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University Medical Center Rotterdam

Quantitative HBV DNA measurements and the management of infected health care workers

A.A. van der Eijk

Department of Virology, Erasmus MC, Rotterdam,

the Netherlands

Introduction

- Worldwide since 1970s, 45 HCWs with HBV transmission described resulting in 434 infected patients.
- ± 500.000 operations per year in the Netherlands
- Since 1970 3 HCWs transmitting HBV identified in NL, resulting in 42 infected patients.

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Public Health Policy

- United States

  Exclusion from performing EPPs is based on the HBeAg status only.

- European consensus group

  Propose a cut-off level of 10^4 HBV DNA copies/ml and recommends that all HBeAg-positive HCWs should be excluded.

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Public Health Policy

- The Netherlands

  All HCWs with HBV DNA > 10^5 geq/ml are excluded. This cut-off minimizes both transmission risk and loss of valuable medical personnel.

- UK

  All HBeAg-positive HCWs are excluded, as well as HBeAg negative HCWs with HBV DNA > 10^3 geq/ml.

IGZ Bulletin: Preventie Iatrogene Hepatitis B, 2002

NHS Executive. Hepatitis B infected health care workers, 2000

Factors associated with transmission risk:

- Serum HBV-DNA level
- HBeAg positivity
- Duration of surgery
- Volume of blood transmitted
- Route of transmission: percutaneous vs. mucosal
- Skill and medical condition of HCW

Buster EHCJ, van der Eijk AA, Schalm SW. Antiviral Research 2003: 60(2):79-85

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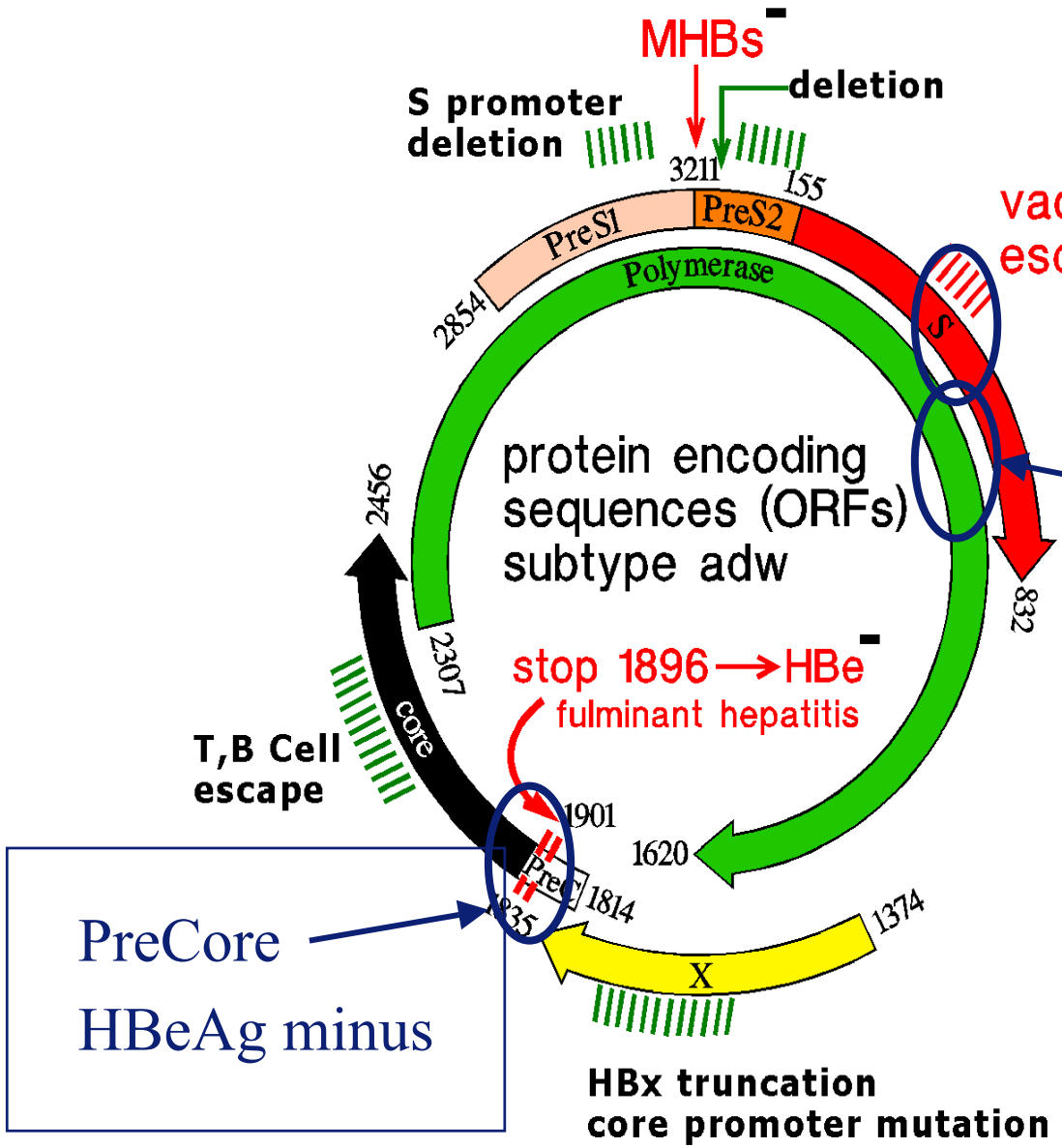
Frequent Mutations in the HBV Genome



