Sanquin

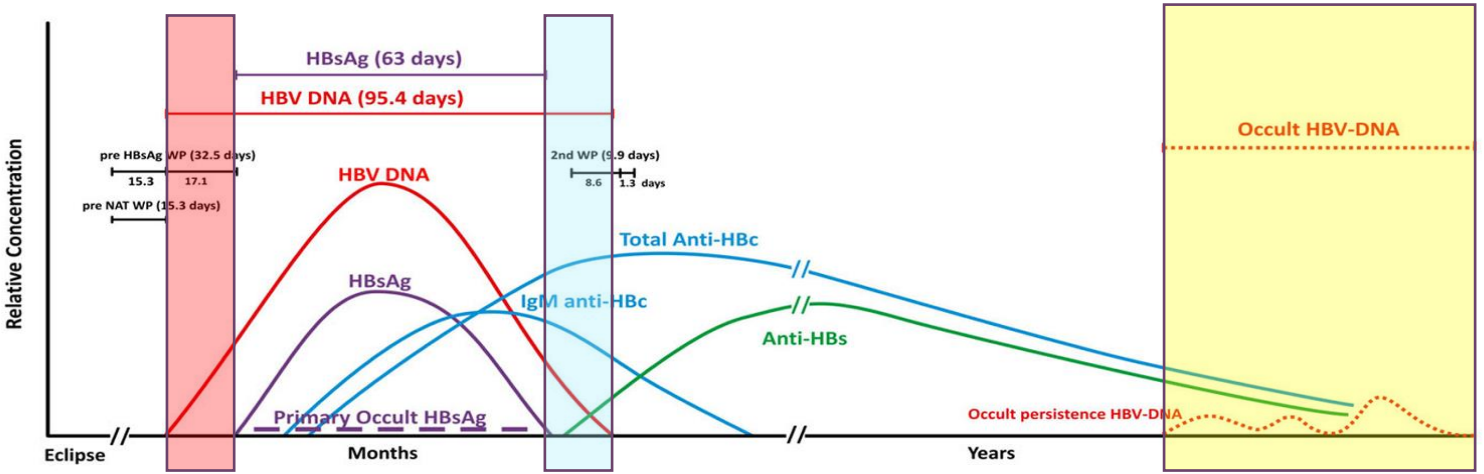
Costs and benefits of anti-HBcore screening

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For Life.



Occult Hepatitis B virus Infection (OBI): HBsAg-negative chronic HBV infection



Very acute HBV (serological window) | **Late acute HBV (trailing window)** | **Chronic OBI**

HBV DNA	+	+	+
HBsAg	-	-	-
Core IgM	-	+	-
Anti-HBcore	-	-	+

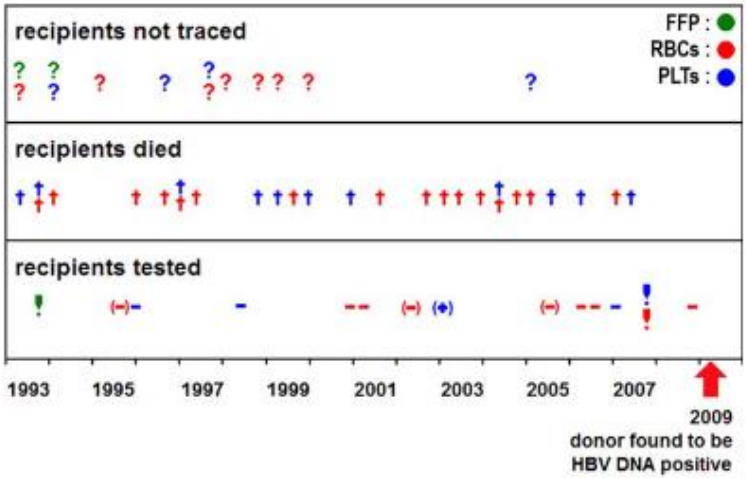
Vermeulen et al Transfusion 2012; 52: 880-92

OBI is transmissible via blood transfusion

		Donor	HBV VL	Anti-HBc	Anti-HBs
D1	F (63)	Repeat	220 IU/ml	POS	Neg
D2	F (60)	Repeat	33 IU/ml	POS	65 IU/L
D3	M (31)	Repeat	23 IU/ml	POS	Neg
D4	M (67)	Repeat	20 IU/ml	POS	23 IU/L
D5	M (51)	Repeat	<12 IU/ml	POS	13 IU/L
D6	F (43)	Repeat	<12 IU/ml	POS	Neg
D7	F (46)	Repeat	<12 IU/ml	POS	Neg
D8	M (53)	Repeat	<12 IU/ml	POS	Neg
D9	M (46)	Repeat	<12 IU/ml	POS	Neg
D10	M (58)	Repeat	<12 IU/ml	POS	Neg
D11	M (64)	Repeat	<12 IU/ml	POS	14 IU/L
D12	M (62)	Repeat	<12 IU/ml	POS	86 IU/L
D13	F (61)	Repeat	<12 IU/ml	POS	31 IU/L
D14	M (58)	New	<12 IU/ml	POS	Neg
D15	F (53)	New	<12 IU/ml	POS	32 IU/L

