

Evaluation of NAT in China and South East Asia.

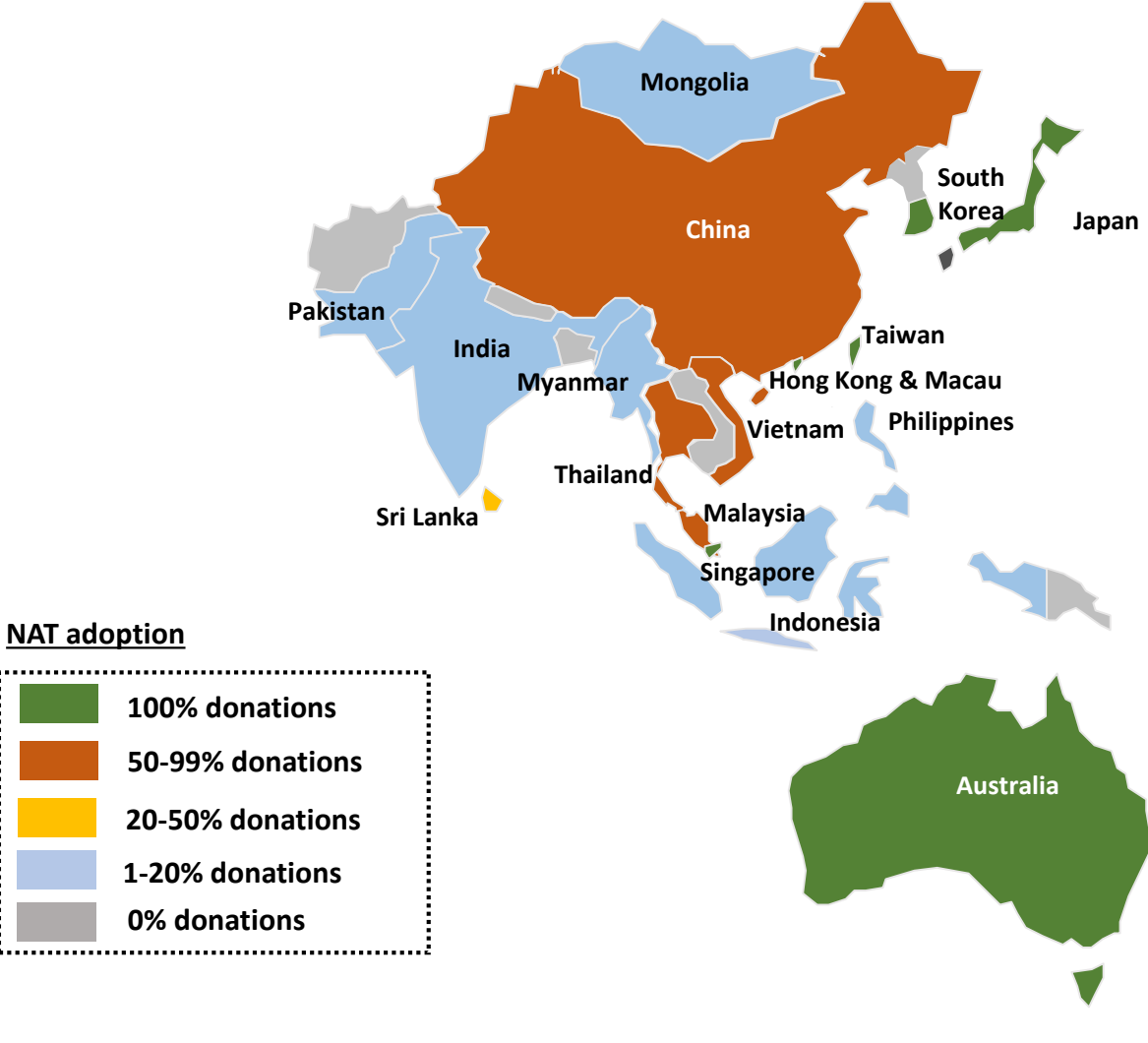
Nico Lelie[^] on behalf of Prof. Lulan Wang
and the multi-center South-East Asian Study Group

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IPFA-PEI 25th Workshop on Surveillance and Screening of Blood Borne Pathogens, May 16th 2018

Asia Pacific Region – NAT Adoption

Update May 2018

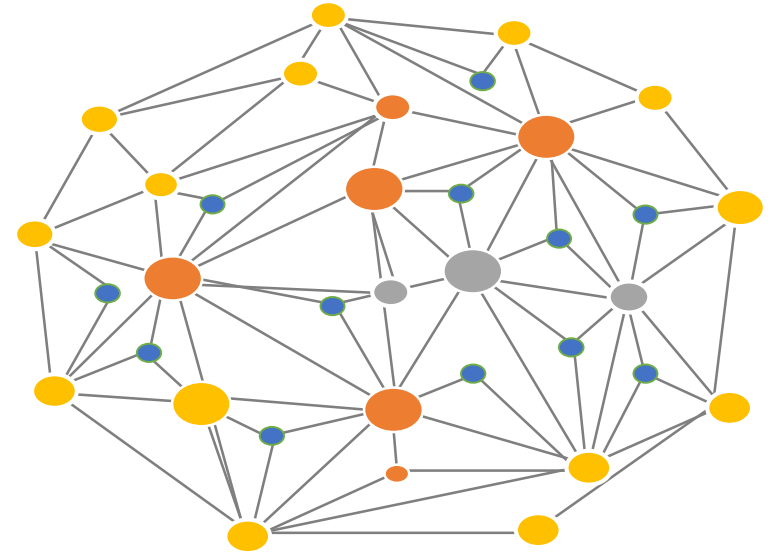


Country	Adopted	Unadopted
Afghanistan	0%	100%
Australia	100%	0%
Cambodia	0%	100%
China	93%	7%
India	12%	88%
Indonesia	15%	85%
Laos	0%	100%
Japan	100%	0%
Macau	100%	0%
Malaysia	72%	28%
Mongolia	>1%	<99%
Myanmar	3%	98%
Nepal	0%	100%
New Zealand	100%	0%
Pakistan	7%	93%
Papua New Guinea	0%	100%
Philippines	6%	94%
Singapore	100%	0%
South Korea	100%	0%
Sri Lanka	26%	74%
Hong Kong	100%	0%
Taiwan	100%	0%
Thailand	98%	2%
Vietnam	63%	37%

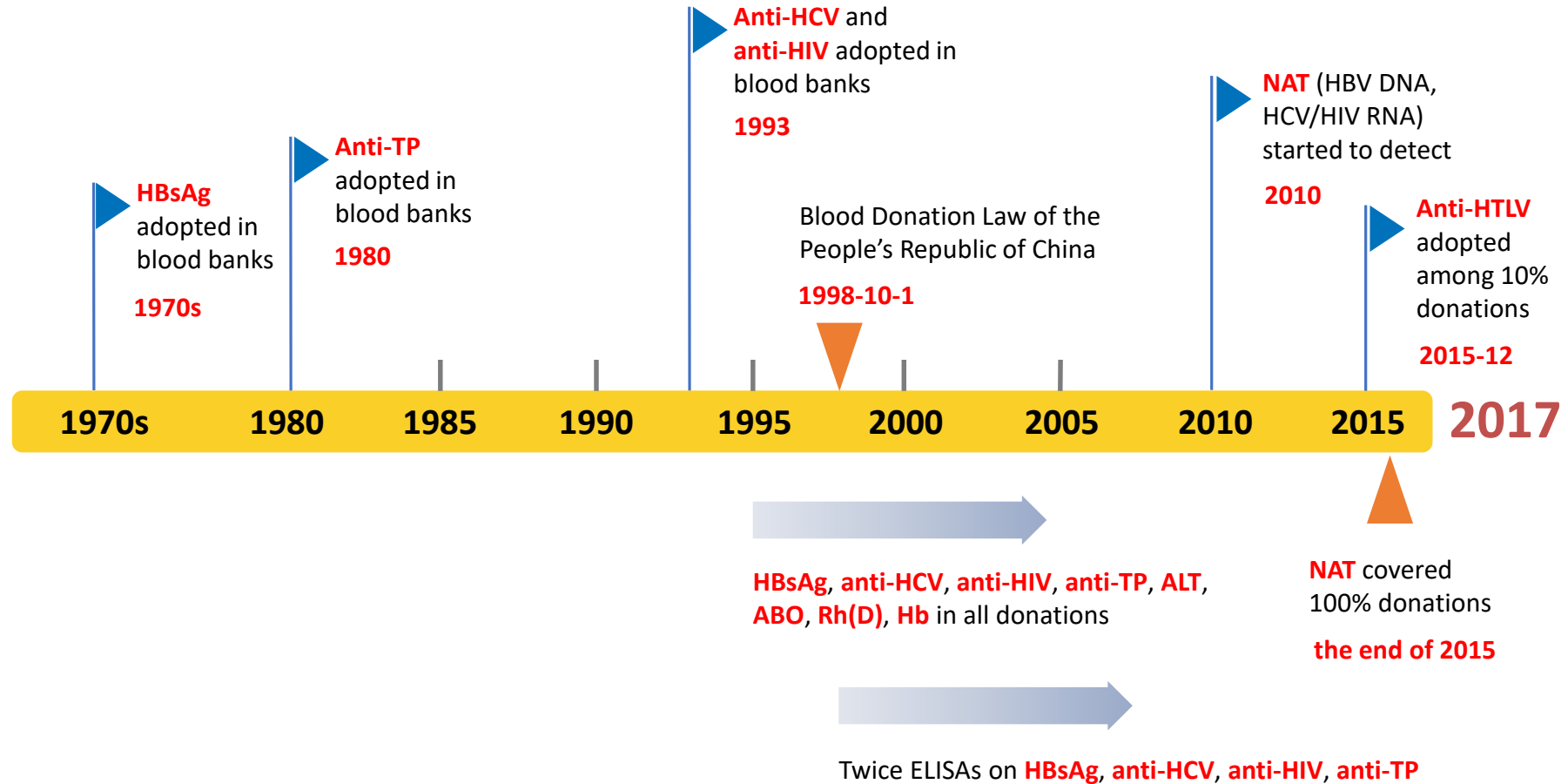
Implementation of National NAT Mandate in China : Opportunities & Challenges

