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

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robertgish.com
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
“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

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>anti-HBc</th>
<th>anti-HBs</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>HBsAg</td>
<td>anti-HBc</td>
<td>positive</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>anti-HBc</td>
<td>negative</td>
<td>Immune due to hepatitis B vaccination</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>Anti-HBc IgM/anti-HBc total</td>
<td>positive</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>Anti-HBc IgM</td>
<td>negative</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>anti-HBc Total</td>
<td>positive</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>Anti-HBs</td>
<td></td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>

Definitions:

**Occult B Infection**

OBI

HBsAg (-)

Anti-HBc(+) or (-)

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

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.

Published online 2017 Jun 21. doi: [10.3390/v9060156](https://doi.org/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

<table>
<thead>
<tr>
<th>Titer</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBeAg</td>
</tr>
<tr>
<td></td>
<td>anti-HBe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weeks after Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 4 8 12 16 20 24 28 32 36 52 100</td>
</tr>
</tbody>
</table>

- **HBsAg**: Presence of HBsAg indicates active viral replication.
- **IgM anti-HBc**: Appearance of IgM anti-HBc indicates acute infection.
- **anti-HBs**: Seroconversion to anti-HBs indicates recovery.
- **Total anti-HBc**: Peaks early in infection and remains high.
- **anti-HBc**: Declines as total anti-HBc peaks.
Progression to Chronic Hepatitis B Virus Infection

Typical Serologic Course

<table>
<thead>
<tr>
<th>Weeks after Exposure</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

- **Acute (6 months)**
  - IgM anti-HBc
  - HBsAg
  - Total anti-HBc

- **Chronic (Years)**
  - HBeAg
  - anti-HBe

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

Prevalence, %

- USA: 5%
- Spain: 12%
- France: 7%
- Japan: 9%
- China: 54%
- Taiwan: 57%

• Multiple Studies
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
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)

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Initial Result (S/CO)</th>
<th>Instrument Flag</th>
<th>Interpretation</th>
<th>Retest Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.00</td>
<td>NONREACTIVE</td>
<td>Nonreactive</td>
<td>No retest required.</td>
</tr>
<tr>
<td>≥ 1.00</td>
<td>REACTIVE</td>
<td>Reactive</td>
<td>Retest in duplicate.</td>
</tr>
</tbody>
</table>

#### Final ARCHITECT Anti-HBc II Interpretation

<table>
<thead>
<tr>
<th>Initial Interpretation</th>
<th>Results with Retest</th>
<th>Final Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreactive</td>
<td>No retest required.</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>Reactive</td>
<td>If two of the three results are &lt; 1.00 S/CO</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>Reactive</td>
<td>If two of the three results are ≥ 1.00 S/CO</td>
<td>Reactive</td>
</tr>
</tbody>
</table>
Table 1: ARCHITECT Anti-HBc II Precision

<table>
<thead>
<tr>
<th>Panel member</th>
<th>n</th>
<th>Mean (S/CO)</th>
<th>Within Run</th>
<th>Total**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>432</td>
<td>0.22</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Positive Control</td>
<td>431</td>
<td>2.97</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Human Plasma Panel 1</td>
<td>144</td>
<td>0.81</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Human Plasma Panel 2</td>
<td>144</td>
<td>1.18</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* 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 \( \geq 99.5\% \) on a blood donor population and \( \geq 98.0\% \) 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

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>IR [%]</th>
<th>RR [%]</th>
<th>Clinical Specificity</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Blood Donors</td>
<td>5141</td>
<td>44 [0.86]</td>
<td>41 [0.80]</td>
<td>99.71% (5098/5113)</td>
<td>99.52 - 99.84%</td>
</tr>
<tr>
<td>Blood Donor Serum</td>
<td>3584</td>
<td>25 [0.70]</td>
<td>22 [0.61]</td>
<td>99.75% (3561/3570)</td>
<td>99.52 - 99.88%</td>
</tr>
<tr>
<td>Hospitalized/ Diagnostic Specimens</td>
<td>260</td>
<td>28 [10.77]</td>
<td>28 [10.77]</td>
<td>100% (231/231)</td>
<td>98.42 - 100%</td>
</tr>
</tbody>
</table>

* 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\textsuperscript{a,*}, Michael Nassal\textsuperscript{b}, Jolanta Vorreiter\textsuperscript{b}, Valeria Falcone\textsuperscript{a}, Dieter Neumann-Haefelin\textsuperscript{a}, Wolfram H. Gerlich\textsuperscript{c}, Marcus Panning\textsuperscript{a}

\textsuperscript{a} Department of Virology, Freiburg University Medical Center, Hermann-Herder-Str. 11, 79104 Freiburg, Germany
\textsuperscript{b} Dept. of Internal Medicine II/Molecular Biology, Freiburg University Medical Center, Hugstetter Str. 55, 79106 Freiburg, Germany
\textsuperscript{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\textsuperscript{®} anti-HBcII, DiaSorin Liaison\textsuperscript{®} anti-HBc, Siemens ADVIA Centaur HBcT\textsuperscript{®}). The percentage of inhibition was calculated as follows:

\[
100 - \left( \frac{RLU(\text{Sample} + Ag)}{RLU(\text{Sample} + PBS)} \right) \times 100
\]

for the ADVIA Centaur HBcT\textsuperscript{®} and the Abbott Architect\textsuperscript{®} anti-HBcII
Development of an advanced anti-HBc antibody detection assay

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


Institute of Transfusion Medicine and Immunohematology, German Red Cross, Johann Wolfgang Goethe University, Frankfurt, Germany
Paul Ehrlich Institute, Langen, Germany

All 112 anti-HBe-reactive samples were also concordantly reactive in the nine anti-HBe 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-HBe assays. One might also consider the S/Co ratio of anti-HBe results as an indication of distinguished true and false-positive anti-HBe results: significantly lower anti-HBe 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 < 0.01).
What is the significance of anti-HBe(+) in HBsAg(-) patients who are also anti-HBc(+)?
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 in 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:

Brian McMahon
Massimo Levrero
Stephen Locarnini

HEPDART: 2017  Ray Schinazi