N=3

N=1



These 16 donors gave 448 blood products after 1992

- 196 (44%) recipients died
- 170 (38%) recipients could not be traced
- 82 (18%) recipients were traced and tested
- 4/82 (5%) recipients got infected

Lieshout-Krikke et al Transfusion 2016; 56: 691-8

Modelled TTI OBI risk versus OBI lookback data

TABLE 5 Comparison of WP and OBI ID-NAT (Ultrio) yield rates in all donations from six geographical regions as well as projected WP and OBI ID-NAT yield rates for Ultrio Plus (using adjustment factors from data in Table S1)

	South-East Asia	South Africa	Egypt	Mediterranean	Central-North Europe	Oceania
All donations	1,051,381	3,571,315	80,631	2,096,732	2,617,682	1,542,480
Ultrio WP NAT yields (rate/million)	25 (23.8)	115 (32.2)	1 (12.4)	20 (9.4)	6 (2.3)	1 (0.6)
Ultrio OBI NAT yields (rate/million)	161 (153.2)	194 (54.3)	8 (99.2)	134 (63.9)	41 (15.7)	45 (29.2)
Ultrio Plus WP NAT yield rate improvement factor[a]	2.70	1.56	2.07	1.56	1.56	1.56
Ultrio Plus OBI NAT yield rate improvement factor[a]	2.99	1.68	5.43	1.68	1.68	1.68
Projected Ultrio Plus WP NAT yield rate (rate/million)	64.2	50.2	25.7	14.7	3.6	1.0
Projected Ultrio Plus OBI NAT yield rate (rate/million)	463.5	91.3	538.7	107.	26.3	49.0
Ultrio Plus OBI to WP NAT yield rate factor	7.2	1.8	21.0[b]	7.3	7.4	48.5[b]
Ultrio Plus WP risk/million with $ID_{50} = 3.16$ (range 1–10) virions	36.3 (25.2–48.0)	28.4 (19.7–37.5)	14.5 (10.1–19.2)	8.3 (5.8–11.0)	2.0 (1.4–2.7)	0.6 (0.4–0.8)
Ultrio Plus OBI risk/million with $MID_{50} = 316$ (range 100–1000) virions	24.1 (8.8–57.0)	4.7 (1.7–11.2)	28.0 (10.2–66.3)	5.6 (0.5–3.2)	1.4 (0.5–3.2)	2.5 (0.9–6.0)
Percent WP risk of overall TT-HBV risk	60 (46–74)%	86 (77–92)%	33 (22–50)%	60 (45–74)%	60 (45–74)%	18 (11–30)%
Percent OBI risk of overall TT-HBV risk	40 (26–54)%	14 (8–23)%	66 (50–78)%	40 (26–55)%	40(26–55)%	82 (70–89)%

Note: Residual risk estimates per million Ultrio Plus-screened RBC transfusions were calculated for different ranges of the ID_{50} by the Weusten models.[4,5]

[a]Factor calculated from sequential Ultrio and Ultrio Plus screening data in the regions and corrected for HBsAg prevalence as described in Appendix S1 and presented in Table S1.

[b]Datasets with only one WP donation are too small for reliable comparison of WP and OBI prevalence. Table S1 presents a dataset from India[27] (where genotype D is prevalent like in Egypt) and prevalence of OBI was 5.5-fold higher than WP.

Lelie et al Transfusion 2021; 61: 3190-3201

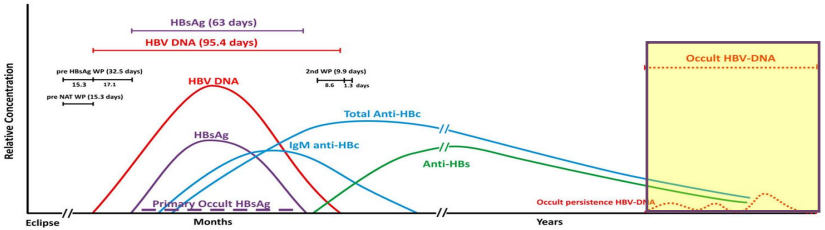
Modelled TTI OBI risk
40% (26-55%) of HBV TTI risk.

Observed TTI OBI risk
2-8% of HBV TTI risk

Infectivity
WP > OBI (anti-HBs-) > OBI (anti-HBs+)

Higher viral load (or viral dose)
Absence of anti-HBs

How to detect/exclude donors with OBI



"An HBsAg negative donor with chronic HBV infection whose donation contains HBV DNA which may or may not be detectable by NAT due to intermittent and/or very low-level viremia".

