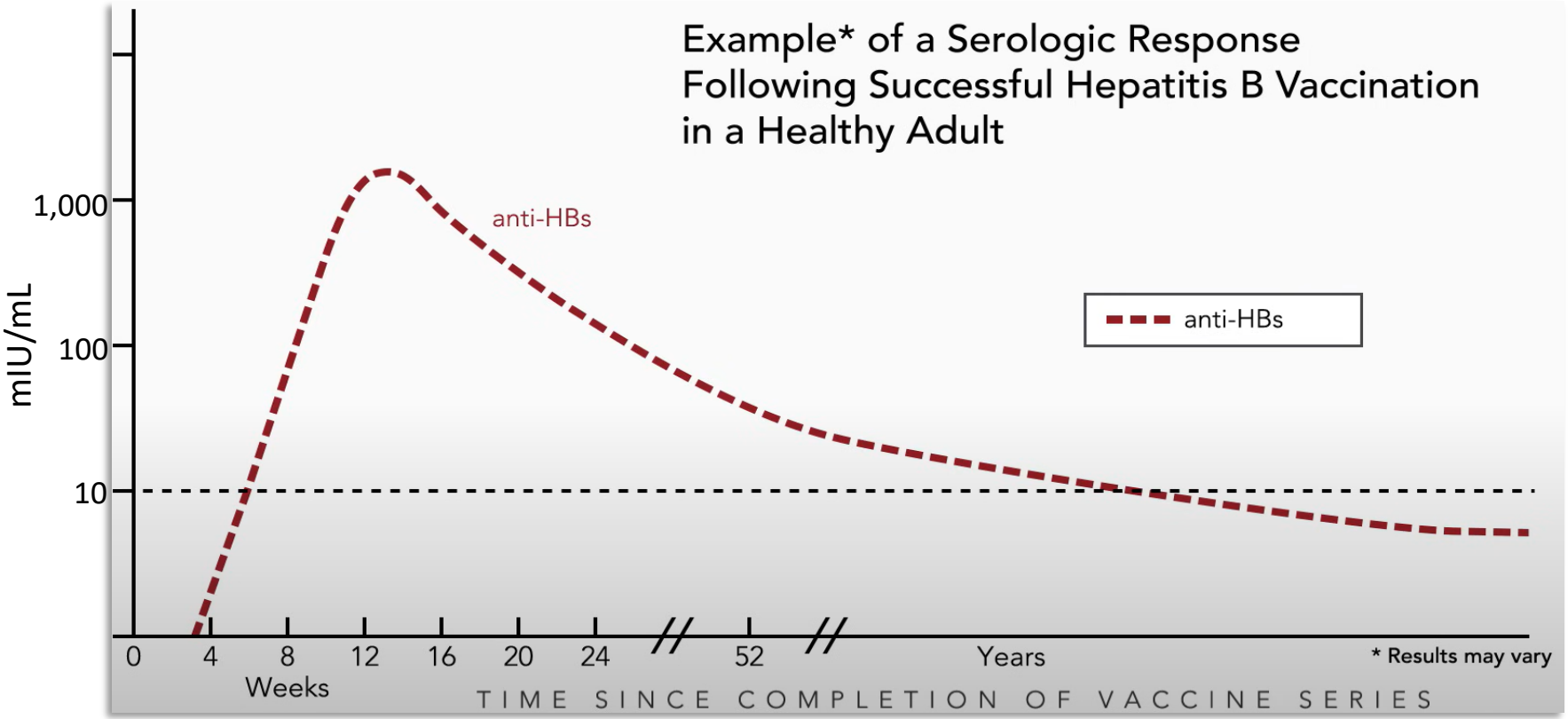
Acute Hepatitis B Virus Infection
Early phase post-exposure: HBsAg/HBV DNA Alone



