Unusual Courses of HBV infection caused by Wildtype Virus and Escape Mutants

Wolfram H. Gerlich
Institute of Medical Virology, Justus Liebig University, Giessen, Germany
HBV infection: always detectable, always preventable?

Wolfram H. Gerlich
Institute of Medical Virology, Justus Liebig University
Giessen, Germany
Overview

• Limitations of current serological HBV tests
• Limitations of current HB vaccines
• Gaps in our prevention strategies
Hepatitis B Virus

Discovered by D.S. Dane 1970
Hepatitis B Virus

The particle

- preS1
- preS2
- S
- HBc
- RT
- PKC
- pr
- DNA
- 52 nm
- MHBs
- LHBs
- SHBs

The genome

- Genotype A
- 3221 Bp
- PreC
- DR2
- X
- 1374
- 1835
- 1814
- 1620
- 1901
- DR1
- 3211
- PreS2
- PreS1
- 2854
- 2307
- 2356
- 822
acute hepatitis B resolving

log titer

HBV DNA PCR

months
Origins of hepatitis B diagnostics

Australia antigen, discovered by B.S. Blumberg, 1963
is the hepatitis B surface antigen HBsAg

Pictures from Harvey Alter, Nat. Med. 2001
Virus and HBsAg Particles in Blood From Highly Viremic HBV Carriers

<table>
<thead>
<tr>
<th>HBV particles</th>
<th>HBsAg filaments</th>
<th>HBsAg 20nm particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^9 - 10^{11}$ /ml</td>
<td>$10^{11} - 10^{12}$ /ml</td>
<td>$10^{12} - 10^{14}$ /ml</td>
</tr>
</tbody>
</table>

From: Doerr & Gerlich  Medizinische Virologie Thieme Verlag, in print
Structural Components of HBV and HBsAg Particles

<table>
<thead>
<tr>
<th>Ratio</th>
<th>PCR</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Det. Limit</td>
<td>100/mL</td>
<td>10,000,000/mL</td>
</tr>
<tr>
<td>Rel. det. Lim.</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>
acut hep B resolving

disease

log titer

HBV PCR

HBsAg
Problems of HBsAg tests

- HBV genotypes react differently
  - WHO standard only genotype A
  - Worldwide genotypes B, C und D predominant
  - Genotype F not detected for years with some tests

- Insufficient sensitivity even for genotype A
  - Best possible ELISA detects 10pg HBsAg/ml
  - This corresponds to $10^6$ HBV particles/ml
  - if excessive HBsAg is not present in early phase

- HBsAg is highly variable under immune selection
  - after post exposure immunisation
  - during reactivation

- Consequence: false negative results
HBsAg-negative, HBV-infectious blood donation: the donor

- **16th July 2002**
  - HBsAg in AxSym **negative**

- **17th September 2002**
  - AxSym (Abbott) **positive** s/co=2.4
  - Enzygnost (Dade Behring) **negative** s/co=0.8

- **21st September 2002**
  - Enzygnost **positive** s/co=1.9
  - HBV-DNA **positive** 3200 ge/mL
  - Anti-HBc total antibody and IgM and HBeAg negative

*Meisel et al. Transfusion Medicine and Hemotherapy, in press*
HBsAg-negative, HBV-infectious blood donation: the recipient

- 22. July 2002
  - receives erythrocyte concentrate from the pre-seroconversion donation
  - HBsAg and anti-HBc negative
  - HBsAg positive, HBV DNA $1.8 \times 10^{10}$ genomes/mL
  - No hepatitis, develops persistent viremia
  - anti-HBc still negative, later positive
- HBV DNA sequence of donor and recipient
  - identical, genotype A, in Germany predominant variant
  - S-gene without mutations
HBsAg-negative, HBV-infectious blood donation: the donor

