Anti-HBc: state of the art
what is the CORE of the issues?

Robert G Gish MD
Adjunct Professor
Stanford University

Medical Director
Hepatitis B Foundation

Hepatitis B Foundation
Cause for a Cure

Disclosures:

See robertgish.com

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or go to my website

robertgish.com

Updated from : Alter, M.; CDC Atlanta, GA, 2002

Epidemiology - Worldwide

2 Billion People have HBV disease defined as HBV DNA / cccDNA in their liver

have serologic evidence of past or present HBV infection, anti-HBc +

257 Million HBsAg(+) Carriers WW and up to 2.2 M in the US

are chronically infected with HBV

1 Million People or more WW

die each year from HBV-related Chronic Liver Diseases

Misinformation (AF/FN)

“HBV is curable” (“functional cure” is an oxymoron) naturally or using current therapies

“You should boost anti-HBs in an isolated anti-HBc (+) patient with vaccine for “protection””

There is a “natural immunity” to HBV (anti-HBc {+} and anti-HBs{+}) (CDC website accessed 2017) (another oxymoron)

“One does not need to test anti-HBc in specific patient populations” (CDC website accessed 2017)

Anti-HBc (+) (HBsAg(-)) patients are often referred to GI/ID/Liver providers for HBV “treatment”

Resolved HBV ≠ Sterilizing cure

HBV testing: the basics

HBsAg(+) = infection

Anti-HBc(+) = exposure

Anti-HBs(+) = immunity {if anti-HBc(-)}

Since HBV is incurable, once a patient is infected, the patient will develop chronic infection or occult disease/resolved disease and anti-HBc is the marker of this persistent life-long infection and production of viral proteins including HBcAg

Interpretation of Hepatitis B Serologic Test Results

HBsAg anti-HBc anti-HBs	negative negative negative	Susceptible
HBsAg anti-HBc anti-HBs	negative positive positive	Immun atural infection
HBsAg anti-HBc anti-HBs	negative negative positive	Immune due to hepatitis B vaccination
HBsAg Anti-HBc IgM/ anti-HBc total Anti-HBs	positive positive positive Negative	Acutely infected
HBsAg Anti-HBc IgM anti-HBc Total Anti-HBs	positive negative positive negative	Chronically infected

Adapted from: A Comprehensive Immunisation Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunisation Practices. Part 1: Immunisation of infants, Children, and Adolescents. MMWR 2005:54 (No.RR-16)

Definitions:
Occult B Infection

OBI

HBsAg (-)

Anti-HBc(+) or (-)

HBV DNA (+) in blood (or in tissue) “Resolved HBV”

OBI

Acute HBV >> Chronic HBV as part of the opioid crisis
Many new HBsAg + cases due to recent IVDA

Anti-HBc+ patients all have HBV in their liver

OBI

Anti-HBc + is 6% of US population

7-10% of anti-HBc (+) will have measurable HBV DNA

7% of those have HBV DNA+

+1.2 M people

Sensitivity of HBsAg assay is 0.05 IU/mL

New assays measure to 0.005 IU/mL
How may of the 6% of the US population who are anti-HBc (+) will have HBsAg by the more sensitive assay?

What data supports that HBV is incurable?

Reactivation with HCV DAAs, immune suppression, chemotherapy, B-cell depletion has a risk up to 70% of reactivation