WANG Lunan

National Center for Clinical Laboratories



Time line for development of blood screening in China

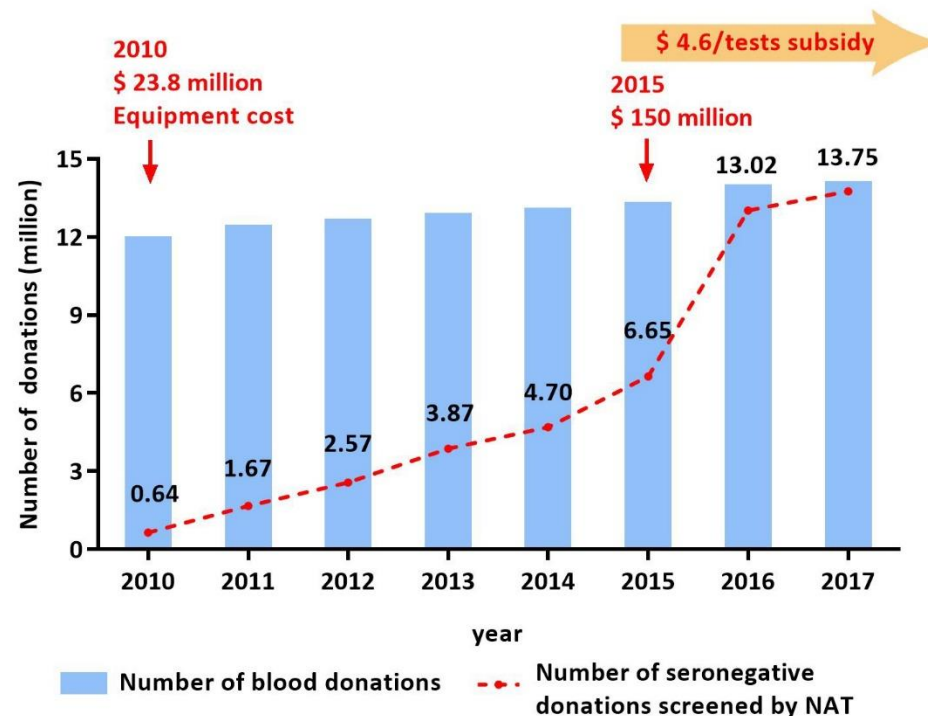


NAT in China from 2010 to 2017

In **2010**, MOH started a **NAT** screening program for HBV, HCV, and HIV infection in **15** blood centers and blood banks from **12** provinces.

By **the end of 2015**, blood donations for clinical use **all** had been screened by NAT.

- **Government funding**
- **Technical guidance**
NCCL have already trained ~3000 technicians and several times of quality monitoring activities.
- **Domestic detection systems**
relatively cheaper; ~ 50% of the market



Blood Centers or Blood Banks in China

- Blood screening

384 Blood Centers and Blood Banks from **31** provinces and autonomous regions

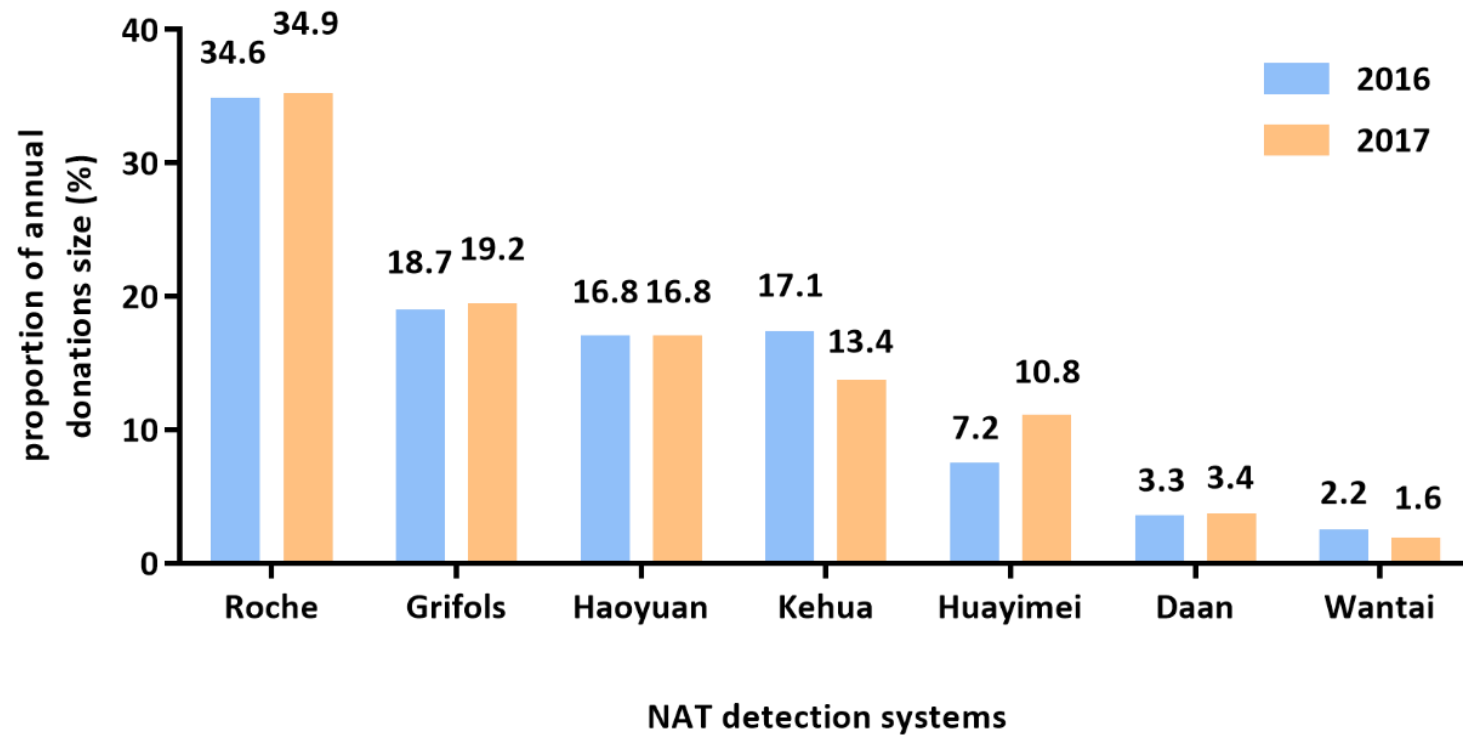
- **274** Blood Centers and Blood Banks have implemented NAT.
- **110** Blood Banks **entrusted** other Blood Banks to perform NAT.