- **16th July 2002**
  - HBsAg in AxSym negative
  - HBV DNA ca 2000 genomes/mL
- **17th September 2002**
  - AxSym (Abbott) positive s/co=2.4
  - Enzygnost (Dade Behring) negative s/co=0.8
- **21st September 2002**
  - Enzygnost positive s/co=1.9
  - HBV-DNA 3200 genomes/mL
  - Anti-HBc total antibody and IgM and HBeAg negative
- **The donor was >60 days infectious without detectable HBsAg**

- **12th May 2003**, no hepatitis noticed
  - HBsAg negative, HBV DNA negative
  - anti-HBc weakly and anti-HBs clearly positive
**Almost silent HBV infection in a plasma donor**

<table>
<thead>
<tr>
<th>Date</th>
<th>anti HBc</th>
<th>HBV DNA Ge/ml *</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.11.97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21.11.97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>05.12.97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.11.98</td>
<td>-</td>
<td>+ 85 Ge/ml ** (S, X-Primers)</td>
</tr>
<tr>
<td>14.05.99</td>
<td>-</td>
<td>•</td>
</tr>
<tr>
<td>25.06.99</td>
<td>-</td>
<td>•</td>
</tr>
<tr>
<td>02.07.99</td>
<td>-</td>
<td>•</td>
</tr>
<tr>
<td>27.08.99</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21.06.99</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

No HBsAg, anti-HBs, anti-HBe, ALT elevation at any time

* Detection limit ca. 100 Ge/ml (50 - 250)

** Genotype D, 3 Exchanges in X to reference strain

**Viremia >9 months before appearance of anti-HBc**
Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay* for hepatitis B surface antigen screening

Minegishi et al. Japanese Red Cross Vox sang 2003 84:287

- >11 million donations tested in pools of 50 (>1000 ge/mL)
- Prescreened with RPHA for HBsAg* and high anti-HBc
- 181 HBV DNA positive donations found (early phase)
- Retested these with single sample PCR

<table>
<thead>
<tr>
<th>copies/mL**</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>14</td>
</tr>
<tr>
<td>&lt;1000</td>
<td>28</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>59</td>
</tr>
<tr>
<td>&gt;10,000</td>
<td>80</td>
</tr>
<tr>
<td>total</td>
<td>181</td>
</tr>
</tbody>
</table>

Conclusion: the true number of HBV DNA positives will be much higher

*Abbotts Prism  
*ca 13500 positive  
**Taqman
Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay* for hepatitis B surface antigen screening

Minegishi et al  Japanese Red Cross  Vox sang 2003  84:287

- >11 million donations tested in pools of 50
- Prescreened with RPHA for HBsAg* and high anti-HBc
- 181 HBV DNA positive donations found (early phase)

<table>
<thead>
<tr>
<th>copies/mL**</th>
<th>N</th>
<th>HBsAg* neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>100 - 1000</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>1000 - 10^4</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>&gt;10^4</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>total</td>
<td>181</td>
<td>76</td>
</tr>
</tbody>
</table>

*Abbotts Prism  *ca 13500 positive  **Taqman
Gaps in detection of early phase HBV infection in blood donors: potential solutions

- Ignore because of rarity
  - Only ca 3% of infected donors were missed
  - In Japan ca 1 in 30 000 donations
  - much less in Europe and North America
    - 0.05 /100 000 in Germany (Roth et al. Transfusion 42:869)

- Highly sensitive NAT screening
  - Detection limit? Costs?
  - Cannot replace HBsAg or anti-HBc testing (?)

- Vaccination of blood donors
  - Could it replace NAT and repeat anti-HBc testing?
  - protection against HBV
    - Long lasting in donors (active)
    - Short term in recipient (passive)

- Escape mutants
What is HBsAg?