Anti-HBcore (specificity issue)

Exclusion of all potentially infectious donors

BUT Unnecessary loss of non-infectious donors, might be very problematic in high(er) HBV endemic areas

1) anti-HBcore positivity with true resolved HBV
2) False positive anti-HBc results

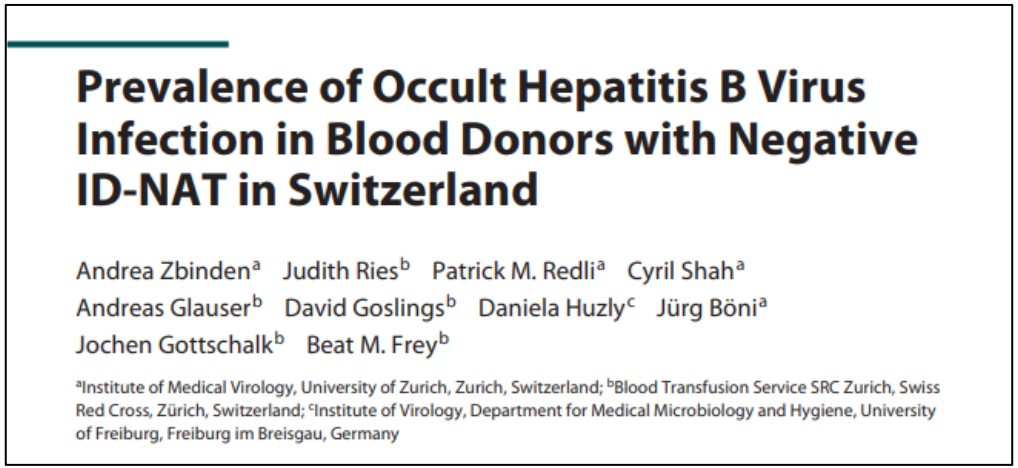
HBV NAT (sensitivity issue)

Not detecting all low-viremic infectious donors.

Minimal infectious dose: 3 IU per product
(20 ml plasma: required LOD HBV NAT = 0.15 IU/ml, which is below the LOD of ID NAT).

Candotti et al Gut 2019; 68: 313-321
Van de Laar et al Transfusion 2021; 61: 2216-24

Donors with confirmed OBI missed by ID NAT



Prevalence of Occult Hepatitis B Virus Infection in Blood Donors with Negative ID-NAT in Switzerland

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Andreas Glauser[b] David Goslings[b] Daniela Huzly[c] Jürg Böni[a]
Jochen Gottschalk[b] Beat M. Frey[b]

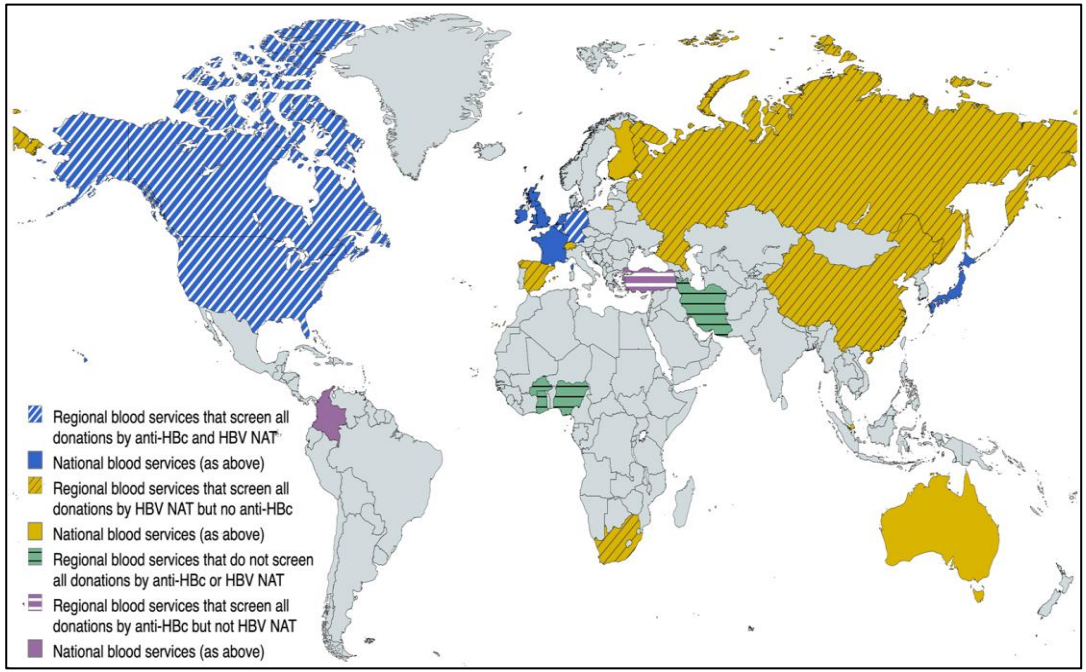
[a]Institute of Medical Virology, University of Zurich, Zurich, Switzerland; [b]Blood Transfusion Service SRC Zurich, Swiss Red Cross, Zürich, Switzerland; [c]Institute of Virology, Department for Medical Microbiology and Hygiene, University of Freiburg, Freiburg im Breisgau, Germany

Zbinden et al Transfus Med Hemother 2022; 49: 338-342

Ultracentrifugation of anti-HB positive donations revealed HBV DNA in **5-6%** of HBV ID NAT negative donations.

More studies have shown similar results.