202 Plasma collection sites

7 Cord blood banks

NAT systems in China

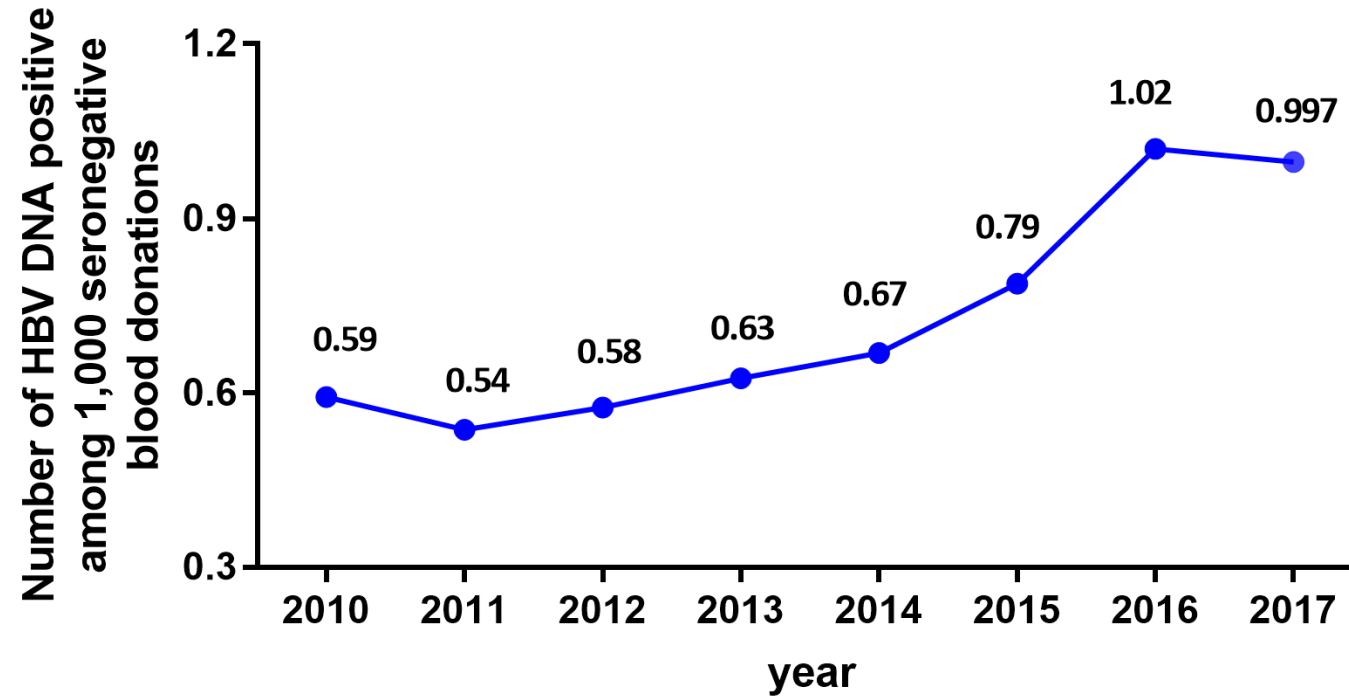
7 NAT systems (2 imported and 5 domestic) approved by CFDA.



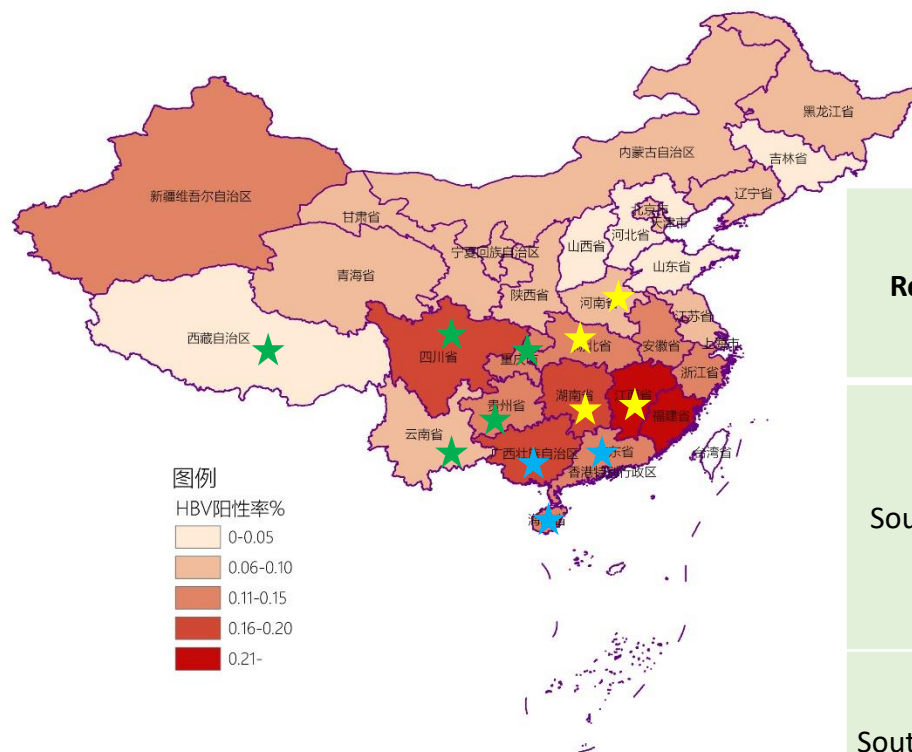
Data from NCCL

HBV DNA positivity Rate in China

According to the data from 2016 to 2017, **99.6%** NAT reactive seronegative donations were **HBV DNA** positive.