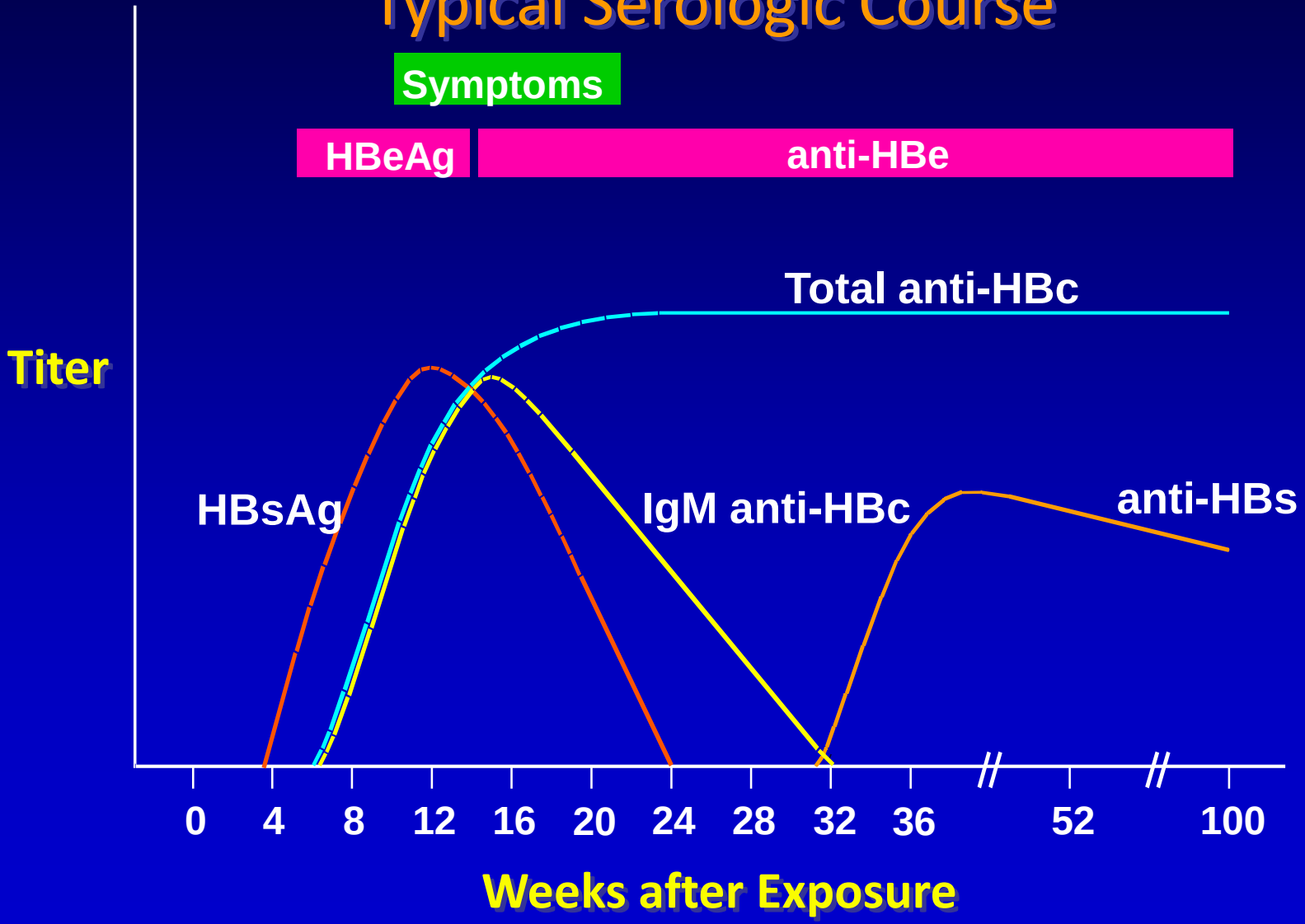
Liver transplant: Donor livers transmit HBV when the donor is anti-HBc(+) up to 100% of cases

Liver Tissue (+) for HBV DNA / cccDNA, biopsy or explant tissue in anti-HBc(+) patients

Viruses. 2017 Jun; 9(6): 156.
Published online 2017 Jun 21. doi: 10.3390/v9060156
PMCID: PMC5490831
The Role of cccDNA in HBV Maintenance
Lena Allweiss and Maura Dandri

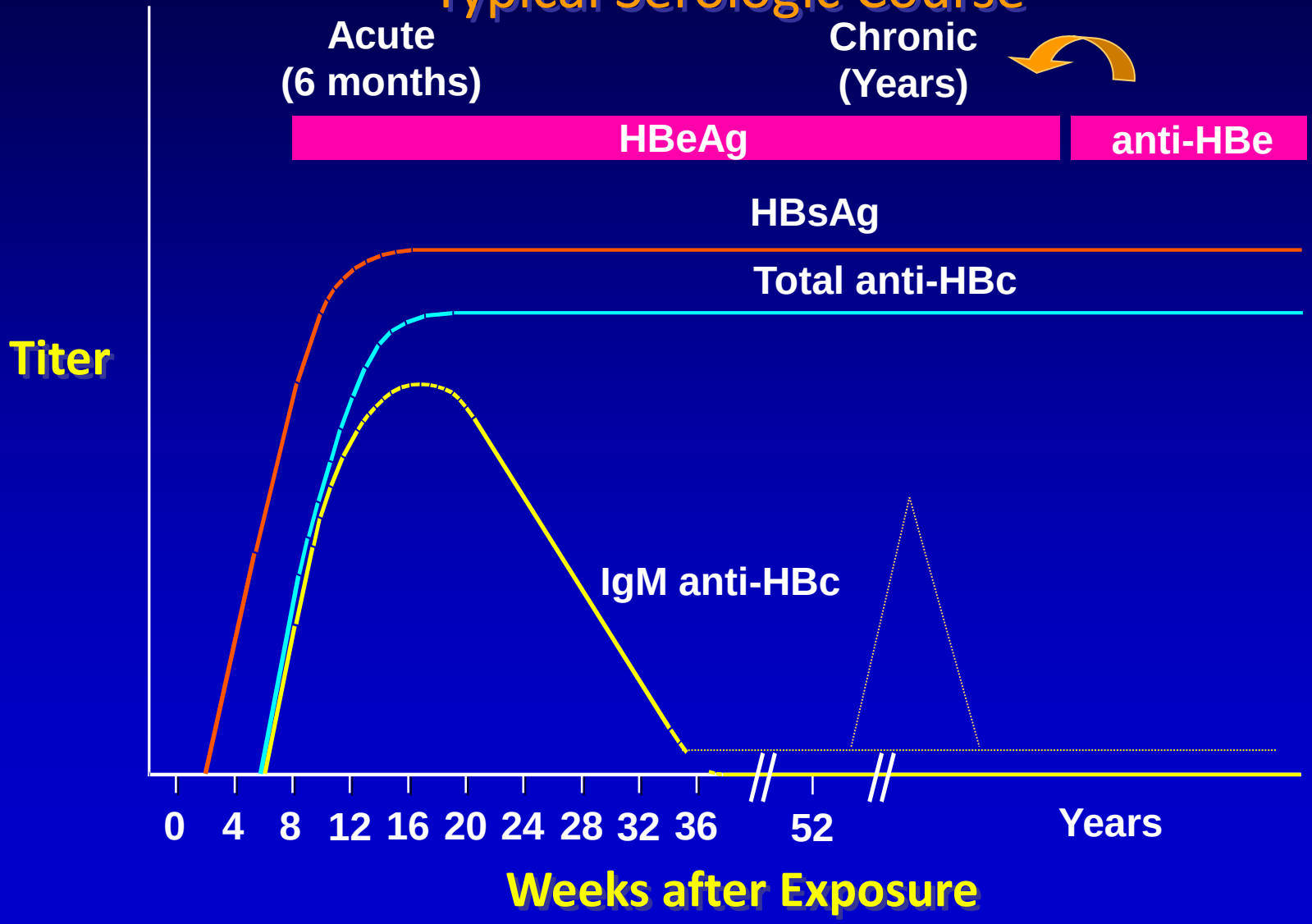
Acute Hepatitis B Virus Infection with Recovery