International review on blood donor screening for OBI



Fu et al Transfusion 2024 - submitted

Population: 12.000.000 blood donors

All countries tested HBsAg

Countries (n=25)

NAT + anti-HBc	44%
NAT only	32%
anti-HBc only	8%
No NAT, no anti-HBc	16%

NAT-only: ID NAT
NAT + anti-HBc: 11/14 pooled NAT (MP6-96)

International review on blood donor screening for OBI

Countries with anti-HBc and NAT screening

	Anti-HBc+ New donors (%)	HBV NAT	95% LOD Per donation (IU/ml)	Confirmed OBI from all donors (%)
France	0.88	ID NAT	1.7	0.0015
Japan	0.57	ID NAT	3.1	0.0014
Ireland	0.06	ID NAT	4.0	0
Netherlands	0.68	MP6	8.4	0.0014
Belgium	1.03	MP6	8.4	0
UK	0.46	MP24	33.6	0.0013
Canada	1.55	MP6-MP16	8.4 - 80	0.0016
USA	0.92	MP16	69 - 74	0.0038
Germany	0.58	MP48-MP96	67 – 154	0

Fu et al Transfusion 2024 - submitted

- Out 12 million donors:
  414 (0.0035%) donors had confirmed OBI.
- Number of anti-HBc positive donors
  is 100 – 300 times higher than the number
  of anti-HBc positive donors with confirmed OBI.
- Implementation of anti-HBc ‘re-entry’ strategies:
  - Algorithms with anti-HBs (>100 IU/L)
  - Algorithms with multiple anti-HBc tests

All countries with (relatively) low anti-HBc prevalence

International review on blood donor screening for OBI

Countries with only HBV NAT screening

	HBV NAT	LOD per donation (IU/ml)	Confirmed OBI from all donors (%)
Switzerland	ID NAT	1.2	0.0051
Finland	ID NAT	1.4	0.0017
Singapore	ID NAT	1.4	0.0154
South Africa	ID NAT	4.3	0.0188
Australia	ID NAT	4.3	0.0044
Spain	ID NAT / MP6	4.3 – 8.4	0.0060
China	ID NAT / MP6	3.3 – 13.4	0.0502
Russia	ID NAT /MP6	1.4 – 25.8	0

- NAT LOD in countries that only use HBV NAT is lower than in countries that also test for anti-HBc (1.4 – 25.8 IU/ml vs 1.7 – 154 IU/ml)
- Confirmed OBI yield in countries with only HBV ID NAT screening was 5x times higher than in countries with MP NAT + anti-HBc screening.

Fu et al Transfusion 2024 - submitted

- ID NAT:
  Higher confirmed OBI rates and no unnecessary donor loss due to false-positive anti-HBc and truly resolved HBV
  Isn't the answer simple then?

Lack of sensitivity HBV NAT screening

		Donor	HBV VL	Donation after start HBV NAT that tested HBV NAT positive
D1	F (63)	Repeat	220 IU/ml	1
D2	F (60)	Repeat	33 IU/ml	5
D3	M (31)	Repeat	23 IU/ml	1
D4	M (67)	Repeat	20 IU/ml	3
D5	M (51)	Repeat	<12 IU/ml	5
D6	F (43)	Repeat	<12 IU/ml	1
D7	F (46)	Repeat	<12 IU/ml	1
D8	M (53)	Repeat	<12 IU/ml	2
D9	M (46)	Repeat	<12 IU/ml	1
D10	M (58)	Repeat	<12 IU/ml	3
D11	M (64)	Repeat	<12 IU/ml	1
D12	M (62)	Repeat	<12 IU/ml	1
D13	F (61)	Repeat	<12 IU/ml	1
D14	M (58)	First-time	<12 IU/ml	2
D15	F (53)	New	<12 IU/ml	1

After implementation of MP6 NAT (not ID NAT) screening in the Netherlands in 2009:

6/15 donors with confirmed OBI were missed at the first 1 – 4 donations after implementation of HBV NAT screening

In total this involved 14 (most likely) HBV DNA positive donations that slipped through.

With anti-HBc screening all 15 donors would be deferred at the first donation, but 6/15 would have lacked OBI confirmation.

In other words, part of the seemingly unnecessary donor loss is not so unnecessary

Introduction of anti-HBc screening in the Netherlands: July 2011

HBV test algorithm:

HBsAg
HBV NAT (MP6)
anti-HBc (if anti-HBc positive then anti-HBs)

Donors with anti-HBs titer >200 IU/L are eligible.
Rationale: enough neutralisation for low level viremia