Hepatitis B Virus Infection / Chronic



Hepatitis B Virus Infection / Vaccination



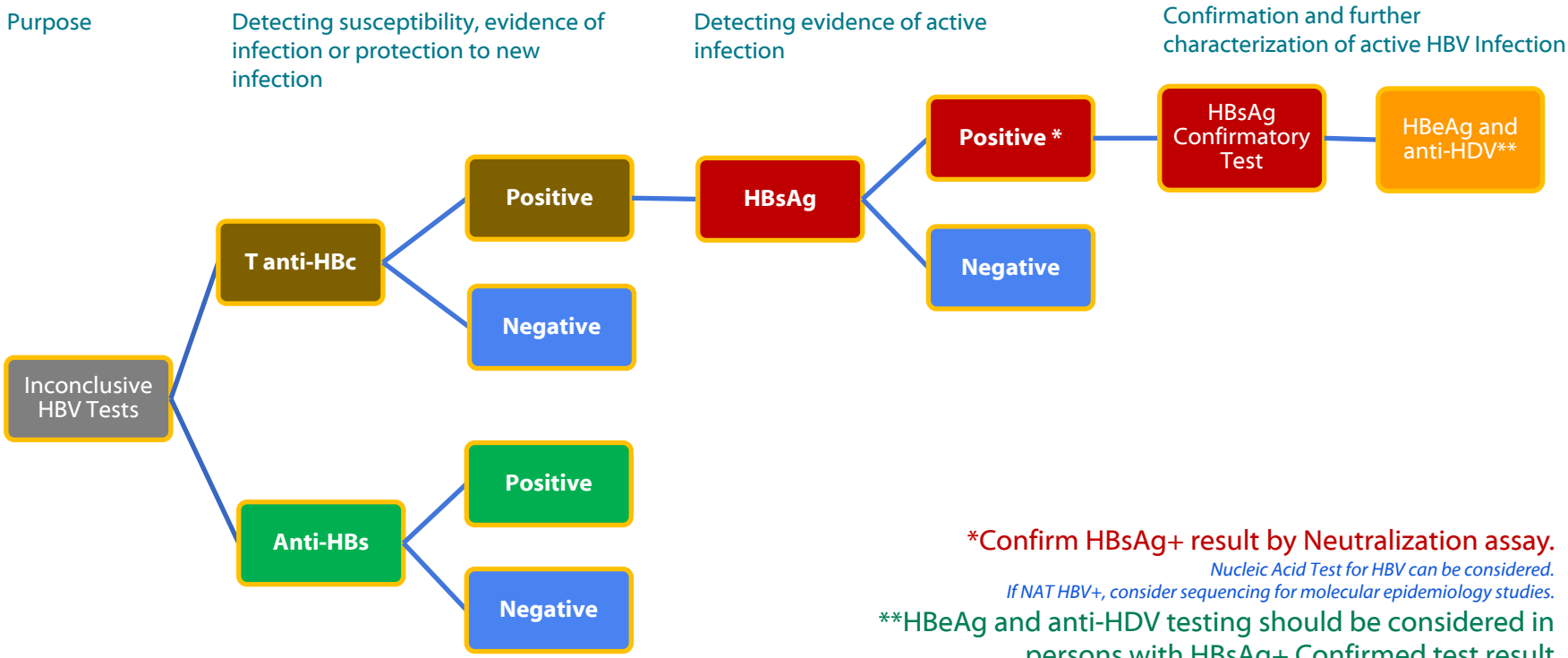
Hepatitis B Virus Infection
Interpretation of Test Results

Serologic Test Result	HBsAg	Total Anti-HBc	IgM anti-HBc	Anti-HBs
Detected following vaccination or for 3-6 months following receipt of HBIG	Negative	Negative	Negative	Positive
False-positive (Susceptible) / Past Infection (Resolved) / "Low level" chronic infection (Unlikely to be infectious)	Negative	Positive	Negative	Negative
Chronic Infection	Positive	Positive	Negative	Negative
Past Infection with recovery (Immunity to new infection)	Negative	Positive	Negative	Positive
Acute Infection	Negative	Positive	Positive	Negative
Early Acute Infection / Receipt of the hep B vaccine within several weeks	Positive	Negative	Negative	Negative
Never infected (Susceptible)	Negative	Negative	Negative	Negative