Typical Serologic Course

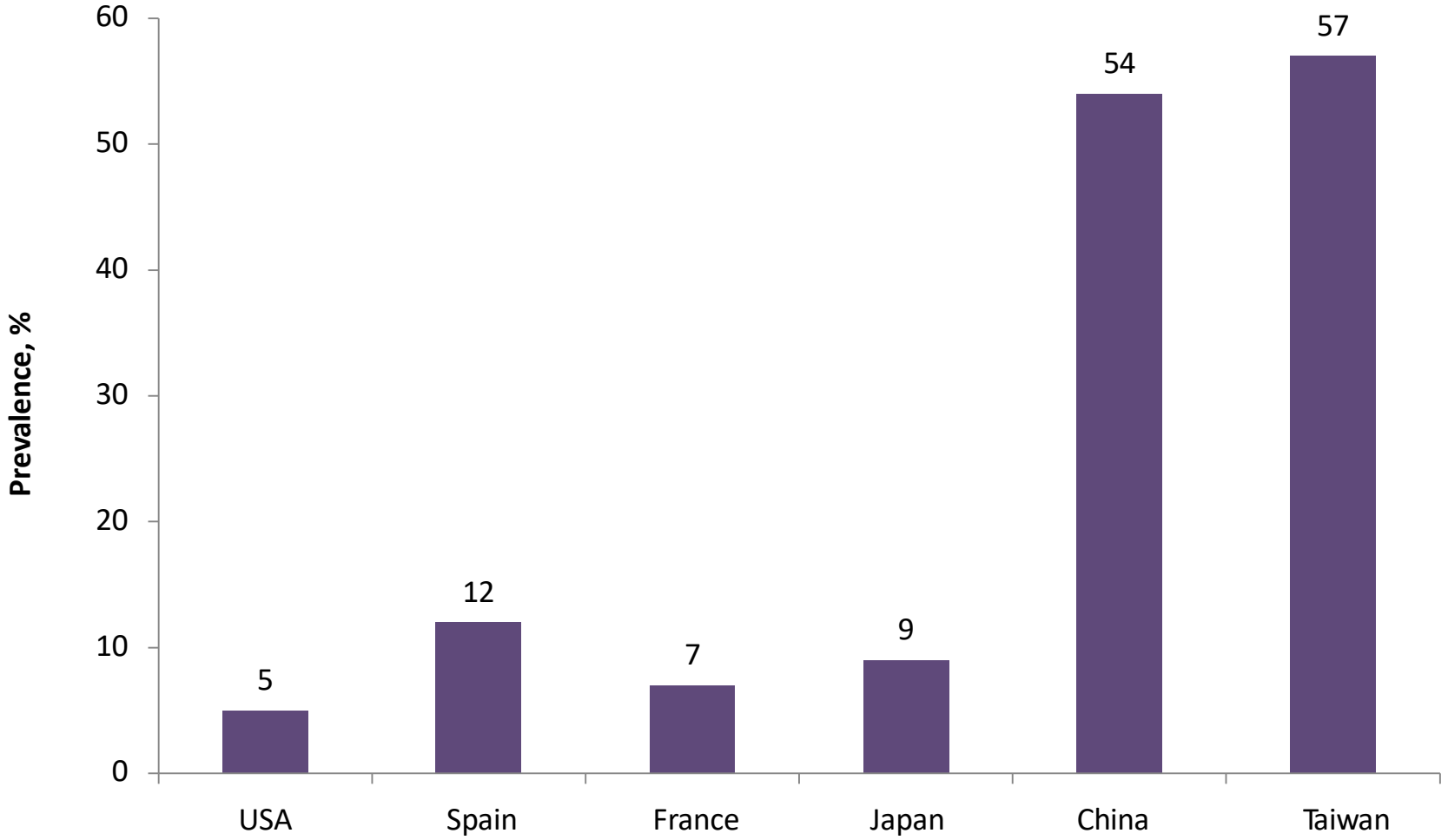


Progression to Chronic Hepatitis B Virus Infection

Typical Serologic Course



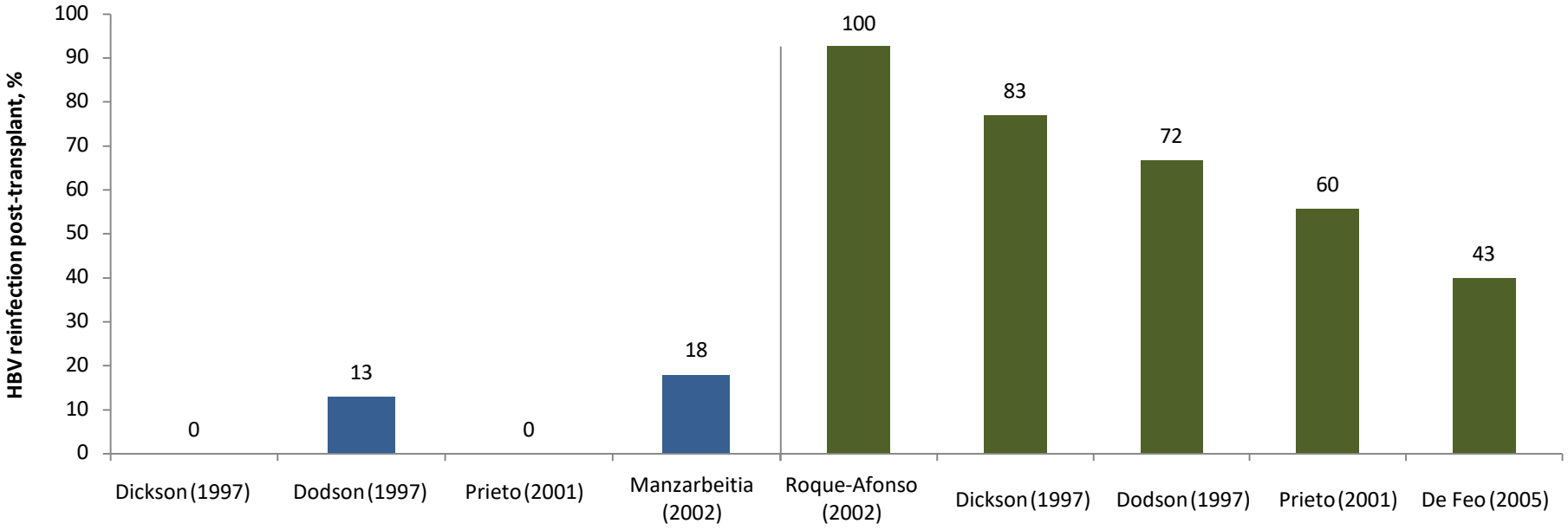
Global Prevalence of Anti-HBc Positivity among Liver Donors (Published Data)



- Multiple Studies
- Cholongitas E, et al. *J Hepatol* 2010;52:272-279.

HBV transmission rates without prophylaxis from anti-HBc(+) donors (in HBV naïve Patients-earlier era experience)