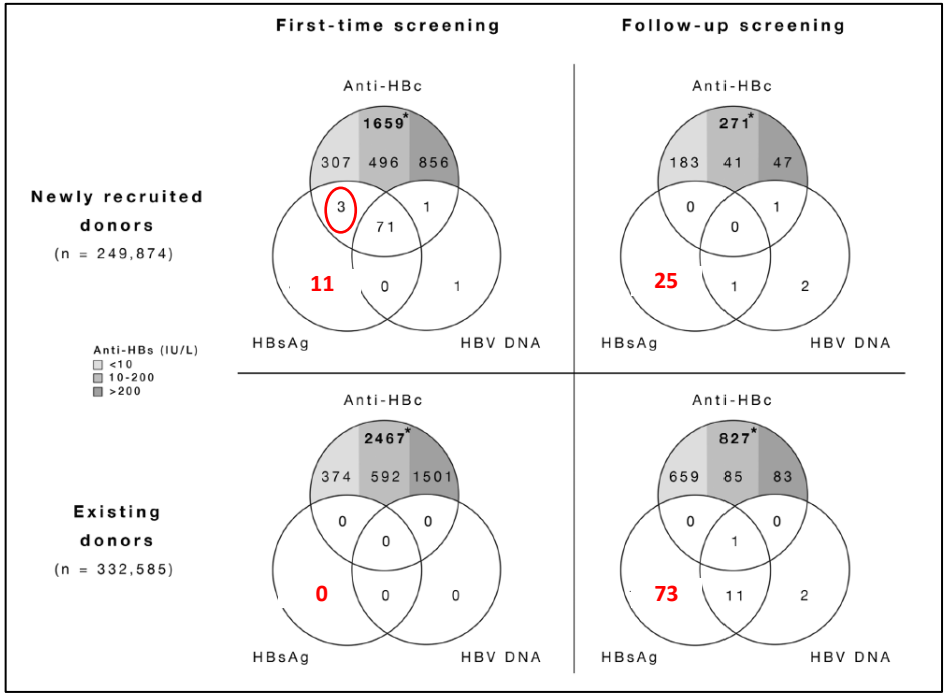
(Most countries use a cut-off of 100 IU/L,
but there has been at case of HBV transmission with anti-HBs titer 100 – 200 IU/L)

Implemented anti-HBc re-entry methods:

- Existing donors that test positive for anti-HBc in the absence of anti-HBs get second chance.
- Donors that seroconvert for anti-HBc in the absence of anti-HBs get a second chance.

Rationale: Highest chance of (temporarily) false-positive anti-HBc test results, and some extra service to our loyal existing donors.

Results of HBV screening in the Netherlands: July 2011 – July 2018

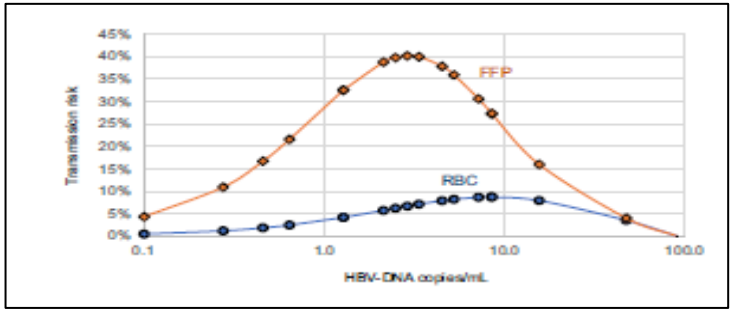


Van de Laar et al Transfusion 2021; 61: 2216-24

In presence of HBV DNA and anti-HBc testing HBsAg screening seems redundant

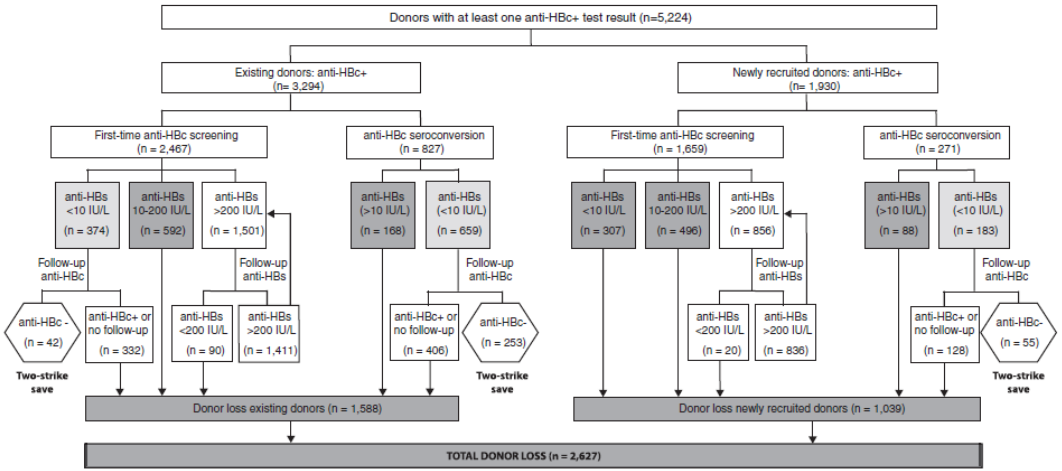
HBsAg only (n=108): all HBV vaccination
HBsAg+/MP6 NAT-/ID NAT+ (n=3): all anti-HBc+

Confirmed in Egypt
High(er) HBV endemic



El Ekiaby et al J Viral Hepatitis 2022; 29: 330-339

Results of HBV screening in the Netherlands: the book keeping



Van de Laar et al Transfusion 2021; 61: 2216-24

Most important question: How unnecessary is this donor loss? How 'true' is anti-HBc, and are these donations potentially infectious?