Hepatitis B e Antigen (HBeAg)

- Within hepatocytes, HBeAg and HBcAg are generated from the same region of the HBV DNA. The newly formed HBeAg is then secreted from the hepatocyte into the blood circulation.
  - In contrast, the HBcAg is assembled into the HBV core and incorporated into the intact virion.
- Although abundant HBeAg is produced, it is not a component of the intact hepatitis B virion or subviral particles, and it is not required for viral infection, assembly, or replication.
- The presence of HBeAg is typically associated with elevated HBV DNA levels and high infectivity, but it is variably present in persons with chronic HBV infection.
  - Certain precore and basal core promoter mutations are associated with reduced or abolished HBeAg protein production.

Testing Algorithm Recommended when the Assessment of HBV Infection based on Incomplete HBV Testing is Inconclusive



*Confirm HBsAg+ result by Neutralization assay.

Nucleic Acid Test for HBV can be considered.
If NAT HBV+, consider sequencing for molecular epidemiology studies.

**HBeAg and anti-HDV testing should be considered in persons with HBsAg+ Confirmed test result

Hepatitis B Virus Infection
Interpretation of Test Results from Testing Algorithm

Serologic Test Result	Total Anti-HBc	Anti-HBs	HBsAg	HBeAg
Never infected (Susceptible)	Negative	Negative	Not Tested	Not Tested
Post-vaccination Immunity to new infection	Negative	Positive	Not Tested	Not Tested
Past Infection with recovery (Immunity to new infection)	Positive	Positive	Negative	Not Tested
False-positive (Susceptible) / Past Infection (Resolved) / Resolving recent infection (Window Period) / "Low level" occult chronic infection (Unlikely to be infectious)	Positive	Negative	Negative	Not Tested
Chronic Infection ("Low level" infection - likely asymptomatic "Carrier")	Positive	Negative	Positive	Negative
Chronic Infection Active replication - likely highly infectious)	Positive	Negative	Positive	Positive

Hepatitis B Virus Infection
Interpretation of Test Results with HBV DNA

Serologic Test Result	Total Anti-HBc	Anti-HBs	HBsAg	HBV DNA
Early Acute Infection Detected mainly during the blood donors screening by NAT in recently infected organ donors; contacts in an actively developing outbreak Follow up test allows for the resolution of quandary	Negative	Negative	Negative	Positive
Follow up test allows for the resolution of quandary- likely confirming Early Acute Infection with HBsAg+ and anti-HBc+	Positive	Negative	Positive	Positive
"Low level" occult chronic infection	Positive	Negative	Negative	Positive

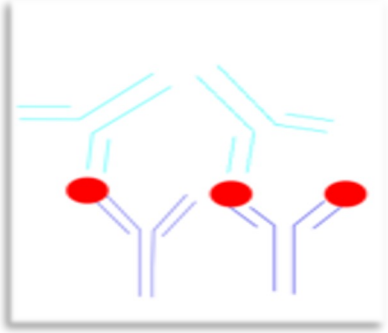
Diagnostic Challenges: Interfering Substances

Marker	Pos	Neg	Biotin Concentration					
			0 ng/ml		300 ng/ml		1200 ng/ml	
			FP	FN	FP	FN	FP	FN
HBsAg[a]	20	20	0	0	ND	ND	ND	ND
IgM anti-HBc	8	42	0	0	0	2 (25%)	0	7 (87.5%)
IgM anti-HAV	30	30	0	0	1 (3%)	0	2 (6.7%)	0
Total anti-HAV	30	30	0	0	0	30 (100%)	0	30 (100%)