Differences between HBV-experienced and HBV-naïve recipients



~ 50 % of Centers in 2001 were not using anti-HBc donors. Burton J et al Liver Transplant 2003

All donors were anti-HBc positive
Cholongitas E, et al. *J Hepatol* 2010;52:272-279.

Rituximab-Associated HBV Reactivation in Lymphoproliferative Disorders

Meta-analysis and review of FDA safety profiles

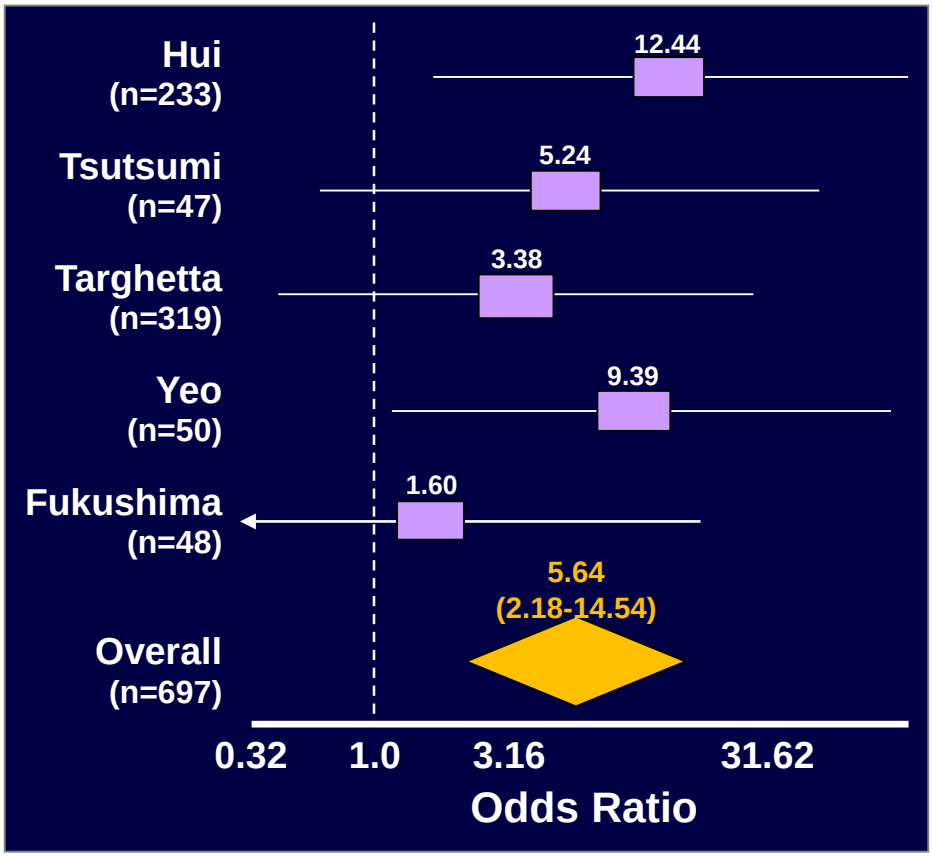
- Case reports (n=27)
- Case series reports (n=156)