Anti-HBc prevalence
New donors: 0.66%
Existing donors: 0.74%

Donors saved by re-entry algorithms:

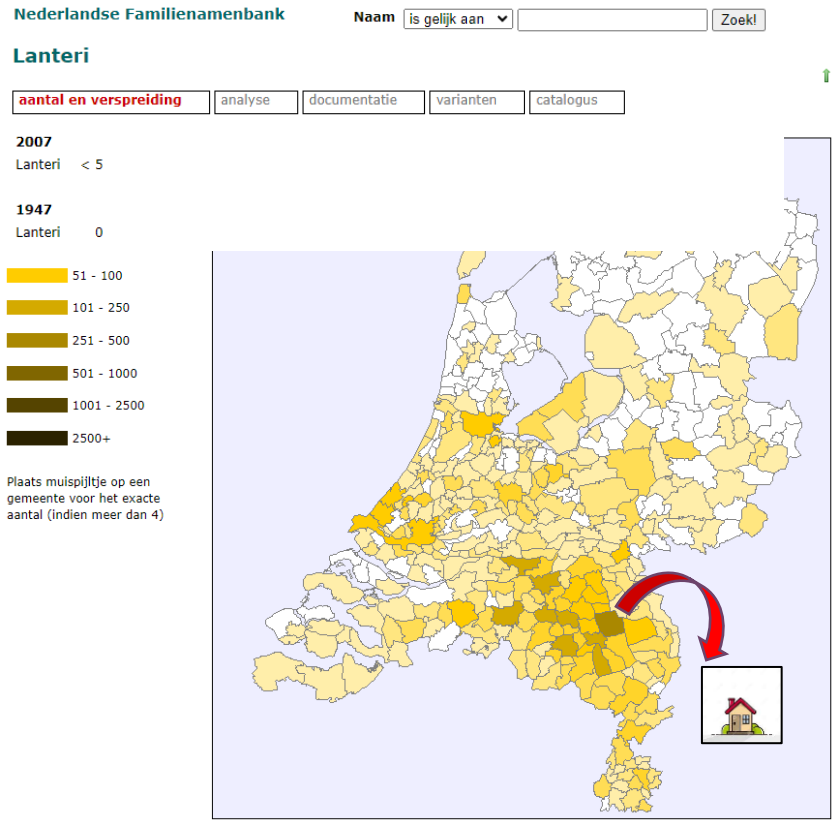
Anti-HBs >200 IU/L (57%)
Seroconversion re-entry (6%)
Existing donor re-entry (<1%)

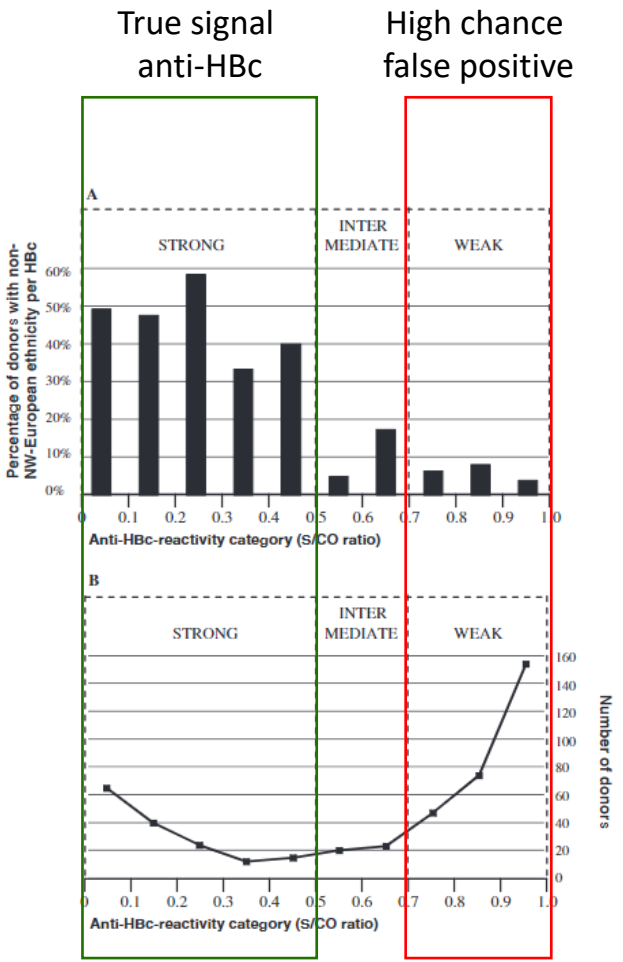
Anti-HBs >100 IU/L (would save 83%)

Total donor loss:

2,627/5,224 (50%) anti-HBc+ donors
2,627/582,459 (0.45%) all donors

Likelihood of false-positive anti-HBc results





Costs and yield of HBV screening in the Netherlands: July 2011 – July 2018

YIELD

- 2 donors with confirmed OBI
  (MP6 NAT negative, ID NAT positive).
- 18 donors with confirmed recent HBV
  (**Korelitz:** True anti-HBc/anti-HBs seroconversion
- 1067 donors truly at increased risk for OBI.
  ('True' anti-HBc signal and anti-HBs < 200 IU/L)