HBV transmission and HBeAg status

- Most cases involved HBeAg positive HCWs
- First described case of transmission by HBeAg negative HCW in 1993
- All e-negative surgeons who transmitted HBV to patients have a precore mutant.
  - G-to-A transition at nucleotide 1896 introducing a stop in codon 28 is most common.
  - No production of HBeAg despite continuing replication.
- HBV DNA levels surgeons: 10^4 - 10^9 geq/ml

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Incident Investigation Teams, NEJM 1997; Corden, J Clin Vir 2002

Quantitative detection methods for HBV

- *Signal amplification systems:*
  - Hybrid Capture System (Digene)
  - Branched DNA assay (Bayer)

- *Target amplification systems*
  - COBAS Amplicor (Roche Diagnostics)
  - NASBA with molecular beacon detection
  - TaqMan or Real Time Detection Assay

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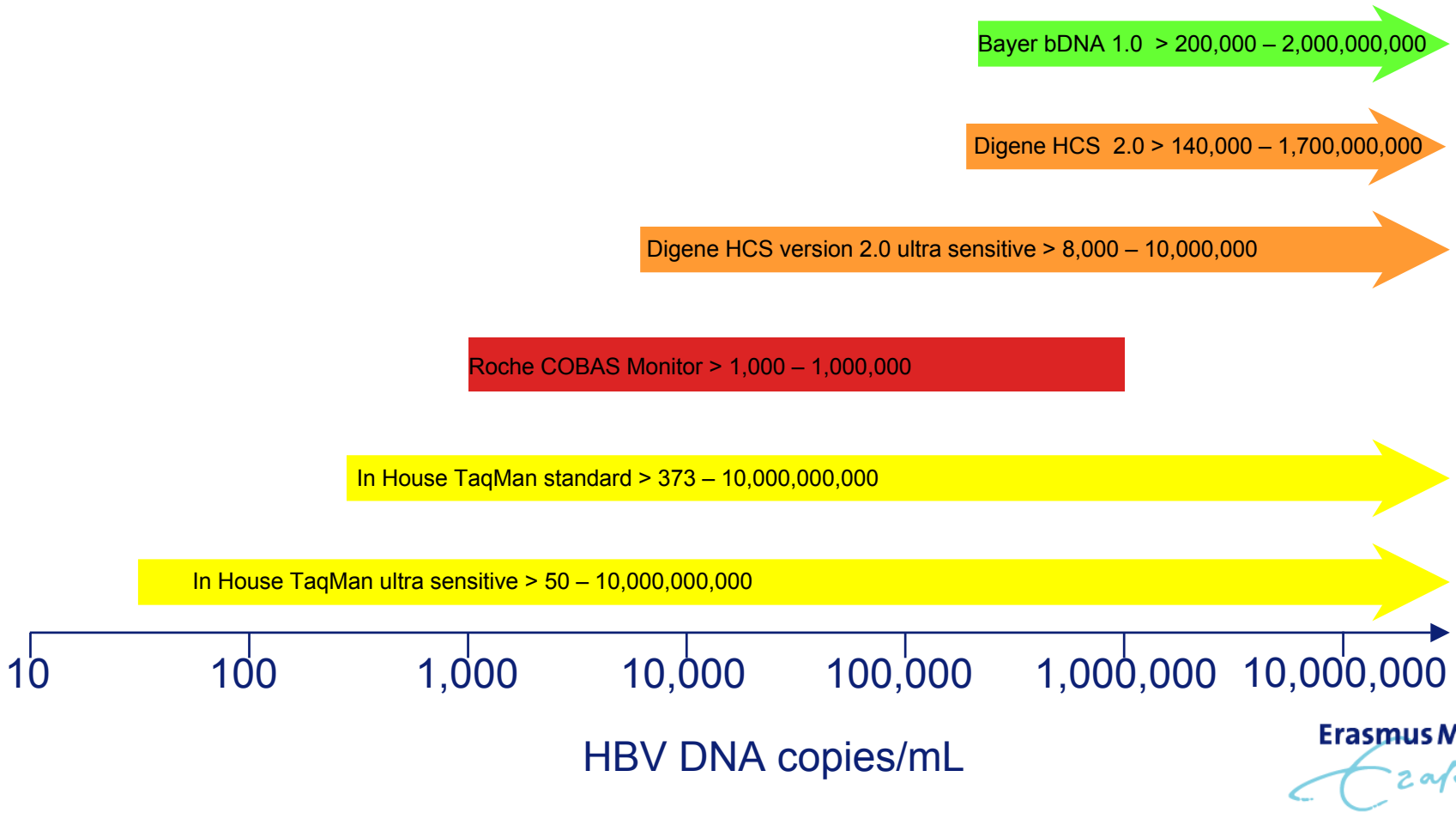
Ranges of HBV DNA assays



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More information becomes available...

Diagnosis usually demonstrated:

- The presence of a specific virus

but now additionally will involve:

- The quantity in which the virus is present

  Quantitative analysis

  - √ *to follow the course of chronic infections*
  - √ *to detect disturbances in host-pathogen interactions*
  - √ *to make soundly based therapeutic decisions*
  - √ *to determine levels of infectivity*

- A more precise description of the virus involved

  Qualitative analysis

  - √ *subtypes, genotypes, variants, mutants, genotypic resistance*
  - √ *increasingly relevant for management of the infection*

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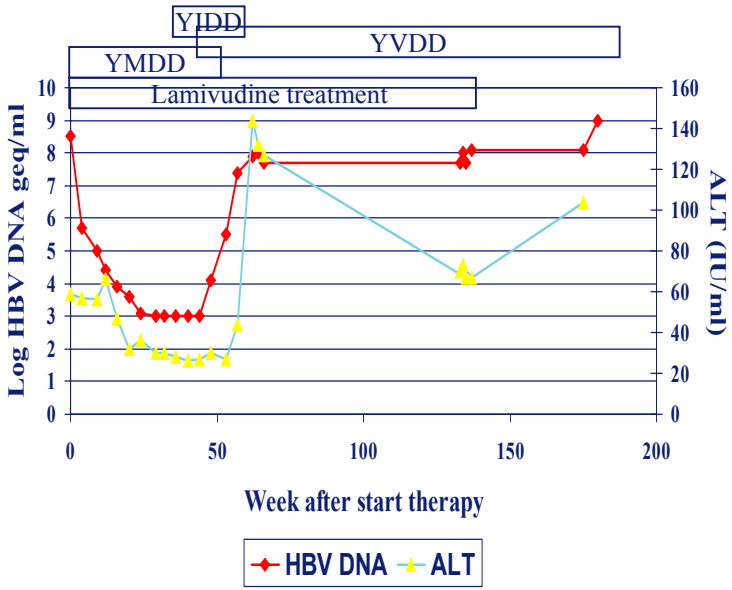
Disease Management