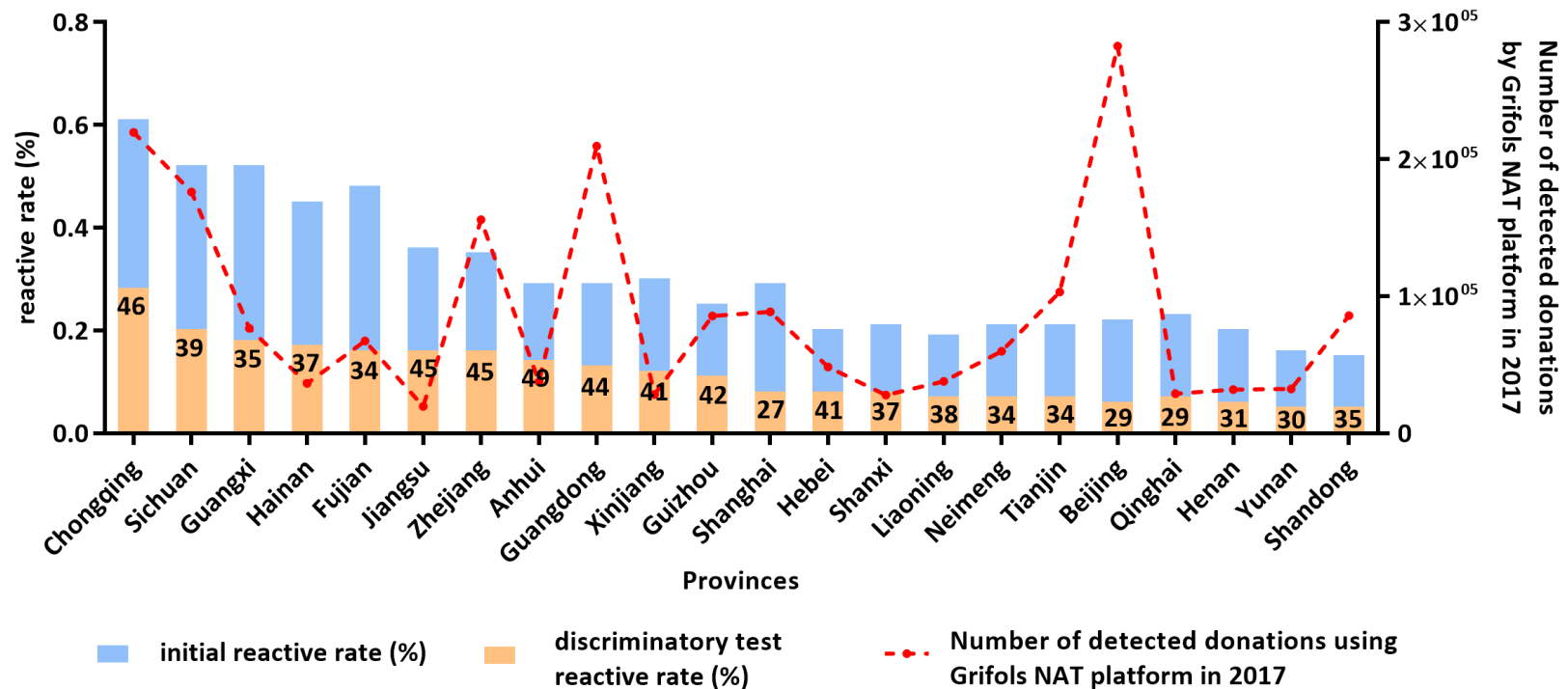
HBV DNA positivity Rate in China



Region	Province	Number of seronegative donations detected by NAT	Number of HBV DNA reactive and seronegative donations	Rate of seronegative HBV DNA reactive (%)
★ Southwest	Sichuan	595,980	1008	0.169
	Guizhou	283,799	318	0.112
	Yunnan	393,851	336	0.085
	Tibet	3815	1	0.026
	Chongqing	243,694	283	0.116
	Total	1,521,139	1946	0.128
★ South China	Guangxi	577,265	961	0.166
	Guangdong	1,217,793	1634	0.134
	Hainan	102,614	149	0.145
	Total	1,897,672	2744	0.145
★ Central China	Hunan	509,121	776	0.152
	Hubei	604,287	699	0.116
	Henan	988,604	681	0.069
	Jiangxi	346,828	760	0.219
	Total	2,448,840	2916	0.119

Grifols NAT in China

- Used in **66** blood screening labs from **22** provinces
- Total detected donations in the first three quarters in 2017: **1,940,561**
- Highest/ Lowest HBV DNA positive rate: **0.22%** in Chongqing / **0.05%** in Shandong
- Proportion of positives by discriminatory test : **27%~49%**



Challenges of implementing NAT in China

- Complexities of HBV DNA positive /seronegative donations

Serological characteristics of the HBV DNA positive /seronegative donations

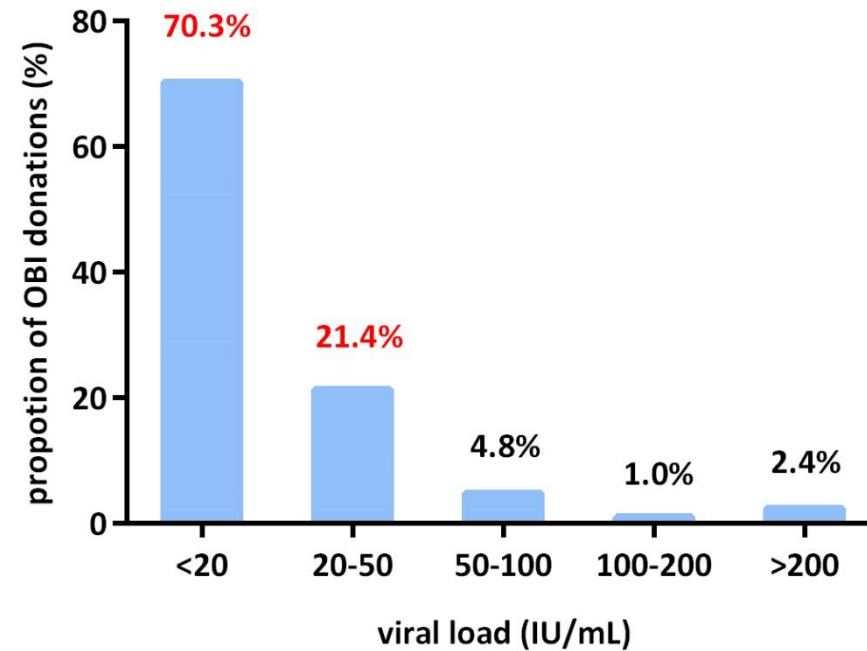
HBsAg	HBsAb	HBeAg	HBeAb	HBcAb	Number(percent)
–	–	–	–	–	587 (12.76%)
–	+	–	–	–	347 (7.54%)
–	+	–	–	+	744 (16.17%)
–	+	–	+	+	528 (11.48%)
–	+	+	–	+	13 (0.28%)
–	–	–	–	+	922 (20.04%)
–	–	–	+	+	969 (21.06%)
–	–	+	–	+	15 (0.33%)
+*	+ or–	+ or–	+ or–	+ or–	381 (8.28%)
Border*	+ or–	+ or–	+ or–	+ or–	95 (2.06%)

*The samples were HBsAg positive or borderline positive tested by electrochemiluminescence immunoassay (ECLIA) or chemiluminescent microparticle immunoassay (CMIA) while they were negative when tested by ELISA.

Challenges of implementing NAT in China

Low viral load in OBI

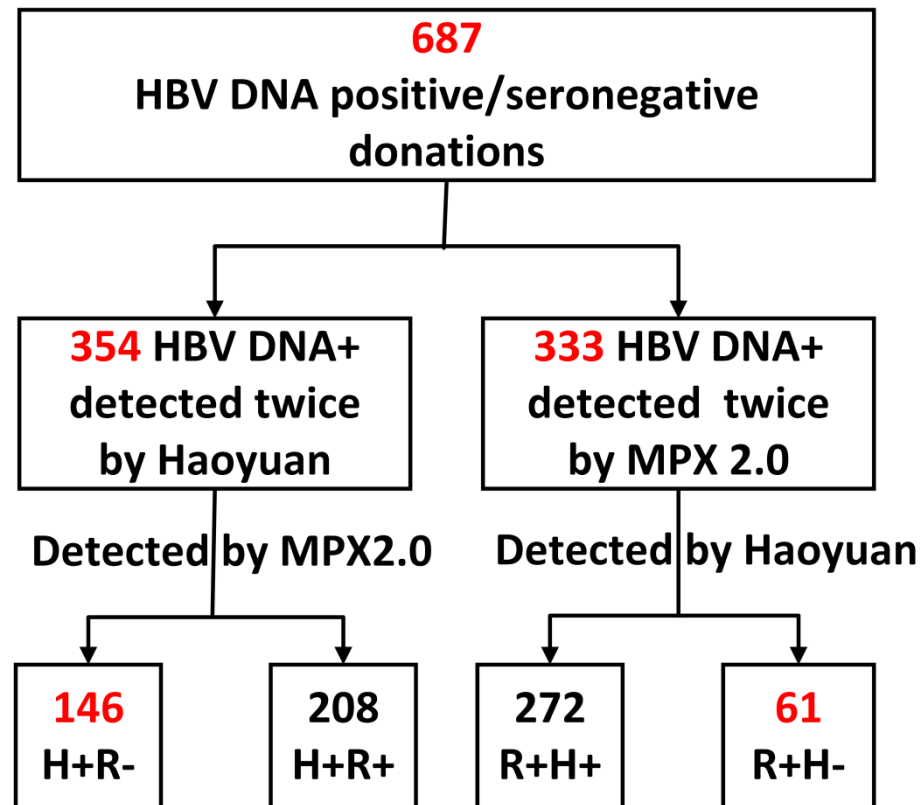
More than **70%** of 998 **OBI** donations had concentration lower than **20 IU/mL**.
More than **90%** were lower than **50 IU/mL**.



