



Updates on Serologic and Molecular Testing for Viral Hepatitis

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Learning objective

1. Review and learn current and new serologic and molecular marker tests for the diagnosis and monitoring of viral hepatitis A, B , C, D, and E.
2. Understand the test methods, clinical utility, and indications for use of the new marker tests.
3. Recognize the pitfalls and resolution approaches to these laboratory tests to determine the definitive infection status.

Conflict of Interests Disclosure

None

Pre-Test Question 1

Which of the following viral hepatitis **does / do not** have FDA-cleared or FDA-approved **serologic tests** available for routine clinical use?

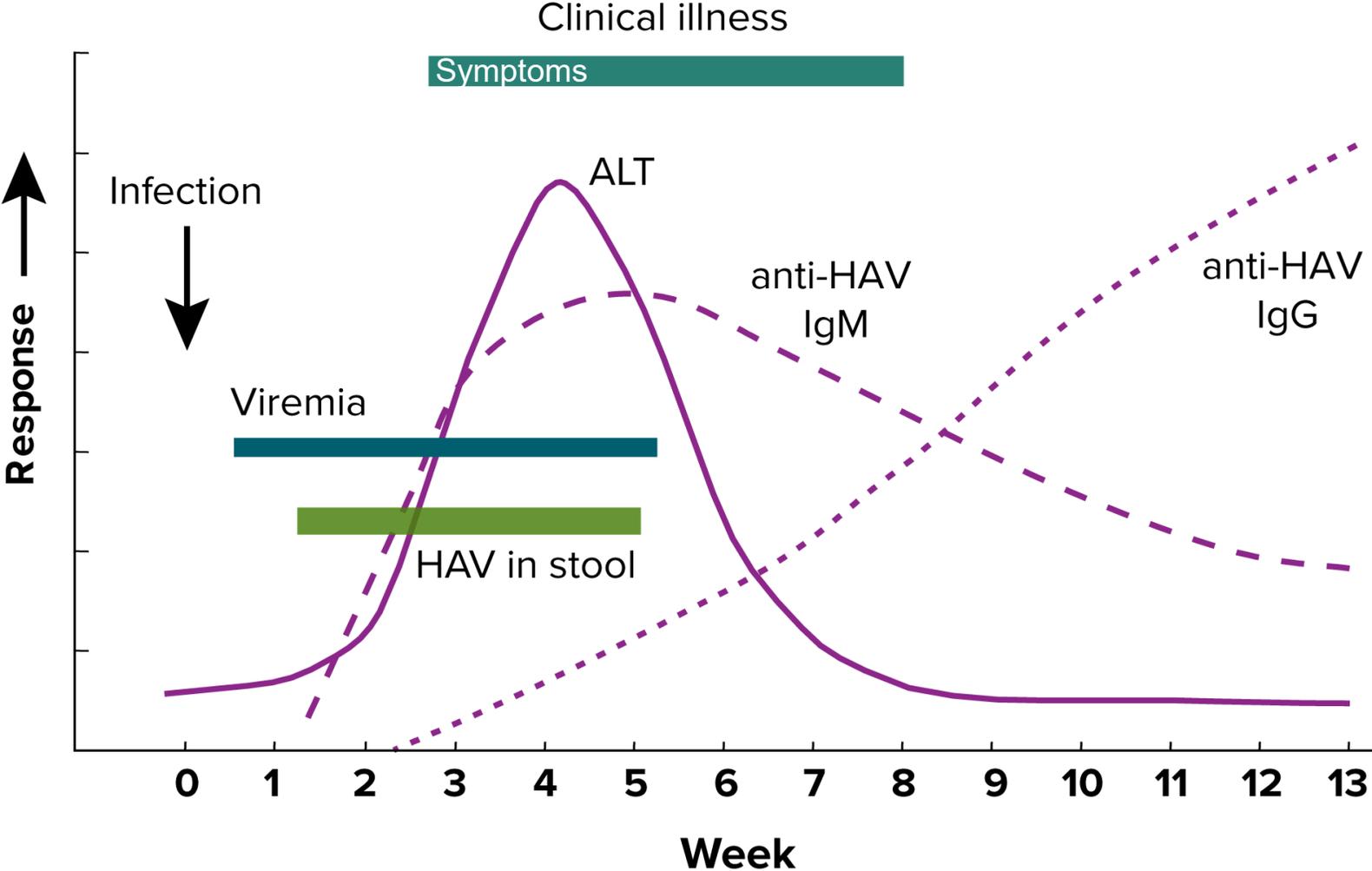
1. Hepatitis A
2. Hepatitis B
3. Hepatitis C
4. Hepatitis D
5. Hepatitis E
6. Combination of above

Pre-Test Question 2

Which of the following viral hepatitis **does / do not** have FDA-cleared or FDA-approved **molecular tests** available for routine clinical use?

1. Hepatitis A
2. Hepatitis B
3. Hepatitis C
4. Hepatitis D
5. Hepatitis E
6. Combination of above

Typical Serologic Profile of Acute Hepatitis A



https://www.cdc.gov/hepatitis/php/surveillance-guidance/hepatitis-a.html#cdc_generic_section_4-interpretation-of-laboratory-test-results
<https://www.cdc.gov/hepatitis/hcp/training/index.html>

Hepatitis A

Currently available laboratory tests

HAV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HAV IgM Ab	EIA, CIA	IVD	86709	\$11.26
HAV IgG Ab	EIA, CIA	IVD	86708 or 86790	\$12.39 or \$12.88
HAV Total Ab	EIA, CIA	IVD	86708	\$12.39
HAV RNA (qual)	PCR	CE-IVD, LDT	87999	?
HAV RNA (quant)	PCR	CE-IVD, LDT	87999	?

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

CPT 86790: Analysis for antibody to virus

CPT 87999: Unlisted microbiology procedure

Current Problems in Laboratory Tests for Hepatitis A

1. False-positive HAV IgM Ab

- CT state (2002 - 2003): 19 / 127 Ab+ (15%) did not meet case definition
- Alaska state (2002 – 2005): 13 / 41 Ab+ (32%) did not meet case definition
- U.S. Viral Hepatitis Sentinel Counties Study (2003): 87 / 140 Ab+ (62%) did not meet case definition

CDC. *MMWR* 2005; 54(18):453-456. Castrodale L, et al. *Clin Infect Dis* 2025; 41:386-e88.

- Potential causes: autoimmune hepatitis, connective tissue disorders, acute CMV or EBV infection, hypergammaglobulinemia, polyclonal B-cell activation states, assay reagent contamination.
- *Recommendations:*
 - A. HAV IgM Ab testing should NOT be the sole diagnostic method, especially with borderline index values.
 - B. Limit testing of HAV IgM Ab to persons with symptoms & signs of acute hepatitis **and** have recent exposure to known HAV-infected person.

Current Problems in Laboratory Tests for Hepatitis A

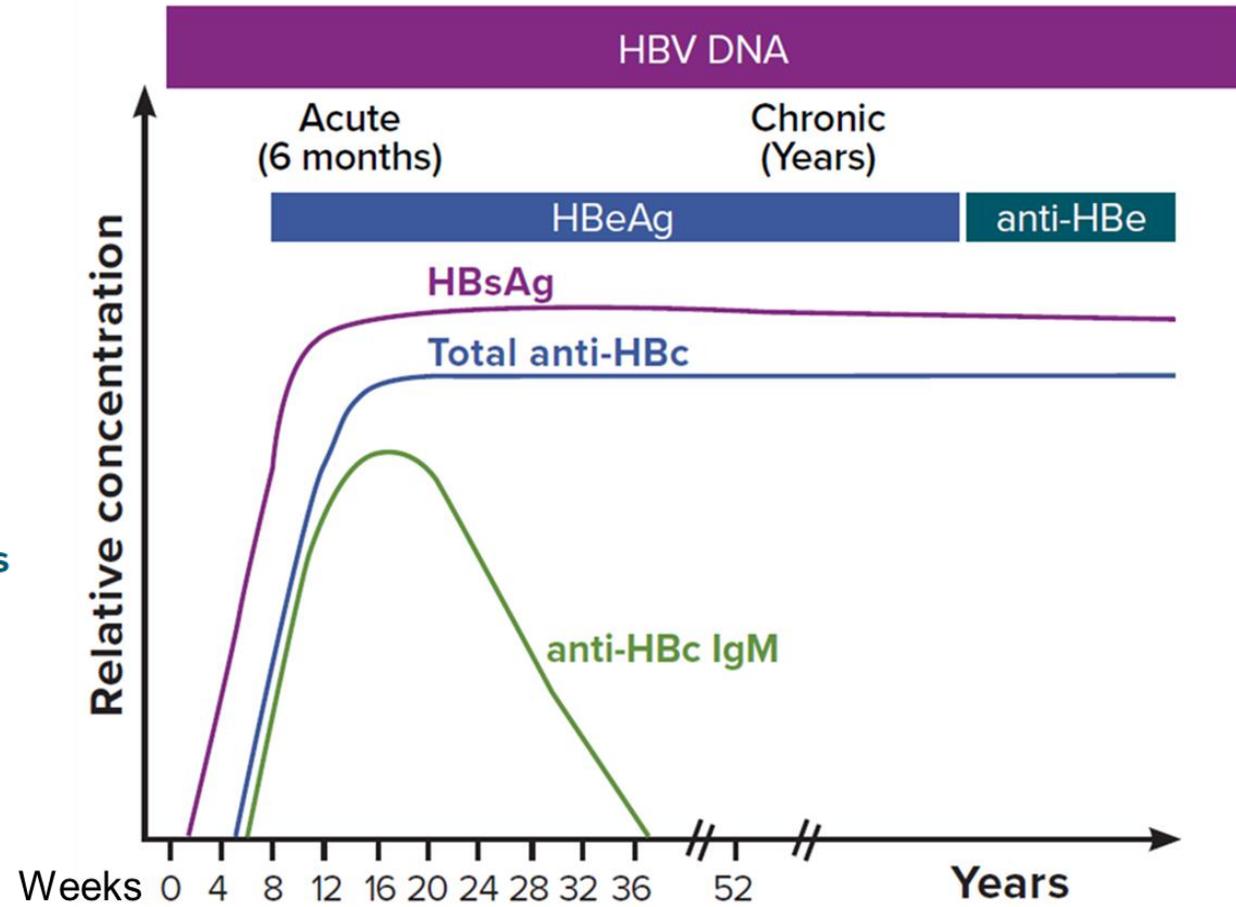
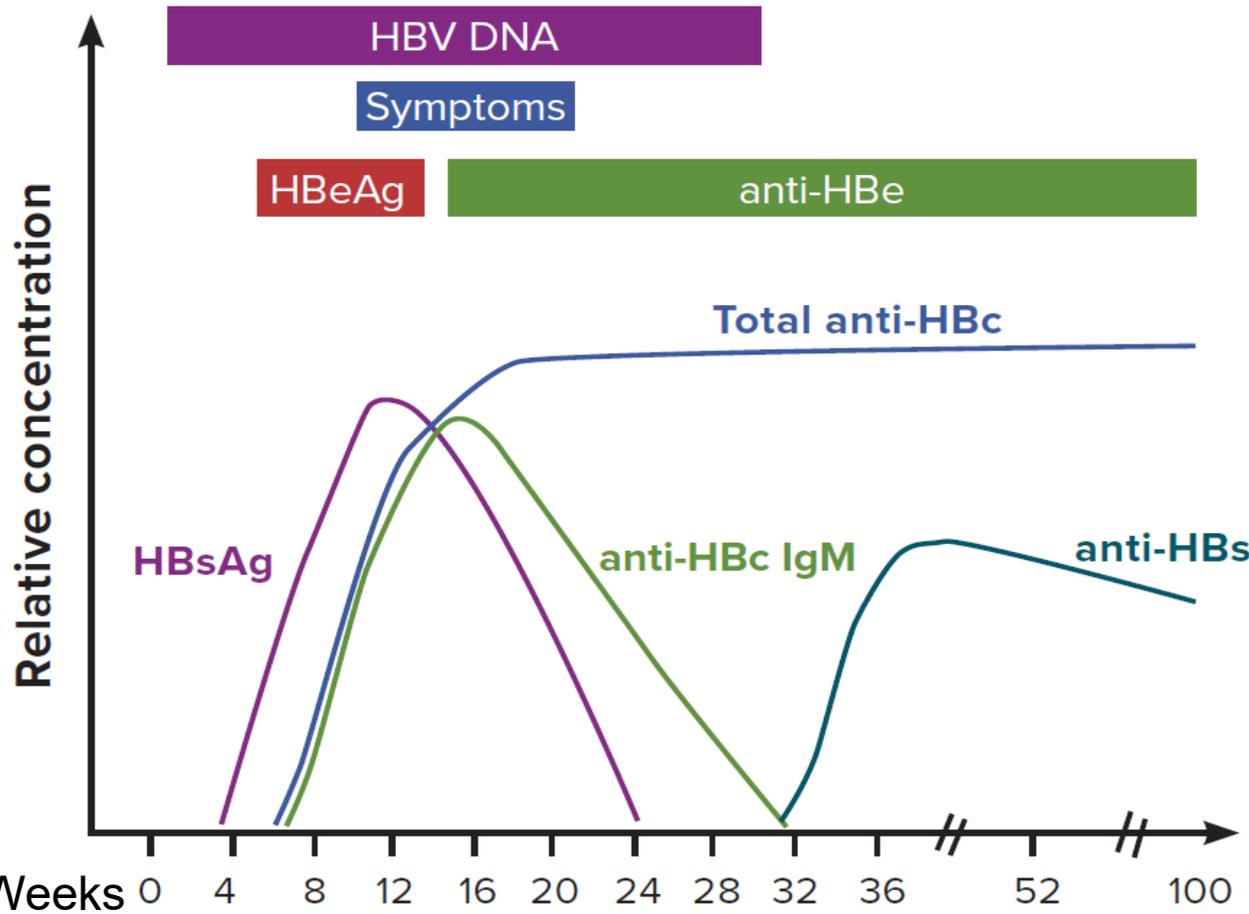
2. False-negative HAV IgM Ab