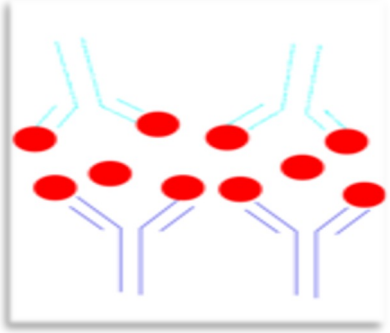
- **Legend:** [a] = no biotin chemistry; FP = false positive; FN = false negative; ND = Not Done
- HBsAg test does not use biotin as a secondary label. Test was not done because there is not expected interference from Biotin for HBsAg.

Kodani et al. 2019:

Diagnostic Challenges: Prozone Effect

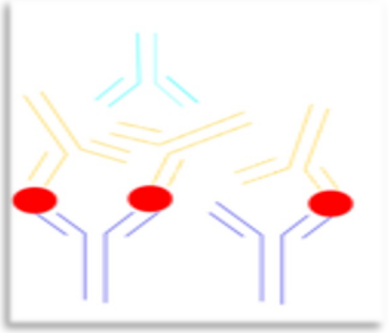


A) In a sandwich immunoassay, the antigen binds to the primary antibody and the secondary labeled antibody forms a complex to release the signal.



B) Excessive antigen binds to primary and secondary antibody, preventing the complex from forming.

- If false negative HBsAg is suspected, dilution may resolve the discordance.



C) In competitive assays (e.g., anti-HBc T), cross reacting substances can compete for the binding site of the detection antibody.

Diagnostic Challenges: Vaccination and Transient HBsAg Positivity

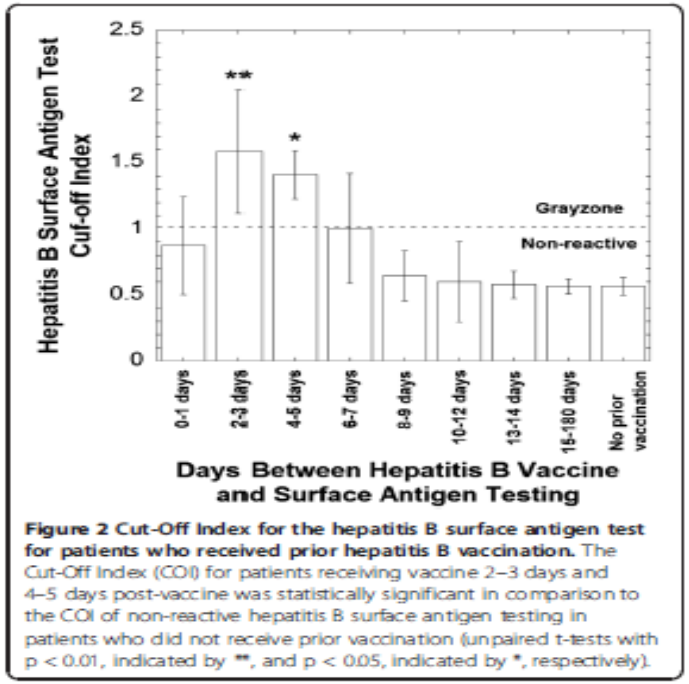


Figure 2 Cut-Off Index for the hepatitis B surface antigen test for patients who received prior hepatitis B vaccination. The Cut-Off Index (COI) for patients receiving vaccine 2–3 days and 4–5 days post-vaccine was statistically significant in comparison to the COI of non-reactive hepatitis B surface antigen testing in patients who did not receive prior vaccination (unpaired t-tests with $p < 0.01$, indicated by **, and $p < 0.05$, indicated by *, respectively).