Onset post last rituximab dose

- Median: 3 months (range: 0-12 months)
- >6 months: 29%

Reactivation in anti-HBc positive patients receiving rituximab versus no rituximab

- Odds ratio: 5.73 (*P*=0.0009)



Evens AM, et al. *Ann Oncol.* 2011;22:1170-1180.

Screening Recommendations for Hepatitis B to Minimize the Risk of HBV Reactivation

Organization	Population	Recommended Testing
CDC , 2008	All needing immunosuppressive therapy, including chemotherapy, immunosuppression related to organ transplantation, and immunosuppression for rheumatologic or gastroenterologic disorders	HBsAg Anti-HBc Anti-HBs
American Academy of Dermatology (AAD), 2008	Hepatitis B reactivation after treatment with TNF inhibitors has been reported; in the appropriate clinical setting, patients should be screened for hepatitis B infection	Not stated
AASLD, 2009	All patients before beginning immunosuppressive therapy	HBsAg Anti-HBc
APASL , 2011	All receiving immunosuppression or chemotherapy including patients who are going to receive biologic agents such as anti-CD20 or anti-TNFa	HBsAg Anti-HBc
EASL, 2012	All candidates for chemotherapy and immunosuppressive therapy	HBsAg Anti-HBc
American Society of Clinical Oncology (ASCO), 2010	Physicians may consider screening patients belonging to groups at heightened risk for chronic HBV infection or if highly immunosuppressive therapy is recommended	Consider HBsAg Consider anti-HBc

HBV/HCV Coinfection

Both HCV and HBV have shared modes of transmission

HCV coinfection among HBsAg carriers

- Global: 5% to 20%, more common in regions where both viruses are endemic
- NHANES III (US): 25% of HCV patients have positive HBV serologic markers

"Occult" HBV infection

- 11.9% to 44.4% in HCV-infected patients
- Up to 52% when liver tissue was examined for HBV DNA
- Clinical significance of the tissue HBV DNA is not defined

Konstantinou D, et al. *Ann Gastroenterol.* 2015;28:221-228.
Caccamo G, et al. *World J Gastroenterol.* 2014;20:14559-14567.
Jamma S, et al. *Curr Hepat Rep.* 2010;9:260-269.
Chu C-J, et al. *J Gastroenterol Hepatol.* 2008;23:512-520.
Potthoff A, et al. *Expert Opin Pharmacother.* 2010;11:919-928.

Spontaneous Post-Marketing Cases of HBV Reactivation With 2nd Generation DAA Regimens

FDA adverse event reporting system database (2013-2015) HBV reactivation with DAA therapy (n=29, 5 within the US)

- Reactivation was temporally related to DAA initiation
  - Occurred within 4-8 weeks (mean time: 53 days)
- Heterogenous in HCV genotype, DAA received, and baseline HBV parameters
  - Anti-HBc data were available in 6/29 cases; all 6 were anti-HBc positive and experienced reactivation

Descriptive Characteristics

	Patients (n=29)
Mean years of age (range)	61 (36-58)
Male (%)	45
Country of report (number)	
USA/Japan/Other	5/19/4
Mean days to event (range)	53 (14-196)
Treatment delay (number)	
Yes/possibly/no delay	7/7/2
No treatment given or not stated	13
HCV genotype	
1/other/not reported (number)	16/2/11
Baseline HBV serology (number)	
HBsAg (+)/(-)/not reported	13/4/12
Anti-HBc (+)/not reported	6/23
HBsAb (-)/not reported	3/26
HBV DNA	
Undetectable/detectable	19/9
Not reported or unclear	4

Bersoff-Matcha SJ, et al. *Ann Intern Med.* 2017;166:792-798.

Spontaneous Post-Marketing Cases of HBV Reactivation With 2nd Generation DAA Regimens

Outcomes of HBV reactivation
- Decompensation (n=3, death in 2, and liver transplantation in 1)

HBV treatment given (n=16)
- Entecavir (n=9), tenofovir DF (n=6), emtricitabine/tenofovir DF (n=1), not reported (n=6)
- Treatment was usually delayed (at least in 7 cases)
- Improvement in HBV DNA and other signs and symptoms

No consistency with regard to DAA received, suggesting a drug-class effect

Outcomes

	Patients (n=29)
HBV treatment delay (number)	
Yes/possibly	7/7
No delay	2
No treatment given or not stated	13
DAA therapy (number)	
Completed	13
Discontinued	10
Not reported	6
Outcomes (number)	
Death	2
Transplant	1
Hospitalization	6
Other	20

Bersoff-Matcha SJ, et al. *Ann Intern Med.* 2017;166:792-798.

How good is the anti-HBc assay?