- HAV IgM Ab- but HAV Total Ab+
- Possible causes: <2 wks from exposure; HAV Total Ab assays more sensitive in detecting IgM Ab; high-dose (≥ 5 g daily) biotin supplement intake (interference in biotin-based EIA & CIA)
- *Recommendations:*
 - A. Retest for HAV IgM Ab in 5 - 7 days after initial negative result
 - B. Confirmatory testing for HAV RNA (qualitative)

HAV RNA can be detected for a mean of 79 days after the peak ALT and remains detectable in 40% of persons with acute hepatitis A for 70 - 127 days after the peak ALT.

Bower WA, et al. *J Infect Dis* 2000; 182:12-17.

Typical Serologic Course of Acute Hepatitis B to Recovery and Chronic Hepatitis B



<https://www.cdc.gov/hepatitis/php/surveillance-guidance/hepatitis-b.html>
<https://www.cdc.gov/hepatitis/hcp/training/index.html>

Hepatitis B

Currently available laboratory tests

HBV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HBs antigen	EIA, CIA	IVD	86340	\$10.33
HBs Ag neutralization	EIA, CIA	IVD	86341	\$10.33
HBs Ab (IgG)	EIA, CIA	IVD	86706	\$10.74
HBc IgM Ab	EIA, CIA	IVD	86705	\$11,77
HBc Total Ab	EIA, CIA	IVD	86704	\$12.05
HBe antigen	EIA, CIA	IVD	87350	\$11.53
HBe Ab (IgG)	EIA, CIA	IVD	86707	\$11.57

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

Hepatitis B

Currently available laboratory tests (*cont'd*)

HBV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HBV DNA (quant)	PCR	IVD	87517	\$42.84
HBs Ag (quant)	EIA, CIA	CE-IVD, LDT	87467	\$25.32
HBcr antigen (total)	EIA, CIA	CE-IVD, LDT	82397	\$14.12
HBV pgRNA (quant)	PCR	CE-IVD, LDT	87799	\$42.84

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

CPT 82397: CIA method

CPT 87799: Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification

Hepatitis B Screening in U.S.

2023 CDC Recommendations

Universal screening *(new)*

- Test at least one during lifetime for all adults with HBsAg, HBs Ab, and HBc Total Ab

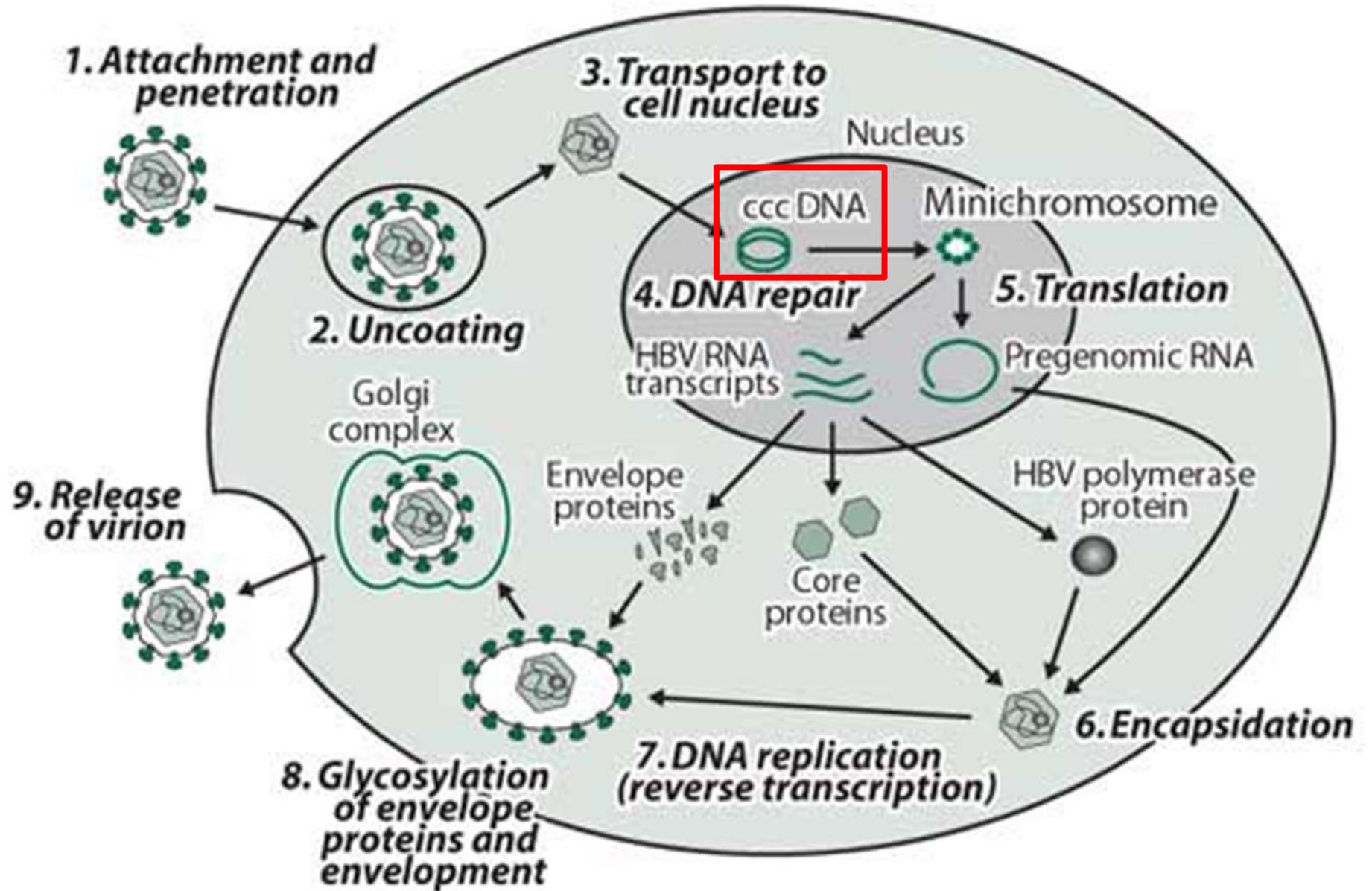
Pregnancy

- Screen at every pregnancy, preferably at first trimester, regardless of HBV vaccination status or testing history:
 - No previous testing: HBsAg, HBs Ab, and HBc Total Ab
 - Previously negative for all 3 markers + no new exposure: HBsAg

Risk-based

- Test all susceptible at-risk individuals, regardless of age – never been infected + incomplete HBV vaccination, vaccine nonresponders
- Periodic testing of above group with ongoing exposure risk

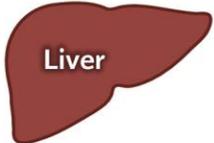
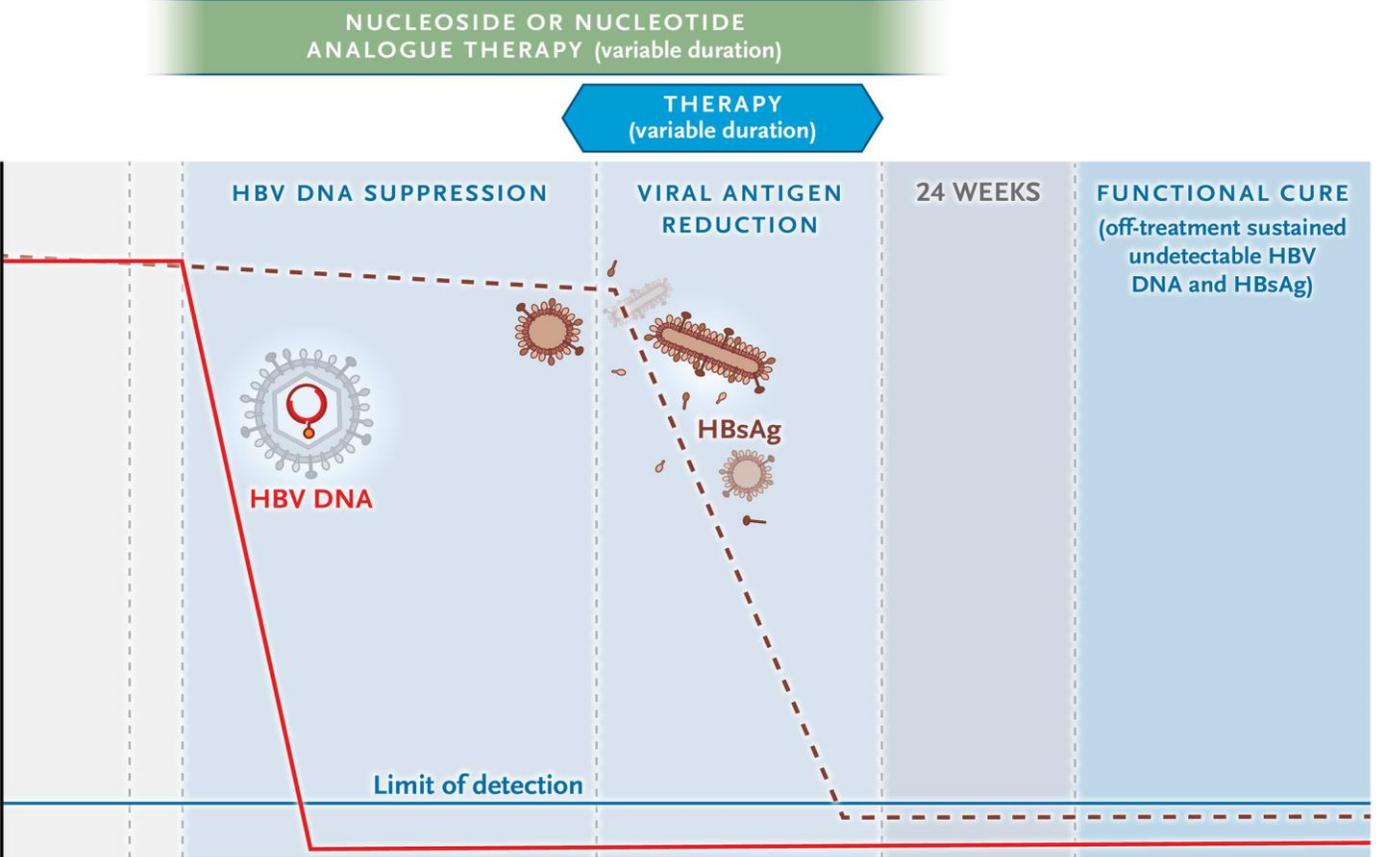
HBV Life Cycle



