VHPB Technical meeting

The impact of viral hepatitis treatment, vaccination non-responders & occult hepatitis on public health

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Residual risk of Hepatitis B virus transfusion-transmission: need for reappraisal of blood safety measures?

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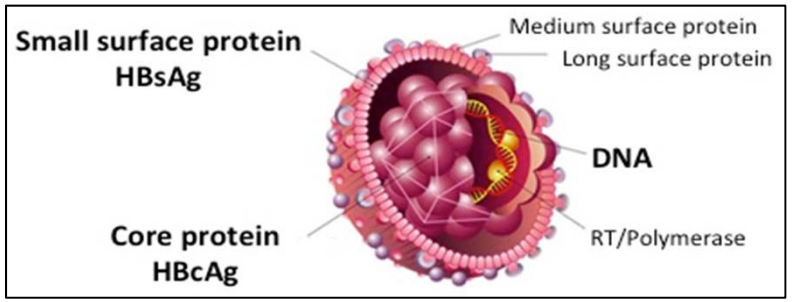
Hepatitis B virus in transfusion

- **Transmission with infected blood or blood products**

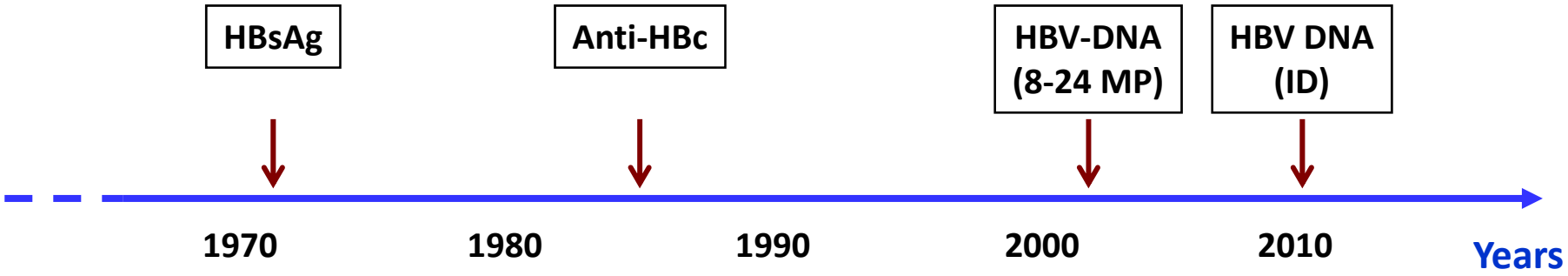
- **Continuous and significant reduction of HBV transfusion-transmission risk over the past decades**

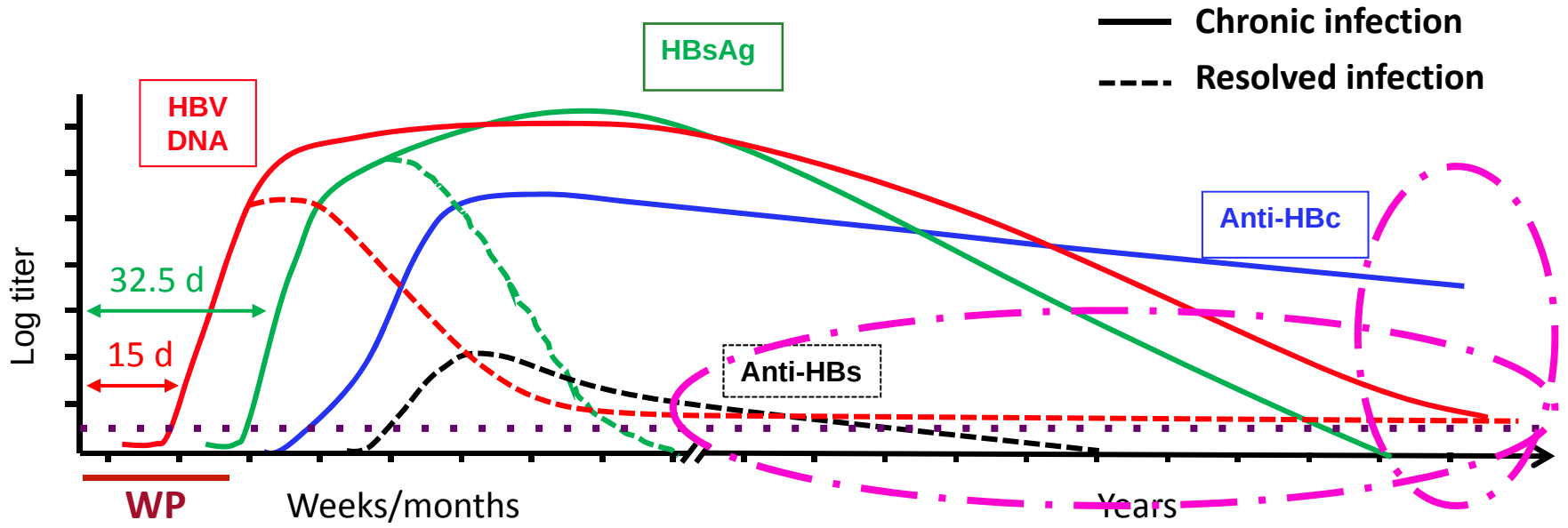
- **Implementation of safety measures:**
  - Donor selection --> evaluation of behavior risks
  - **Serological screening --> HBsAg and anti-HBc Ab**
  - **Molecular screening --> HBV DNA**
  - Pathogen reduction procedures



- **Hepatitis B remains the most frequent transfusion-transmitted viral infection**

HBV screening in blood donors





Residual risk of HBV transfusion-transmission

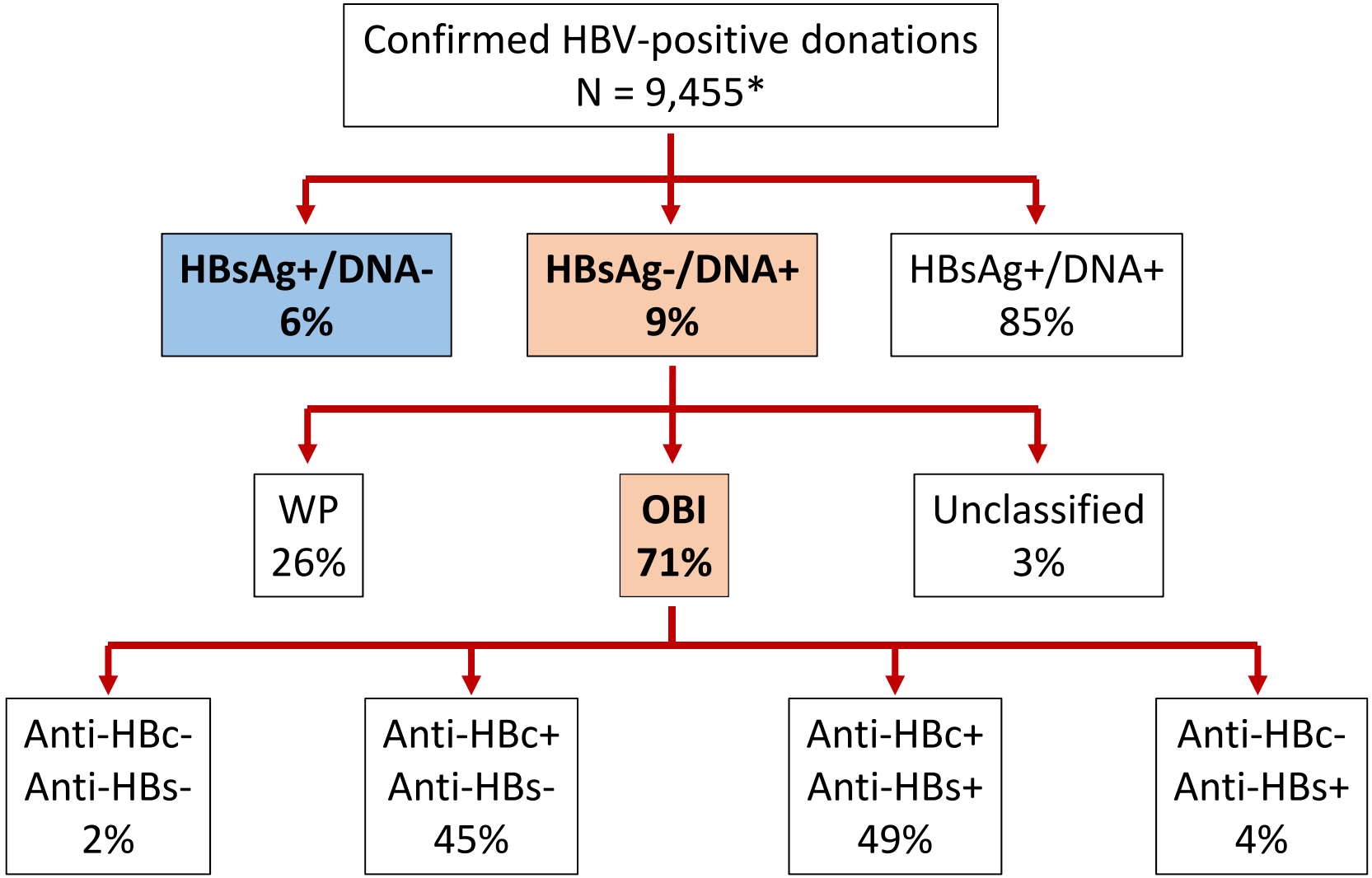
- **Residual risk depends on several factors**
  - HBV epidemiology
  - Donor populations
  - Screening strategies

- **Estimation of residual risk**
  - Low endemic settings: <1 – 1.4 per million donations
  - High endemic settings: 16 – >100 per million donations

- **Limits**
  - Mathematical models used
  - Lack of recent and liable HBV epidemiology data in blood donors

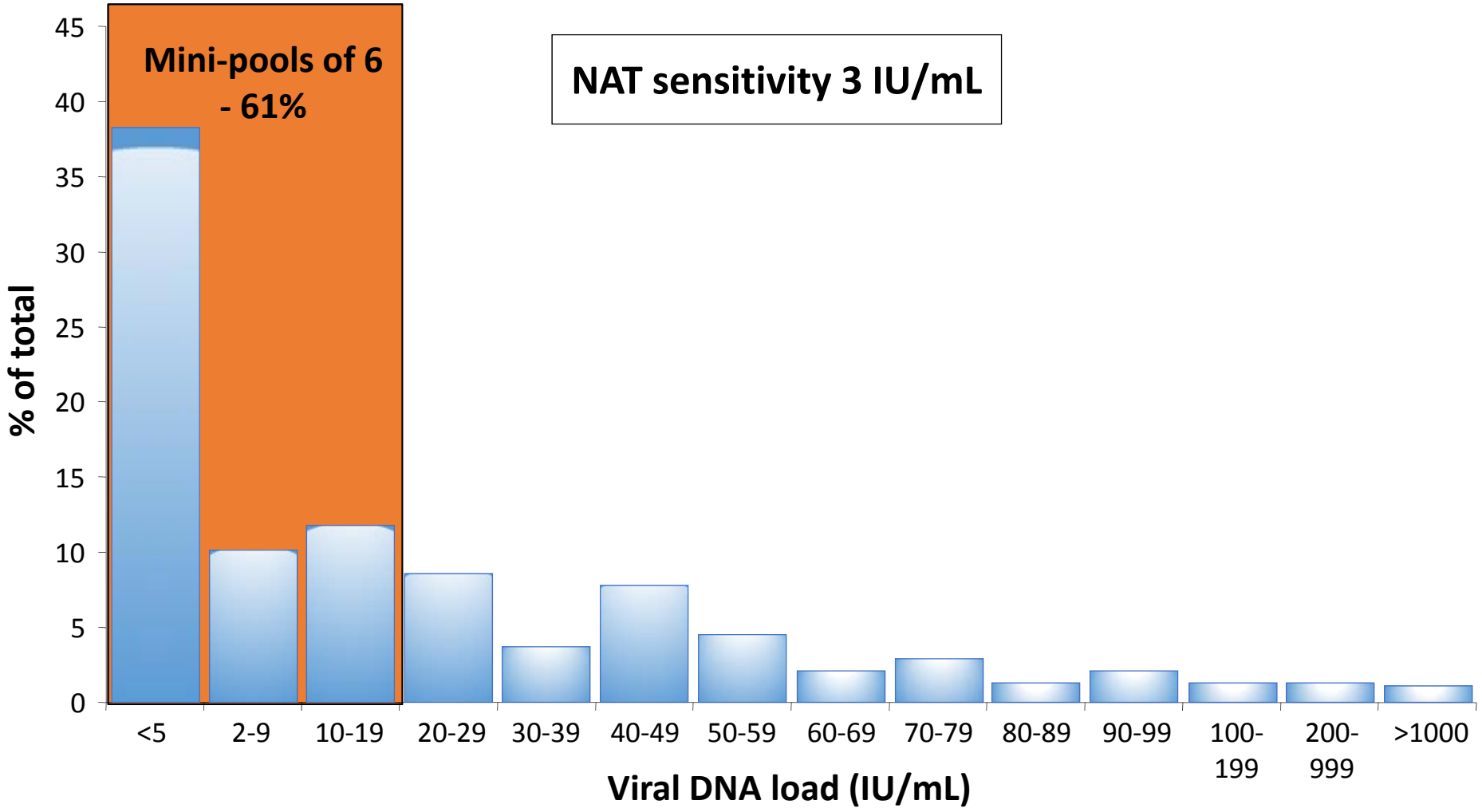
- **Risk mainly related to failure of serological and/or molecular screening**
  - Pre-seroconversion window period (acute infection)
  - Late chronic infection --> **occult HBV infection (OBI)**

HBV markers in 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.

HBV DNA load distribution in 191 OBI donors



OBI vs NAT non-repeatable reactive donations

- **Definition according to assay used and users testing algorithm**
- **NRR rates of 0.09% - 0.29% for Ultrio, Ultrio Plus, and Cobas MP-6**

(Candotti & Allain. Blood Transfus 2012; Deng *et al.* Unpublished)

- **NRR NAT testing frequently associated with seronegative donations**

NRR rates in seronegative/NAT IR Chinese blood donors

Screening assays	NAT yield IR	dHBV R	dHCV R	dHIV R	NRR
Ultrio	224	58 (26%)	1 (0.4%)	1 (0.4%)	**164 (73.2%)**
Ultrio Plus	1,224	389 (32%)	0	1 (0.08%)	**834 (68%)**

L. Wang, personal communication

Confirmation of OBI/NRR NAT donors

- **Follow-up to monitor seroconversion and exclude acute infection**
- **Re-testing from initial plasma bag to resolve sample cross-contamination**
- **Re-testing with different NAT assays**
  - Different analytical sensitivity between assays

Commercial HBV NAT assay reactivity of 52 anti-HBc reactive donors

MPX	Ultrio Plus	Ultrio	SuperQuant
34 (65%)	26 (50%)	5 (9.6%)	7 (13.5%)

Enjalbert *et al.* Transfusion 2014;54:2485-95.

- **Multiple repeats of the screening and/or discriminatory test**
  - Poisson distribution principle but how many repeats?
    i.e. HBV reactive in 3/23 tests (Candotti *et al*. Gut 2018)
  - Costly & Time consuming
  - Large volume of sample

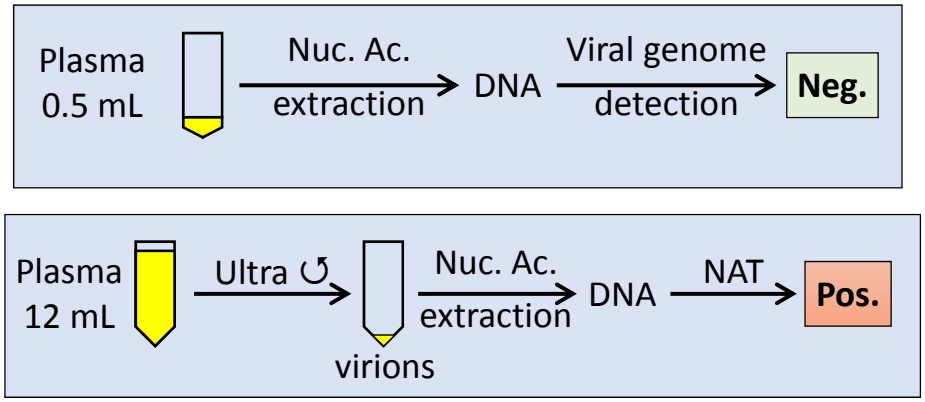
Increasing HBV DNA detection sensitivity

- **Developing highly sensitive alternative in-house real-time qPCR and nested PCR assays**
  - Increased amplification efficiency of short regions
  - Sequencing of amplified products --> definitive confirmation

- **Increasing the volume of plasma extracted (0.2 mL --> 5 mL)**

- **Concentrating viral particles from large volume of plasma (10-20 mL)**
  - Immuno or chemical (i.e. heparin) capture
  - Precipitation with PEG
  - **Ultracentrifugation**

Extremely low viral load but potential infectious risk



HBsAg assay sensitivity & OBI status

- **Development of HBsAg assays with improved analytical sensitivity**

  - Enzymatic immunoassays (EIA) --> LoD: 0.013 – 1 IU/mL

  - Chemiluminescent enzyme immunoassay (CLIA) --> LoD = 5 mIU/mL

  - Immune complex transfer CLIA (ICT-CLIA) --> LoD = 0.5 mIU/mL

HBsAg reactivity of 36 previously characterized OBI donors.

	HBsAg Liaison-XL (DiaSorin; LoQ: 0.05 IU/mL)	**Lumipulse G HBsAg-Quant** (Fujirebio; LoQ: 0.005 IU/mL)
Reactive samples	0/32*	**7/36**
%	-	**19.4%**

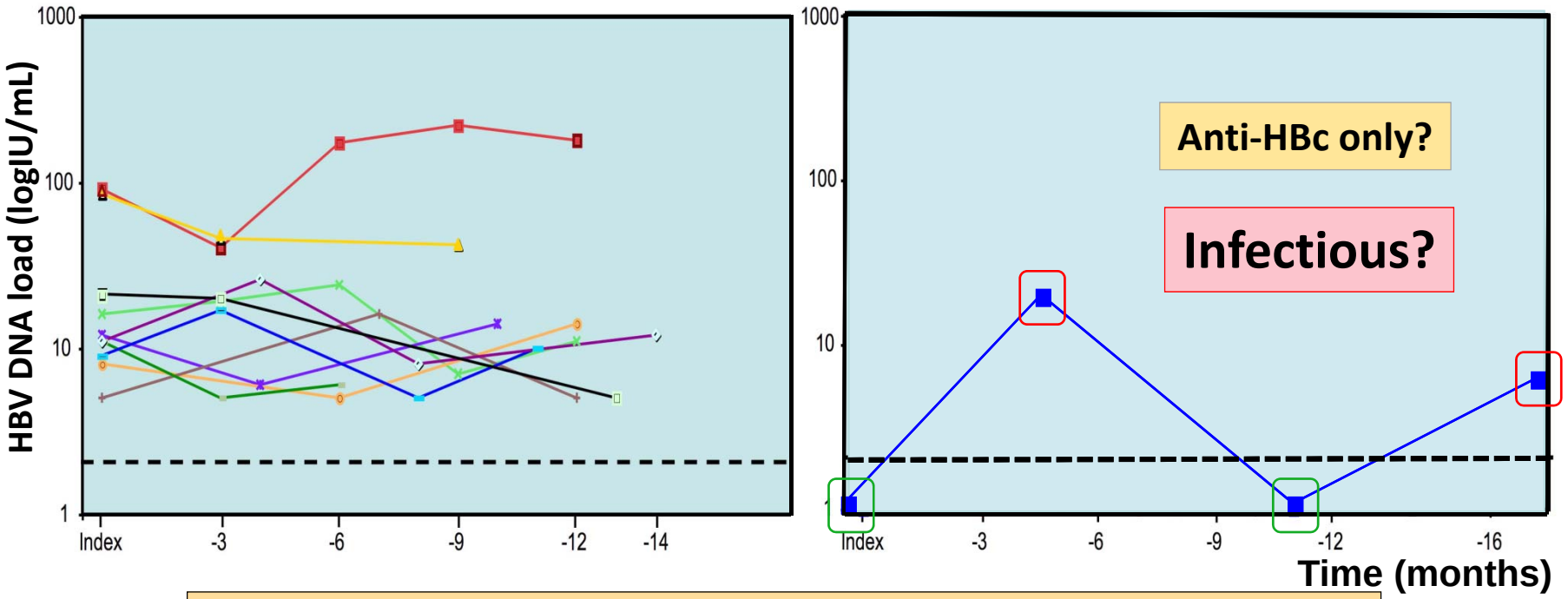
*4 not tested due to insufficient volume but non-reactive with the Roche HBsAg qualitative assay.

Pronier *et al.* 2018 submitted.

Overtime fluctuation of HBV DNA load in OBI donors

Group 1
(48%)

Group 2
(52%)



Clinical observations

Transmission by blood products tested anti-HBc only

Viral reactivation in immuno-suppressed patients anti-HBc only

HBV transmission from anti-HBc only OBI donor
6 cases documented

(Candotti *et al.* Gut 2018)

	Recipient 2-1 Female 69y, dialysis & cirrhosis FFP transfused Hepatitis 8 months post-tx		**Recipient 2-2** Male 67y, trauma surgery FFP transfused (x+3) 16 months post-tx	
	Pre-tx	Post-tx	Pre-tx	Post-tx
HBsAg	-	**+**	-	**+**
HBV DNA	ND	**6E+07 IU/mL**	ND	-
Anti-HBc	-	**+**	-	**+**
Anti-HBs	-	-	-	**+**

Donor 2

Male, 53y, repeat donor (x21; 2009-2014)

HBsAg - & DNA -

(follow-up: 3xR & 20xNR)

Anti-HBc +

Ultra ↺ 20 mL plasma → DNA pos

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	Pre-tx	**Post-tx**	Pre-tx	Post-tx	Pre-tx	Post-tx
HBsAg	**-**	**+**	-	**+**	-	**+**
HBV DNA	ND	**6E+07 IU/mL**	ND	-	ND	**+**
Anti-HBc	-	**+**	-	**+**	ND	**+**
Anti-HBs	-	-	-	**+**	ND	-

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HBsAg	-	**+**	-	**+**	-	**+**
HBV DNA	ND	**6E+07 IU/mL**	ND	-	ND	**+**
Anti-HBc	-	**+**	-	**+**	ND	**+**
Anti-HBs	-	-	-	**+**	ND	-

?

3,257-nt sequences
Genotype D2
99.9% identity

Donor 2
Male, 53y, repeat donor (x21; 2009-2014)
HBsAg - & DNA -
(follow-up: 3xR & 20xNR)

Anti-HBc +
Ultra ↺ 20 mL plasma → DNA pos

Unaware of infection
No clinical sympt.
VL: 10^9 IU/mL

HBV infection in 31 recipients transfused with HBsAg neg/HBV DNA neg blood products

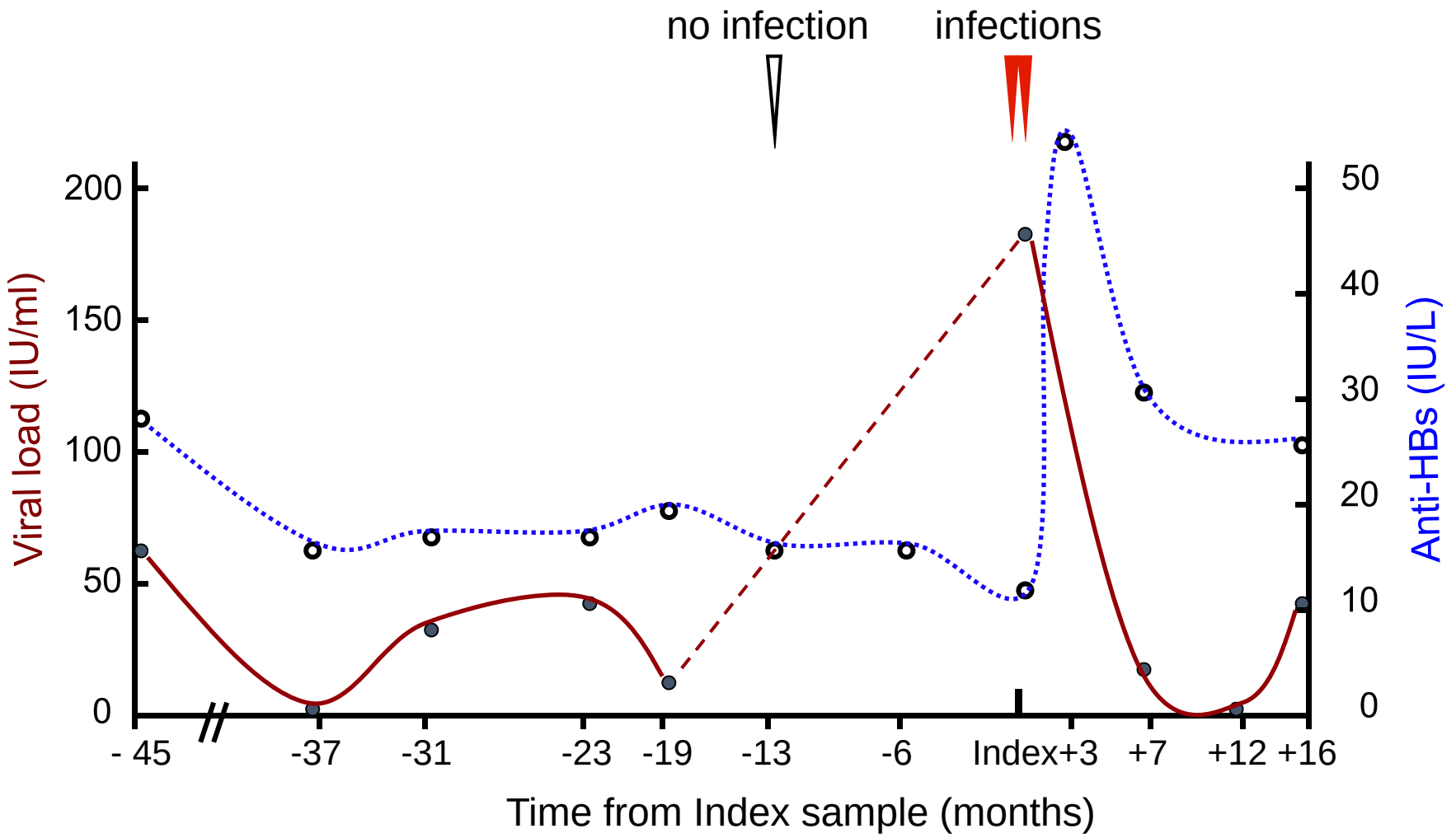
Recipients	Blood products			
	FFP	RBC	PLT*	Total
Anti-HBs pos	-	0/4	0/3	0/7
Anti-HBs neg	7/11 (64%)	2/10 (20%)	0/3	9/24 (37.5%)

* Pathogen reduction treatment of platelet concentrates.

TTI residual risk associated with HBsAg neg/HBV DNA neg/anti-HBc pos donations: 3% (RBC) - 14% (FFP)

Anti-HBs+ OBI transfusion transmission

(Levicnik Stezinar *et al.* J Hepatol 2008)



Can anti-HBc testing prevent OBI transmission?

- **Anti-HBc testing improves blood safety but limited by HBV prevalence --> blood shortage**
- **Anti-HBc negative OBI donors**

Anti-HBV markers	OBI donor origins		
	Dalian (China)	South East Asia*	Worldwide**
N	294	111	604
Anti-HBc +/anti-HBs +	108 (36.7%)	43 (39%)	296 (49%)
Anti-HBc +/anti-HBs -	160 (54.5%)	53 (48%)	272 (45%)
Anti-HBc -/anti-HBs +	**25 (8.5%)**	15 (13%)	24 (4%)
Anti-HBc -/anti-HBs -	1 (0.3%)	0 -	12 (2%)

*Hong Kong (n=75), Malaysia (n=3), Singapore (n=11), and Thailand (n=22) (Candotti *et al.* Gut 2012).

** 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) (Lelie *et al.* Transfusion 2017).

Conclusions (1)

- **Desirable risk zero goal yet to be achieved despite accumulation of biosafety measures (utopic?)**

- **OBI remains the main risk of TTID but identification depends on:**
  - Archived sample availability --> limited volume
  - Extended serologic and molecular testing of donor & recipient
  - Analytical performance of serlogic and molecular assays used
  - Genetic characterization of viral strains infecting donor & recipient

- **Anti-HBc-only donations can be infectious in immunocompetent recipients**
  - FFP (28.6% - 50%) > RBC (0% - 4.5%)
  - **81% recipients not infected**
  - Transmission rate: 9.5% (molecular confirmation) – 19% (indirect evidences)

- **Estimated HBV infectious dose: 16-160 virions (<600 IU) per transfusion**
  - Related to residual plasma volume in blood products
  - Protective role of anti-HBs in donor and/or recipient **(non-optimal?)**
  - Immune status of recipient

Conclusions (2)

- **Anti-HBc testing improves blood safety but limited by HBV prevalence**
- **Infected donations tested false-negative with serology and/or NAT still persist**
- **Frequency of exposure to HBV-infected blood products and transfusion-transmission underestimated?**
- **Debates on apparent redundancy of markers and blood testing cost reduction**
  - HBsAg testing removal when NAT and HBcAb testing in place
  - Large scale studies needed to evaluate impact on blood safety
- **HBV screening strategy should be decided according to local epidemiology, infectious risk estimate, and resources**
- **Perspectives:**
  - Universal HBV vaccination (?) --> OBI reported in vaccinated blood donors
  - Pathogen reduction technology --> in dvlpt, cost, clinical impact?

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