The entity of all possible surface epitopes on HBV particles which are detected by mammalian B lymphocytes

or

What the HBsAg assays detect?

or

What the hepatitis B vaccines are made of?
Structural Components of HBV and HBsAg Particles

- **virus**
- **preS1**
- **preS2**
- **filaments**
- **LHBs**
- **SHBs**
- **MHBs**
- **spheres**
- **length variable**
- **52 nm**
- **25 nm**
- **3.2kb DNA**
- **RT**
- **pr**
- **HBc**
Nature of the currently predominating hepatitis B vaccines

- Small hepatitis B surface protein SHBs
  - *No preS-containing surface proteins*
- HBsAg subtype adw2
- Expressed in transformed yeast cells
  - Incomplete folding, no secretion
  - Neutralising conformational epitopes in vitro only partially generated
- Moderately immunogenic (3 doses of 20 µg)
PreS1 dependent binding and infection of primary Tupaia hepatocyte cultures with human hepatitis B virus

Dieter Glebe¹, Mehriar Aliakbari¹, Peter Krass¹, Eva Knoop¹, Stephan Urban² and Wolfram H. Gerlich¹

¹Institute of Medical Virology
Justus Liebig University of Giessen, Germany

²Department of Molecular Virology
Otto-Meyerhof-Zentrum
University of Heidelberg, Germany

J Virol 2003; 77:9511-21
Tupaia (tree shrews)

- natural habitat: tropical forests in southeast Asia
- small mammals (10-15 cm)
- insectivores.
- complex behaviour
- order: Scandentia, Tupaiidae
- 19 recognised species

Tupaia belangeri
Tupaia (tree shrews)

- Considered as an animal model in primate-related research
- More related to Lagomorpha (rabbits) than primates (Schmitz et al. 2000)
- Can be infected with HBV in vivo and in vitro (Li et al. 1994, Walter et al. 1996, Köck et al. 2001)
Isolation of primary tupaia hepatocytes

Tupaia belangeri
(Asian tree shrew)
Infection of primary Tupaia hepatocytes with HBV

Formation of cccDNA

LightCycler PCR specific for HBV DNA without nick and gap
gel electrophoresis of PCR products
Methods

Infection experiments

- infection of tupaia hepatocytes with purified HBV from plasma of HBV-infected patients

- quantification of purified input HBV (ge) using real-time PCR

- quantification of HBV cccDNA and mRNAs using real-time PCR and RT-PCR

- quantification of secreted HBeAg and HBsAg in supernatant
Titration of input virus

HBeAg production.

S/CO HBeAg vs. days p.i.

Cut-off = 1
Neutralising activity of preS1, preS2 and S-specific monoclonal antibodies

Preincubation of HBV with MA18/7

Infection of primary Tupaia hepatocytes

HBeAg secretion

Preincubation of HBV with MA18/7

Infection of primary Tupaia hepatocytes
Nature of the currently predominating hepatitis B vaccines

• Small hepatitis B surface protein SHBs
  - *No preS-containing surface proteins*

• HBsAg subtype adw2
  - *No other HBsAg subtypes or HBV genotypes*

• Expressed in transformed yeast cells
  - Incomplete folding, no secretion
  - Neutralising conformational epitopes in vitro only partially generated

• Moderately immunogenic (3 doses of 20 µg)
Structural elements of Small HBs Protein (SHBs)

122 K/R d/y

N glyc

HBs antigenic region

NH₂

transmembrane α-helices

COOH 226

Internal hydrophilic loop
## HBsAg Subtype Determinants: Amino Acids and Genotypes

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino Acid</th>
<th>Determinant</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>122</td>
<td>Lys</td>
<td>d</td>
<td>A, B, C, F</td>
</tr>
<tr>
<td></td>
<td>Arg</td>
<td>y</td>
<td>A – D, E</td>
</tr>
<tr>
<td>126</td>
<td>Thr</td>
<td>t</td>
<td>A - F</td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>i</td>
<td>C</td>
</tr>
<tr>
<td>127</td>
<td>Phe*</td>
<td>w1</td>
<td>A, B</td>
</tr>
<tr>
<td></td>
<td>Pro</td>
<td>w2*</td>
<td>A – D</td>
</tr>
<tr>
<td></td>
<td>Thr</td>
<td>w3</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>Leu / Ile</td>
<td>w4</td>
<td>E, F</td>
</tr>
<tr>
<td>160</td>
<td>Lys</td>
<td>w</td>
<td>A – F</td>
</tr>
<tr>
<td></td>
<td>Arg</td>
<td>r</td>
<td>C</td>
</tr>
</tbody>
</table>