How do develop a high performance test

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Anti-HBc II assay is a two-step immunoassay for the qualitative determination of anti-HBc in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, assay diluent, specimen diluent, and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in the sample binds to the rHBcAg coated microparticles.
2. The reaction mixture is washed and anti-human acridinium-labeled conjugate is added.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-HBc in the sample and the RLUs detected by the ARCHITECT iSystem optics.

The presence or absence of anti-HBc in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-HBc.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

Antibody testing to HBc: what are the current details?

Interpretation of Results

Initial ARCHITECT Anti-HBc II Results

Initial Result (S/CO)	Instrument Flag	Interpretation	Retest Procedure
< 1.00	NONREACTIVE	Nonreactive	No retest required.
≥ 1.00	REACTIVE	Reactive	Retest in duplicate.

Final ARCHITECT Anti-HBc II Interpretation

Initial Interpretation	Results with Retest	Final Interpretation
Nonreactive	No retest required.	**Nonreactive**
Reactive	If two of the three results are < 1.00 S/CO	**Nonreactive**
Reactive	If two of the three results are ≥ 1.00 S/CO	**Reactive**

Table 1: ARCHITECT Anti-HBc II Precision

Panel member	n	Mean (S/CO)	Within Run		Total**	
			SD	%CV	SD	%CV
Negative Control	432	0.22	0.01	6.52	0.02	7.57
Positive Control	431	2.97	0.08	2.63	0.09	2.87
Human Plasma Panel 1	144	0.81	0.02	2.73	0.03	3.24
Human Plasma Panel 2	144	1.18	0.03	2.52	0.03	2.87

* Representative data; results in individual laboratories may vary from these data.

** Total is an accumulation of within run, between run and between day.

Specificity

The ARCHITECT Anti-HBc II assay is designed to have an overall specificity of ≥ 99.5% on a blood donor population and ≥ 98.0% on a hospitalized/diagnostic population. A study was performed at one internal and two external evaluation sites. A total of 5141 serum and plasma specimens collected from five blood-donation centers and 260 hospitalized/diagnostic specimens were evaluated to assess specificity.

Table 2: ARCHITECT Anti-HBc II Specificity

Category	N	IR [%]	RR [%]	Clinical Specificity	95% Confidence Interval
Overall Blood Donors	5141	44 [0.86]	41 [0.80]	99.71% (5098/5113)	99.52 - 99.84%
Blood Donor Serum	3584	25 [0.70]	22 [0.61]	99.75% (3561/3570)	99.52 - 99.88%
Blood Donor Plasma	1557	19 [1.22]	19 [1.22]	99.61% (1537/1543)	99.16 - 99.86%
Hospitalized/ Diagnostic Specimens	260	28 [10.77]	28 [10.77]	100% (231/231)	98.42 - 100%

* Representative data; results in individual laboratories may vary from these data.

Sensitivity

A total of 406 anti-HBc positive specimens from patients with acute, chronic and recovered HBV infection and signs and symptoms of HBV infection were tested, resulting in a sensitivity of 100% (406/406), 95% confidence interval: 99.10% - 100%. (Representative data; results in individual laboratories may vary from these data).

Are many of the anti-HBc (+) tests false positives ?

No: current tests have a false (+) rate < 0.6 %

Abbott PRISM: false +: 3/1000

how did the antibody tests change ? Improve?

Development of an improved anti-HBc antibody

Simple confirmatory assay for anti-HBc reactivity

Daniela Huzly[a,*], Michael Nassal[b], Jolanta Vorreiter[b], Valeria Falcone[a], Dieter Neumann-Haefelin[a], Wolfram H. Gerlich[c], Marcus Panning[a]

[a] Department of Virology, Freiburg University Medical Center, Hermann-Herder-Str. 11, 79104 Freiburg, Germany
[b] Dept. of Internal Medicine II/Molecular Biology, Freiburg University Medical Center, Hugstetter Str. 55, 79106 Freiburg, Germany
[c] Institute for Medical Virology, Justus Liebig University Giessen, Frankfurter Str. 107 35392 Giessen, Germany

The amino acid sequences 1–183 of the rHBcAg used for inhibition are based on cloned HBV DNA of HBV genotype D, HBsAg subtype ayw3 (GenBank accession number J02203). Briefly, rHBcAg was expressed as previously described, except that in addition bacterial endotoxin was depleted. Intact and >95% pure core protein as judged from Coomassie Blue-stained SDS-PAGE was isolated by sedimentation through 10–60% sucrose gradients. Purified rHBcAg

Four different anti-HBc-reactive sera from patients with a history of hepatitis B infection were used for the development of the confirmatory assay. All sera were low positive for anti-HBc and had index values below 2.0 in the Siemens Centaur system (cut-off at 0.5). A 200 µg/ml stock solution of rHBcAg was serially diluted in PBS and in an anti-HBc-negative serum pool. Ten microliters of each dilution were then incubated for 1 h at room temperature (RT), at 4 °C and at 37 °C with 190 µl of anti-HBc reactive sera. As negative control, 10 µl PBS or anti-HBc-negative pool serum was used. After incubation, anti-HBc reactivity was determined in samples with rHBcAg and in control samples by means of three different commercial assays (Abbott Architect i1000® anti-HBcII, DiaSorin LIAISON® anti-HBc, Siemens ADVIA Centaur HBcT®). The percentage of inhibition was calculated as follows:

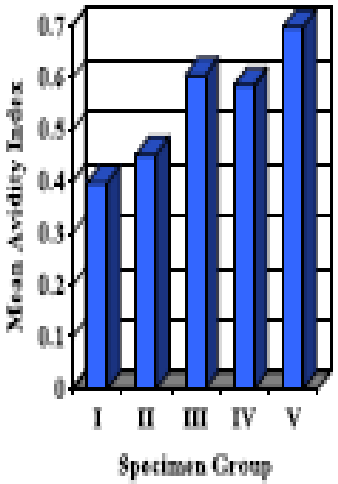
$$100 - \left(\frac{RLU(Sample + Ag)}{RLU(Sampe + PBS)}\right) \times 100$$

for the ADVIA Centaur HBcT® and the Abbott Architect® anti-HBcII and

$$100 - \left(\frac{RLU(Sample + PBS)}{RLU(Sampe + Ag)}\right) \times 100$$

Development of an advanced anti-HBc antibody detection assay

Evaluation of Specimens Using Anti-HBc Avidity Assay



- The relative avidity of the anti-HBc antibody in samples from 5 groups of specimens was assessed:
  - Group I (N = 10): past infection, ARCHITECT CORE(+) Centaur HBcT(-)
  - Group II (N = 13): past infection, ARCHITECT CORE(+) Centaur HBcT Low Pos.
  - Group III (N = 6): past infection, ARCHITECT CORE(+) Centaur HBcT(+)
  - Group IV (N = 11): HBsAg(+) seroconversion samples
  - Group V (N = 7): HBsAg(-) anti-HBs(+) seroconversion samples
- Specimens that were ARCHITECT CORE positive but negative or low positive by Centaur HBcT (Groups I and II) had a lower mean avidity compared to the other specimen groups.

This avidity assay was strictly a research assay based on similar methods published in the literature. It is not in our assay development pipeline.

Summary and Conclusions

- Anti-HBc continues to be a clinically important marker for current and past HBV infection. Persons with past HBV infection are at an elevated risk of hepatitis B reactivation if treated with immunosuppressive therapies; the CDC recommends anti-HBc testing to identify these patients.
- Anti-HBc assays differ in their ability to detect anti-HBc in samples from persons with past HBV infection. Lack of detection may be due to lower sensitivity for anti-HBc or it may be related to assay formats with decreased detection of lower avidity anti-HBc antibodies.
- An anti-HBc research panel was developed. The panel is comprised of extensively characterized anti-HBc positive samples from individuals with past HBV infection and includes samples with low, medium, and high levels of anti-HBc. This panel may be useful in the evaluation of anti-HBc assay performance.

Kuhns ISVLSD 2009

Vox Sanguinis (2006) **91**, 237–243

DOI: 10.1111/j.1423-0410.2006.00818.x

ORIGINAL PAPER

Anti-HBc screening of blood donors: a comparison of nine anti-HBc tests

M. Schmidt,[1,*] C. M. Nübling,[2,*] H. Scheiblauer,[2] M. Chudy,[2] L. A. Walch,[1] E. Seifried,[1] W. K. Roth[1] & M. K. Hourfar[1]

[1] *Institute of Transfusion Medicine and Immunohematology, German Red Cross, Johann Wolfgang Goethe University, Frankfurt, Germany*

[2] *Paul Ehrlich Institute, Langen, Germany*

All 112 anti-HBe-reactive samples were also concordantly reactive in the nine anti-HBc assays, providing strong evidence for anti-HBe as the most specific marker for a past HBV infection. Figure 2 shows S/Co values for each group according to the different anti-HBc assays. One might also consider the S/Co ratio of anti-HBc results as an indication of distinguished true and false-positive anti-HBc results: significantly lower anti-HBc signals were obtained with the anti-HBs- and/or anti-HBe-negative samples compared with anti-HBs- and/or anti-HBe-reactive samples (Fig. 2, $P < 0{\cdot}01$).

What is the significance of anti-HBe(+) in HBsAg(-) patients who are also anti-HBc(+)?