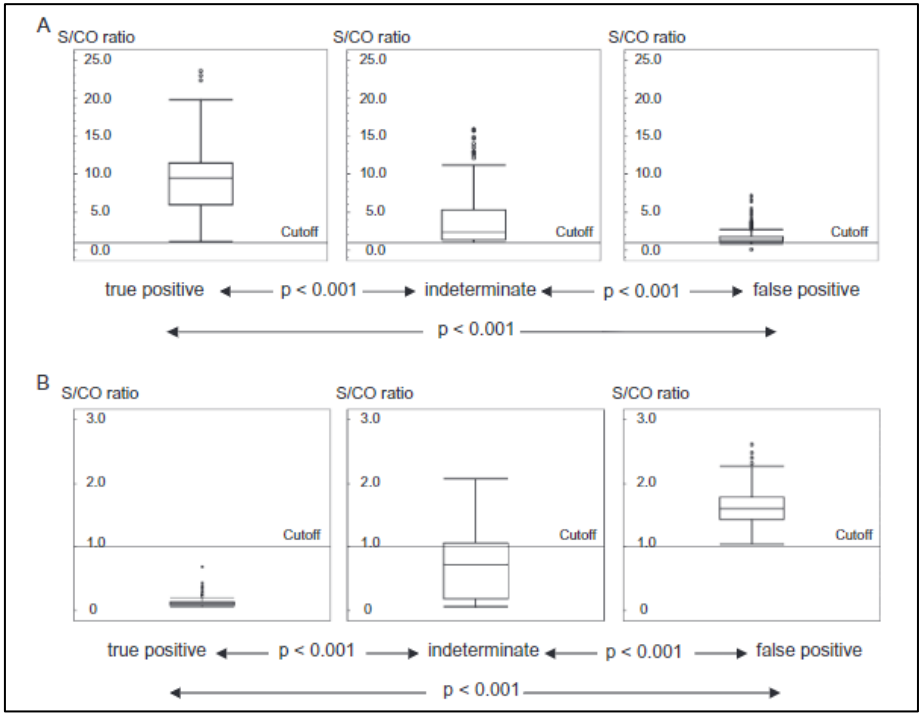
41% (but again how infectious are these donations, especially those with detectable anti-HBs)

COSTS

- 110 donors with waning anti-HBs <200 IU/L
- 1430 donors with presumably false-positive anti-HBs

59% (probably non-infectious)

Alternative re-entry algorithms: multiple anti-HBc assays



Juhl et al Transfusion 2011; 51: 1477-1485

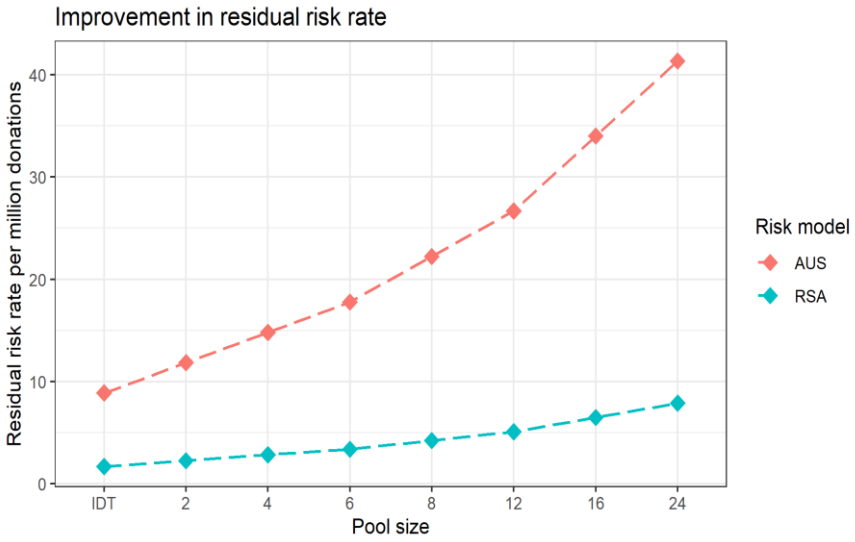
All anti-HBc positive donors with test A (Architect) were retested with test B (Axsym).

Only 36% of donations tested positive with Both test A and B.

Not suprisingly:
There is a clear association with S/CO ratio,

Powerful tool to limit unnecessary anti-HBc loss

HBV screening UK: introduce anti-HBc testing or reduce HBV NAT poolsize (MP24)



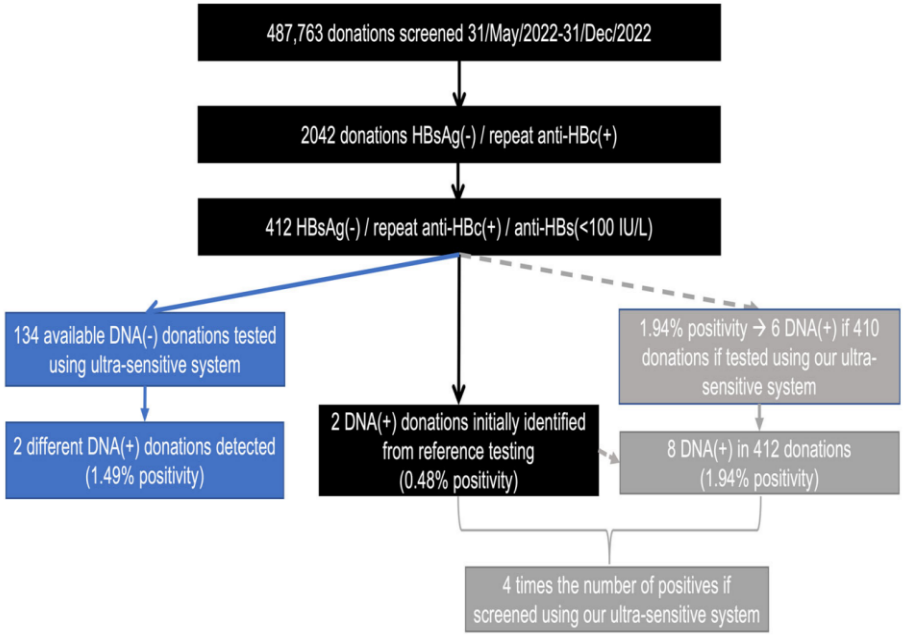
Note – residual cases do NOT equal zero not even with ID NAT