*For w2 additional Arg 122, Phe 134 and/or Ala 159 necessary according to Magnus and Norder, 1994*
# Estimated prevalences of HBV genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>% prevalence</th>
<th>Number (millions)</th>
<th>in</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td>5</td>
<td>300</td>
<td>World</td>
</tr>
<tr>
<td>A*</td>
<td>0.5</td>
<td>3</td>
<td>N. Europe, USA</td>
</tr>
<tr>
<td>A´</td>
<td>?</td>
<td>?</td>
<td>S.&amp;E. Africa</td>
</tr>
<tr>
<td>B/C</td>
<td>8</td>
<td>240</td>
<td>East Asia</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>40</td>
<td>Middle East, Mediterranean</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>20</td>
<td>West Africa</td>
</tr>
<tr>
<td>F</td>
<td>Low</td>
<td>2 ?</td>
<td>C.&amp;S. America</td>
</tr>
</tbody>
</table>

*used for HBsAg vaccines and reference samples
Genotypes of Hepatitis B Virus and other Hepadnavirus Species

From Stephan Schaefer
Nature of the currently predominating hepatitis B vaccines

- Small hepatitis B surface protein SHBs
  - No preS-containing surface proteins
- HBsAg subtype adw2
  - No other HBsAg subtypes or HBV genotypes
- Expressed in transformed yeast cells
  - Incomplete folding, no secretion
  - Only conformational epitopes are neutralising
- Moderately immunogenic (3 doses of 20 µg)
Structural elements of Small HBs Protein (SHBs)

HBs antigenic region

Transmembrane α-helices

Internal hydrophilic loop

122 K/R d/y

N glyc

NH₂
A vaccinated anti-HBs positive thrombapheresis donor seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc</th>
<th>Anti-HBc</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2</th>
<th>PCR 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td></td>
<td>&gt;1000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.2002</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
A vaccinated anti-HBs positive thrombapheresis donor\textsuperscript{a} seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc\textsuperscript{b}</th>
<th>Anti-HBc\textsuperscript{c}</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2\textsuperscript{d}</th>
<th>PCR 3\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td>&gt;1000</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.2002</td>
<td>-</td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04.2003</td>
<td>+/-</td>
<td></td>
<td>390</td>
<td>-/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05.2003</td>
<td>+</td>
<td></td>
<td>380</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2003</td>
<td>+</td>
<td></td>
<td>490</td>
<td>-/-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Donor was always HBsAg negative (AxSym)
A vaccinated anti-HBs positive thrombapheresis donor\(^a\) seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc(^b)</th>
<th>Anti-HBc(^c)</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2(^d)</th>
<th>PCR 3(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td>&gt;1000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.2002</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04.2003</td>
<td>+/-</td>
<td>390</td>
<td>-/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05.2003</td>
<td>(+)</td>
<td>380</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2003</td>
<td>(+)</td>
<td>490</td>
<td>-/-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Donor was always HBsAg negative (AxSym)
\(^b\) AxSym (Abbott)
\(^c\) Enzygnost (Dade Behring)
\(^d\) Detection limit >50 ge/mL
\(^e\) with ultracentrifugation > 3 ge/mL
A vaccinated anti-HBs positive thrombapheresis donor\textsuperscript{a} seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc\textsuperscript{b}</th>
<th>Anti-HBc\textsuperscript{c}</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2\textsuperscript{d}</th>
<th>PCR 3\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td>&gt;1000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.2002</td>
<td>-</td>
<td>+/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04.2003</td>
<td>+/-</td>
<td>390</td>
<td>+/-</td>
<td>-</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>05.2003</td>
<td>(+)</td>
<td>380</td>
<td>+/-</td>
<td>-</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>06.2003</td>
<td>(+)</td>
<td>490</td>
<td>+/-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Donor was always HBsAg negative (AxSym)

\textsuperscript{b} AxSym (Abbott)