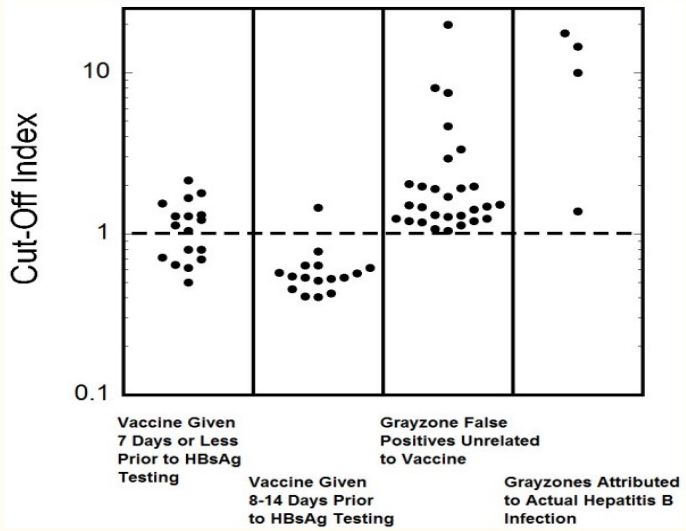


Figure 3

Dot plots of Cut-Off Index. The Cut-Off Index is plotted for four populations: patients who received hepatitis B vaccine 7 days or less prior to HBsAg testing, patients who received vaccine 8–14 days prior to HBsAg testing, patients with grayzone HBsAg reactions not attributed to vaccine or to actual hepatitis B virus infection, and patients with grayzone reactions who appear to have actual hepatitis B virus infection. The dashed line is at the Cut-Off Index of 1 which divides non-reactive from grayzone.

Rysgaard et al. BMC Clinical Pathology 2012, 12:15

Diagnostic Challenges: HBsAg Mutations

TABLE. HBsAg lab results for the case patient by facility and testing instrument

Date collected	Laboratory facility	Testing instrument	Result
November 11, 2010	A*	Advia Centaur XPT	Negative†
December 8, 2016	A*	Advia Centaur XPT	Negative†
December 9, 2016	B*	Advia Centaur XP	Positive
December 14, 2016	C*	Advia Centaur XP	Positive
January 5, 2017	A*	Advia Centaur XPT	Negative†
February 2, 2017	A*	Advia Centaur XPT	Negative†
March 2, 2017	A*	Advia Centaur XPT	Negative†
March 2, 2017	C*	Advia Centaur XP	Positive
May 7, 2017	CDC	Vitros Eci	Negative†
May 7, 2017	CDC	Abbott ARCHITECT	Positive
May 23, 2017	NPHL	Advia Centaur XP	Positive
July 25, 2017	D*	ETI-MAK-2 PLUS	Positive
July 25, 2017	E*	Vitros 3600	Negative†

Abbreviation: NPHL = Nebraska Public Health Lab.
* Deidentified commercial laboratory.
† False-negative result.

MMWR Notes from the Field describe False-Negative HBsAg test result in a Hemodialysis Patient

Testing at CDC:

- High HBV DNA levels (14,200,000 IU/mL), anti-HBs levels of 114 mIU/ml indicative of immunity, and
- HBsAg positivity in one assay but negative in another.
- Sequencing of the S gene identified an G145R surface antigen mutation

Mutations in HBsAg can result in false-negative HBsAg results

MMWR 2018 Mar 16; 67(10): 311–312

Why Other Tests besides HBsAg are Needed?

Table IV. Interpretation of initial reactive HBsAg from pregnant women with discrepant HBsAg results

Resolution of initial HBsAg-result	No. (%)	Additional information	No. (%)
Probable true positive	14 (9.8)	Positive total anti-HBc	11 (78.6)
		Positive IgM anti-HBc	2 (14.3)
		Vaccination 3 days before HBsAg testing*	1 (7.1)
Probable false positive	75 (52.8)	Negative total anti-HBc	67 (89.3)
		No HBV DNA detected	6 (8.0)
		Technical error reported by laboratory	2 (2.7)
Unresolved	53 (37.4)	No additional serology, no history of vaccination	28 (52.8)
		Negative IgM anti-HBc	17 (32.1)
		Negative IgM anti-HBc, negative anti-HBs	3 (5.7)
		Negative IgM anti-HBc, negative HBeAg	1 (1.9)
		Negative anti-HBs	1 (1.9)
		Negative anti-HBs, negative HBeAg	2 (3.8)
		Positive anti-HBs, unknown vaccination history	1 (1.9)