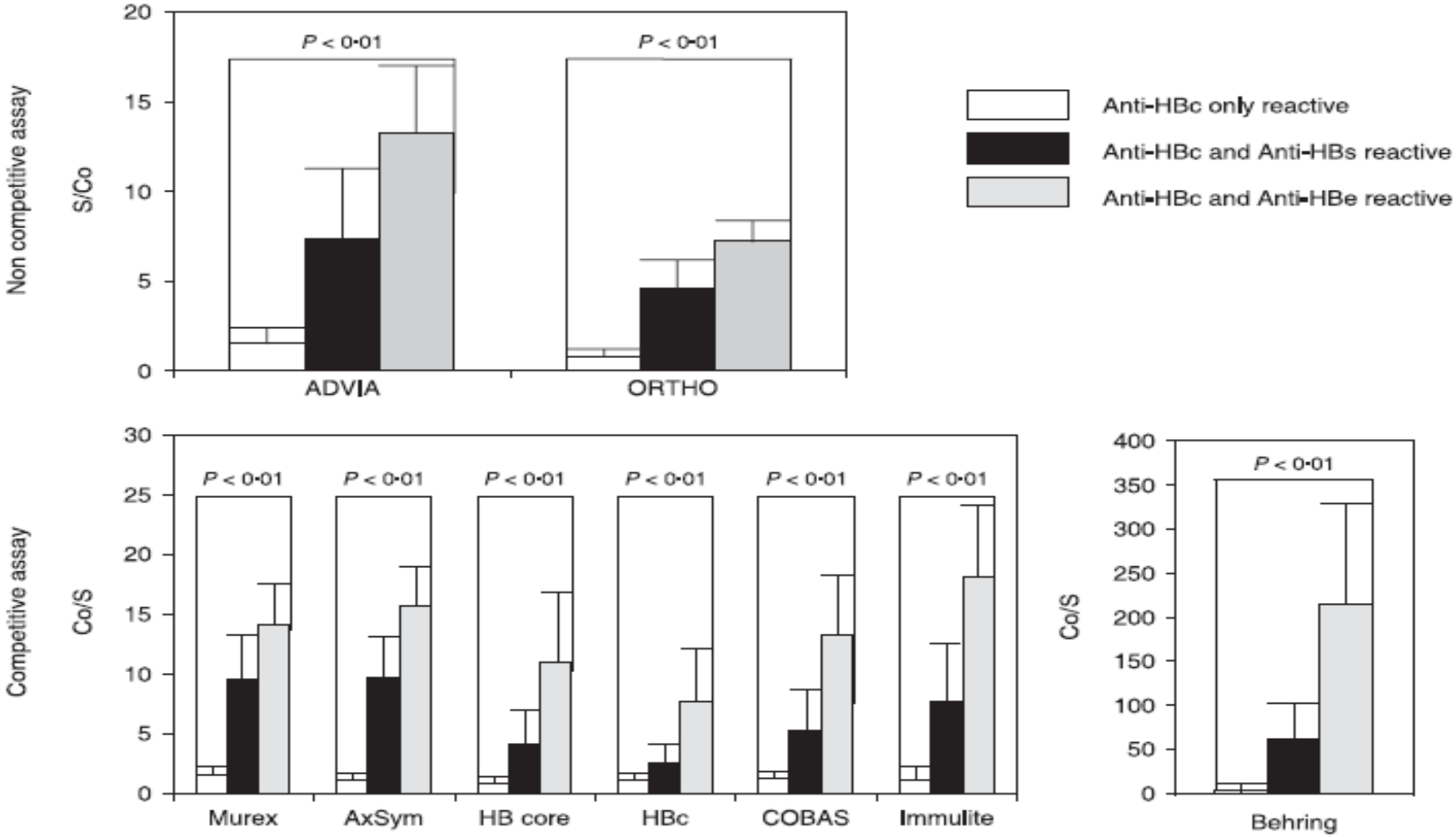


Fig. 2 Analysis of 188 antibody to hepatitis B core antigen (anti-HBc)-reactive samples: comparison between nine anti-HBc assays. Anti-HBc assays were divided into competitive (Murex, AxSYM, PRISM® HBcore, PRISM® HBc, COBAS Immulite and Behring) and non-competitive (ADVIA and Ortho) assays. Sample cut-off values (S/Co) differed significantly between anti-HBc-only reactive samples and anti-HBc + antibody to hepatitis B envelope antigen (anti-HBe)-reactive samples.

Summary of the improvements of anti-HBc test performance

1) Use a recombinant HBcAg that has a broadly prevalent serotype such as ayw

2) Use well characterized serum specimens of patients with known past HBV infection

3) Choose appropriate S/CO levels

4) Consider confirming with anti-HBe when developing new antibody testing to prove high specificity (or in the clinic)

5) WHO now has the first international standard for anti-HBc which is derived from the PEI (Paul Erlich Institute) standard

Anti-HBc
Summary and Conclusions

- Anti-HBc testing is an essential part of assessing all patients for their HBV status
- When you see an anti-HBc test: you can believe the patient has been exposed to HBV and currently has cccDNA in their liver
- In patients who are isolated anti-HBc(+): consider testing for HBV DNA quant (occult HBV infection)
- Patients with occult/resolved HBV: educate patients about risk for reactivation and monitor for reactivation is special settings
- Anti-HBc titers: research opportunities in the setting of new therapies
- Anti-HBc and interaction with HBcrAg to be determined
- There is no evidence we should “boost” patients who are anti-HBc(+) with HBV vaccine

Real Facts, The Truth

HBV is not curable, reduce cccDNA ≠ cure

You should not boost anti-HBs in an isolated anti-HBc (+) patient with vaccine for since there is no data that this provides any “protection”

There is no “natural immunity” to HBV (anti-HBc {+} and anti-HBs{+}): let us replace this with the term “immune control” and “resolved disease”

One does need to test anti-HBc in all patient populations where you are performing HBV Screening

Anti-HBc: Additional notes and comments:

Can we use anti-HBc titers to help document disease control and eventually a sterilizing cure?

Do we need to search for HBsAg mutants in anti-HBc positive patients with active liver disease?

We need better highly accurate cccDNA tissue assays to document when we reach a sterilizing cure

Will HBcrAg be an accurate test for the presence of cccDNA? Or just cccDNA activity ?

Cure = no cccDNA on biopsy, no transmission of HBV with liver transplant, no reactivation with potent immune suppression, anti-HBc titer decline or < LOD

Thank you to:

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Massimo Levrero

Stephen Locarnini

HEPDART: 2017   Ray Schinazi