\textsuperscript{c} Enzygnost (Dade Behring)

\textsuperscript{d} Detection limit >50 ge/mL

\textsuperscript{e} with ultracentrifugation > 3 ge/mL
A vaccinated anti-HBs positive thrombapheresis donor seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Anti-HBc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2&lt;sup&gt;d&lt;/sup&gt;</th>
<th>PCR 3&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td>-</td>
<td>&gt;1000</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>Specific or</td>
<td>Specific or</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Contamination?</td>
<td>Contamination?</td>
</tr>
<tr>
<td>11.2002</td>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04.2003</td>
<td>+/-</td>
<td>390</td>
<td>+/-</td>
<td>-</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>05.2003</td>
<td>(+)</td>
<td>380</td>
<td>+/-</td>
<td>-</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>06.2003</td>
<td>(+)</td>
<td>490</td>
<td>+/-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Donor was always HBsAg negative (AxSym)
b) AxSym (Abbott) 
c) Enzygnost (Dade Behring )
d) Detection limit >50 ge/mL 
e) with ultracentrifugation > 3 ge/mL
A vaccinated anti-HBs positive thrombapheresis donor seroconverts to anti-HBc: viremia?

<table>
<thead>
<tr>
<th>Date</th>
<th>Anti-HBc&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Anti-HBc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Anti-HBs</th>
<th>PCR 1</th>
<th>PCR 2&lt;sup&gt;d&lt;/sup&gt;</th>
<th>PCR 3&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.1999</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2000</td>
<td>-</td>
<td>-</td>
<td>&gt;1000</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01.2001</td>
<td>-</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03.2002</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06.2002</td>
<td>-</td>
<td>++</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.2002</td>
<td>-</td>
<td>++</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04.2003</td>
<td>+/-</td>
<td>++</td>
<td>390</td>
<td>+/-</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>05.2003</td>
<td>(+)</td>
<td>++</td>
<td>380</td>
<td>+/-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>06.2003</td>
<td>(+)</td>
<td>++</td>
<td>490</td>
<td>+/-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

- **Specific or Contamination?**

---

a) Donor was always HBsAg negative (AxSym)
b) AxSym (Abbott)  
c) Enzygnost (Dade Behring )
d) Detection limit >50 ge/mL  
e) with ultracentrifugation > 3 ge/mL
HBV DNA* in plasma extracts from a vaccinated anti-HBs thrombapheresis donor who seroconverted very slowly to anti-HBc

- HBsAg subtype ? y w3 137
- HBV/D PGSSTTSAGPCRTCTTTTAOGTSMYPSC
- Donor PGSSTTSAGTCRTCTTTAOGTSMYPSC
- Vaccine PGSTTTSTGPCKTCTTTPAOGNNSMFPSC
- HBsAg subtype d w2

* 3 or 11 GE/mL: escape mutant?
HBV DNA* in plasma extracts from a vaccinated anti-HBs thrombapheresis donor who seroconverted very slowly to anti-HBc

- HBsAg subtype: ? y w3 137
- HBV/D: PGSSSTTSAGPCRTCTTTTAQGTSMYPSC
- Donor: PGSSSTTSAGTCRTCTTTTAQGTSMYPSC
- Vaccine: PGSTTTTSTGPCKTCTTPAQPQGNSMFPSC
- HBsAg subtype: d w2 w2
HBV DNA* in plasma extracts from a vaccinated anti-HBs thrombapheresis donor who seroconverted very slowly to anti-HBc

- HBsAg subtype * y w3 ? w3 137
- HBV/D PGS STTSAGPCR TCTTT TAQGT SMY PSC
- Donor PGS STTSAGTCR TCTTT TAQGT SMY PSC
- Vaccine PGSTTTSTGPKT CTTP AQGN SMFPSC
- HBsAg subtype d w2 w2

- * causes vaccine escape
Speculations* on a case of anti-HBc seroconversion after successful vaccination