Challenges of implementing NAT in China

- Low viral load in OBI

Low repeat detection rate in donations with low viral load



Challenges of implementing NAT in China

- Low viral load in OBI

Low repeat detection rate in donations with low viral load

Quantitative analysis results on R+H- and H+R- samples

Number	ID Ct by Haoyuan	ID Ct by MPX2.0	Viral load by m2000 (IU/mL)	Viral load by HBV2.0 (IU/mL)
5HA12002	ND	35.2	54	<20
5SR11001	ND	34.5	27	<20
6CD02003	ND	36	<10	<20
5SR11005	ND	36.4	<10	ND
6SX04006	ND	37.8	12	ND
6XZ05002	ND	29.8	139	97
6SX04007	ND	36.3	<10	ND
6SX03015	ND	35.9	<10	<20
6QD06005	35.71	ND	ND	<20
5SY05005	35.47	ND	ND	ND
5QN05002	35.35	ND	ND	ND
5SY04002	36.26	ND	14	ND
LOD IU/mL	6.23	2.3	5	5

Challenges of implementing NAT in China

Low viral load in OBI

TaqScreen 1.0 MP6-NAT study ² in 826,044 donations			Ultrio ID-NAT study ³ in 1,205,796 donations		
NAT result (proportion)	% anti-HBc+	% anti-HBc+/ anti-HBs-	NAT result (proportion)	% anti-HBc+	% anti-HBc+/ anti-HBs-
MP-	598/1605 (37.3%)*	98/1605 (6.1%)	ID-	1571/4317 (36.4%) *	260/4317 (6.0%) ‡
MP+/ID- 35.8%	61/127 (48%) * †	13/127 (10.2%) ‡	ID+/dHBV- 70.4%	703/1028 (68.4%) *	320/1028 (31.1%) ‡ §
MP+/ID+ 64.2%	504/619 (81.4%) †	334/619 (54.0%) ‡	ID+/dHBV+ 27.6%	287/409 (70.17%)	210/409 (51.3%) §

* p=0.0161

† p<0.0001

‡ p<0.0001

* p<0.0001

‡ p<0.0001

§ p<0.0001

Anti-HBc in screening resolution algorithm?

2. Wang L, et al. What is the meaning of a nonresolved viral nucleic acid test-reactive minipool? Transfusion, 2015, 55(2): 395-404.

3. Gou H, et al. Evaluation of an individual-donation nucleic acid amplification testing algorithm for detecting hepatitis B virus infection in Chinese blood donors. Transfusion, 2015, 55(9): 2272-81.

HBV NAT yield rate in first time and repeat donations in Hong Kong (2007-2011)^

	First time donations		Lapsed and repeat donations	
	Ultrio	Ultrio Plus	Ultrio	Ultrio Plus
Donations tested	92,551	80,565	424,521	318,761
All HBV infections (%)	1,714 (1.85%)	945 (1.17%)	228 (0.054%)	224 (0.070%)
Acute HBV NAT yields (rate)*	4 (1:23,138)	5 (1:16,113)	11 (1:38,593) [‡]	18 (1:17,709) [‡]
Chronic HBV NAT yields (rate)*	12 (1:7,713) [†]	27 (1:2,984) [†]	89 (1:4,770) [§]	136 (1:2,344) [§]