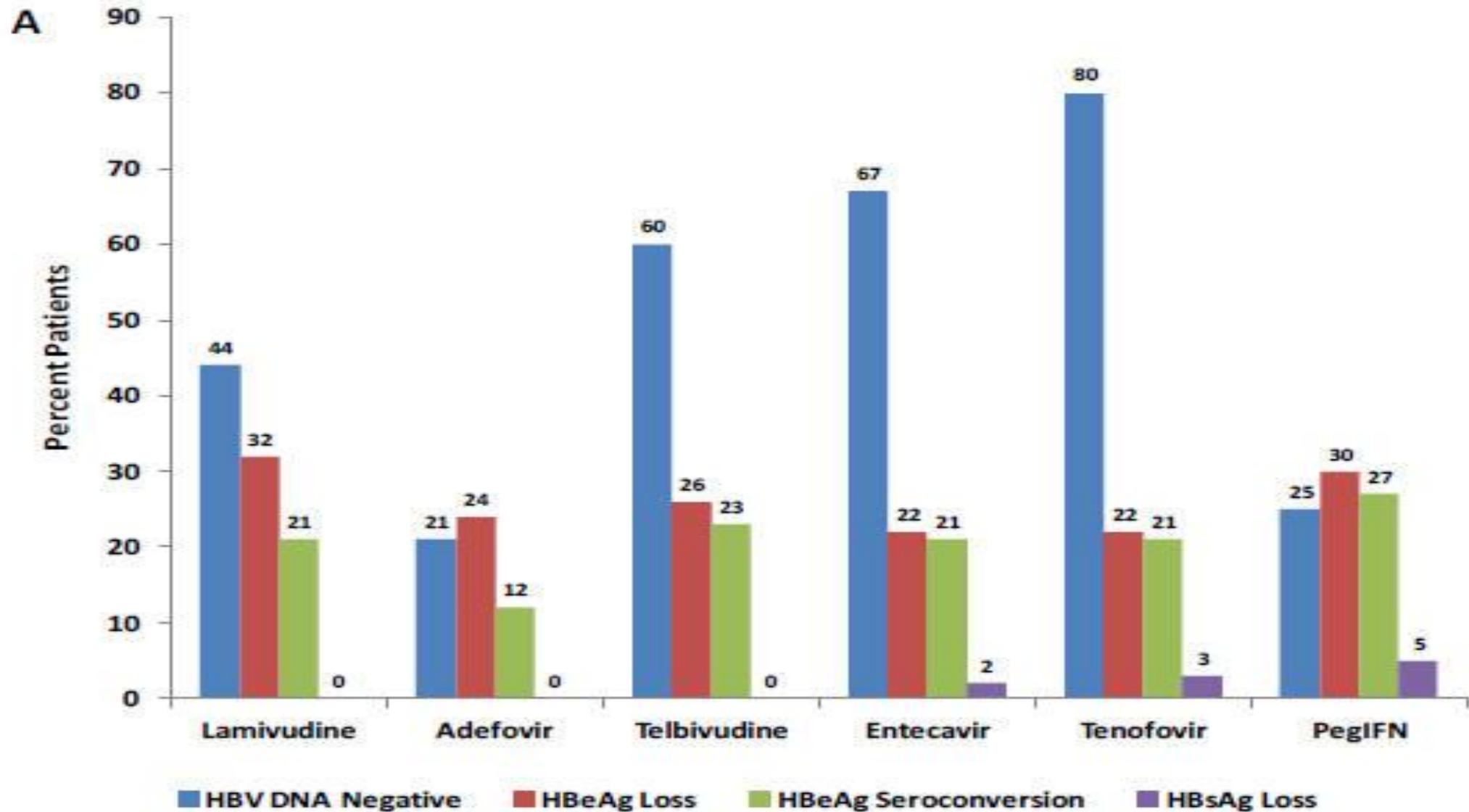
HBV Markers during Therapeutic Course of Chronic Hepatitis B Therapy



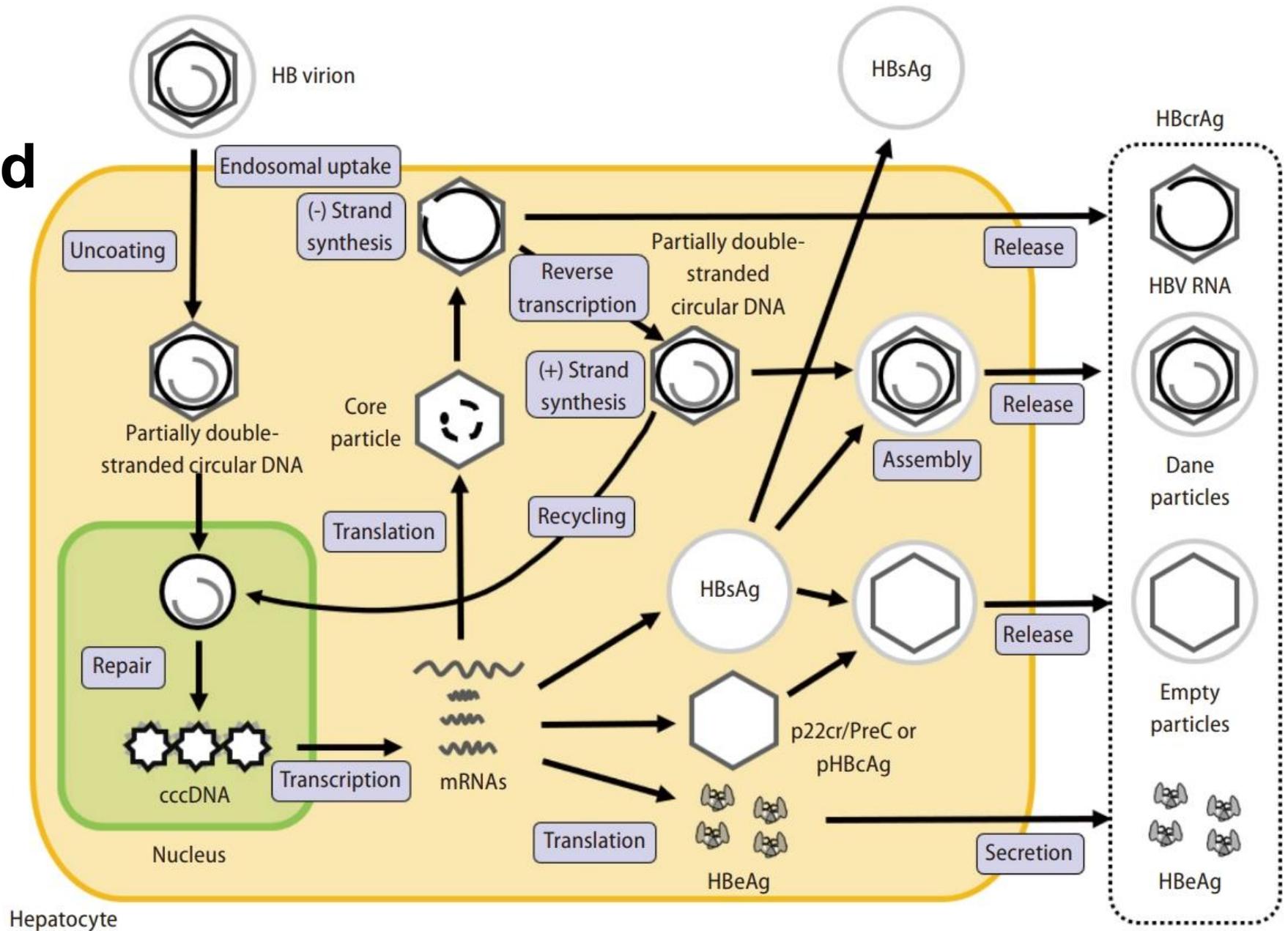
HBsAg and HBV DNA



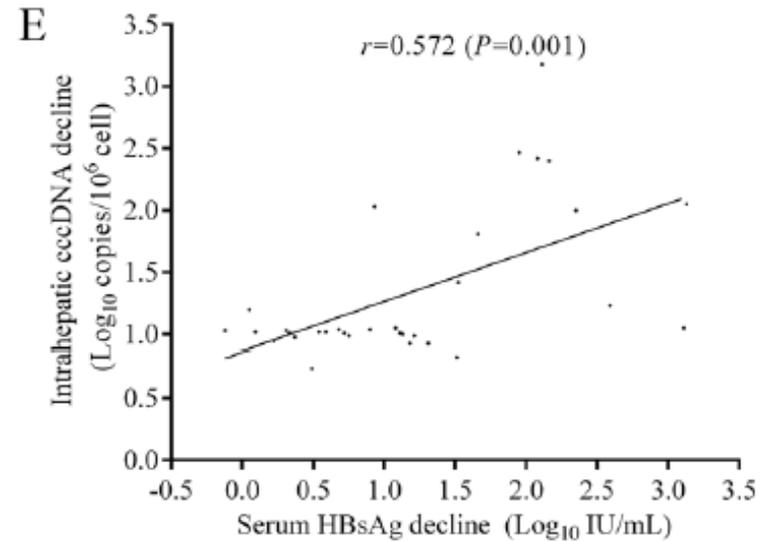
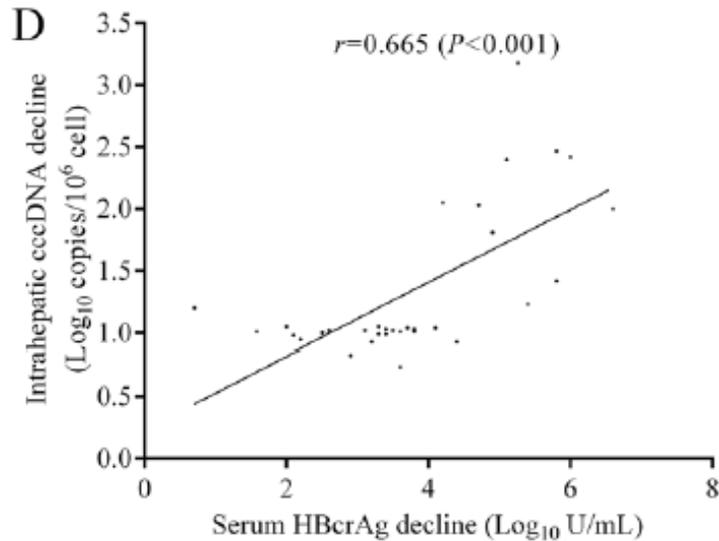
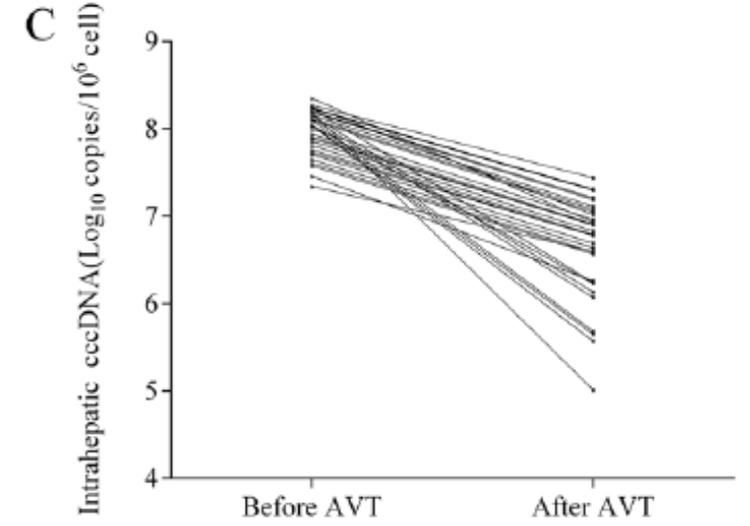
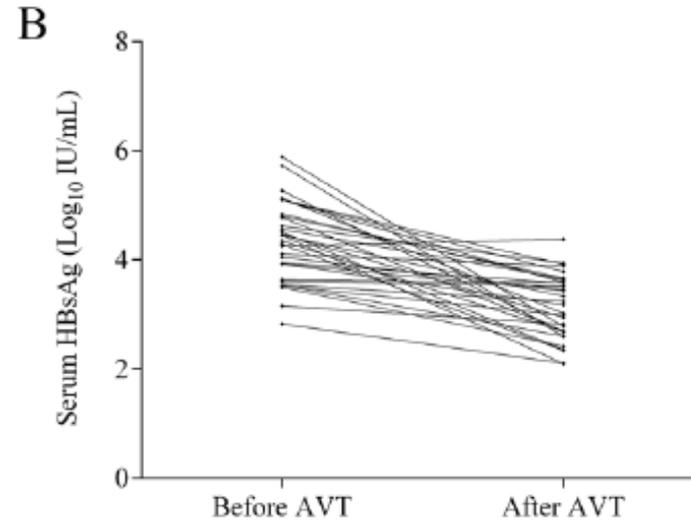
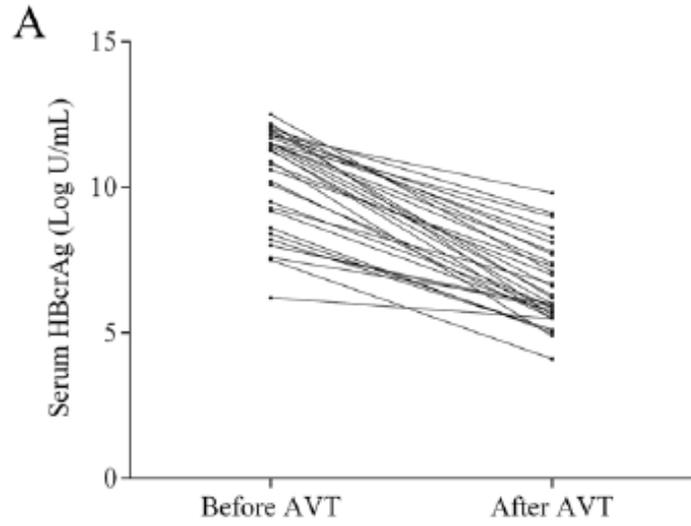
Efficacy of Current FDA-approved CHB Rx



HBs antigen, HBV core-related antigen, total (HBcr Ag or tHBcrAg), HBV pgRNA

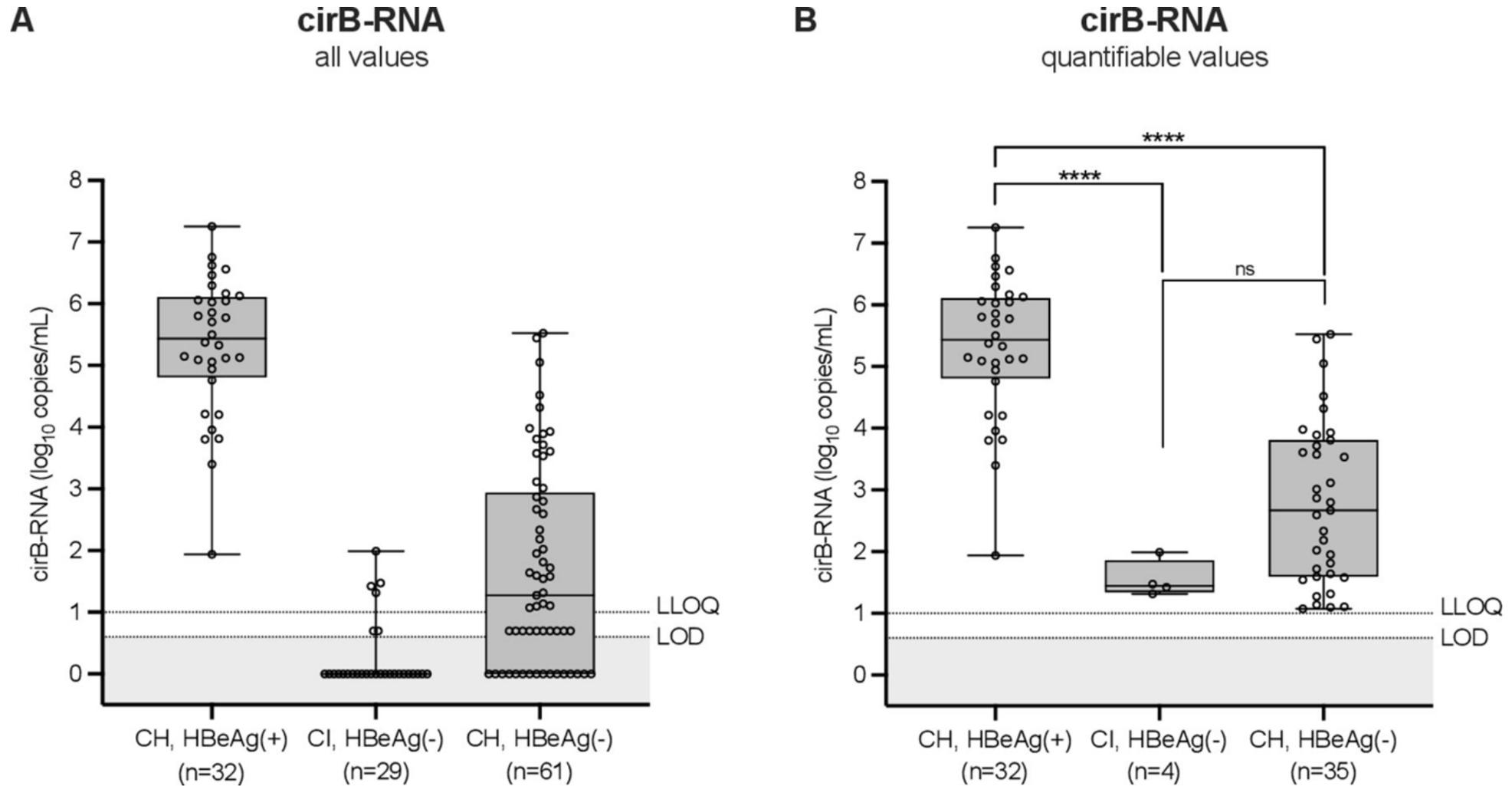


Correlation of HBsAg Quant and HBcrAg Quant with HBV cccDNA



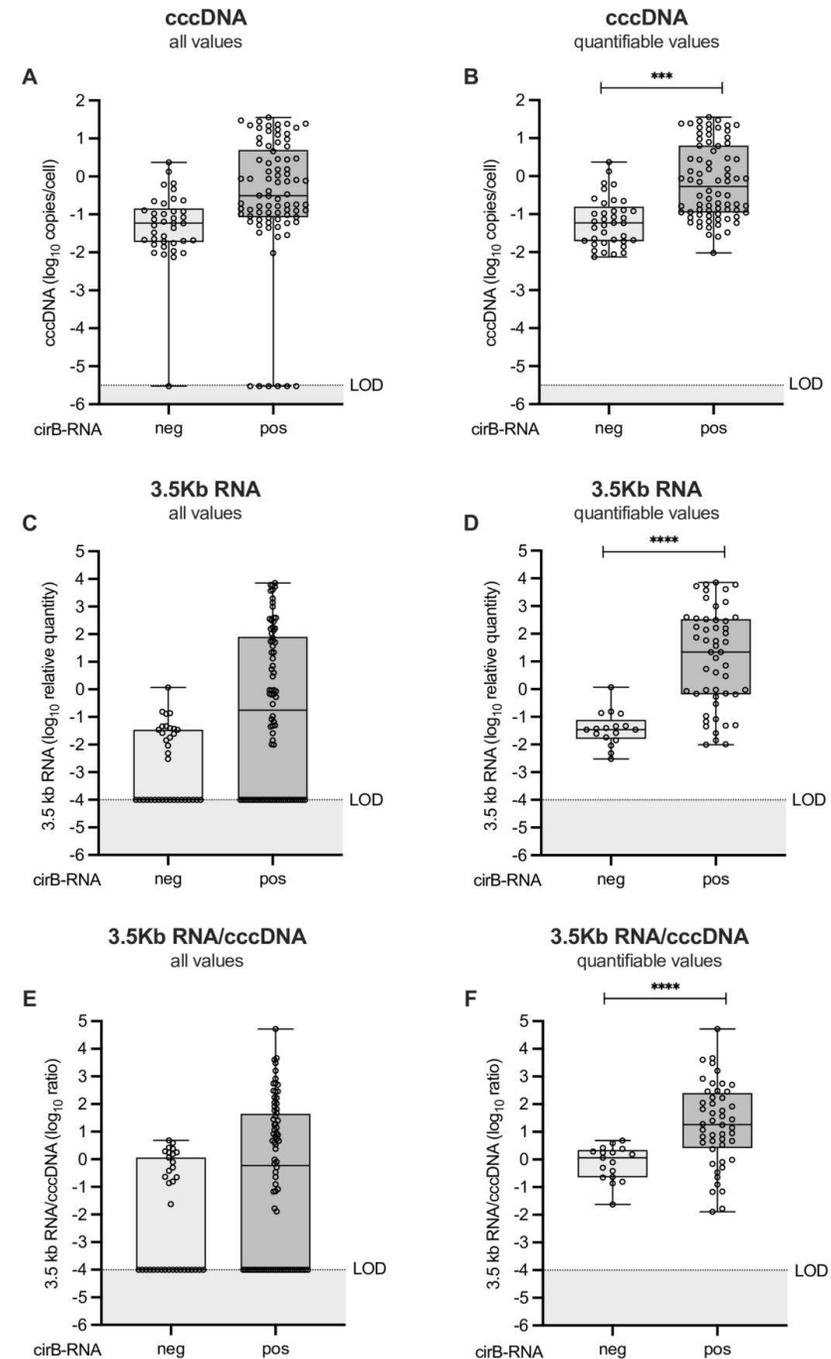
Chen E-Q, et al. *Sci Rpt* 2017; 7:173.
<https://www.nature.com/articles/s41598-017-00111-0>

HBV pgRNA Distribution per HBeAg Status in Untreated CHB



Testoni B, et al. *Gut* 2023; 73:659-667. <http://dx.doi.org/10.1136/gutjnl-2023-330644>

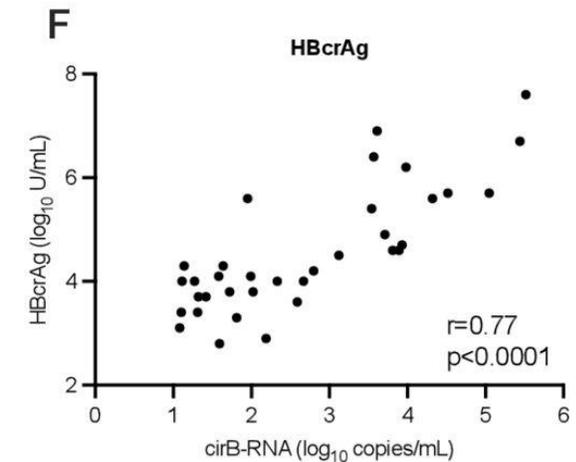
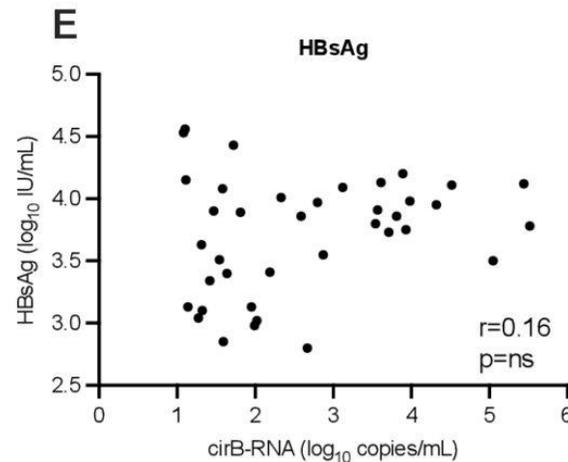
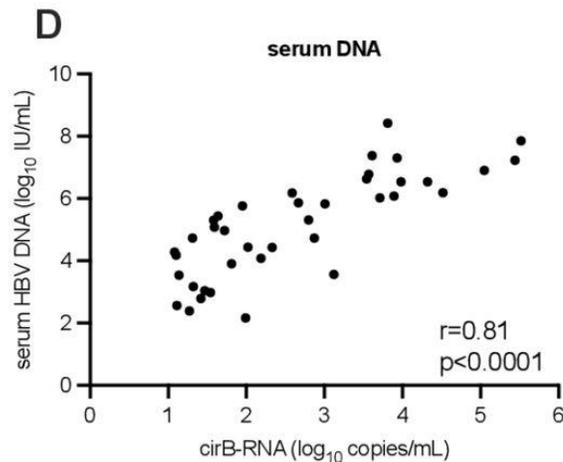
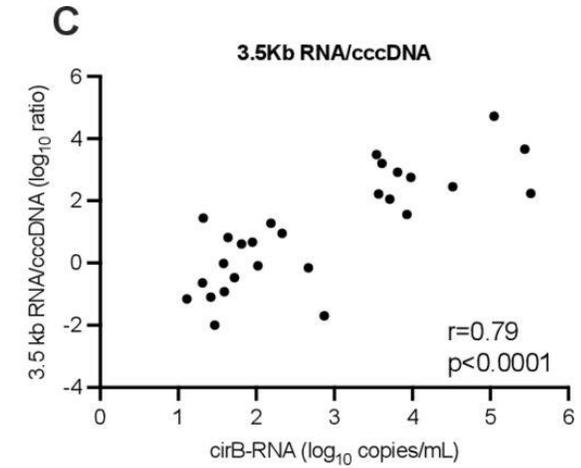
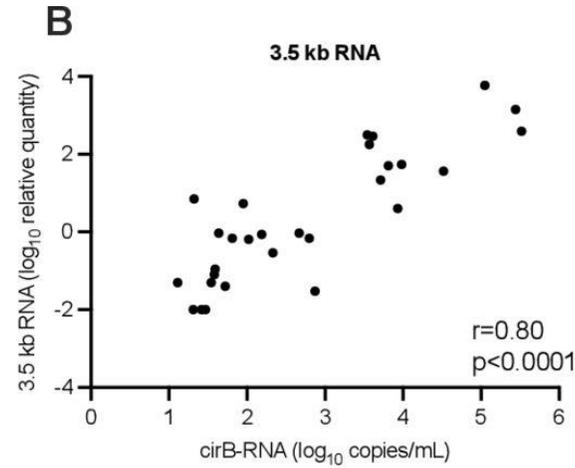
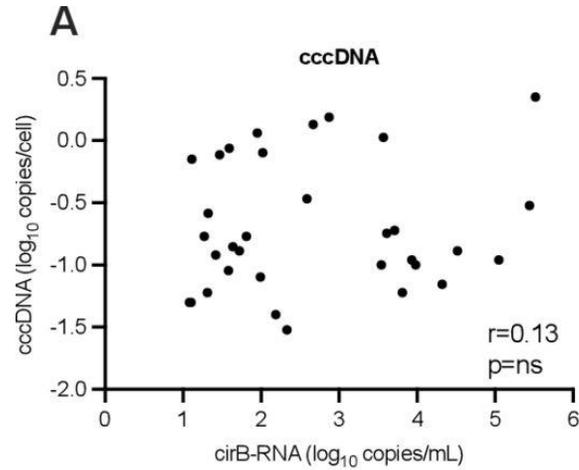
Correlation of Intrahepatic HBV Markers (cccDNA & 3.5 kb RNA) to Circulating HBV pgRNA in Untreated CHB



Testoni B, et al. *Gut* 2023; 73:659-667.

<http://dx.doi.org/10.1136/gutjnl-2023-330644>

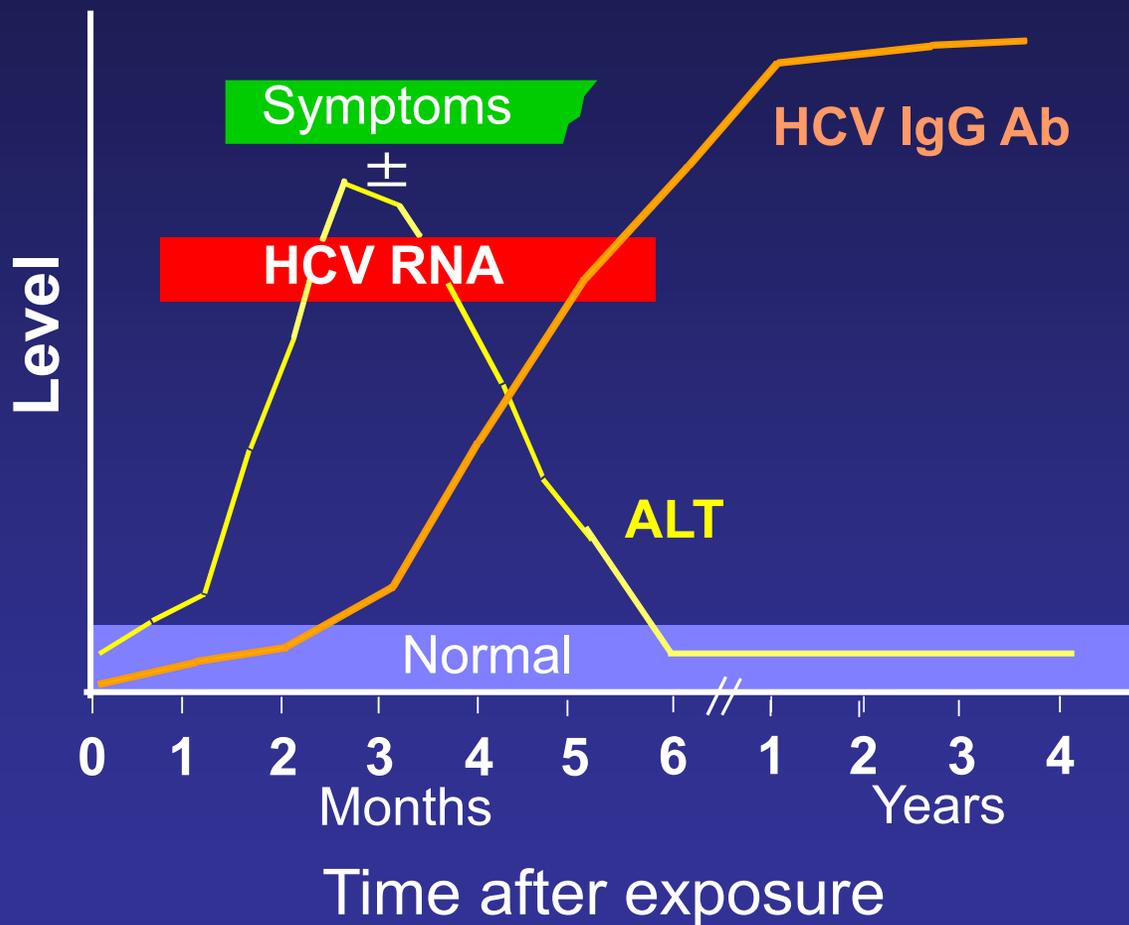
Correlation of circulating HBV pgRNA to cccDNA, 3.5 kb RNA, HBsAg, HBcrAg in Untreated HBeAg(-) CHB



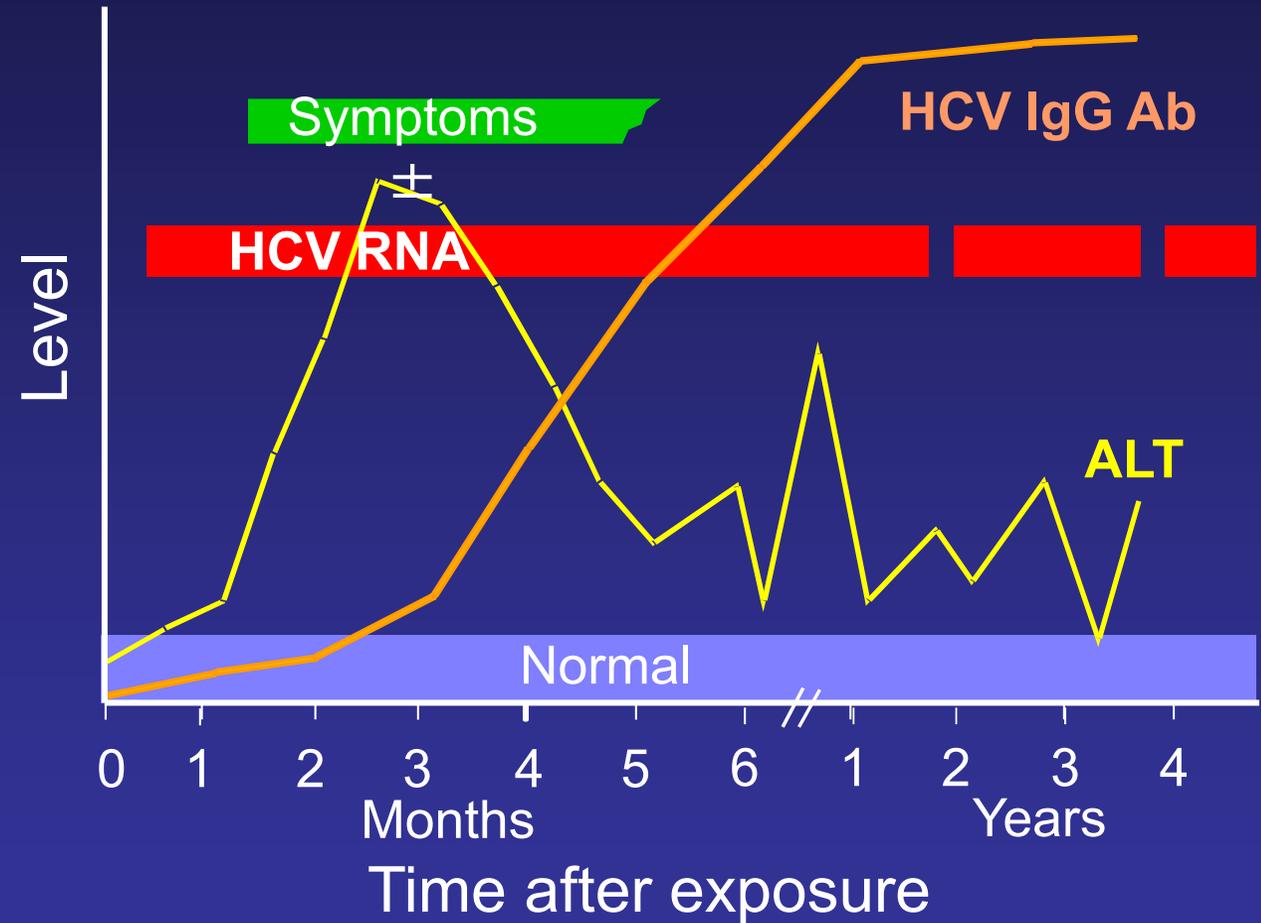
Testoni B, et al. *Gut* 2023; 73:659-667. <http://dx.doi.org/10.1136/gutjnl-2023-330644>

Outcome of Acute Hepatitis C

Spontaneous resolution in 25%



Chronic infection in 75%



Hepatitis C

Currently available laboratory tests

HCV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HCV Ab (IgG or total)	EIA, CIA	IVD	G0472 or 86803	\$46.35 \$14.27
HCV RNA (qual)	PCR (PoCT)	IVD	G0567	\$35.09
HCV RNA (quant)	EIA, CIA	IVD	87522	\$12.39
HCV core antigen	EIA, CIA	CE-IVD	N/A	N/A
HCV cAg + IgG Ab	CIA	CE-IVD	N/A	N/A

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

HCPCS G0472, G0567: HCV screening for high-risk individuals and other covered indication(s) under current CMS coverage policy.

Current HCV Screening Recommendations in U.S.

AASLD-IDSA (2022-10-24) ^a

- One-time, routine opt-out testing in all aged ≥ 18 years
- HCV Ab testing with reflex to HCV RNA

CDC (2020-4) ^b

- Universal screening (at least once in a lifetime) in all aged ≥ 18 years
- Single-visit blood collection for HCV Ab testing with reflex to HCV RNA ^c

U.S. Preventive Services Task Force (2020-3) ^d

- Screen all asymptomatic adults aged 18 to 79 years
- HCV Ab testing with reflex to HCV RNA

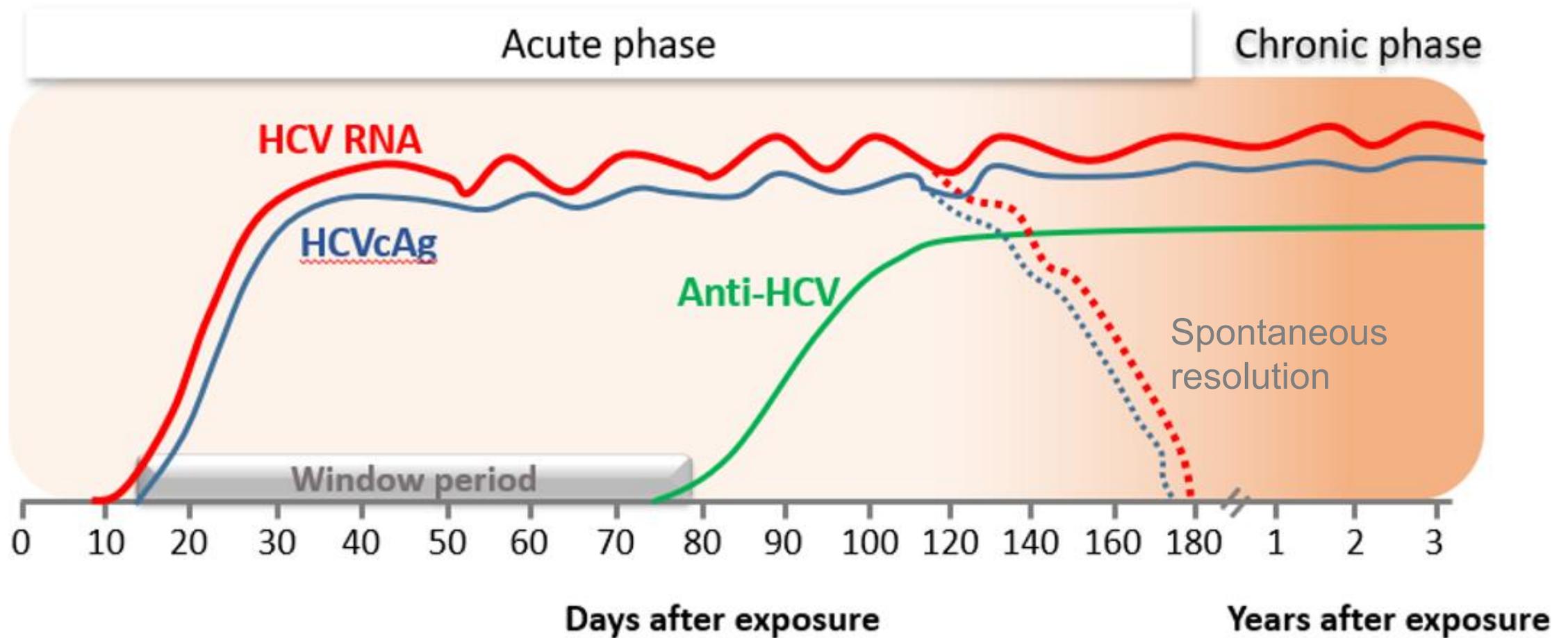
^a <https://www.hcvguidelines.org/evaluate/testing-and-linkage>

^b CDC. *MMWR Recomm Rep* 2020; 69(No. RR-2):1-17. <https://www.cdc.gov/mmwr/volumes/69/rr/rr6902a1.htm>

^c CDC. *MMWR* 2023; 72(28):766-768. <https://www.cdc.gov/mmwr/volumes/72/wr/mm7228a2.htm>

^d Owens DK, et al. *JAMA* 2020; 323:970-975. <https://doi:10.1001/jama.2020.1123>

Temporal profile of HCV infection markers



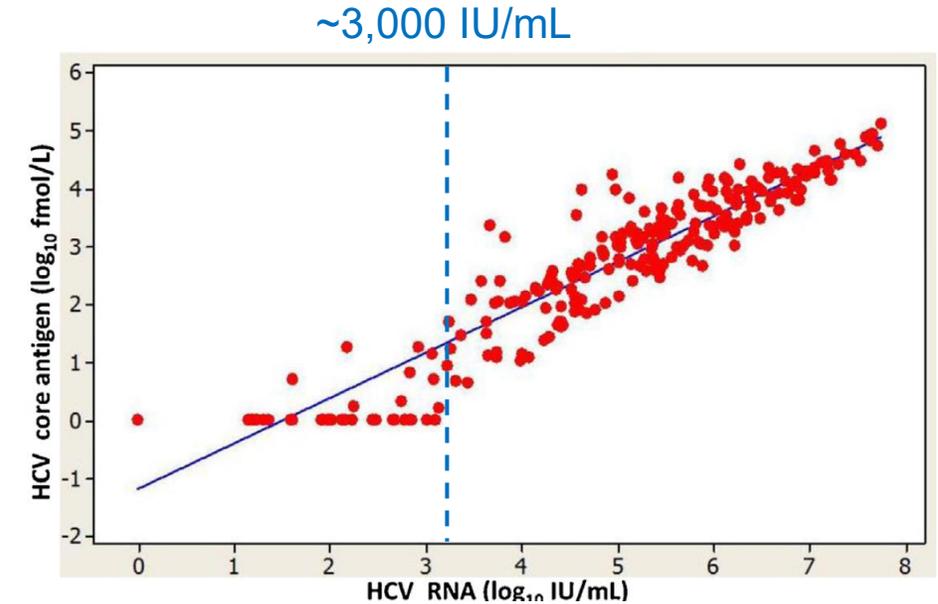
Courtesy of Nathan W. Furukawa, M.D., M.P.H., Centers for Disease Control and Prevention, Atlanta, GA.

HCV core Ag assays

Test performance

Systematic review of HCV cAg vs RNA detection

HCV RNA status	HCV cAg (+)	HCV cAg (-)
RNA (+)	3,339 (41.04%)	286 (3.52%)
RNA (-)	46 (0.57%)	4,461 (54.83%)
Indeterminate	4 (0.05%)	



- False (-) HCV cAg results may be due to poor sensitivity or interference from HCV Ab during seroconversion
- Good correlation between HCV cAg and HCV RNA down to ~3,000 IU/mL of HCV RNA. Sensitivity dropped to <25% at <3,000 IU/mL (Abbott Architect HCV cAg CE-IVD).

Khan H, et al. *Open Forum Infect Dis* 2017; 4(2):ofw252 <https://doi.org/10.1093/ofid/ofw252>

Mixson-Hayden T, et al. *J Clin Virol* 2015; 66:15-18. <http://dx.doi.org/10.1016/j.jcv.2015.02.015>

HCV cAg + Ab Combination Assays

Detection of HCV cAg and Ab with Elecsys HCV DUO ECLIA)

Standard of care HCV results	Ab-/RNA-		Ab+/RNA+		Ab-/RNA+		Ab+/RNA-	
	N = 671		N = 260		N = 7		N = 403	
Elecsys HCV Duo results	Detected	Not detected	Detected	Not detected	Detected	Not detected	Detected	Not detected
Overall detection rates, n (% total)	1 (0.1) ^a	670 (99.9)	260 (100)	0	5 (71)	2 (28.6)	374 (93)	29 (7) ^e
Detected only on Ab component, n (% total)	1 (0.1) ^a	0	65 (25) ^b	0	3 (43) ^c	0	372 (92)	0
Detected only on Ag component, n (% total)	0	0	0	0	0	0	0	0
Detected on both Ab and Ag, n (% total)	0	0	195 (75)	0	2 (29)	0	2 (0.5) ^d	0

^aNonreactive on repeat testing.

^bAn additional 10/65 samples were reactive for both Ab and Ag on repeat testing.

^cOne of three samples were not detected on repeat testing.

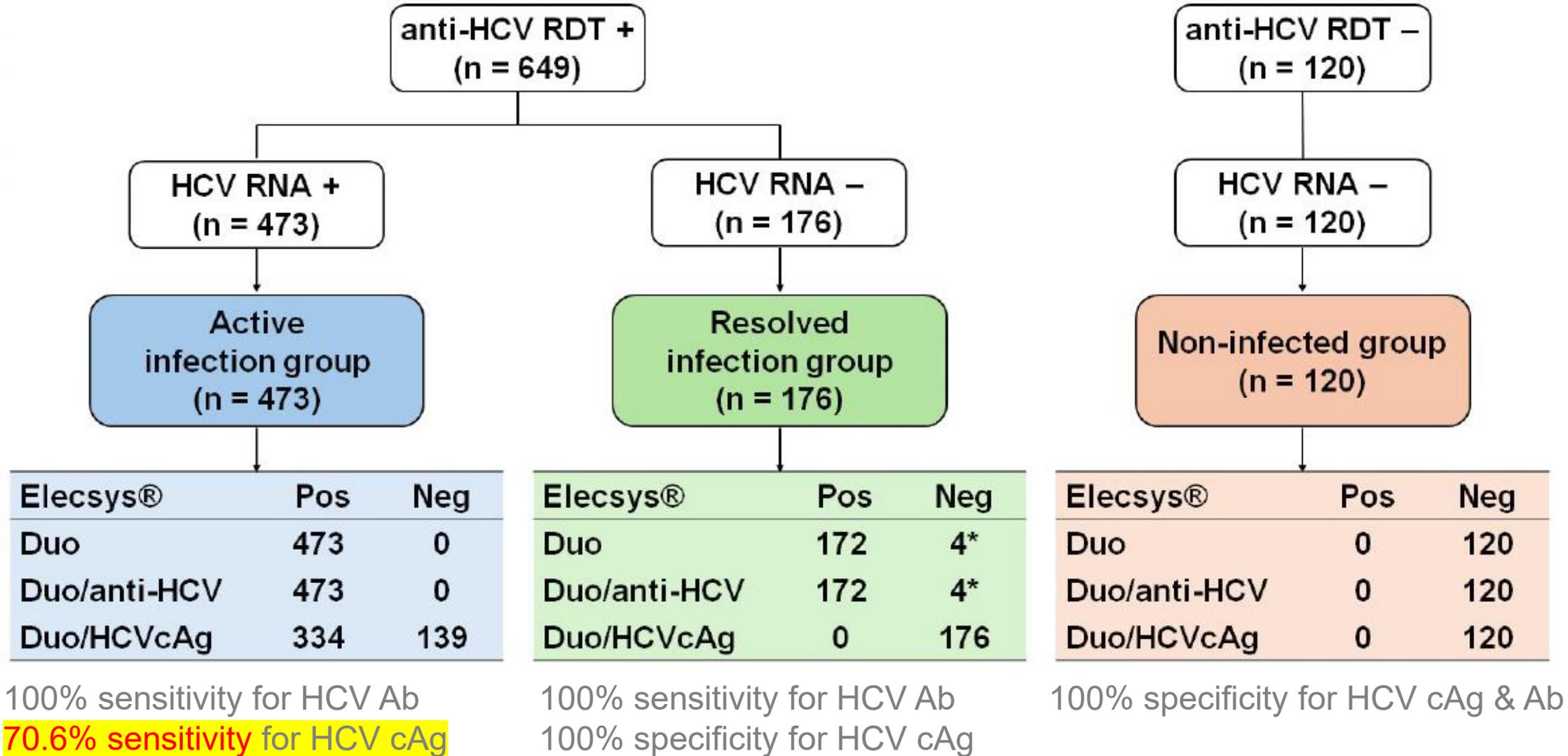
^dThese two samples were Ab reactive but Ag nonreactive upon repeat testing.

^eOne of 29 samples were reactive for Ab on repeat testing.

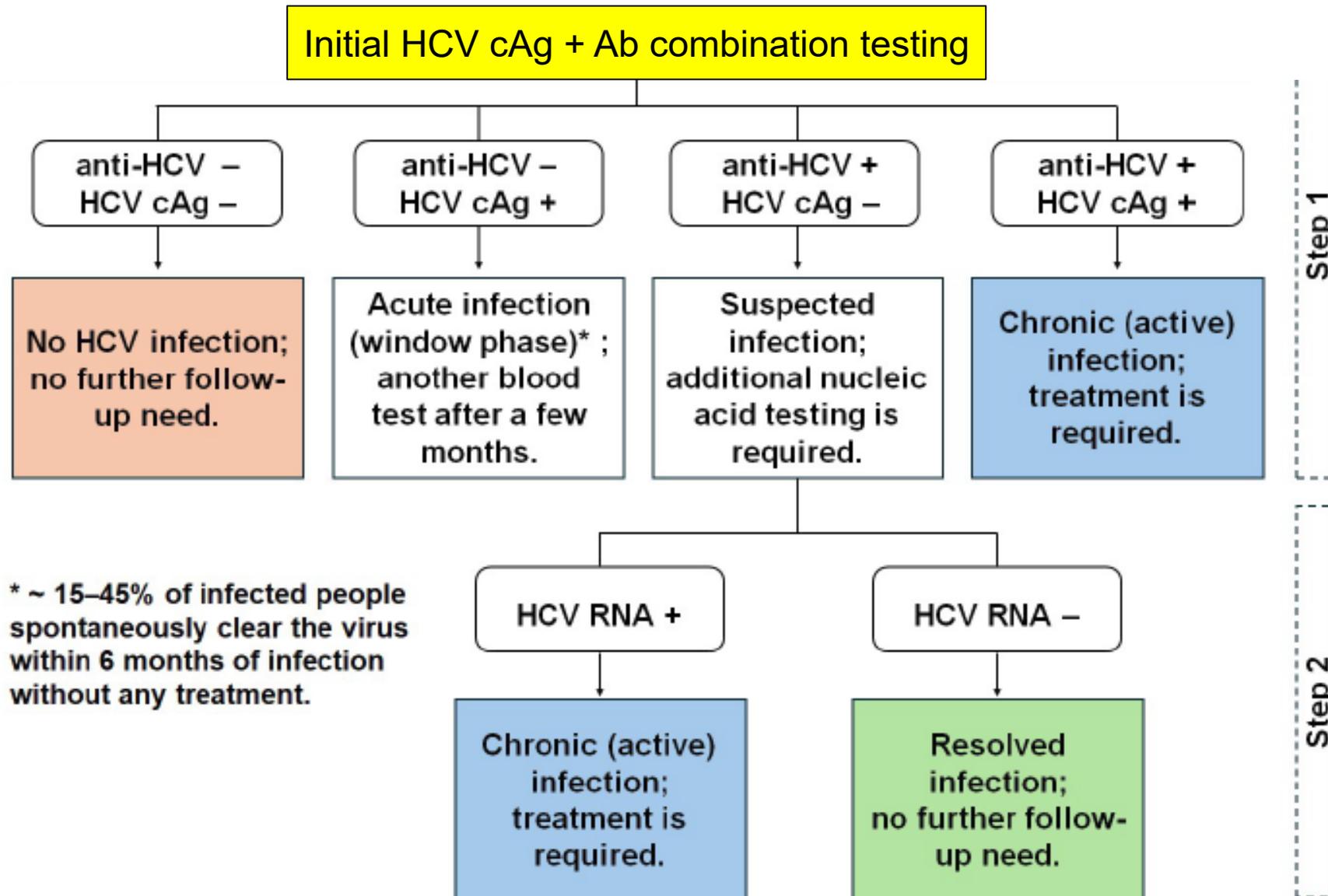
Result agreement	COI cutoff ≥ 1.00		COI cutoff ≥ 0.70	
	HCV Ab	HCV cAg	HCV Ab	HCV cAg
PPA	95.6%	73.5%		81.5%
NPA	99.1%	99.8%		99.7%

Bui T, et al. *J Clin Microbiol* 2024; 62(10):e99832-24. <https://journals.asm.org/doi/10.1128/jcm.00832-24>

HCV cAg + Ab Combination Assays



Diagnostic Use of HCV cAg + Ab Combination Assays



HCV RNA (Qualitative) Assay for HCV Screening

HCV genotype	Xpert® HCV LoD in fingerstick whole blood (IU/mL)	95% CI
1a	35.0	22.3 – 54.9
1b	41,5	29.8 – 57.9
2b	89.2	47.7 – 166,8
3a	58.2	39.0 – 86.7
4	32.2	21.1 – 49.1
5	136.4	83.2 – 223.4
6	83.8	57.2 – 122.8

HCV RNA assay *	Manufacturer	LoD in serum (IU/mL)
Alinity m HCV	Abbott Molecular Inc.	7.96 (5.06 – 25.52)
Aptima HCV Quant Dx	Hologic Inc.	3.4 (2.3 – 5.2)
cobas HCV	Roche Molecular Systems, Inc.	9.60 (8.70 – 10.95)

https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN240016.pdf

HCV RNA (Qualitative) Test for HCV Screening

Table 14. Xpert HCV test result according to PIS

		Patient Infection Status		
		HCV Positive ^a	HCV Negative ^b	Total
Xpert HCV test	HCV detected	114	2	116
	HCV not detected	8	858	866
Total		122	860	982
PPA*		93.44%; 95% CI (87.59% – 96.64%)		
NPA*		99.77%; 95% CI (99.16% – 99.94%)		

^a Active chronic or acute infection.

^b Past/resolved infection or not infected.

PIS = Patient infection status

https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN240016.pdf

Hepatitis D (Delta Hepatitis)

Clinical presentation

Coinfection

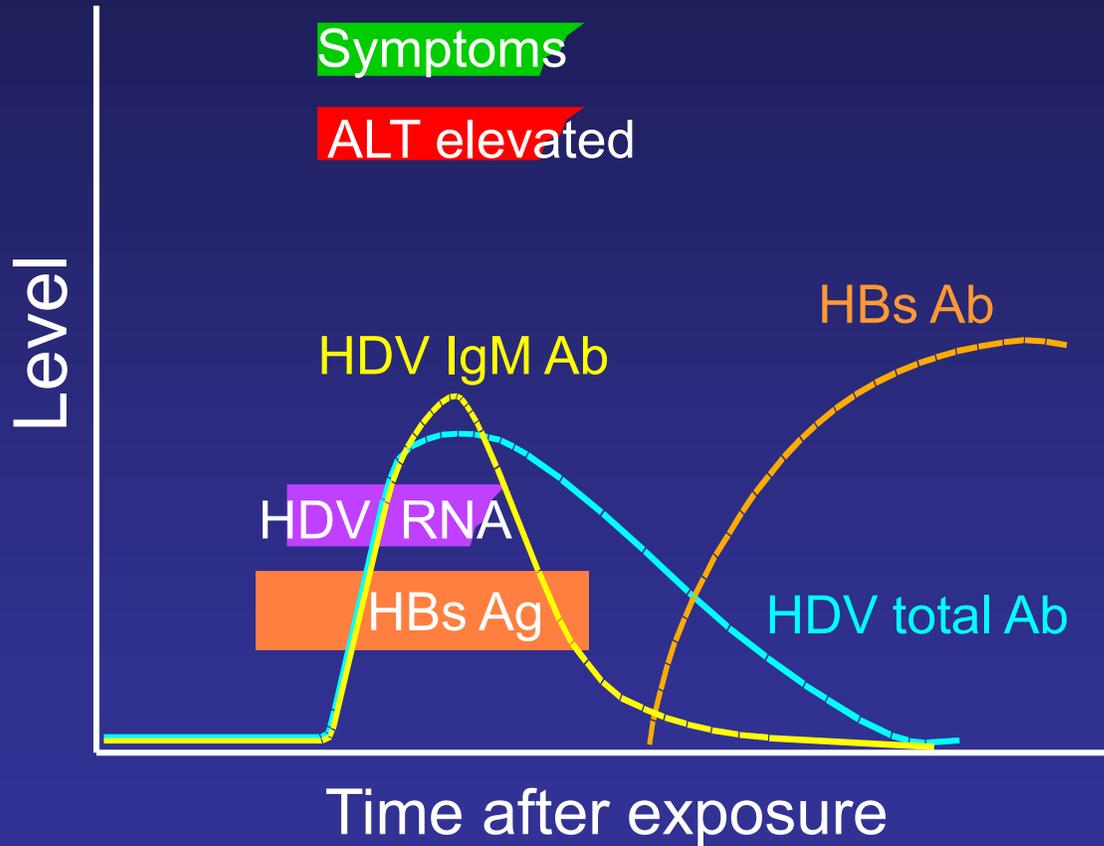
- Acute infection acquired together with acute hepatitis B
- 5% → chronic HDV

Superinfection

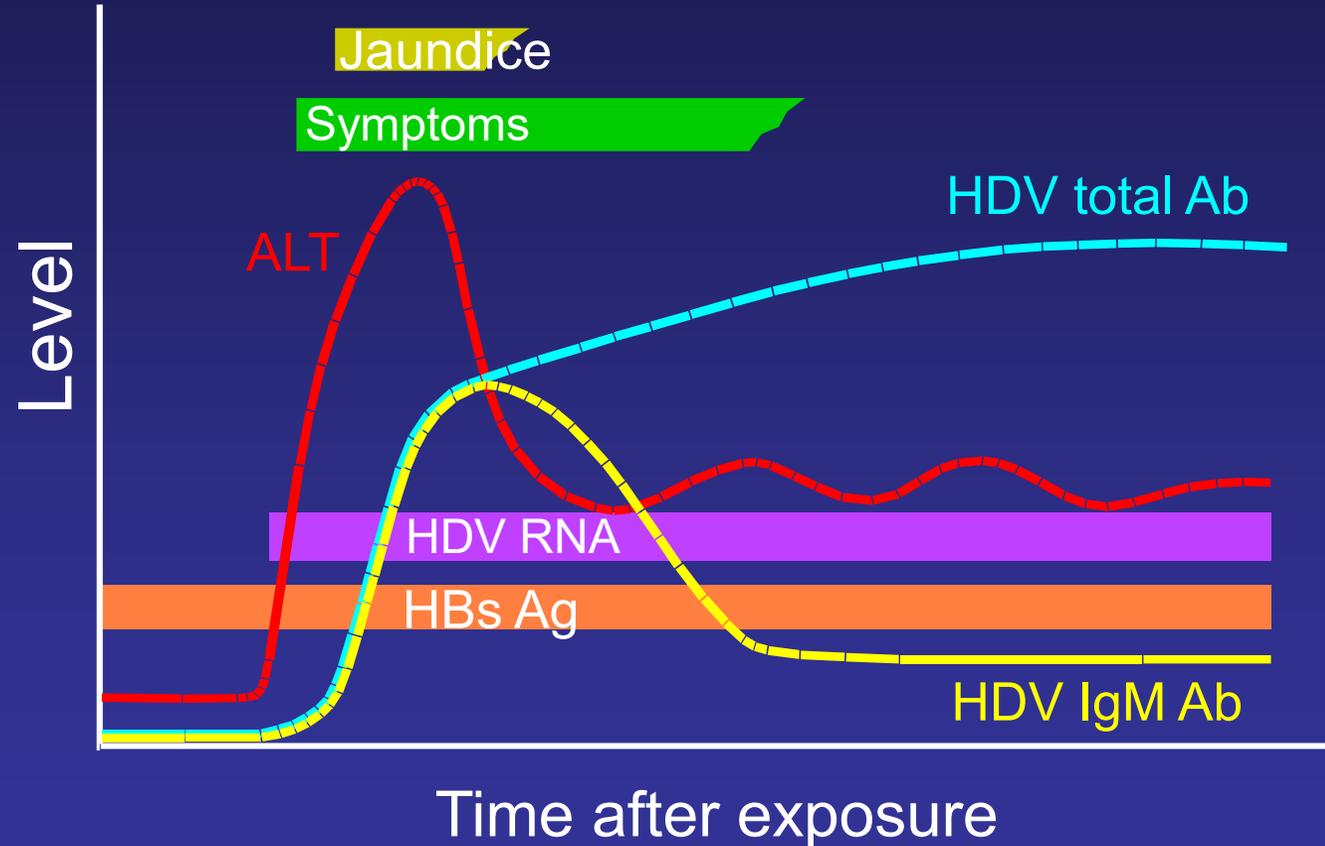
- New onset of fatigue, icterus, declining liver function in known chronic hepatitis B
- 90% → chronic HDV

Profiles of HDV Infection Markers

HBV - HDV coinfection



HBV - HDV superinfection



Hepatitis D

Currently available laboratory tests

HDV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HDV antigen	EIA, CIA	CE-IVD, LDT	87380	\$18.36
HDV IgM Ab	EIA, CIA	CE-IVD, LDT	86692	\$17.16
HDV IgG Ab	EIA, CIA	CE-IVD, LDT	86692	\$17.16
HDV Total Ab	PCR	CE-IVD, LDT	86692	\$17.16
HDV RNA (quant)	PCR	CE-IVD, LDT	87523	\$42.84

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

CPT 86692: Analysis for antibody to hepatitis D virus

Current Problems in Laboratory Tests for Hepatitis D

- No FDA-cleared / -approved serologic or molecular tests
- Wide differences in assay sensitivity & specificity: HDV Ag assays are not as sensitive as HDV RNA testing → acute or chronic HDV may be missed
- Automatically / reflexively test all HBs Ag+ serum / plasma specimens for HDV total Ab and subsequently HDV RNA ?
 - APASL 2025; EASL 2025: All HBsAg+ individuals be tested for HDV
 - WHO 2024; AASLD 2018 & 2025: High-risk HBsAg+ (IDU, HCV- or HIV-infected, hemodialysis pts, MSM, commercial sex workers, cirrhosis, persistent 2x ALT levels with low or undetectable HBV DNA

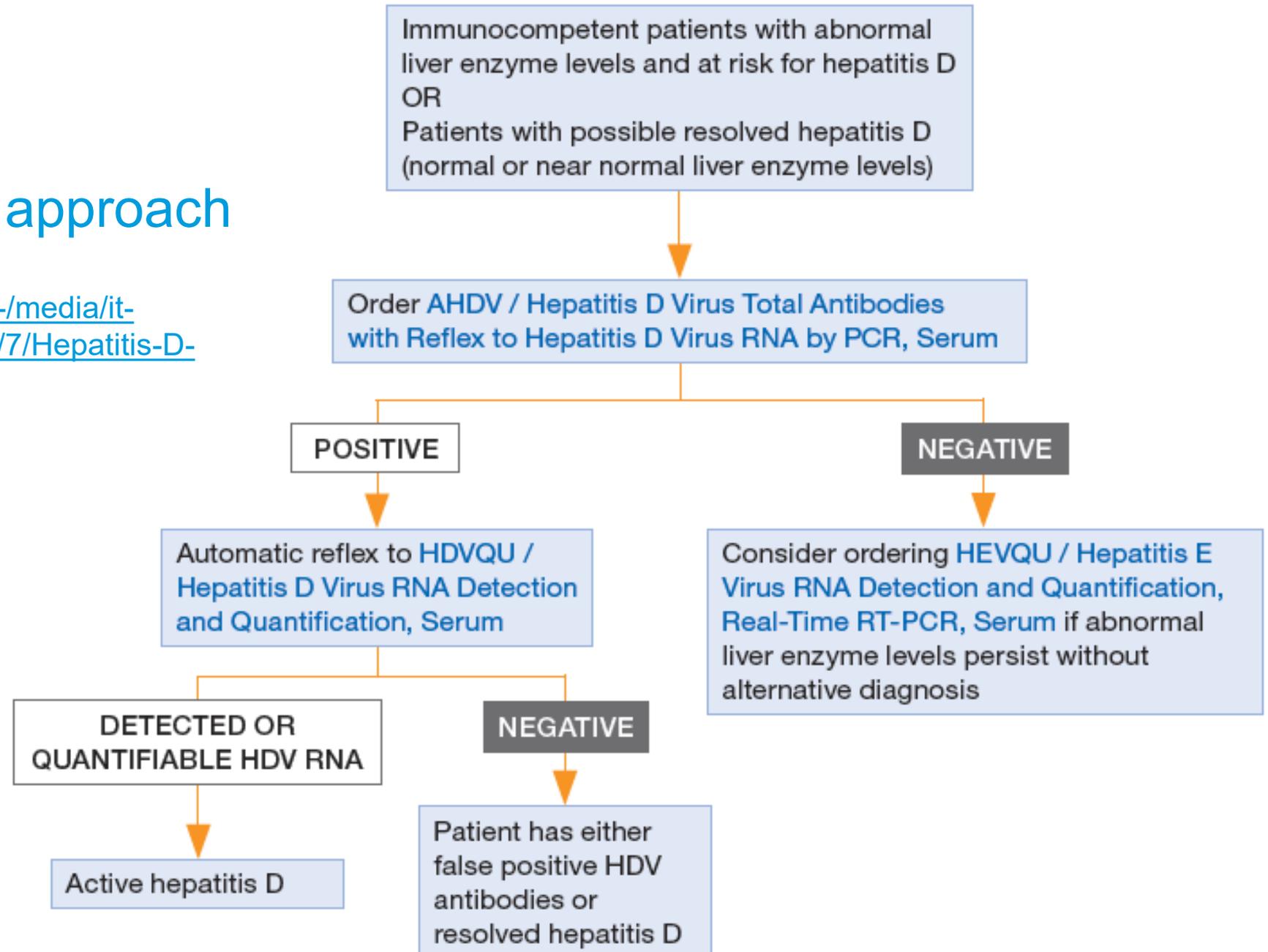
https://journals.lww.com/hep/fulltext/9900/aasld_isda_practice_guideline_on_treatment_of.1416.aspx

<https://iris.who.int/server/api/core/bitstreams/34470cc8-af90-4d7b-a949-ef27e5d0726f/content>

Hepatitis D (Delta Hepatitis)

Laboratory testing approach

<https://www.mayocliniclabs.com/-/media/it-mmfiles/Special-Instructions/E/B/7/Hepatitis-D-diagnostic-testing-algorithm>



Hepatitis E

Clinical features

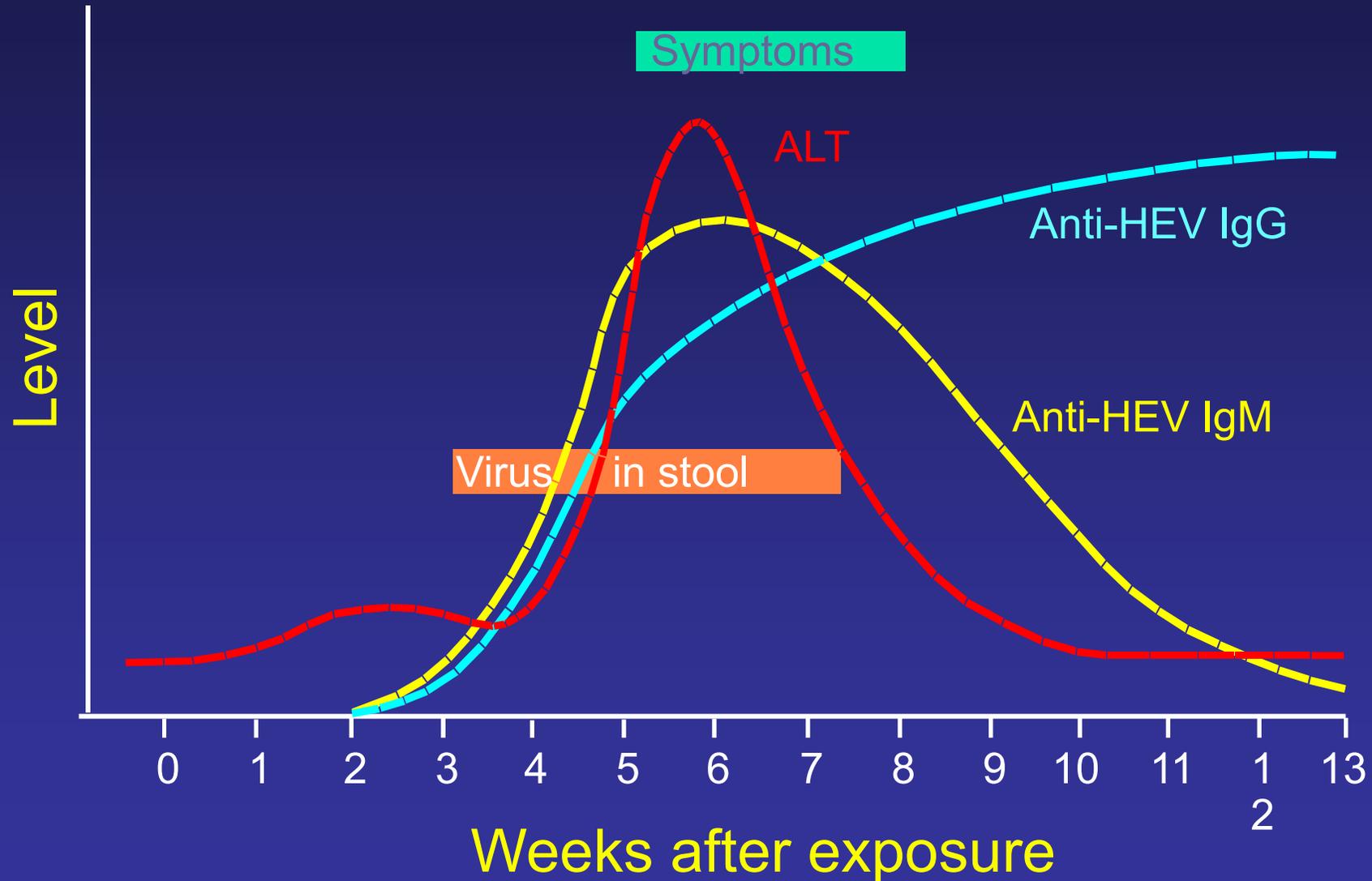
- Risk factors:** Exposure to contaminated food & water
Consumption of poorly cooked pork
- Illness severity:** Asymptomatic in 50% cases
Severity ↑ with age at time of infection –
neurologic, renal, & hematologic abnormalities
- Case-fatality rate:** Overall 1 - 3%
Pregnant women ~30%
- Chronic sequelae:** Lifelong immunity in immunocompetent
Chronic infection in organ Tx recipients

<https://www.who.int/news-room/fact-sheets/detail/hepatitis-e>

<https://www.cdc.gov/hepatitis-e/about/index.html>

Hepatitis E Virus Infection

Typical Serologic Course



Hepatitis E

Currently available laboratory tests

HEV markers	Test method	Device regulatory status	CPT code	CMS reimbursement rate (1Q 2026)
HEV IgM Ab	EIA, CIA	CE-IVD, LDT	86790	\$12.88
HEV IgM Ab confirmation	EIA, CIA	CE-IVD, LDT	86790	\$12.88
HEV IgG Ab	EIA, CIA	CE-IVD, LDT	86790	\$12.88
HEV RNA (quant)	PCR	CE-IVD, LDT	87799	\$42.84

EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay.

CE-IVD, Conformité Européenne – In Vitro Diagnostic; LDT, laboratory-developed test

CPT 86790: Analysis for antibody to virus

Laboratory Testing for Hepatitis E

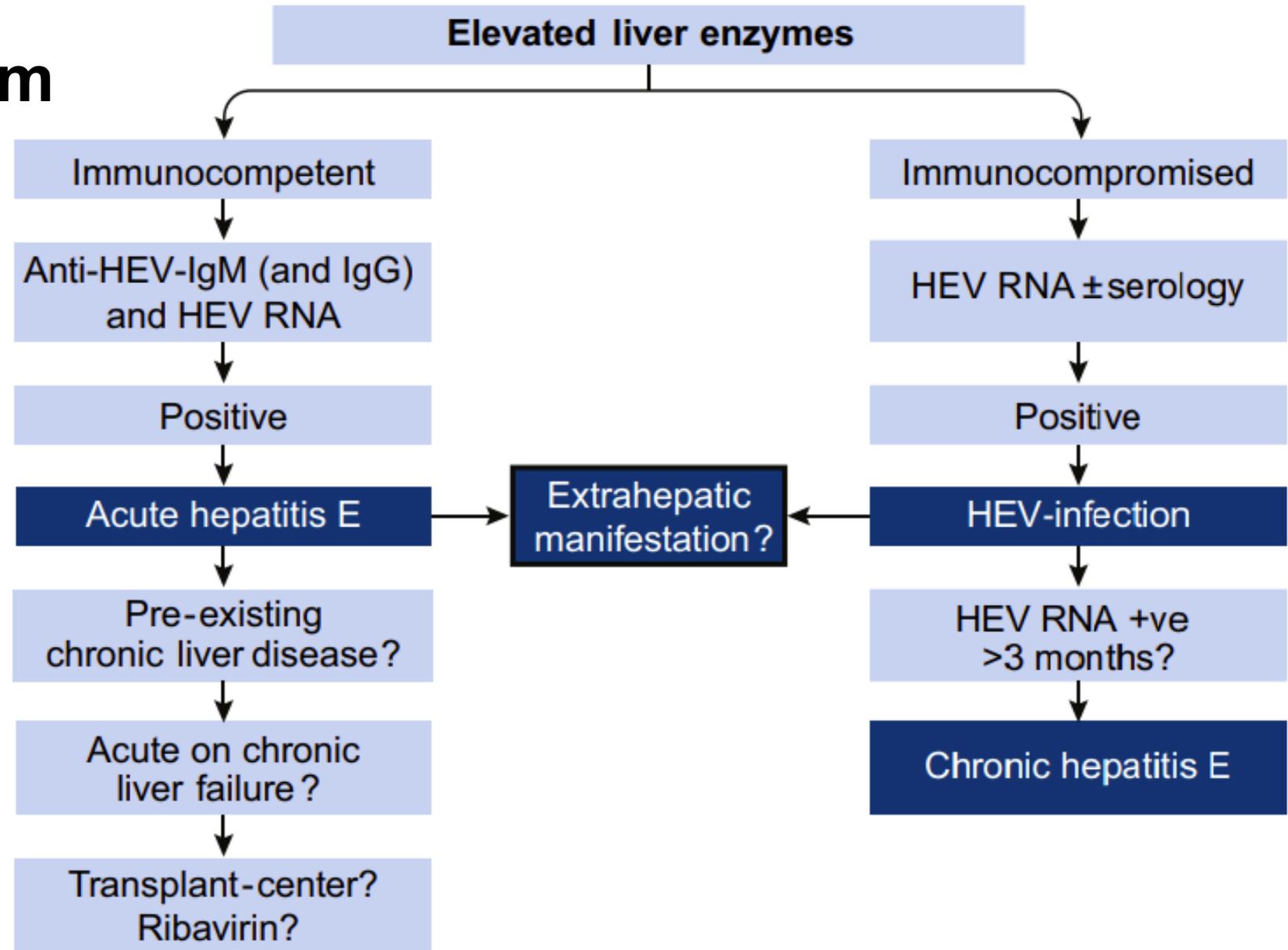
- No FDA-cleared / -approved serologic or molecular tests
- Wide differences in sensitivity and specificity among current serologic assays
- *Recommendations*
 - EASL 2018: Chronic liver disease with unexplained flares, all immunocompromised individuals with unexplained elevated ALT

<https://www.journal-of-hepatology.eu/action/showPdf?pii=S0168-8278%2818%2930155-7>

<https://www.who.int/news-room/fact-sheets/detail/hepatitis-e>

<https://www.cdc.gov/hepatitis-e/about/index.html>

2018 EASL Diagnostic Algorithm for Hepatitis E



Summary

- Beware of false-positive HAV IgM Ab results and possible causes
 - Testing for HAV IgM Ab should be based on presence of acute hepatitis presentation + epidemiologic exposure history
- New, upcoming HBV markers to help monitor and determine functional and possible virologic cure: HBs Ag quant, HBcr Ag quant, HBV pgRNA
- Uncertain future availability of HCV cAg / Ab combo test for screening in U.S.; viral-first testing to screen asymptomatic individuals ?
- Individuals with chronic hepatitis B and at risk for HDV should be screened for hepatitis D; all HDV total Ab+ specimens should auto-reflex to HDV RNA.
- Immunocompromised Individuals with unexplained elevated ALT should be tested for HEV IgM Ab + HEV RNA

Post-Test Question 1

Which of the following viral hepatitis **does / do not** have FDA-cleared or FDA-approved **serologic tests** available for routine clinical use?

1. Hepatitis A
2. Hepatitis B
3. Hepatitis C
4. Hepatitis D
5. Hepatitis E
6. Combination of above

Post-Test Question 2

Which of the following viral hepatitis **does / do not** have FDA-cleared or FDA-approved **molecular tests** available for routine clinical use?

1. Hepatitis A
2. Hepatitis B
3. Hepatitis C
4. Hepatitis D
5. Hepatitis E
6. Combination of above

Questions & answers