- HBV infection may occur inspite of a high anti-HBs titer
- Anti-HBc seroconversion may be very slow
- Anti-HBc assays may have very different sensitivity
- Viremia may persist at very low levels
- The current small S gene, genotype A vaccine from yeast may not protect against certain genotype D variants
- Hepatitis B vaccinations may not reliably improve HBV
- Safety of blood products

*Conclusions may change after completion of the study
Problems of HBsAg tests

• HBV-Genotypes react differently
  - WHO standard only genotype A
  - Worldwide genotypes B,C und D predominant
  - Genotype F not detected for years with some tests

• Insufficient sensitivity
  - Best possible ELISA detects 10pg HBsAg/ml
  - Or $10^6$ HBV particles/mL
  - if excessive HBsAg is not present in very early phase

• HBsAg is highly variable under immune selection
  - after post exposure immunisation
  - during reactivation

• Consequence: false negative results
Reactivation of hepatitis B during immunosuppressive lymphoma therapy

- Patient from Lebanon
  - 15 years ago resolved hepatitis B
  - Before therapy: anti-HBc +, anti-HBs >1000 IU/L, HBsAg -
  - Receives in 2002 for four months chemotherapy (CHOP),
  - Thereafter two months anti-B cell mab Rituximab

- Develops acute hepatitis B
  - $4 \times 10^9$/mL HBV genomes, anti-HBs 880 IU/L
  - Patient dies inspite Lamivudine therapy after 2 weeks

- HBsAg in AxSym highly positive, in VIDAS negative
  - PEI finds 2 further out of 5 tests which fail
  - sample in one further test weakly +, but not inhibitable

- HBsAg sequence shows 4 escape mutations
  - L109R, Y134S, P142L, D144A, and HBsAg subtype change R122K

University clinics B. Franklin, Berlin  T.Westhoff et.al, blood, in press
Genotype- and vaccine-escape induced specific exchanges in the α determinant of SHBs

Rituximab associated escape mutant

Conserved
Genotype-specific exchanges
Variable
Vaccine-induced exchanges
Subtype-specific alleles

S. Schaefer, modified
Schaefer, 2001
Reactivation of hepatitis B during immunosuppressive lymphoma therapy

• Patient from Lebanon
  - 15 years ago resolved hepatitis B
  - Before therapy in 2000:
    - anti-HBc +, anti-HBs >1000 IU/L, HBsAg -
    - Receives in 2002 for four months chemotherapy (CHOP),
    - Thereafter two months anti-B cell mab Rituximab

• Develops acute hepatitis B
  - 4x10^9/mL HBV genomes, anti-HBs 880 IU/L
  - Patient dies inspite Lamivudine therapy after 2 weeks

• HBsAg sequence shows 4 escape mutations
  - L109R, Y134S, P142L, D144A, and HBsAg subtype change R122K

• Same sequence present at 50 ge/mL before therapy!
Reactivation of hepatitis B during immunosuppressive lymphoma therapy II

- Italian patient with B cell lymphoma since 1989, several therapies
- 1996 HBsAg neg., anti-HBc neg.
  - anti-HBs 612 IU/L; no vaccination
- In 2003 4 cycles of chemotherapy
- July 2003 under chemotherapy
  - HBsAg AxSym highly pos., not inhibitable
  - HBsAg VIDAS neg.
  - Anti-HBc neg., anti-HBs 93 IU/L
  - No hepatitis
- Nonspecific HBsAg or reactivated HBV?

Communicated by R. Kaiser and H. Pfister, University Cologne
Acute resolving hepatitis B

- Anti-HBs
- Low level persistence
- HBsAg
- HBV DNA PCR
- Anti-HBc
- Acute resolving hepatitis B
Reactivation of hepatitis B during immunosuppressive lymphoma therapy II

- Italian patient with B cell lymphoma since 1989, several therapies
- 1996 HBsAg neg., anti-HBc neg.
  - anti-HBs 612 IU/L; no vaccination
- In 2003 4 cycles of chemotherapy
- July 2003 under chemotherapy
  - HBsAg AxSym highly pos., not inhibitable
  - HBsAg VIDAS neg.
  - Anti-HBc neg., anti-HBs 93 IU/L
  - No hepatitis
  - HBV DNA $1.8 \times 10^8$ copies/mL
- Lamivudine therapy started
- Escape mutations: L109R, C137W, G145R