Pharmaceuticals

Laboratory



Diagnostics

"An *information-based*, *integrated* approach to managing a disease, to *optimize* clinical and economic outcomes".

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Percutaneous injury rates in surgery

- Surgeon's percutaneous injury: 7% of operative procedures in general, gynecological, cardiac, orthopedic, or trauma surgery.
- Recontact to patient's wound by sharp object causing surgeon's injury: 32% of observed injuries to surgeon.
- Potential transmission of viral hepatitis: 2-3%.

Tokars, JAMA 1992

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Estimation of infectious particles transmitted by needlesticks with a HBV DNA level of 10^5 geq/ml

Event	µl serum transmitted[1]	Infectious particles transmitted*
Suture needle		
▪ 0.33 mm needle, 5 mm penetration	0.03	<1
▪ 1.12 mm needle, 5 mm penetration	0.23	2
Hollow needle		
▪ 1.07 mm needle, 2 mm penetration	0.14	1
▪ 1.07 mm needle, 5 mm penetration	0.44	4

* Calculation infectious particles: volume of serum in ml x HBV DNA concentration in geq/ml x 0.10 (a)

a) Number of infectious HBV particles ≈ 10% of total number of HBV particles[2]

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[1]Bennett, J Am Coll Surgeons 1994; [2]Heermann, J Clin Microbiol 1999

Infectious particles transmitted by maternal-fetal transfusion and risk of transmission.

HBV DNA level (geq/ml)	Transmission risk	Infectious particles transmitted*
> 10^8	22%	6400
10^7	4%	640
10^6	1%	64
10^5	≈ 0%	6

0.64 µl serum transmitted during delivery by maternal-fetal transfusion[2]

* Calculation infectious particles: volume of serum in ml x HBV DNA concentration in geq/ml x 0.10 (a)

a) Number of infectious HBV particles ≈ 10% of total number of HBV particles[3]

[1]Xu, J Med Virol 2002; [2]Brossard, Vox Sang 1996; [3]Heermann, J Clin Microbiol 1999

Cases of doctor-to-patient of HBV

Author	HCW's profession	HBV DNA (geq/ml)	Quantification technique	Time sample taken
(Harpaz et al., 1996)	Thoracic surgeon	1.0×10^9	Semiquantitative PCR dot-blot hybridization, with serum containing 10^8 chimpanzee-infectious particles as comparison.	4 months after transmission
(The Incident Investigation Teams, 1997)	(1) General surgeon (2) Gynaecologist (3) Gynaecologist (4) General surgeon	1.0×10^7 4.4×10^6 5.5×10^6 2.5×10^5	Liquid Hybridization and enzyme-linked oligonucleotide assay	12 weeks after transmission Unknown Unknown 12 weeks after transmission
(Molyneaux et al., 2000)	Surgeon	1.03×10^6	Lightcycler PCR	Unknown
(Spijkerman et al., 2002)	Surgeon	5.0×10^9	Limited dilution PCR	1 year after identification first infected patient.
(Corden et al., 2003)	(1) Surgeon (2) Surgeon (3) Surgeon (4) Surgeon (5) Surgeon (6) Surgeon	1.12×10^8 2.55×10^5 6.72×10^5 6.35×10^4 4.20×10^8 9.47×10^8	Chiron Quantiplex Branched DNA assay and Roche Amplicor HBV DNA monitor assay	At least 3 months after transmission in all surgeons.

EHJC Buster, AA van der Eijk et al, Antiviral Research 2003;60(2):79-85

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Implications of choosing a HBV DNA level as a cut-off level

- Variability in time of HBV DNA levels in HBV carriers

- Reliability and reproducibility of the molecular diagnostic tests.

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HBV DNA levels in HBeAg negative patients

- Martinot-Peignoux et al. J Hepatol (2002);36:543-46
  - Quantitative HBV DNA levels in inactive HBsAg carriers
  - Mean HBV DNA concentration 1300 copies/ml
  - 98% of sera of inactive HBeAg negative carriers contained HBV DNA levels below 10^5 copies/ml

- Tedder et al. J Med Virology (2002);68:505-512
  - evidence for fluctuations in HBV DNA levels in HBeAg negative HBV carriers

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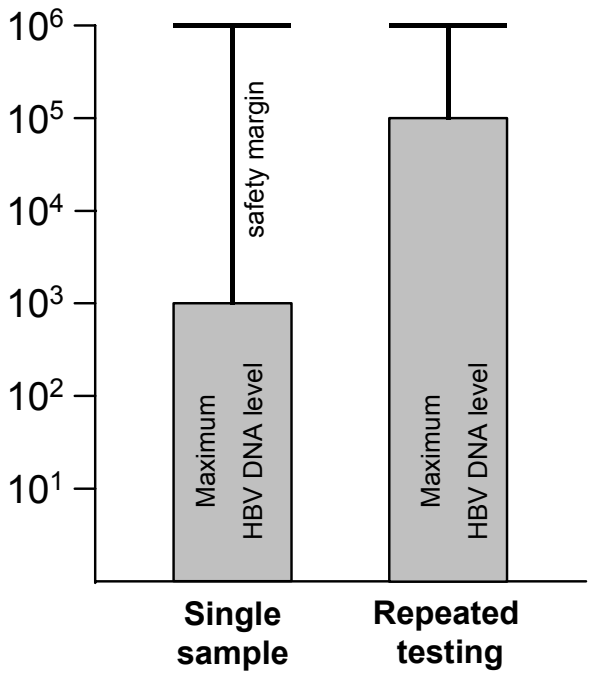
Single sampling vs repeated testing

- Frequency of testing in relation to maximum HBV DNA level and the safety margin needed to account for fluctuations in HBV DNA level and variability of assay used for quantifying HBV DNA.



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Reliability and reproducibility

- The need for internationally defined reference standards
- Since December 7, 2003 the use of an internal calibration standard is mandatory to standardise the commercial as well as commercial kits
- A standard is developed for HBV. However this standard is developed only for genotype A

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Geographic distribution of HBV genotypes

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Reliability and reproducibility

- Qualitative and quantitative assays must yield reproducible results
- inter- and intra-assay variability is more profound in samples with low HBV DNA level
- The use of an internal control is imperative to monitor the quality of extraction and amplification

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Discussion

1. Should HBV DNA be measured instead of HBeAg ?

- Active replication of HBV is associated with the presence of HBeAg
- Knowledge of HBV DNA levels in HBeAg-negative persons makes exclusion of HCW solely based on HBeAg status only obsolete

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Discussion

2. Which level of HBV DNA is acceptable to prevent transmission of HBV from HCW to patient during EPPs ?

- Choosing a low level (10^3 copies/ml) it must be realized that
  - inter- and intra-assay variability is more profound in samples with low HBV DNA level
  - repeated testing will lead to a greater proportion of exclusion
- Regular monitoring of HBV DNA levels can narrow safety margin
- Introduction of internationally defined reference standards for all genotypes as well as participation in international quality control programs is required

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Discussion

3. To what extend is the loss of valuable HCWs acceptable ?

- Vaccination against HBV is safe and should be mandatory
- Each HCWs who carries HBV must be referred to a hepatologist
- Antiviral therapy may reduce the viral load and thus may prevent unnecessary exclusion of valuable medical personnel

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Discussion