IPFA/PEI 30th International Workshop on
Surveillance & Screening of Blood-borne Pathogens

15-16 May 2024 | Aarhus, Denmark

Occult HBV infection and blood safety: focus on HBV-endemic East Asia

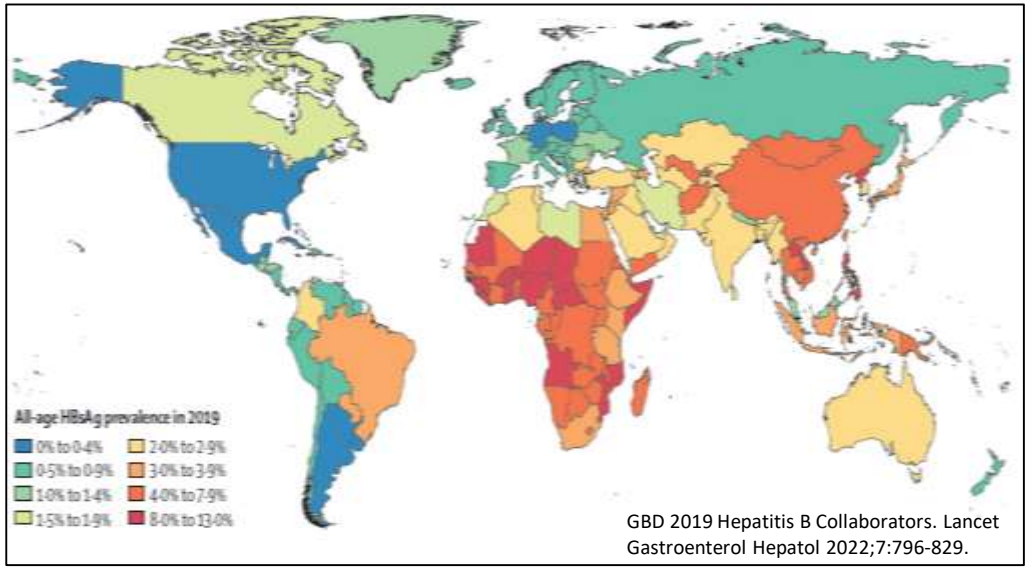
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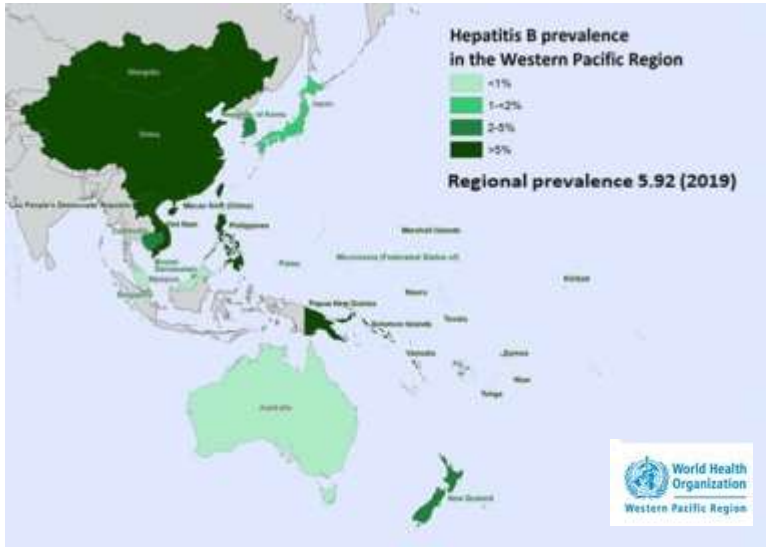
HBV epidemiology

- **WHO estimates that ~2 billions individuals have been infected**
- **~296 million of people are chronic carriers of HBsAg**
- **1.5 million new infections/year**
- **HBV infections account for >820 000 deaths per year**

  transient silent infection → chronic hepatitis → hepatocellular carcinoma



GBD 2019 Hepatitis B Collaborators. Lancet Gastroenterol Hepatol 2022;7:796-829.

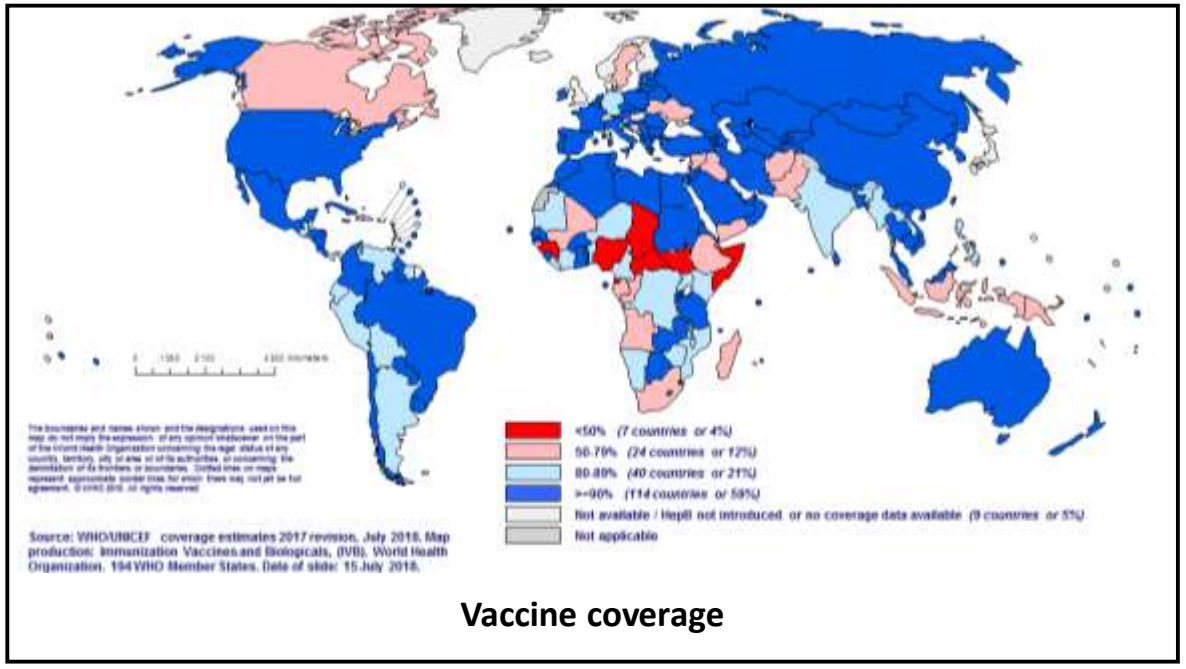


HBV in South-East Asia	
People living with HBV infection	61.4 million
New infections/year	266 000
Deaths/year	218 000
% diagnosed people	2.8%
% treated diagnosed people	3.5%
% treated all people with HBV	0.1%

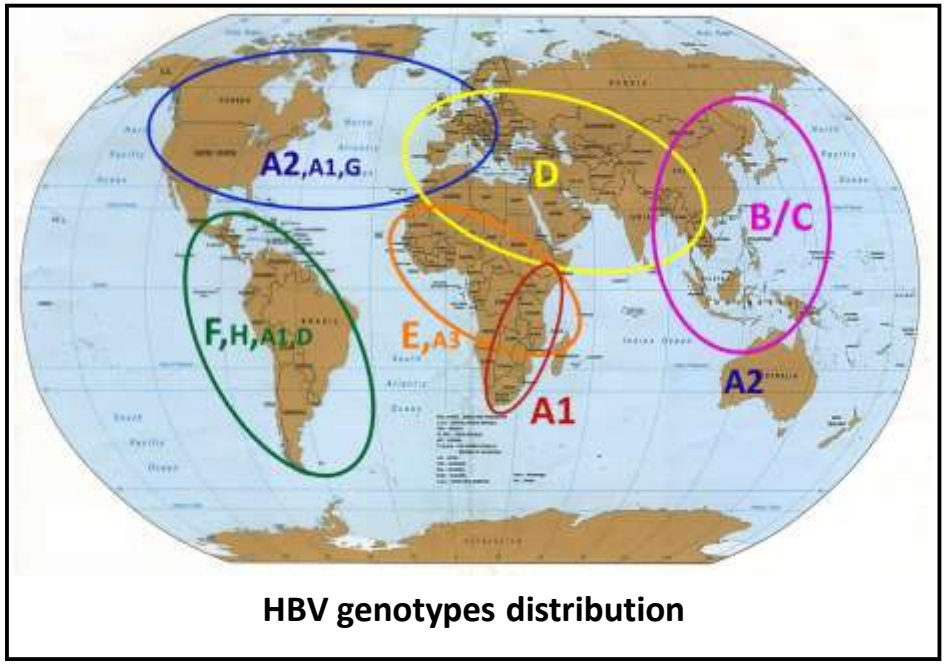
WHO. Global hepatitis report 2024.

HBV vaccine & treatments

- **HBV vaccine**
  - >20 years national vaccination programs in Southeast Asia:
  - Reduction chronic infection prevalence , liver diseases incidence, and efficacy to prevent vertical transmission



Vaccine coverage



HBV genotypes distribution

- **Anti-HBV treatments → functional cure but no HBV eradication**
  - Sustained loss of detectable HBsAg with or without anti-HBs
  - Undetectable serum HBV DNA
  - Persistence of cccDNA with low or no transcriptional activity

Natural persistent form of HBV infection: OBI?

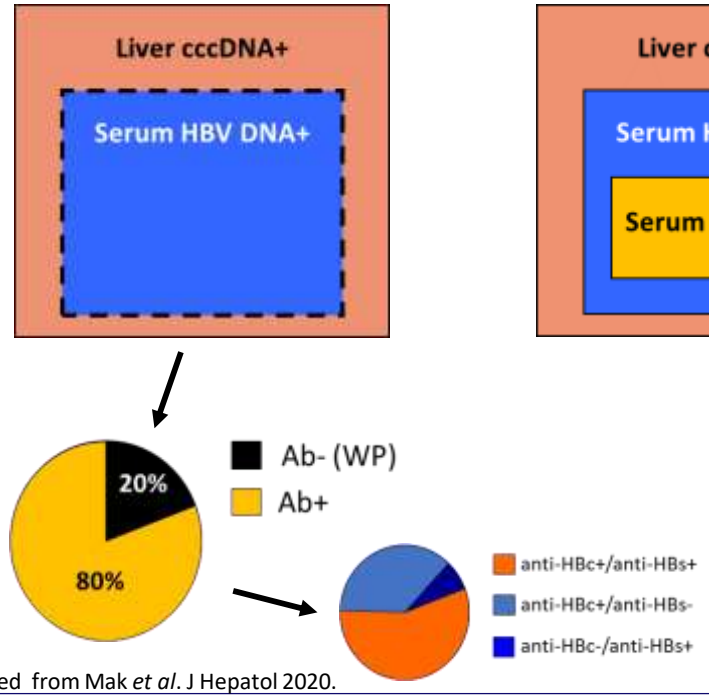
Occult HBV infection (OBI)

Updated statements of Taormina Consensus Conference 2018

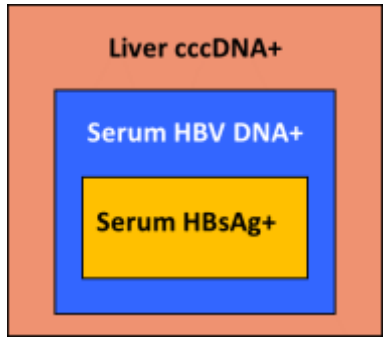
(Raimondo *et al*. J Hepatol 2019)

Presence of replication competent HBV DNA in liver and/or HBV DNA in blood of persons testing HBsAg negative by currently available assays with/without anti-HBc or anti-HBs

Occult HBV infection



Overt HBV infection



Adapted from Mak *et al*. J Hepatol 2020.

OBI genesis

- HBsAg decline to a sub-detectable level during long-term chronic carriage
- Persistence of low levels viral replication in recovered infection (VL<200 IU/mL)
- Imperfect immune control of HBsAg variants
- S mutations impairing HBsAg production/secretion
- Mutations/epigenetic factors affecting HBV replication and/or viral gene expression

HBV NAT yield in blood donors

NAT-positive and NAT-yield donations by region (NAT implementation to 2019)

(Adapted from Faddy *et al*. Vox Sang 2024)

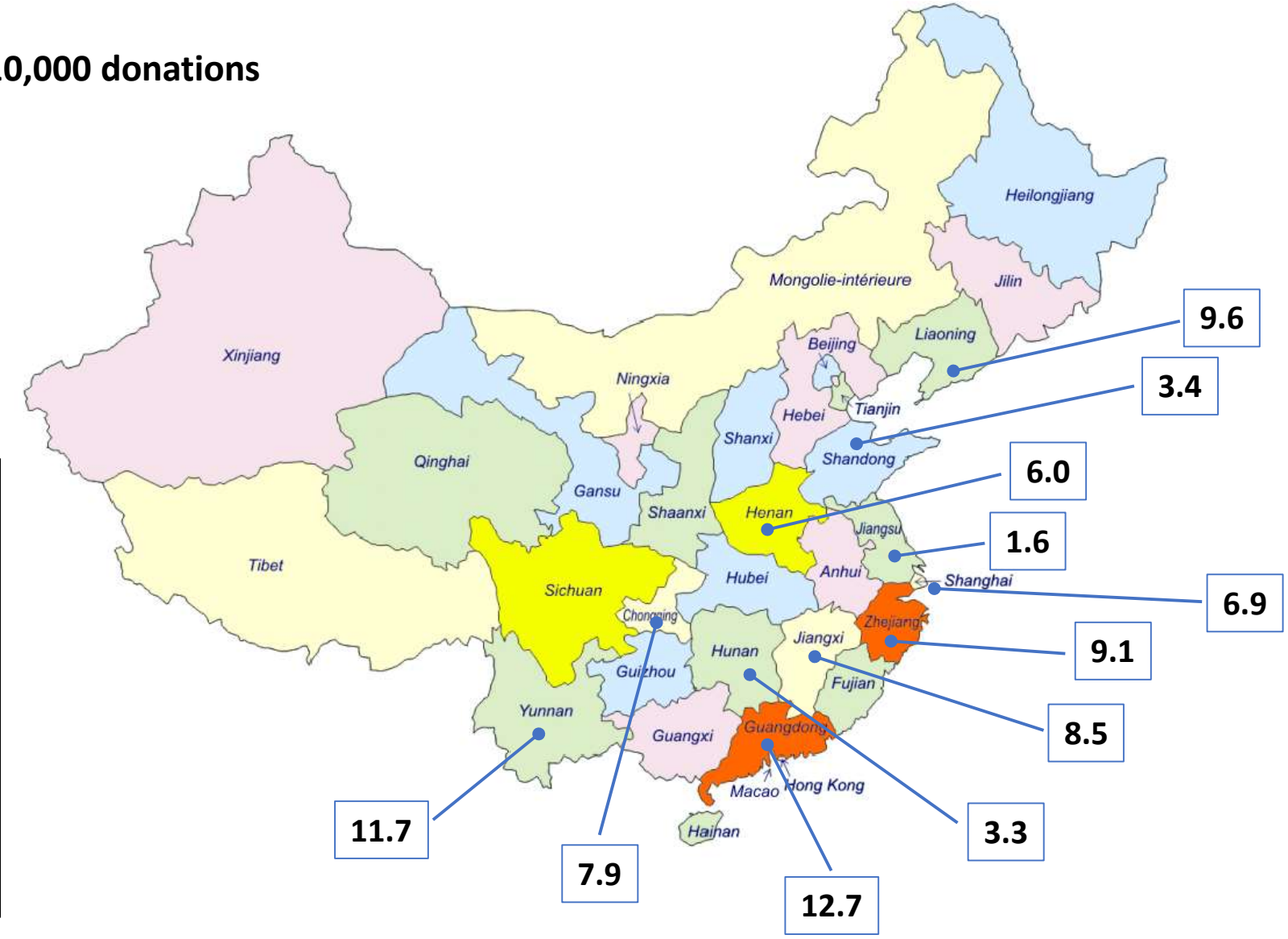
	Africa	Asia Western Pacific	Europe	North America	South America	Total
Donations tested (n)	8,372,857	152,221,471	102,928,968	103,272,077	2,418,536	369,213,909
NAT-positive donations (n)	12,462	33,093	15,975	5,852	714	68,096
NAT positivity (rate)*	1488.38	217.40	155.20	56.67	295.22	184.44
NAT yield donations (n)**	**2,318**	**11,116**	**897**	**124**	**10**	**14,465**
NAT yield positivity (rate)*	**276.85**	**73.03**	**8.71**	**1.20**	**4.13**	**39.18**

*Rate is expressed per 1,000,000 donations.

**NAT yield refers to samples NAT-reactive only and includes WP and OBI samples.

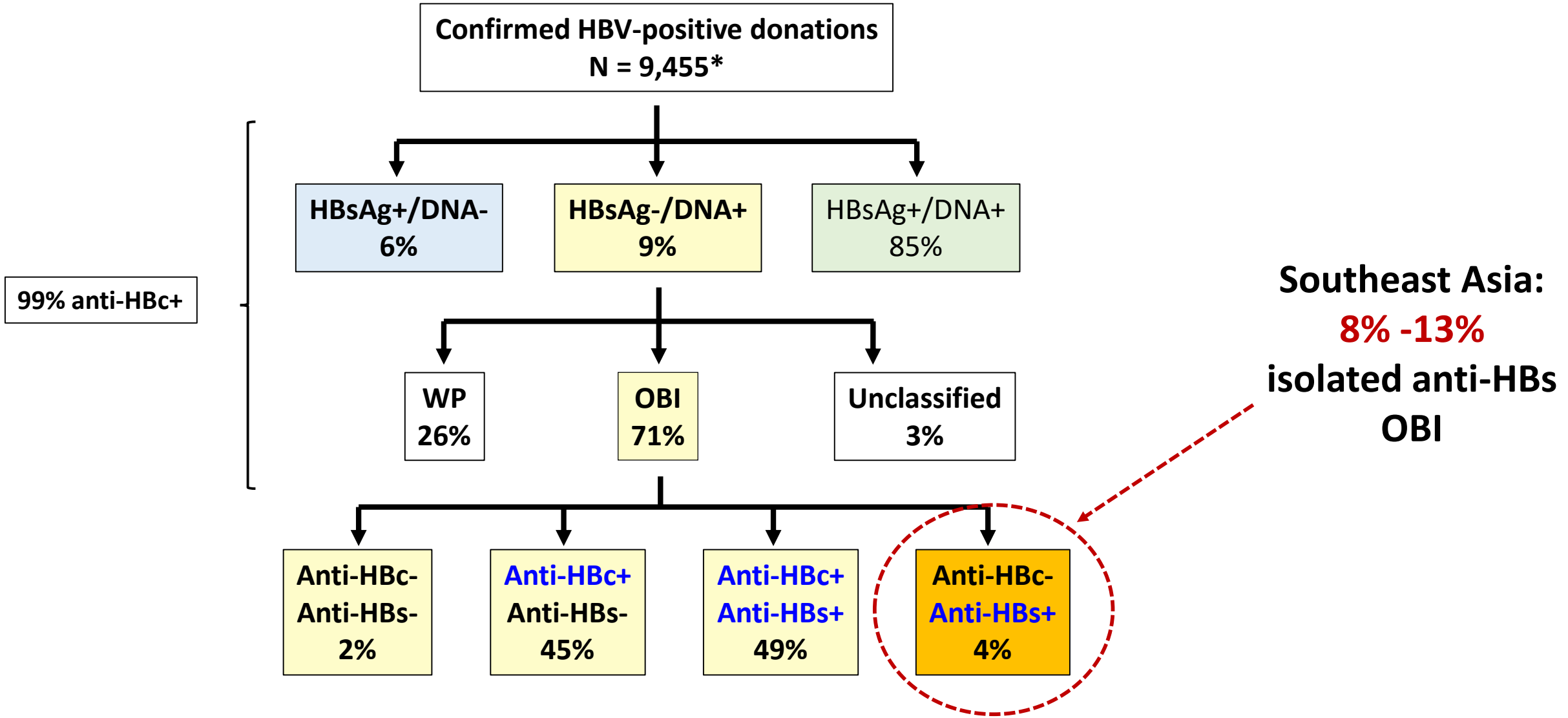
OBI yield in Chinese blood donors

OBI yield rate expressed per 10,000 donations



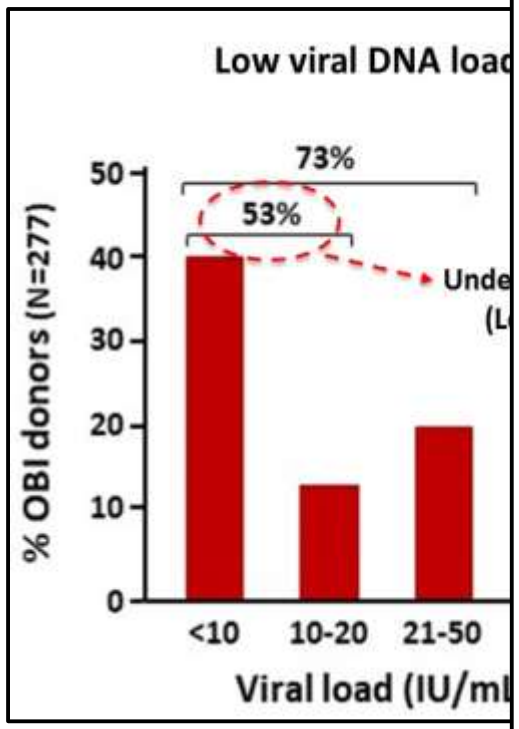
- HBsAg assay diversity: 8
- NAT assay diversity: 8
  - 38% MP-8
  - 40% MP-6
  - 22% ID
- HBV reactivity confirmation: 82%
  - Follow-up
  - in-house NAT
  - alternative commercial NAT
  - National reference laboratory

Serological markers in HBV-infected blood donors

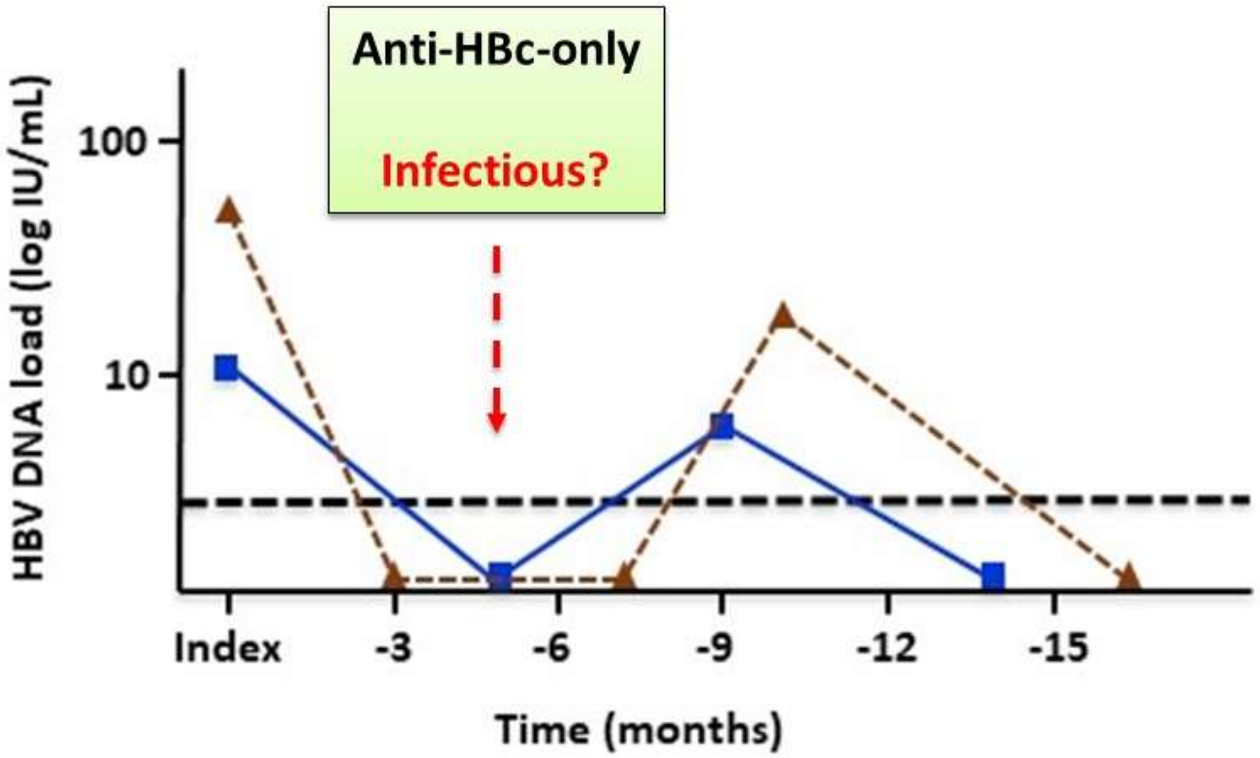


* Donors from South Africa (n=3,416), the Mediterranean region (n=1,608), Central & Northern Europe (n=503), **South East Asia (n=3,754)**, and Oceania (n=174); ID-NAT screening with Procleix Ultrio (Grifols) and HBsAg with Abbott PRISM or ARCHITECT assays. Data from Lelie et al. Transfusion 2017.

Detectability of HBV DNA in blood donors

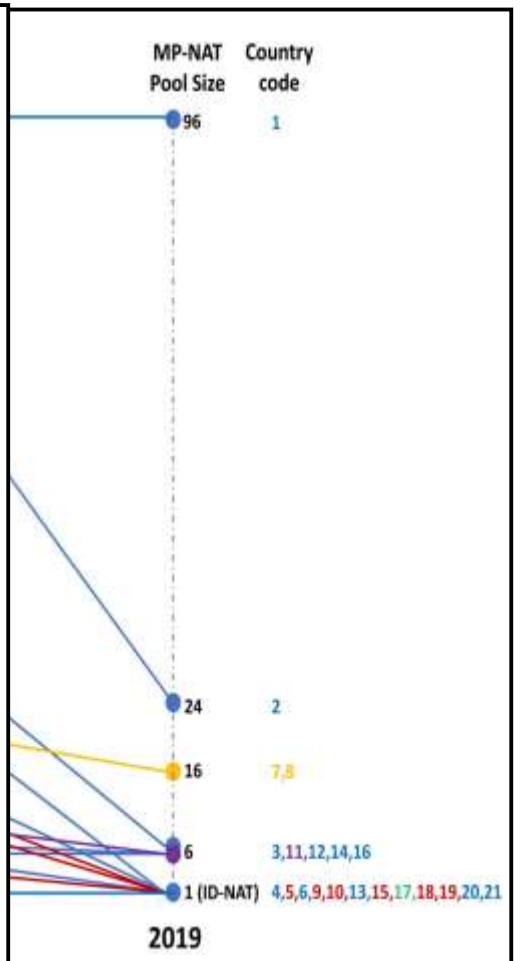


Intermittently detectable HBV viraemia



55% of 51 OBI donors from Hong Kong

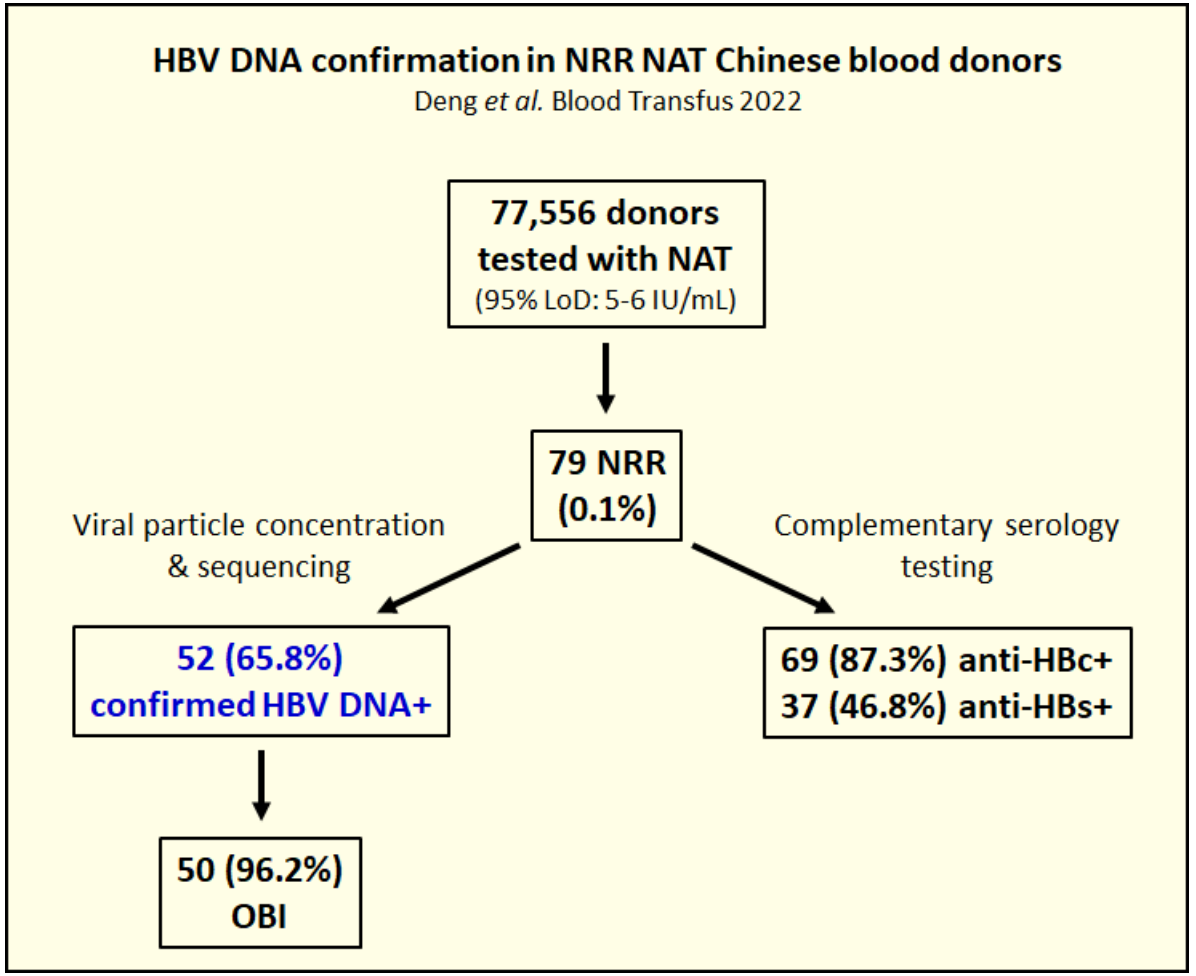
Candotti *et al.* Gut 2012



Adapted from Faddy *et al*. Vox Sang 2024

OBI vs NAT non-repeatable reactive donations

- **NRR rates of 0.09% - 0.29%**
  - Definition according to assays used & users testing algorithms
- **NRR NAT frequently associated with seronegative donations**
  - Alternative biomarkers (i.e. HBcrAg; HBV RNA)?
- **Complex confirmatory procedures**
  - Donor follow-up
  - Re-testing from initial plasma bag --> cross-contamination
  - Re-testing with different NAT assays --> analytical sensitivity?
  - Multiple repeat testing
  - Viral nucleic acid detection assays with improved sensitivity
    - Viral particle concentration from enlarged sample volume
      --> i.e. PEG precipitation; ultracentrifugation
    - Alternative in-house sensitive molecular assays & **sequencing**
- **Majority of NRR donors carried OBI**
  - Potential residual threat
  - Support NRR donors permanent deferral



HBV transfusion-transmission residual risk

- **Residual risk mainly associated with ID-NAT non-reactive OBI donations with/without anti-HBc**

  (Vermeulen *et al.* Vox Sang 2014; Candotti *et al*. Gut 2019; Satake *et al*. Transfusion 2023)

- **Minimal infectious dose by transfusion: 16 – 160 geq (<600 IU)**

  --> screening NAT should reach 0.15 IU/mL limit of sensitivity

- **Estimation of residual risk**
  - **3% with RBC and 3%-14% with FFP** (Seed et al. Vox Sang 2015; Weusten et al. Transfusion 2017)
  - 1 in ~4 millions donations excluding anti-HBs+ donations (Seed et al. Vox sang 2017)
  - Mathematical models used
  - Limited look-back studies --> underestimation of HBV transmission?

- **Residual risk depends on several factors**
  - HBV epidemiology
  - Donor populations selection & screening strategies

- **Transmission related to:**
  - Volume of plasma in transfused blood products: FFP (200 mL) > RBC (20 mL)
  - Anti-HBV immune status of donor & recipient
  - Replicative properties of infecting virus

Reduction of HBV residual risk

- **Anti-HBc testing**
  - Not suitable in high endemic settings (i.e. 30-40% seroprevalence in general Chinese population) --> blood shortage
  - Real anti-HBc prevalence in blood donors?
  - Assay specificity and confirmation --> 16–75% false-reactivity rates reported
  - WP and anti-HBc nonreactive OBI (**8-13%** of OBI in Asia)

- **Pathogen reduction procedures**
  - Efficacy of 2 - >5 log reduction in HBV infectivity but not elimination of infectious risk (HBV VL: <6 - >10^9 IU/mL)
  - Not available for all blood products --> RBC still in development
  - Controversies regarding impact on treated products clinical properties, albeit generally satisfactory
  - Cost & practical sustainability in settings where serology and NAT are already limited

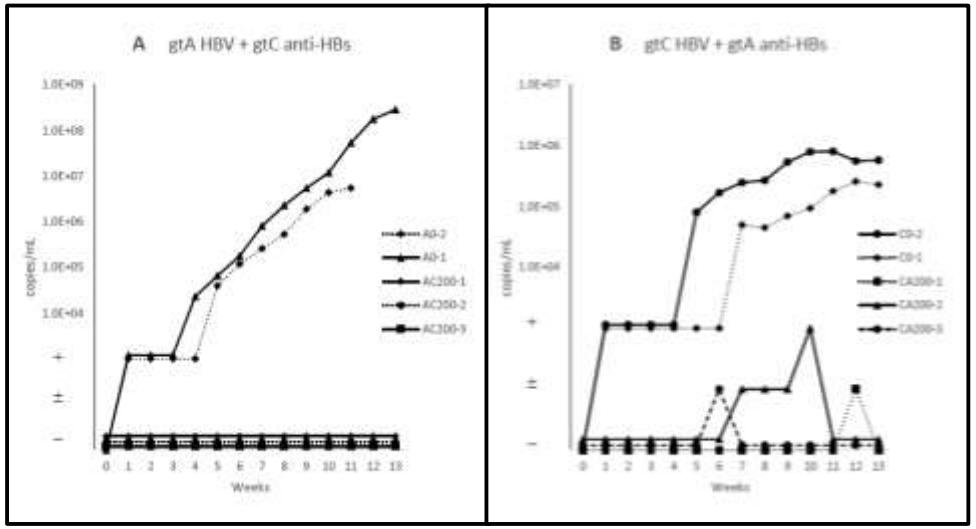
- **Universal HBV vaccination**
  - 10-18% of vaccinated subjects failed to mount an adequate antibody response
  - Waning immunity over time but immune memory retained in 90% of subjects (Lai et al. Clin Microbiol Infect 2019)
  - Prophylactic or therapeutic vaccine?

What level of protection do anti-HBs offer?

- **Natural & vaccine anti-HBs prevent HBV chronic infection & liver diseases**

- **Vaccinated individual with anti-HBs < 100 IU/L:**
  - Semi-abortive acute infection with attenuated symptoms --> persistent infection?

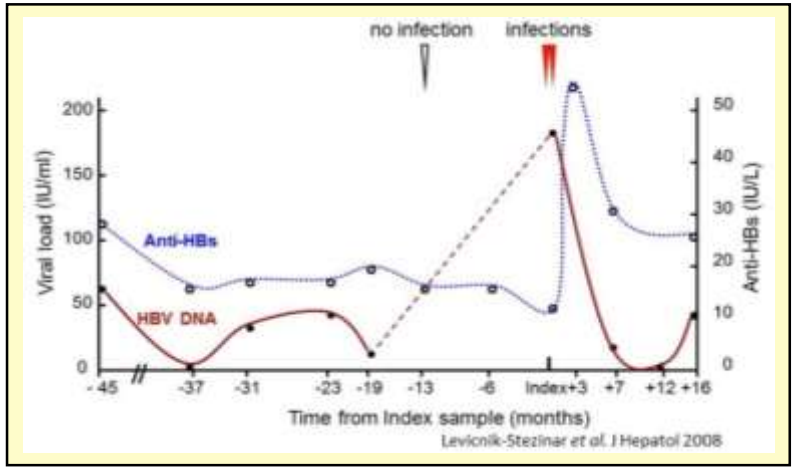
- **Suboptimal cross-protection according to HBV genotypes**
  - Vaccinees infected with $HBV_{B\text{-}D,F}$ strains
    (Stramer *et al*. N Engl J Med 2011)
  - 67% vaccine effectiveness in population exposed to HBV_C
    (Cheah *et al*. Vaccine 2018)
  - Anti-HBV_{A2} less effective at neutralizing HBV_C than HBV_{A2} strains
    (Hoshi *et al*. Transfusion 2019)

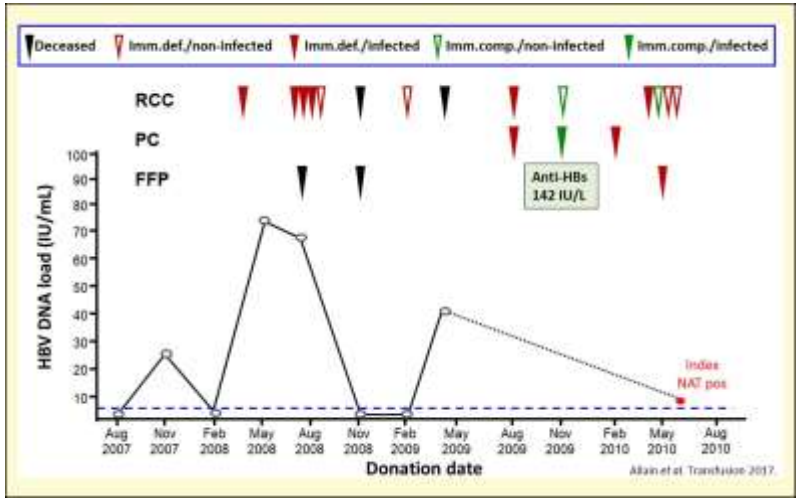


- **Anti-HBc-only blood eligible if anti-HBs ≥ 200 IU/L in Japan**

What level of protection do anti-HBs offer?

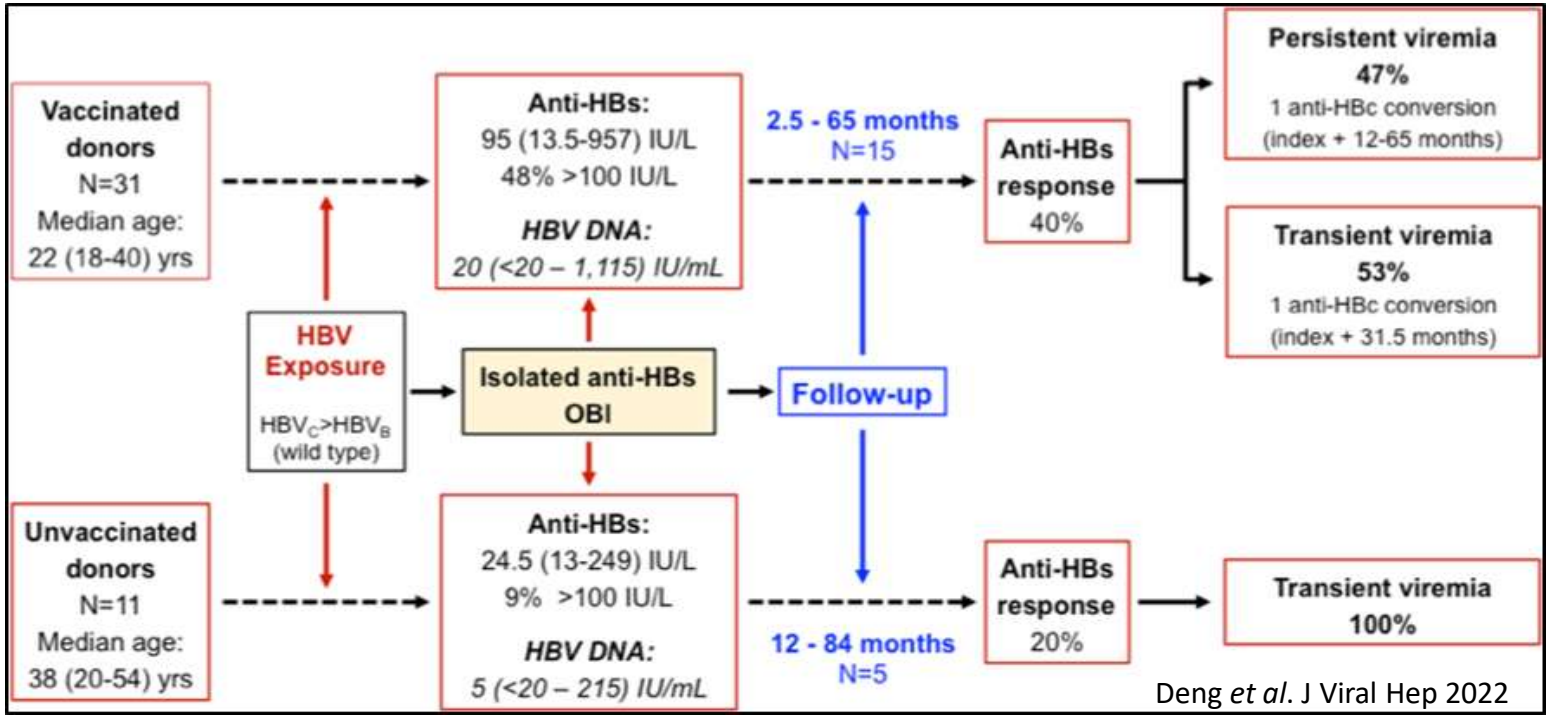
- **40-50% of OBI associated with low level of anti-HBs**
- **6-22% anti-HBc+ in donors born after mandatory vaccine program in Eastern Asia**
  - 88-92% anti-HBc+ Chinese donors carried anti-HBs --> 0.2-0.5% HBV DNA+ (Zheng et al. Transfusion 2015)
  - OBI in vaccinated children (<20y):
    - 1-3% China (Su *et al*. 2017; Zhuge *et al*. 2020)
    - 4-5% Taiwan (Hung *et al*. 2019; Lai *et al*. 2022)
    - 12% Thailand (Poovorawan *et al*. 2011)
- **Rare HBV transmission from anti-HBs+ OBI donors & infection of anti-HBs+ recipients**
- **Anti-HBs IUs/mL value did not strictly correlate with *in vitro* neutralization activity**
  - No evidence of anti-preS1 and anti-preS2 Ab contribution to neutralization (Aronthippaitoon *et al*. Vaccines 2023)





Isolated anti-HBs OBI donors

- **8-13% of OBI in Asia (4% worldwide)**
- **Lasting in time with:**
  - Transient viremia
  - Fluctuating persistent low viremia
- **72% young vaccinated donors (China)**
  - Average age: 24y
    --> 55% at birth & 39% 6-16y
  - Complete vaccine course?
  - Type/HBV genotype specificity of vaccine?
- **HBV transfusion-transmission with isolated anti-HBs / NAT nonreactive blood donation reported in Japan**



Deng *et al*. J Viral Hep 2022

Donation year	ID-NAT (3.1 IU/mL)	HBsAg (0.05 IU/mL)	Anti-HBc (0.4-0.5 PE IU/mL)	Anti-HBs (mIU/mL)
2019-1	Neg	Neg	Neg	1.3
2019-2	Neg	Neg	Neg	0.3
Dec 2019 (index)	Neg	Neg	Neg	10.7
2020	Pos (<20 IU/mL)	Neg	Neg	95.8

Adapted from Satake *et al*. Transfusion 2023

NAT reactivity versus HBV infectious particles

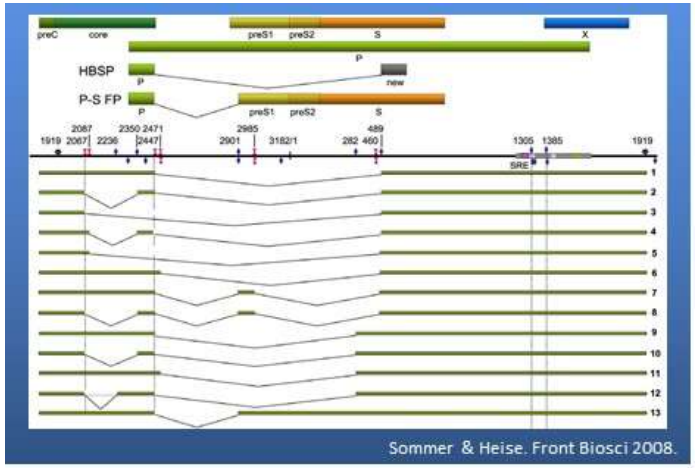
- **Detection of small viral nucleic acid fragment(s)**
- **Indirect evidence of potentially infectious virions from OBI characterization and transfusion-transmission studies**

BUT…

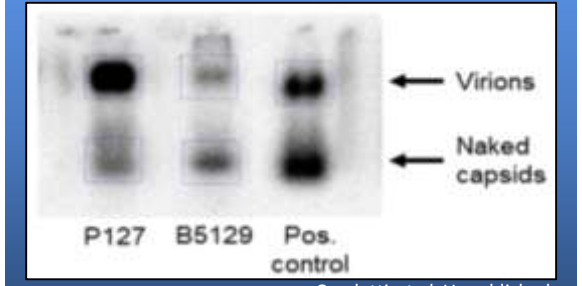
- **HBV pgRNA splicing --> encapsidation of defective genomes**
- **Non-enveloped capsids containing HBV DNA**

Whole HBV genome amplification (3,200 bp)		
Asia	Europe	South Africa
35.5% (49/138)	22% (16/74)	26% (14/54)

Allain & Candotti. Blood Transfus 2009



Sommer & Heise. Front Biosci 2008.



Candotti *et al*. Unpublished.

In summary

- **OBI defined by undetectable HBsAg and concomitant presence of low HBV DNA level**
  - **Natural late phase of HBV subclinical persistent infection**
  - **Prevalence associated with the general local HBV epidemiology**
  - **Multifactorial mechanisms --> including human interventions?**

    --> suboptimal vaccination and/or treatments

- **OBI remains the main risk of hepatitis B transfusion-transmission**
  - **Low infectious dose by transfusion**
  - **Barely detectable viremia --> extremely sensitive assays needed**
  - **Diversity of (unusual) serological patterns:**

    --> Impaired detection

    --> Confirmation requiring complex testing algorithms --> issues for donor re-entry
  - **Virus fitness**

In summary

- **Protective role of anti-HBs in donors and/or recipients**
  - **Effective in preventing OBI transfusion-transmission**
  - **Effective in controlling HBV infection progression**
  - **Suboptimal efficacy in preventing hepatocyte infection?**
    - **--> low anti-HBs level versus HBV genetic/antigenic diversity**
    - **--> need to adapt vaccine strategies (design, boosters,…) according to HBV molecular epidemiology?**
    - **--> risk of developing anti-HBc-negative OBI**
  - **Ineffective of eliminating HBV genome (integrated DNA, cccDNA) from infected hepatocytes**
    - **--> retain pro-oncogenic properties**
    - **--> inform OBI donors about non-specific clinical risk**
      - **long-term limited(?) risk of liver disease including HCC**
      - **HBV reactivation risk if immunosuppression**

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Blood Donors & Recipients worldwide

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C Niederhauser
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Supports