*In house PCR confirmed

† p<0.005

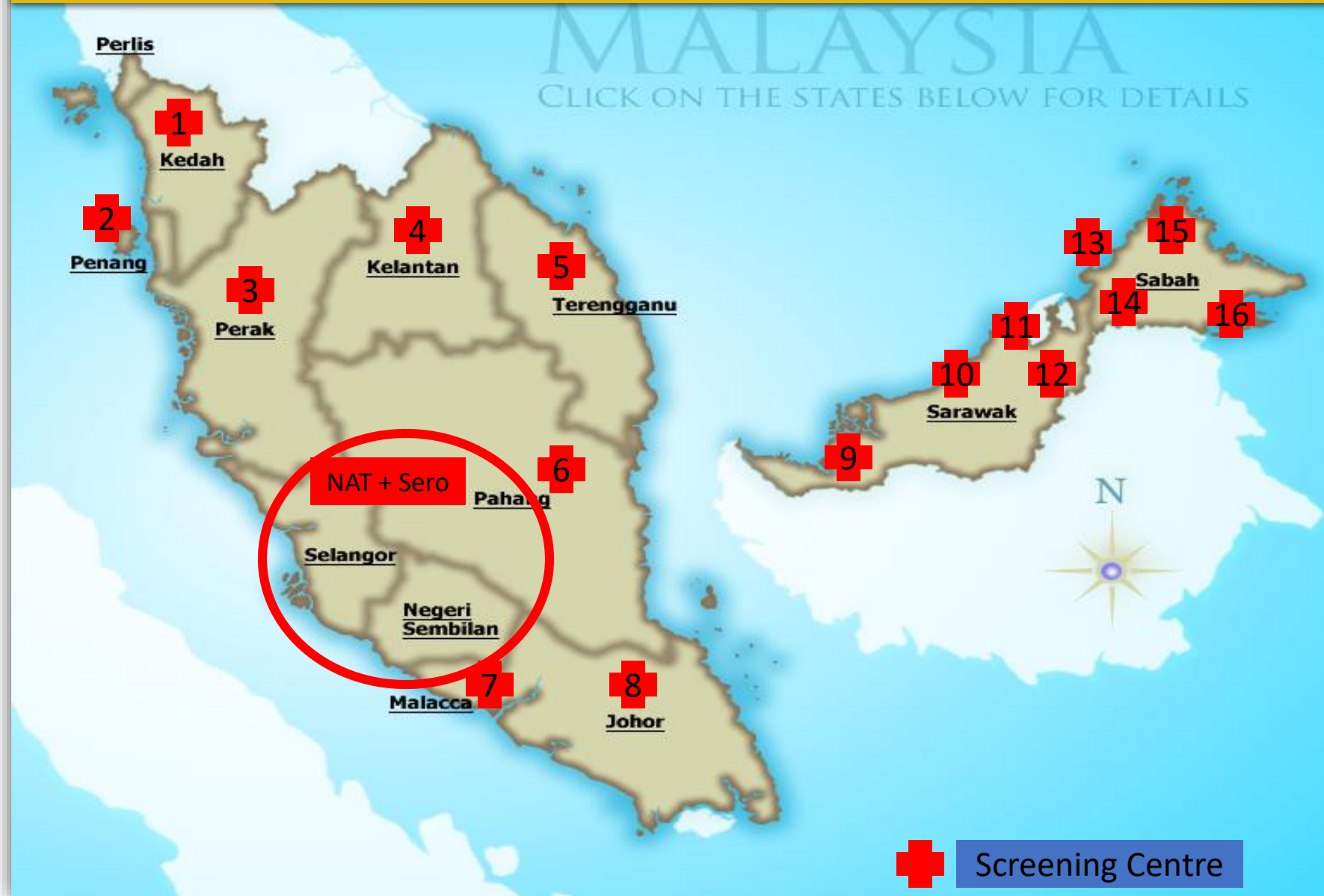
§ p<0.0001

‡ p<0.05

No difference in HBV NAT yield rate between first time and lapsed/repeat donors

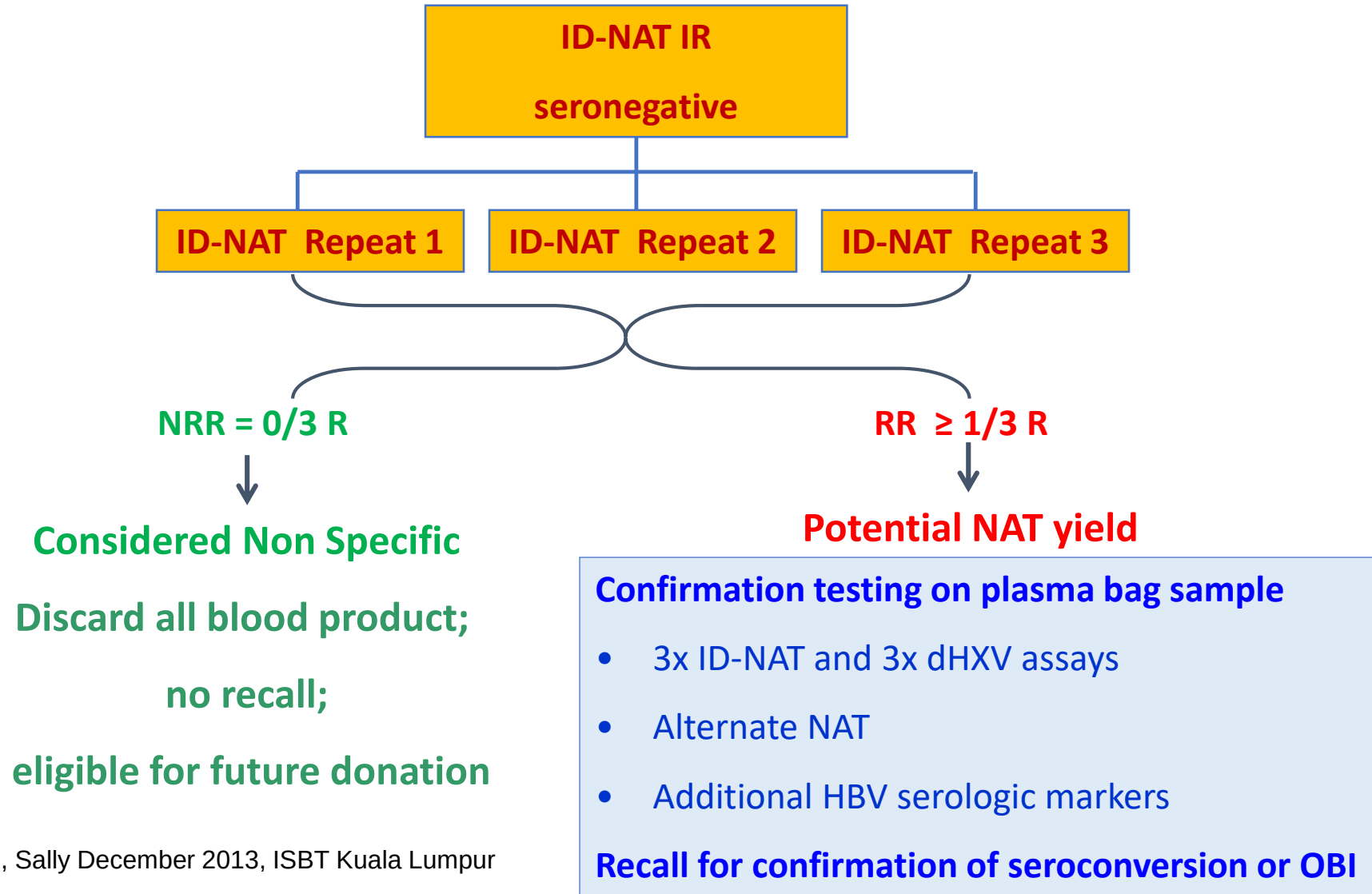
^Tsoi et al. Transfusion 2013;53:2477-2400

Distribution of Blood Screening Centres in Malaysia



NBC KL's ID-NAT Screening Algorithm (Malaysia)

in SeroNEGATIVE Donations



HIV infection rate in first time and repeat donations in Central Malaysian Region (2008 - 2013)^

Donations / Classification	Donations PDN				NBC Districts
	First time	Lapsed	Repeat	All	All
	383,926	145,418	371,510	900,854	1,240,399
All HIV infections (rate)	237 (1:1,620)	103 (1:1,412)	45 (1:8,256)	386 (1:2,334)	494 (1:2,511)
HIV-RNA+ WP (NAT yield rate)	4* (1:95,982)	6 (1:24,236)	10* (1:37,151)	20 (1:45,043)	23 (1:53,930)
HIV-RNA+ and anti-HIV+	233	97	35	366	471
HIV-RNA- and anti-HIV+	0	0	0	0	0

* p=0.096

^ data presented in poster by Mah E. et al, ISBT (2014) in Seoul, manuscript in preparation

HIV window period NAT yield rate in donation categories in multi-regional study[^]

Region	n all donations	% first time donations	WP NAT yields (rate per million)	
			First time	Lapsed and repeat
South Africa	4,449,173	11%	53 (111) [†]	117 (29) [†]
South-East Asia	1,051,381	31%	1 (3)	4 (5.5)
Mediterranean Europe	1,994,494	12%	0	5 (2.9)
Central & Northern Europe	2,617,682	11%	0	1 (0.4)
Oceania	1,542,480	10%	0	1 (0.7)

[†] p<0.0001

[^] Bruhn et al, Transfusion 2013;53:2399-2412

HIV window period NAT yield rates in categories of donations over 10 years in South-Africa^

Ethnic Group		First Time	Lapsed	Repeat
All	n donations WP NAT yields (rate/million)	956,389 137 (143) [†]	882,452 44 (50)	5,923,026 301 (51) [†]
Black	n donations (%) WP NAT yields (rate/million)	427,721 (45%) [†] 130 (304)	154,192 (17%) 37 (240)	975,727 (16%) [†] 247 (253)
Non-black	n donations (%) WP NAT yields (rate/million)	508,824 (53%) [†] 7 (14)	719,653 (82%) 7 (10)	4,912,253 (83%) [†] 54 (11)

[†] p<0.0001

^ Vermeulen et al, manuscript submitted

Viral load in HIV Ag/Ab positive and negative Malaysian WP NAT yield samples^

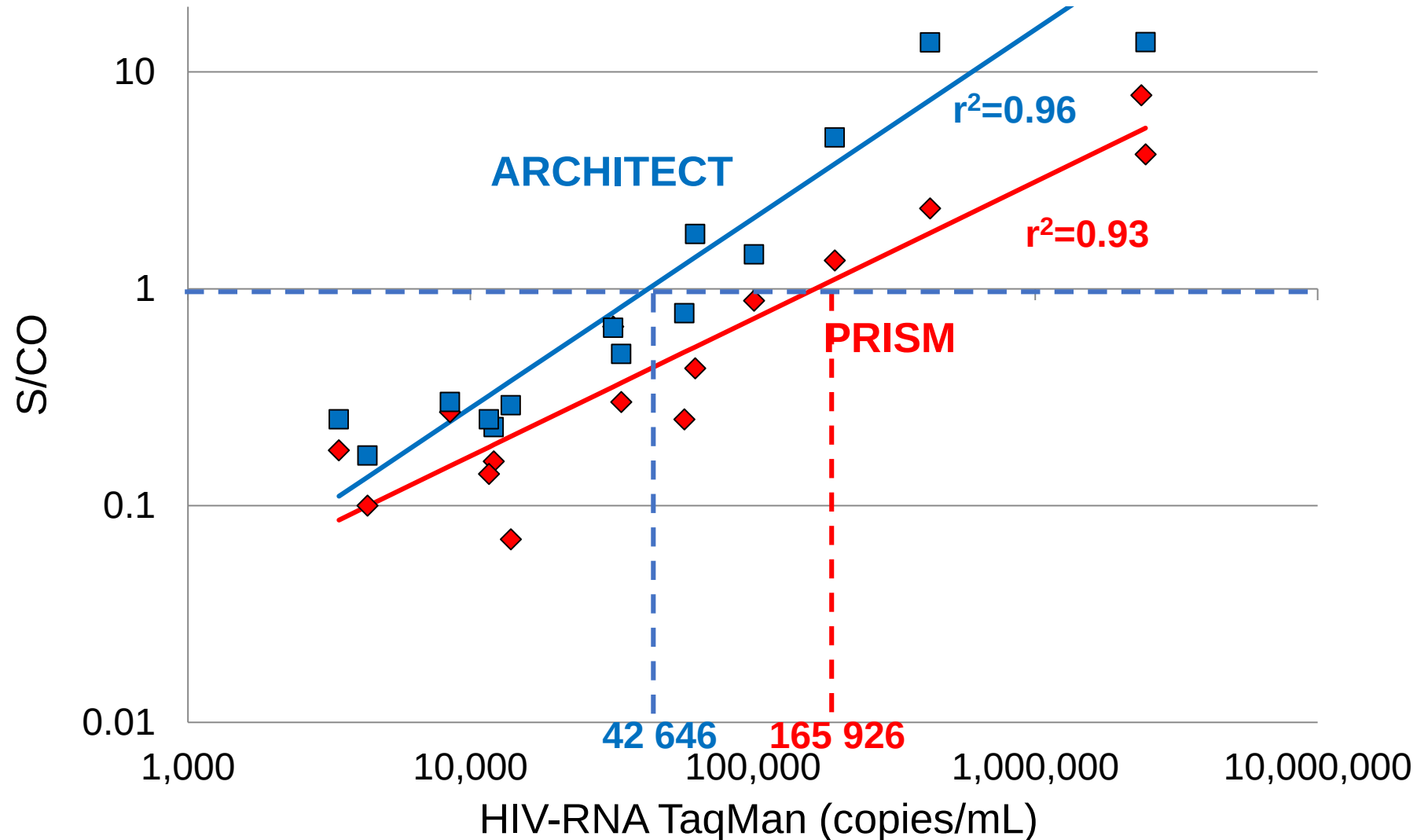
Don or	PRISM HIV-Ag/Ab S/CO	Architect HIV-Ag/Ab S/CO	HIV-RNA (cp/mL)	Don or	PRISM HIV Ag/Ab S/CO	Architect HIV Ag/Ab S/CO	HIV-RNA (cp/mL)
3	0.16*	0.43	2 461 680	6	0.30	0.50	34 220
12	4.17	13.71	2 458 670	11	0.67	0.66	32 030
2	7.80*	89.74	2 376 620	21	0.07	0.29	13 908
1	N.T#*	20.84	N.T#	23	0.16	0.23	12 098
5	2.35*	13.66	424 310	10	0.14	0.25	11 640
17	1.35	4.98	195 047	13	0.27	0.30	8460
18	1.00	3.28	N.T#	8	0.10	0.17	4320
9	0.88	1.44	100 970	14	0.18	0.25	3420
19*	0.43	1.79	62 443	16	0.06	0.25	710
20*	0.38	1.07	N.T#	4*	0.08	0.17	<40
7	0.25	0.77	57 180	15	0.06	0.23	23
				22	0.08	0.23	<20