In 2019 (HBsAg, HBV NAT MP24) the residual risk (RR) related to OBI is 5 – 40 x higher than the RR of acute HBV window infections (depending on the model used).

	ID NAT		Anti-HBc	
Model	AUS	RSA	AUS	RSA
Before: RR per 1 million donors)	37.7	7.2	37.7	7.2
After: RR per 1 million donors	8.9	1.7	0	0
Cost (£) per detected OBI	200K	1000K	11K	56K

SaBTO
Advisory Committee on the Safety of Blood, Tissues and Organs

Sensitivity and specificity issues OBI testing: anti-HBc testing UK (2022)



HBsAg negative / anti-HBc positive:
2,042/487,763 (0.41%) donations.

Anti-HBc positive / anti-HBs < 100 IU/L
412/487,763 (0.08%) donations

80% reduction in loss

HBV NAT positive (routine, MP 24):
2/412 (0.5%)

HBV NAT positive (ultrasensitive):
8/412 (1.9%)

4 fold increase in yield

Potential unnecessary donor loss due to anti-HBc:
404/587,763 (0.08%)

Fu et al J Med Virol 2023; 95:e29144

Anti-HBc testing in higher endemic areas: the Philippines

HBsAg negative / anti-HBc positive:
307/1602 (19.2%) donations.

Anti-HBc positive / anti-HBs (<10 IU/L)
60/307 (19.7%) donations

HBV ID NAT positive (LOD 1.7 IU/L):
4/307 (1.3%)

Potential unnecessary donor loss due to anti-HBc:
58/1602 (3.6%)



80% reduction in loss similar to UK

Similar to UK

True reduction is less if similar to UK anti-HBs titers <100 IU/L are deferred Possibly as low as 45%

2 HBV NAT+ had anti-HBs > 100 IU/L Not OBI but vaccination breakthrough?

With deferral of anti-HBs <100 IU/L Donor loss might rise to >10%

Yu et al Vox Sang 2024; 119: 252-256

Anti-HBc seronegative OBI

Longstanding HBsAg-/anti-HBc- chronic HBV
HBV viral load app 6 IU/ml (genotype A2)

No confirmed transmissions, but
14/17 transfusions could not be investigated

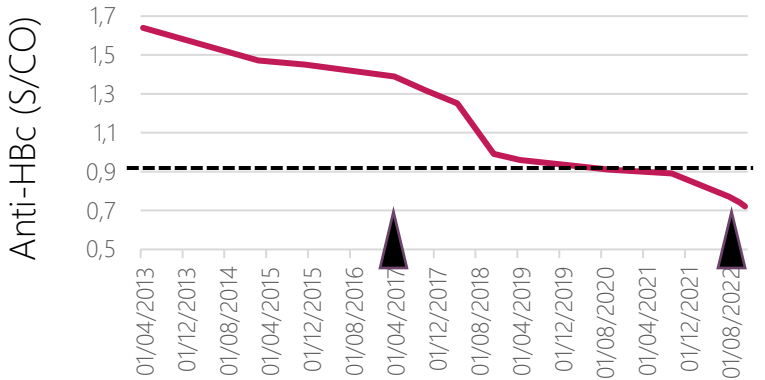
Cappy et al Viruses 2022; 14:2507

5 anti-HBc seronegative OBI cases in
12.000.000 donors

1 confirmed HBV transmission
Possible HBV vaccination breakthrough infection

Fu et al Transfusion 2024 - submitted

Should we maintain anti-HBs testing for ever anti-HBc positive donors



Dutch donor:

Donor with waning anti-HBcore
anti-HBs > 300 IU/L (also gradually waning)
Two blips op HBV DNA

Risk: Born Surinam (Blood transfusion 1975)

Discussion

- OBI exists and if not detected can result in transfusion transmitted HBV, but the true frequency of HBV transmission from components of OBI donors remains unclear (viral load, anti-HBs).

- Risk reduction strategies could be anti-HBc testing or HBV ID NAT, but neither is perfect (sensitivity (ID NAT), specificity (anti-HBc), usability (HBV endemicity) and depends on resource availability.

- Additional consequences of implementing anti-HBc screening:
  What to tell the donor: increased HCC and HBV reactivation risk
  When to do look-back: (almost) no TT HBV from ID NAT-, anti-HBs+ OBI donations

- What to do with anti-HBc negative OBI? Is that a real thing and are these donations potentially infectious? If so, also anti-HBc screening does not result in OBI RR of zero.

Sanquin

Thank you for listening

Sanquin Research: BLOOD-BORNE INFECTIONS

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