Communicated by R. Kaiser and H. Pfister, University Cologne
Genotype- and vaccine-escape induced specific exchanges in the α determinant of SHBs

Rituximab associated escape mutant

Conserved  Genotype-specific exchanges
Variable  Vaccine-induced exchanges  Subtype-specific alleles

Schaefer, 2001
HBsAg signals of a dilution series from a patient serum without and with neutralisation with anti-HBs sera

Dilution of the patient serum (<1µg HBsAg/mL?)
Inhibition of HBsAg signals of a patient serum after neutralisation with anti-HBs sera from aHB-vaccinated donor (650 000 IU/L) or from a Sheep immunised with wt HBsAg genotypes A, D, C (40 000 IU)
Quantitative assay of HBsAg by electroimmunodiffusion
Laurell electrophoresis (QIE)

- Slides covered with antibody-containing agar
- Anti-HBs/α antiserum raised with subsequent injections of HBsAg genotypes A and D
- Wells are filled with HBsAg pos. serum
- HBsAg migrates to anode
- At equivalence antigen/antibody precipitates
- Length of figures increases with antigen

Gerlich and Thomssen, Devel Biol Standard 1975
Gerlich et al, 2003    Hepatitis B Virus Protocols Book, in press
Laurell Electrophoresis: no detection of an HBsAg Escape Variant Genotype D with a multivalent anti-HBs sheep antiserum raised with the genotypes A and D

Anti-wtA,D

2x10^6 IU/L

<table>
<thead>
<tr>
<th>HBsAg serum µg/ml</th>
<th>A</th>
<th>Esc</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>?</td>
<td>20</td>
</tr>
<tr>
<td>&lt;1µg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Escape mutations: L109R, C137W, G145R
Laurell Electrophoresis: successful detection of an HBsAg Escape* Variant Genotype D with a multivalent anti-HBs sheep antiserum raised with the genotypes A, D and C

<table>
<thead>
<tr>
<th>Anti-wtA,D</th>
<th>Anti-wtA,D,C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x10^6 IU/L</td>
<td>4x10^4 IU/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HBsAg serum µg/ml</th>
<th>A</th>
<th>Esc*</th>
<th>D</th>
<th>A</th>
<th>Esc*</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>ca 50</td>
<td>20</td>
<td>100</td>
<td>1:3</td>
<td>20</td>
</tr>
</tbody>
</table>

*Escape mutations: L109R, C137W, G145R

Anti-HBs of the patient neutralised by HBsAg A or D, not C
Conclusions on "unusual" HBV infections

- Early infectious phase of inapparent wt infection may be >60 days HBsAg negative
- Viremia may precede anti-HBc seroconversion >9 months
- HBV infection may occur inspite of high vaccine-induced anti-HBs titer; transmission of an escape mutant?
- Reactivation may occur in presence of high levels of anti-HBs: escape mutants
- HBsAg tests detect escape mutants less sensitive or not at all or non-inhibitable
- Persistent reactivatable infection may be anti-HBc negative, anti-HBs positive
- Genotype C antibodies detect genotype D escape mutant

- Are these cases indeed unusual or usually overlooked?
The message as question and answer

• Do we need to revisit laboratory diagnosis of HBV infections?
  - Yes, supersensitive HBV DNA assays are the reference test, not anti-HBc or HBsAg

• Does Anti-HBs protect against (re-) infection?
  - Not reliably, neither against breakthrough nor reactivation

• Are escape variants dangerous?
  - Occasionally yes, pathogenic and infectious

• May we need better HB vaccines?
  - Possibly, preS1, several genotypes, conformation

• Should we wait for better vaccines?
  - No, current vaccines protect better than no vaccine
No Question

Thank you for your patience and tolerance to listen to an unconventional view of hepatitis B diagnosis and prevention.