HBeAg, hepatitis B e antigen.
*HBsAg in vaccine is reported to be detectable for ≥18 days after vaccination.

Lab Results Review Triade

Interpret lab results in the Clinical, Populational and Epidemiologic Context

- Comorbidities and Inappropriate testing without a thorough review of history contributes to an increased rate of false-positives
- Using an Appropriate algorithm helps eliminate false-positive results
  - E.g., isolated low level HBsAg in a non-risk subject, without anti-HBc is likely a FP.
  - HBeAg in an HBsAg-neg person is a lab algorithm deviation and HPC should retest

Consider Test specificity

- Variability in performance of test from different manufacturers: Design, Automated vs Manual vs Rapid POC tests
- Manual test-formats have an increased potential of human error
- The specificity of the test varies in the general vs high risk population

Review Processed or Raw Data

True positive results are likely to have high SCR or OD values

Case Study 1

Test #	HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	HBV DNA	HBeAg	Other
1 (5/1/23)						+	
2 (5/18/23)	-	-	-	+(33.85 mIU/mL)	-		
3(5/24/23)	-					+	

- ***Mom with no history of hepatitis B, unaware of HepB vaccine status.***
  - *Came to US from Peru; EDD: 8/5/2023*
- **Interpretation:** The tests order on 5/18/23 was informative. Although negative HBV DNA and IgM anti-HBc were reassuring, they were rather unnecessary.
  - With a consistent negative HBsAg result, HBeAg testing is not recommended.
- **PHBPP Action:** To rule out a lab error, a follow up triple screening (HBsAg, anti-HBc total, anti-HBs) can be recommended before the EDD

Case Study 2

Test #	HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	HBV DNA	HBeAg	Anti-HBe
1 (1/19/23)	+					+	
2 (2/3/23)	-	-		+(51.15 mIU/mL)		-	-

- **Mom with unknown HepB vaccine status**
  - Baby received HBIG and HepB at birth.
- **Interpretation:** Decision to Vax+HBIG was correct due to presence of markers of active infection during pregnancy. But lack of anti-HBc total make the results dynamic inconsistent.
- **PHBPP Action:** To rule out a lab error, a follow up triple screening (HBsAg, anti-HBc total, anti-HBs) can be recommended

Case Study 3

Test #	HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	HBV DNA	HBeAg	Anti-HBeAg
1 (1m>Pr)	+	-			-		
2 (Pr)	+	-			-	+	

- **Mom fully vaccinated in childhood**
- **Interpretation:** Lack of anti-HBc total and HBV DNA inconsistent with HBsAg and HBeAg results.
- **PHBPP Action:** Since there is *evidence suggesting maternal HBV infection, administer Hep B vaccine and HBIG.*
  - To rule out a lab error, a follow up triple screening (HBsAg, anti-HBc total, anti-HBs) can be recommended.

Case Study 5

- State Bureau of Disease Prevention and Control received a ***HBsAg quantitative*** monitor test from a provider who was testing an infant followed in the perinatal hep B program.
- **Interpretation:** Per 2018 AASLD Hepatitis B Guidance, HBsAg quantitation can be useful in managing patients receiving peg-IFN therapy. However, HBsAg quantitative monitor test is not an FDA-approved diagnostic test and cannot be used to diagnose the presence or absence of HBV infection.

PHBPP Action: *Triple screening (HBsAg, anti-HBc total, anti-HBs) can be recommended*

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov