

Transmission of occult hepatitis B

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Occult HBV infection (OBI)

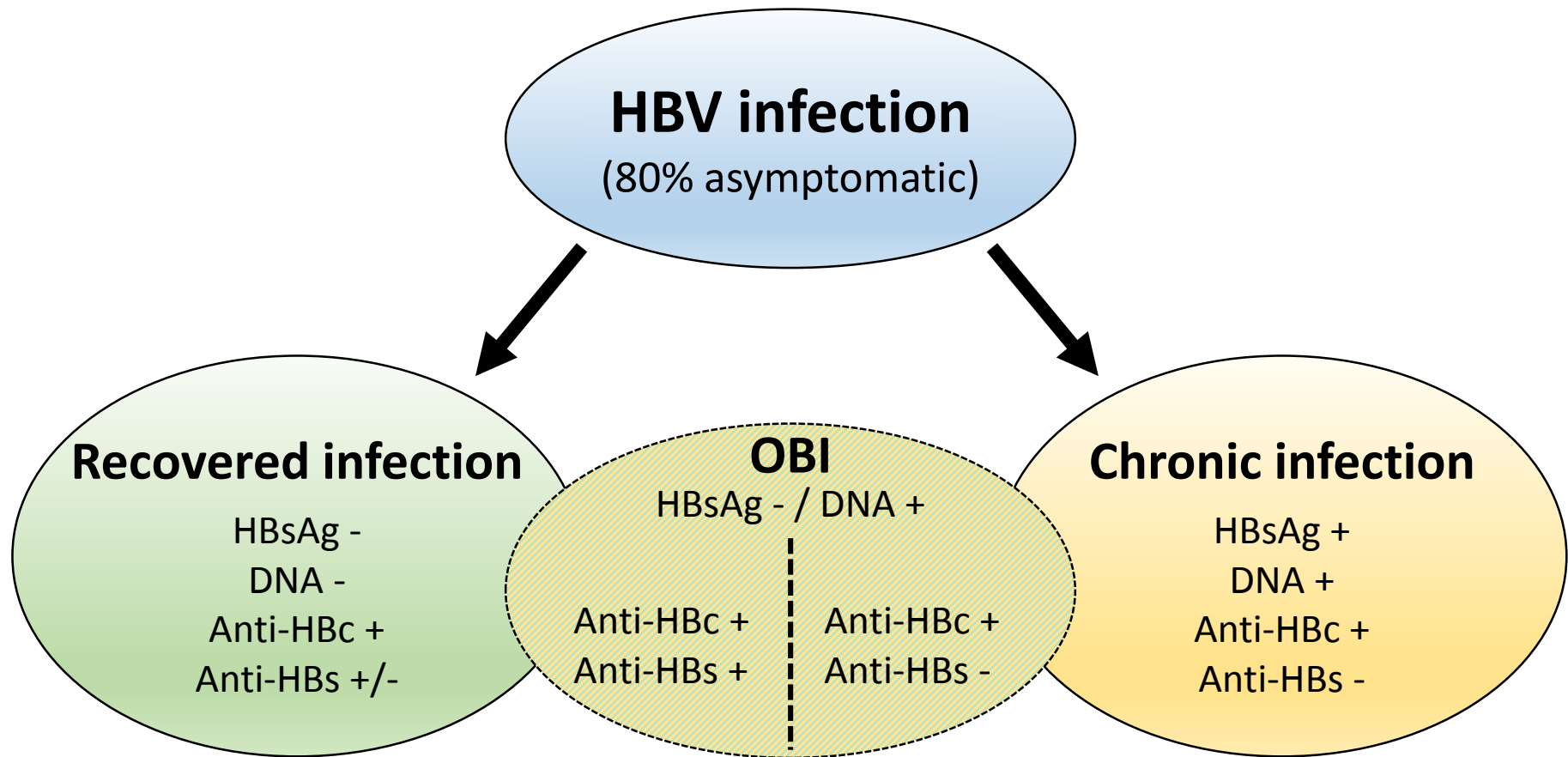
Updated statements of Taormina Consensus Conference

(Raimondo *et al.* J Hepatol 2019)

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

- 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 associated with or without HBsAg variants
- Mutations/epigenetic factors affecting HBV replication and/or viral gene expression

Occult infection in HBV natural history



Technical or biological entity?

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)
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Reactive samples	0/32	7/36
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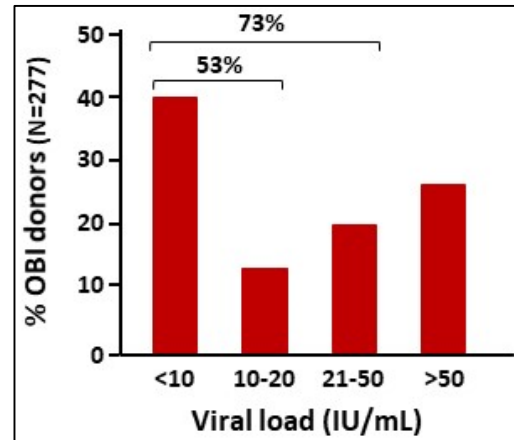
%	-	19.4%
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*4 not tested due to insufficient volume but non-reactive with the Roche HBsAg qualitative assay.

Pronier *et al.* 2018. EASL

Detectability of HBV DNA in OBI donors

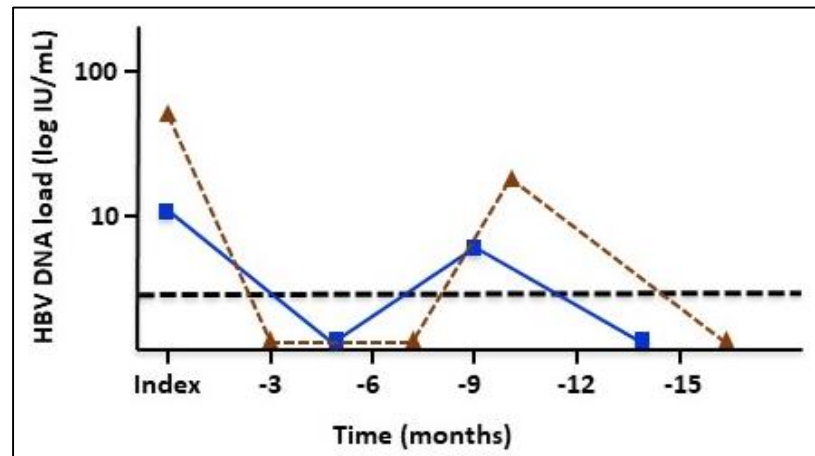
- Low viral DNA load



- NAT non-repeatable reactive

--> NRR rates of 0.09% - 0.29% (Ultrio, Ultrio Plus, & Cobas MP-6 [Deng et al; Unpublished])

- Intermittently detectable



Confirmation of OBI

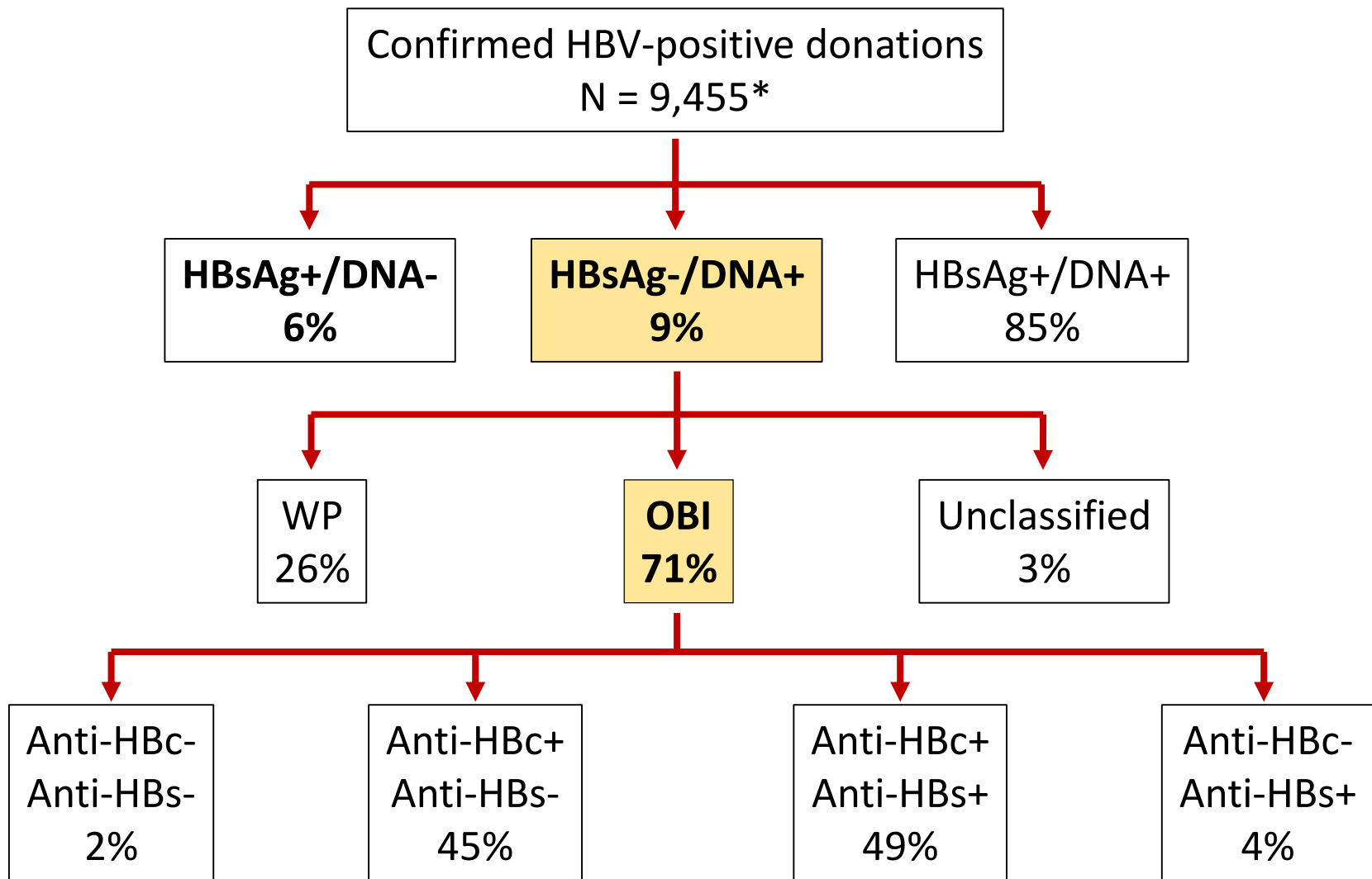
- **Follow-up to monitor seroconversion and exclude acute infection**
- **Re-testing from initial plasma bag to resolve sample cross-contamination**
- **Re-testing with different NAT assays**
 - Different analytical sensitivity between assays

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?
--> costly, time consuming, large volume of sample needed
- **Concentrating viral particles from large volume of plasma (>10 mL) + alternative highly sensitive in-house NAT assays**
 - Ultracentrifugation, PEG precipitation, Immuno/chemical (i.e. heparin) capture
 - Sequencing of amplified products --> definitive confirmation

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.

OBI prevalence in blood donations

Regions	Repeat Donors	1st time Donors	Total
North & Central Europe ¹	1:67 000	1:50 000	1:62 500
Mediterranean ²	1:17 000	1:12 500	1:16 700
Egypt	NA	1:10 000	NA
South Africa	1:33 000	1:3 850	1:20 000
South-East Asia ³	1:6 250	1:7 700	1:6 700
Oceania ⁴	1:33 000	1:33 000	1:33 000
Total	1:25 000	1:8 300	1:20 000

Data adapted from Lelie *et al.* Transfusion 2017. All donations (n=9,455) have been tested with Ultrio and/or UltrioPlus assays.

¹Denmark, Finland, Ireland, Poland, Slovenia, and Switzerland; ²Italy & Spain; ³Hong Kong, Malaysia, and Singapore; ⁴Australia & New Zealand.

HBV transfusion-transmission from OBI donors

Studies	Nb of TTI cases/Nb of transfused components				Comments
	FFP	RBC	PC	Total	
Satake <i>et al.</i> 2007	1/15 (7%)	0/17	0/1	0/17 (3%)	Established
O'Brien <i>et al.</i> 2007	NA	NA	NA	7/279 (2.5%)	No pre-Tx data
Yuen <i>et al.</i> 2011	0/8	1/21 (5%)	0/16	1/45 (2%)	Established
Su <i>et al.</i> 2011	NA	NA	NA	4/12 (33%)	Probable/ established
Allain <i>et al.</i> 2013	16/19 (84%)	23/74 (31%)	6/12 (50%)	45/105 (43%)	Probable/ Established
Seed <i>et al.</i> 2015	7/55 (13%)	11/163 (7%)	2/36 (6%)	20/254 (8%)	Probable/ Established
Lieshout-Krikke <i>et al.</i> 2016	1/1 (100%)	2/56 (4%)	1/23 (4%)	4/80 (5%)	Established

ID-NAT non-reactive OBI transmissions

(1) South African case

Vermeulen *et al.* Vox Sang 2014

	Donor				Recipient	
Drawing date	25/1/12	11/4/12¥	11/5/12	11/6/12	4/7/12#¥	31/1/13§
Status	RBC Issued	lookback	Follow up	Follow up		
Ultrio Plus S/CO	Neg	15.3/15.5/15.1	15.6/14.9	Neg	Pos	
dHBV S/CO		22.7	neg			
HBsAg	Neg	Neg	Neg	Neg	Pos	Neg
Anti-HBc IgM	Neg	Neg			Pos	Pos
Anti-HBc total	Pos	Pos			Pos	Pos
Anti-HBs titre	<2	2.6			<10	284 IU/L
Viral load	1.6 cps/mL*	43 cps/mL				

¥ 100 % homology of whole HBV genotype D genome sequence of donor sample 11/4/12 and recipient sample 4/7/12 (analysis kindly performed by Dr Marco Koppelman, Sanquin, Amsterdam)

* Estimated from 5/20 reps Ultrio Plus reactive by probit analysis

--> 5.1% probability of transmission estimated with ID₅₀ of 316 virions

ID-NAT non-reactive OBI transmissions

(2) Slovenian cases

Candotti *et al.* Gut 2019

- **6 cases of post-transfusion acute hepatitis B reported by hospitals**
 - Recipient pre-transfusion samples available
 - 5 FFP + 1 RBC from 3 anti-HBc+ repeat donors suspected
 - Look-back investigations & molecular analysis of donors/recipients viral strains

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.

HBV transmission from anti-HBc only OBI donor

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 U 20 mL plasma → DNA pos

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16 months post-tx

Recipient 2-3

Male 79y, gastro surgery
FFP transfused (x-57)
78 months post-tx

	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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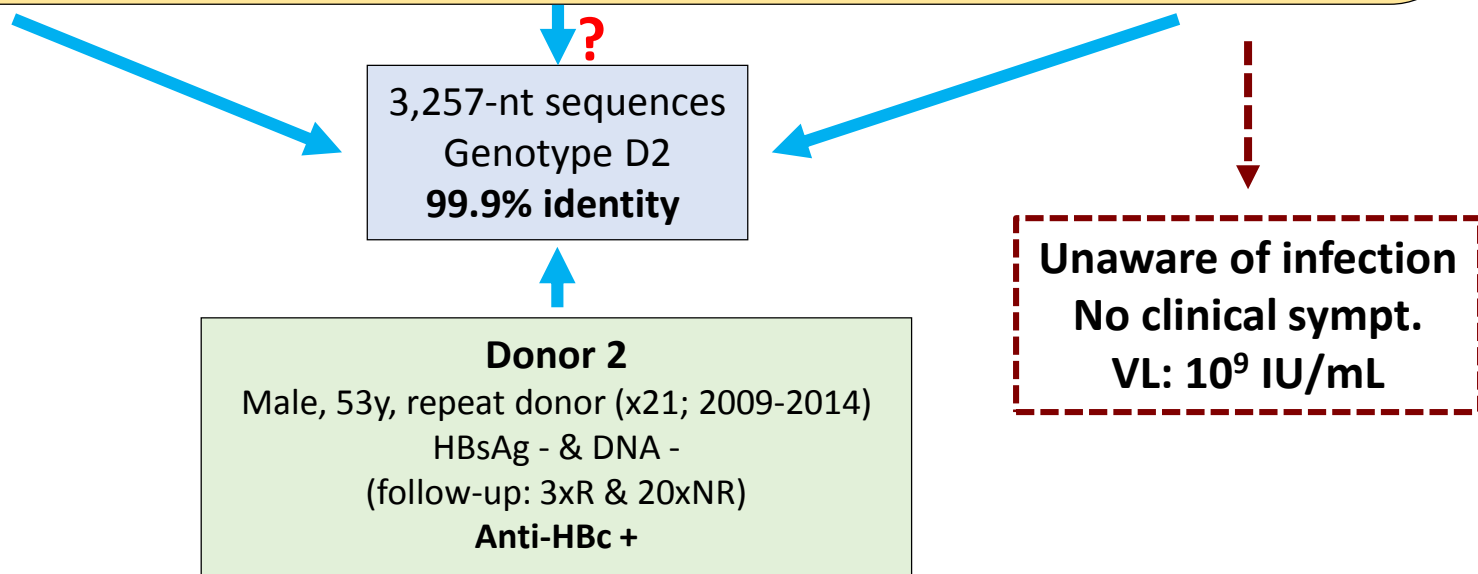
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78 months post-tx

	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	-



Estimated HBV infectious dose by transfusion: 16-160 virions (<600 IU) per transfusion

Residual risk of HBV transfusion-transmission

- **Residual risk associated with ID-NAT non-reactive OBI donations**
- **Estimation of residual risk**
 - 3% with RBC and 14% with FFP (Weusten *et al.* Transfusion 2017; Seed *et al.* Vox Sang 2015)
 - > 1 in ~1 million donations
 - > 1 in ~4 millions donations excluding anti-HBs+ donations (Seed *et al.* Vox sang 2017)
- **Residual risk depends on several factors**
 - HBV epidemiology
 - Donor populations
 - Screening strategies
 - Mathematical models used
 - Limited look-back studies
 - Lack of pre-transfusion recipient sample
 - Donor archive sample rarely available and/or limited volume
 - Delay of investigations --> Resolution of infection --> antibodies as only markers
 - Cost, ethic, and legal issues

Reduction of the HBV residual risk?

(1) Anti-HBc testing

- Not suitable in high endemic settings --> blood shortage
- Assay specificity and confirmation?
- Anti-HBc neg/Anti-HBs pos OBIs

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).

Follow-up of 12 anti-HBc negative OBI donors from Dalian, China

Donor ID	Follow-up (months)	HBV DNA	Anti-HBs (IU/L)	Anti-HBc CLIA*		
				Assay 1	Assay 2	Assay 3
DL32	Index	Pos	37	Neg	Neg	Neg
	2	Pos	44	Neg	Neg	Neg
	12	Pos	36	Neg	Neg	Neg
	54	Pos	41	Pos	Pos	Pos
DL70	Index	Pos	24	Neg	Neg	Neg
	7	Pos	43	Neg	Neg	Neg
DL102	Index	Pos	62	Neg	Neg	Neg
	4	Pos	78	Neg	Neg	Neg
DL127	Index	Pos	53	Neg	Neg	Neg
	2	Pos	33	Neg	Neg	Neg
W2	Index	Pos	190	Neg	Neg	Neg
	4	Neg	1000	Neg	-	-
	6	Neg	616	Neg	-	-
	9	Neg	320	Neg	-	-
	12	Pos	228	Neg	Neg	Neg
W12	Index	Pos	110	Neg	Neg	Neg
	4	Pos	96	Neg	Neg	Neg
	15	Neg	42	Neg	Neg	Neg

Donor ID	Follow-up (months)	HBV DNA	Anti-HBs (IU/L)	Anti-HBc CLIA*		
				Assay 1	Assay 2	Assay 3
DL58	Index	Pos	164	Neg	Neg	Neg
	9	Neg	711	Neg	Neg	Neg
	49	Neg	160	Neg	Neg	Neg
	56	Neg	159	Neg	Neg	Neg
DL79	Index	Pos	40	Neg	Neg	Neg
	3	Neg	85	Neg	Neg	Neg
	18	Neg	54	Neg	Neg	Neg
DL117	Index	Pos	NA	Neg	Neg	Neg
	2	Neg	314	Neg	Neg	Neg
	8	Neg	455	Neg	Neg	Neg
	45	Neg	146	Neg	Neg	Neg
DL128	Index	Pos	64	Neg	Neg	Neg
	6	Neg	72	Neg	Neg	Neg
W8	Index	Pos	27	Neg	Neg	Neg
	7	Neg	766	Neg	Neg	Neg
	31	Neg	25	Neg	Neg	Neg
	51	Neg	20	Neg	Neg	Neg
W10	Index	Pos	16	Neg	Neg	Neg
	25	Neg	30	Neg	Neg	Neg
	47	Neg	12	Neg	Neg	Neg

*Assay 1: Anti-HBc Elecsys (Roche); Assay 2: Architect Anti-HBc II (Abbott); Assay 3: HISCL Anti-HBc assay (Sysmex).

Infectious risk?

Reduction of the HBV residual risk?

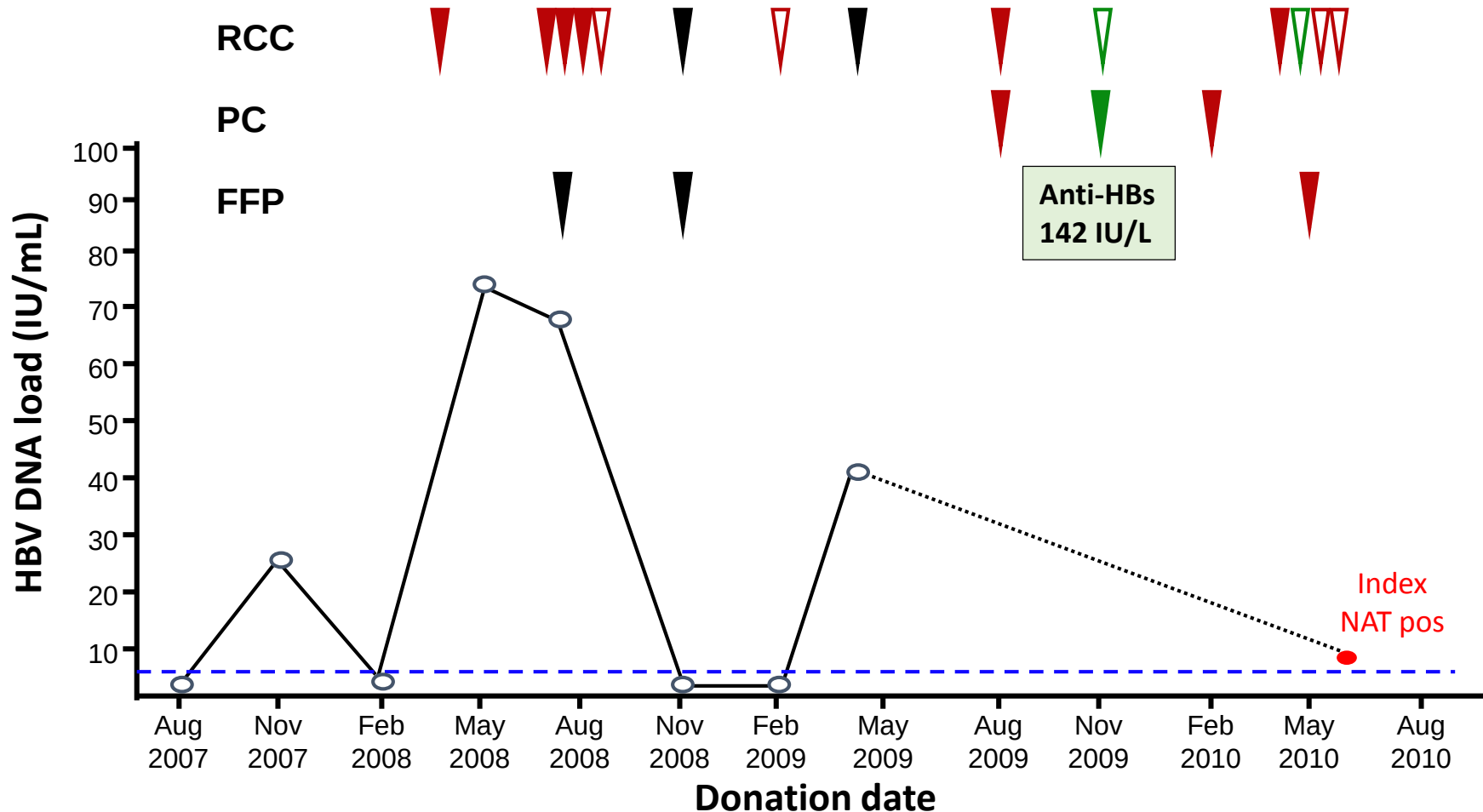
(2) Universal vaccination

- Vaccine efficient to prevent chronic infection & liver diseases
- 1:6 to 1:700 OBI in vaccinated adults according to studies
- 40-50% of OBI donors carry anti-HBs
- Anti-HBs levels >200 IU/L protect against HBV transfusion-transmission
(Hoshi *et al.* Transfusion *in press*)
- Reported cases of HBV transmission from OBI donors despite presence of anti-HBs (10-200 IU/L) in donors or recipients
- Reduced efficacy of vaccine antibodies against non-A HBV genotypes?

Look-back of 20 recipients transfused with blood products from an OBI donor

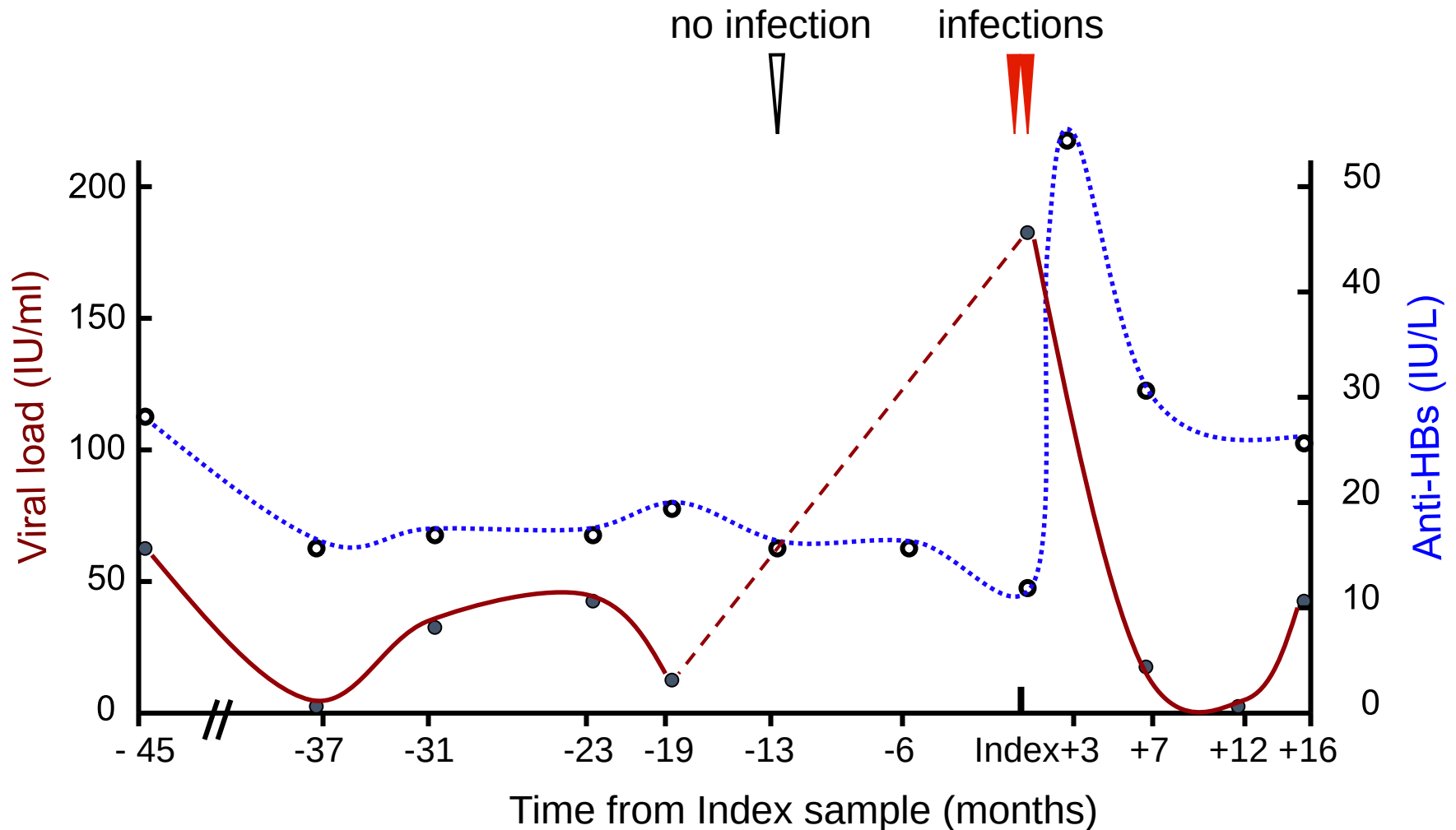
(Allain *et al.* Transfusion 2013)

▼ Deceased ▼ Imm.def./non-infected ▼ Imm.def./infected ▼ Imm.comp./non-infected ▼ Imm.comp./infected



Anti-HBs+ OBI transfusion transmission

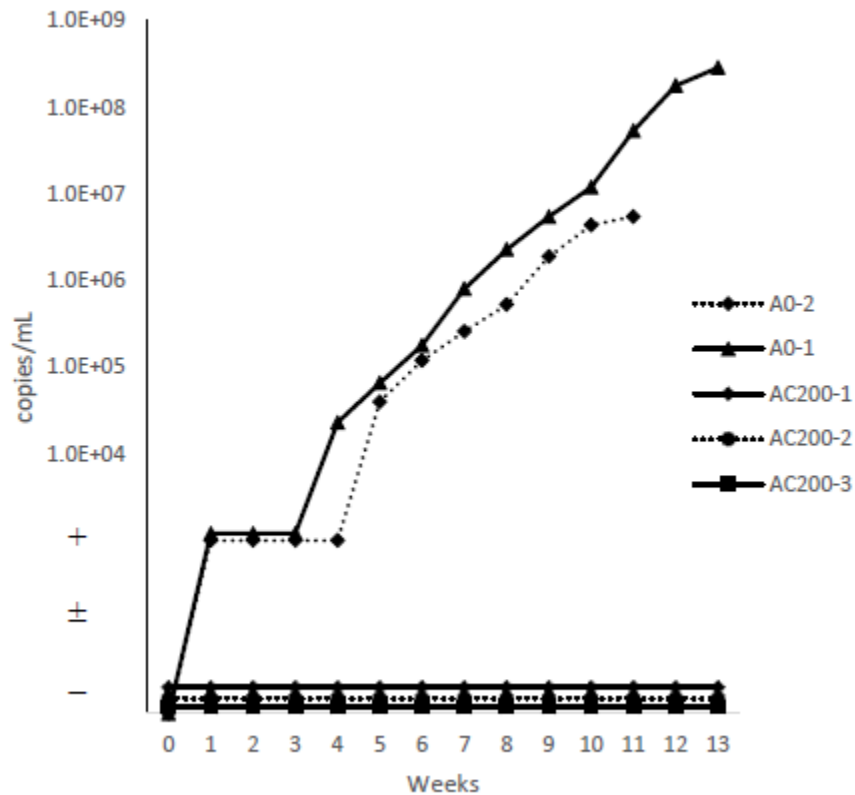
(Levicnik-Stezinar *et al.* J Hepatol 2008)



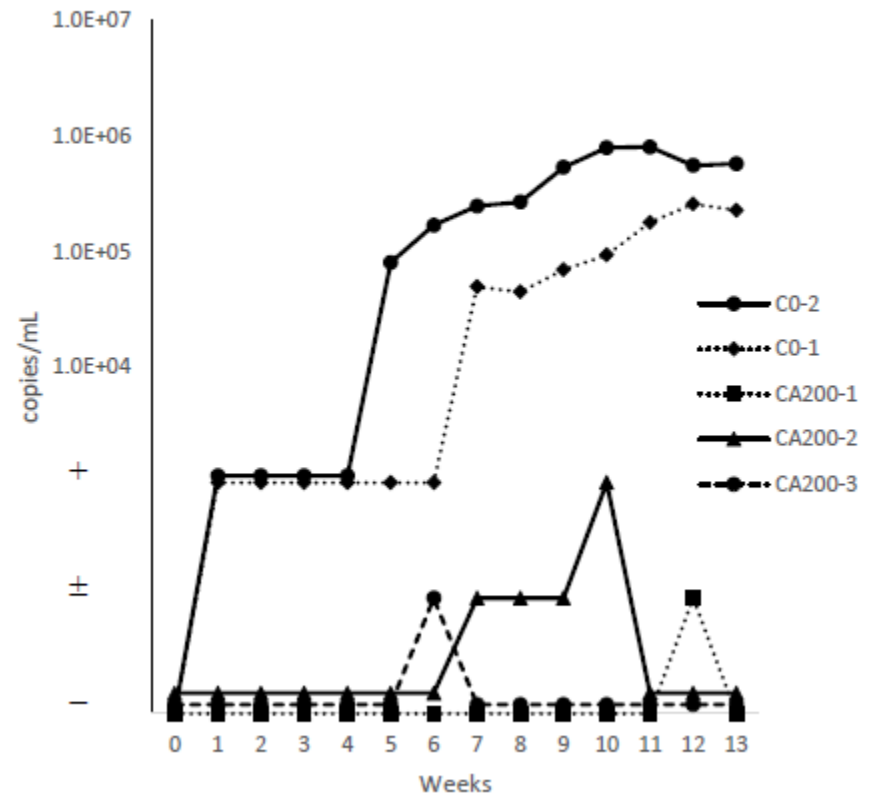
Effect of anti-HBs of discordant genotype on HBV replication in human liver cell-repopulated mice

(Hoshi *et al.* Transfusion *in press*)

A gtA HBV + gtC anti-HBs



B gtC HBV + gtA anti-HBs



Reduction of the HBV residual risk?

(3) Pathogen reduction procedures

- **Efficacy of 2 to >5 log reduction in HBV infectivity (Ware et al. 2017)**
--> HBV infectious risk diminished but not eliminated (VL: undetectable - $>10^9$ 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**

Conclusions

- **OBI and anti-HBc only status depends on the sensitivity of serological and molecular screening assays --> alternative confirmation tests needed**
- **OBI remains the main risk of TTID but identification depends on:**
 - Archived sample availability --> limited volume
 - Extended serological and molecular testing of donor & recipient
 - Genetic characterization of viral strains infecting donor & recipient
--> HBV transfusion-transmission under-estimated?
- **Residual risk associated with ID-NAT non-reactive OBI donations: 3% (RBC) - 14% (FFP)**
- **Estimated HBV infectious dose by transfusion: 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
 - Virus fitness?
- **Perspectives:**
 - Anti-HBc testing improves blood safety but limited by HBV prevalence
 - Universal HBV vaccination --> OBI reported in vaccinated blood donors
 - Pathogen reduction technology --> in dvlpt, cost, clinical impact?

Acknowledgements

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