* Sample nonreactive in PRISM anti-HIV assay

not tested, archive sample not available

^ data presented in poster by Mah E. et al, ISBT (2014) in Seoul, manuscript in preparation

Regression analysis on S/CO values in HIV-Ag/Ab assays and VL in Malaysian HIV WP ramp up samples[^]



[^] data presented in poster by Mah E. et al, ISBT (2014) in Seoul, manuscript in preparation

HIV WP transmission risk with ID-NAT and HIV Ag/Ab assays in Malaysia (2008 – 2013)^

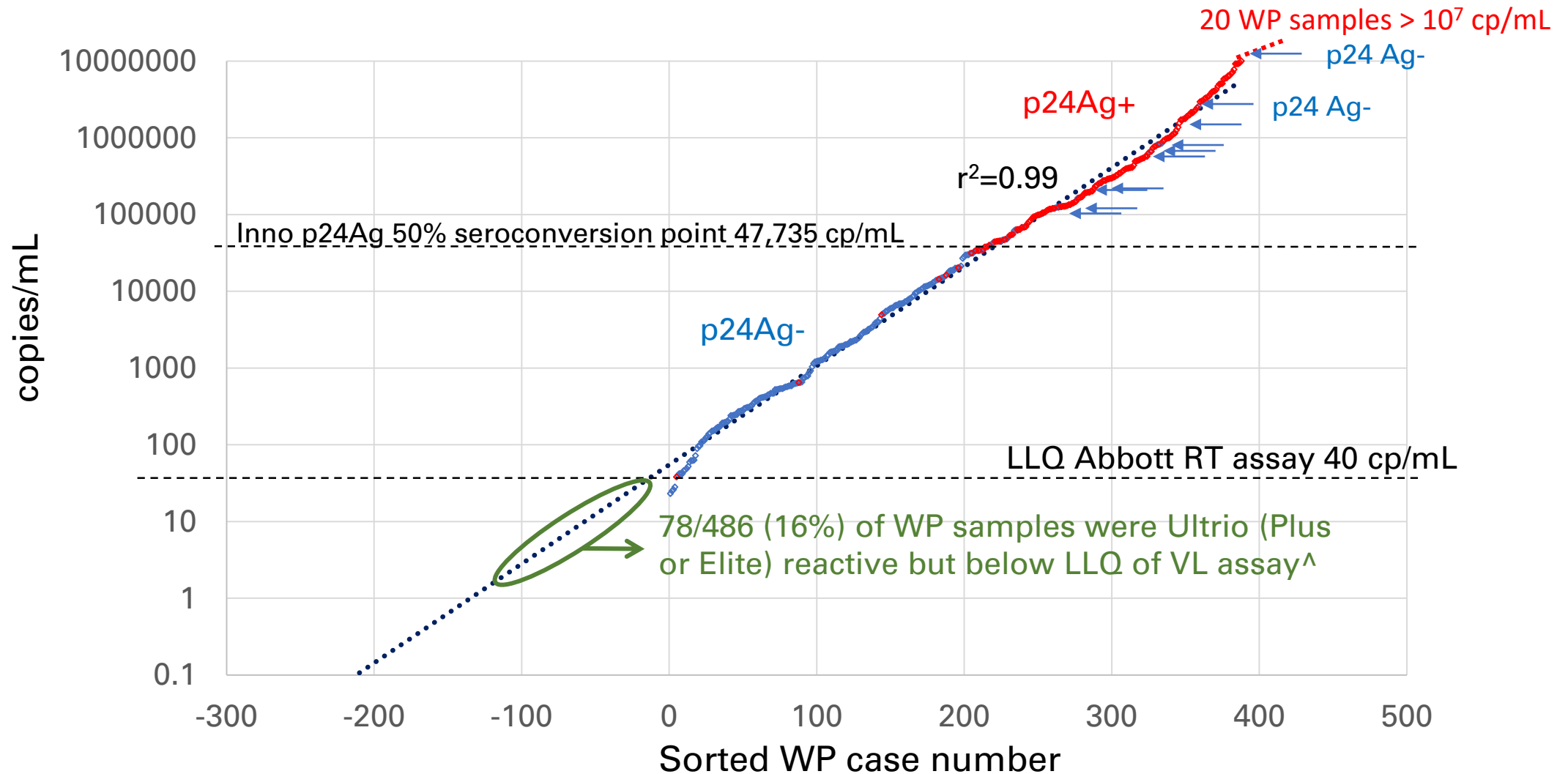
HIV Assay	estimated 50% seroconversion point (copies/mL)	WP risk day equivalents (ID ₅₀ 32 virions)	Calculated Residual risk per million*	Observed p24Ag negative WP NAT yields per million
ULTRIO ID-NAT	2.7	0.86	0.67	-
ARCHITECT HIV-Ag/Ab	42 600	11.9	9.3	11.3#
PRISM HIV-Ag/Ab	166 000	13.6	10.6	13.7§

* For lapsed and repeat donations taken together. Estimated risk for lapsed and repeat donations was almost identical (data not shown).

14 cases and § 17 cases in 1,240,399 donations.

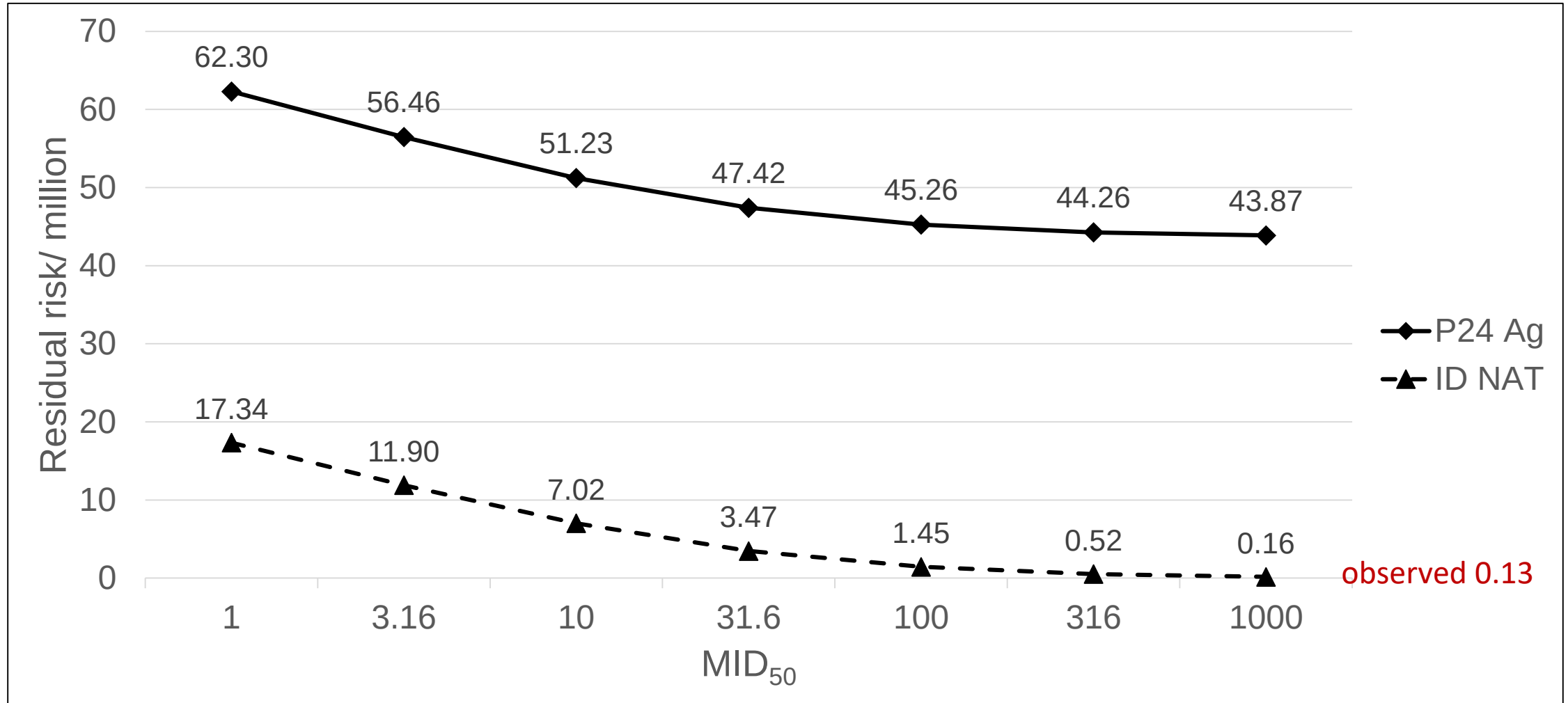
^ data presented in poster by Mah E. et al, ISBT (2014) in Seoul, manuscript in preparation

Viral load distribution in HIV WP donations in South Africa



VL can be estimated by probit analysis using reactivity rate by replicate tests on WP sample and on HIV-1 subtype C standard dilution panels [Vermeulen et al. Transfusion 2013;53:2384-2398]

Impact of HIV infectivity on window period risk over 10 year period in South Africa^



^ Vermeulen et al, manuscript submitted

HIV NAT breakthrough transmission cases

Test Failure Breakthrough Cases												
Country	Blood Product	Stor. time days	Screening Assay	50% LOD cp/mL	95% LOD cp/mL	Pool size	Cp/mL	Viral load method	Approx plasma mL	Estimated copies transfused	Recipient Infected\$	Reference
Germany	RBC		In-house			96	6400	TaqMan	20	128,000	Yes	Schmidt M et al. Transfusion 2009;49:1836-1844
Germany	RBC		In-house	4.2	22.1	96	12800	TaqMan	20	256,000	Yes	Chudy M et al. Transfusion 2012;52:431-9
Viral Load Below Detectable Level												
Japan	FFP		TaqScreen	4.1	28.0	20	4.1	50% LOD	240	984	Yes	Shinohar N. Transfusion 2014;54:2361-2
USA	FFP		Duplex	2.1	15.6	16	33.6	50% LOD	200	6720	Yes	Phelps R et al. Transfusion 2004;44:929-933
USA	FFP		Duplex	2.1	15.6	16	33.6	50% LOD	200	6720	Yes	Stramer S et al. Transfusion 2003;43:Supplement :40-41A
Spain	FFP-MB		Ampliscr	4.3	22.1	44	135	Ampl Monitor	261	35235	Yes	Alvarez et al. Transfusion 2016;56:831-836
Germany	PLT		In-house			24	0	TaqMan	240	0.001	No	Kalus U et al. , Transfusion 2009;49:435-439
Singapore	PLT		No NAT				50	Probit	25	1250	Yes	Ling AE et al. JAMA 2000; 28:4:210-214
Denmark	PLT		In-house			96	246	Abbott RT	25	6150	Yes	Harritshoj Let al. Transfusion 2008;48:2026-28
Spain	PLT	2	Ampliscr	4.3	22.1	44	135	Ampl Monitor	65	8775	Yes	Alvarez et al. Transfusion 2016;56:831-836
S. Africa	RBC		Ultrio Plus	2.7	18.4	1	2.7	50% LOD	20	54	Yes	Vermeulen M manuscript submitted 
Japan	RBC		TaqScreen	4.1	28.0	20	4.1	50% LOD	20	82	No	Shinohar N. Transfusion 2014;54:2361-2
Brazil	RBC	11	in house		22.6	24	4.4	probit	20	87	Yes	Salles NA. Transfusion 2013;53:2593-5
Greece	RBC		No NAT	2.1	15.6	4	8.4	50% LOD	20	168	Yes	Hatzakis et al. (personal communication, 2006)
Germany	RBC		Gfe Blut			96	23.6		20	472	No	Muller B. Transfusion. 2013;53:2422-30
Thailand	RBC		TaqScreen	4.1	28.0	6	24.6	50% LOD	20	492	Yes	Rujirojindakul P. Vox Sang 2015:109 Suppl 1 226 P-408 
S. Africa	RBC		No NAT				31	Probit	20	620	No	Ferriera MCet al. Transfusion 2006;46:156-157
S. Africa	RBC		No NAT				31	Probit	20	620	No	Ferriera MCet al. Transfusion 2006;46:156-157
USA	RBC		Duplex	2.1	15.6	16	33.6	50% LOD	20	672	Yes	Phelps R et al. Transfusion 2004;44:929-933
USA	RBC		Duplex	2.1	15.6	16	33.6	50% LOD	20	672	No	Stramer S et al. Transfusion 2003;43:Supplement :40-41A
USA	RBC		Duplex	2.1	15.6	16	33.6	50% LOD	20	672	Yes	Laffoon et al, MMWR 2010;59:1335-6
France	RBC		Nucl-Ampl	1.9	15.5	24	45.6	50% LOD	20	912	Yes	Najiouallah F et al. J Med Virol 2004;73:347-349
Singapore	RBC		No NAT				50	Probit	20	1000	Yes	Ling AE et al. JAMA 2000; 28:4:210-214
Italy	RBC		No NAT				98	TaqMan	20	1960	No	Zanetti et al. Transfusion 2007;47:1328-1329
Spain	RBC	28	Ampliscreen	4.3	22.1	44	135	Ampl Monitor	20	2700	No	Alvarez et al. Transfusion 2016;56:831-836
USA	RBC		In-house			24	180	NGI ^b	20	3600	Yes	Delwart ELet al. Vox Sang 2004;86:171-177
Denmark	RBC		In-house			96	246	Abbott RT	25	4920	Yes	Harritshoj Let al. Transfusion 2008;48:2026-28
Japan	RBC		AmpliNAT	10.4	43.6	50	520	50% LOD	20	10400	Yes	Satake M et al. Journal of Hematology 2004;80:306-10

\$ MID₅₀ estimated at ~ 50 (....) virions by probit analysis

Summary (HBV-NAT)

- Increasing NAT adoption in Asia (100% in China)
- Complexities with low VL HBV confirmation (70% < 20 IU/mL)
- Enhanced anti-HBc prevalence in MP+/ID- and ID+/dHBV- donations
- Different ID-NAT confirmation algorithm in Malaysia than in China/Hong Kong/South-Korea
- Highest HBV-NAT yield rates found in China
 - Ultrio: 1:2500 [up to 1:500 in some regions] and 1:4400 in Hong Kong
 - TaqScreen 1.0 MPX: 1:1600
- Two-fold increase of HBV-NAT yield rate after introduction Ultrio Plus in Hong Kong (and Malaysia)
- No difference in HBV-NAT yield rate between first time and repeat donors in Hong Kong

Summary (HIV-NAT)

- HIV WP NAT yield rate was 1:16,200 in South Africa, 1:54,000 in Malaysia *and* 1:88,000 Thailand (MP6-NAT)[^]
- No difference in HIV WP NAT yield rate between first time and repeat donations within ethnic groups
- One observed ID-NAT breakthrough case in South Africa and one with MP6-NAT in Thailand
- One HIV Ag/Ab combo CLIA and some p24-Ag ELISA detection failures in high VL WP donations
- HIV-Ag/Ab combo seroconversion point at 43.000 - 166.000 cp/mL
- 16% of South African HIV-NAT yields had VL<40 cp/mL
- ~130 pre-ID-NAT WP donations (>1 virion/RBC) projected in South Africa but no evidence of transmission
- HIV infectivity likely 10-100 fold reduced in stored RBCs (MID₅₀ estimated at 30-300 virions)

[^]reported by Tasanee Sakuldamrongpanich, IPFA, Rome