IPFA/ PEI 24th workshop Zagreb 2017

Erhard Seifried

PI + NAT + serology: Eliminating old tests?

An integrated approach

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GRC Blood Transfusion Service Baden-Württemberg - Hessen

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Blood donations worldwide

Estimated blood donations by WHO region, 2013

Region	Estimated whole blood donations (millions)	Estimated apheresis donations (millions)	Total (millions)
Africa	5.6	0.03	5.6
Americas	20.4	2.0	22.4
Eastern Mediterranean	9.9	0.04	9.9
Europe	26.5	6.1	32.5
South-East Asia	16.6	0.06	16.7
Western Pacific	21.6	3.7	25.3
Global (rounded totals)	100.6	11.9	112.5

Source: WHO Report 2016

NAT market: calculated with >$3,0 billion/year;
Serology calculated with >$1,0 billion/year;

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Blood donor screening strategies

	14 NAT	9 Serology
Classic parameter	HAV	
	HBV	HBV
	HCV	HCV
	HIV-1	HIV-1
	HIV-2	HIV-2
	B19	
		Syphilis
	CMV	CMV
New parameter	WNV	
	Dengue	
	Malaria	Malaria
		Chagas
	HTLV	HTLV
	HEV	
	Chikungunya	
	Zika	

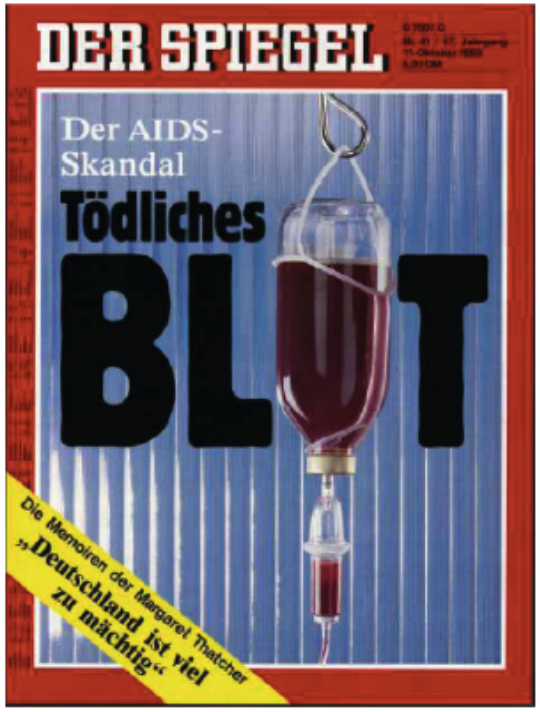
Blood donor screening by serology methods is less important than blood donor screening by NAT

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Blood AIDS scandal in Germany

The German AIDS scandal



Headline "Der Spiegel" 1993

- numerous HIV-1- and HCV-transmissions by blood products
- reduced willingness to donate blood
- legal reorganisation of transfusion medicine
- enactment of the "Transfusionsgesetz" (TFG)

  (Tranfusion act)

⇒ **TFG mandates use of state-of-the-art screening methods**

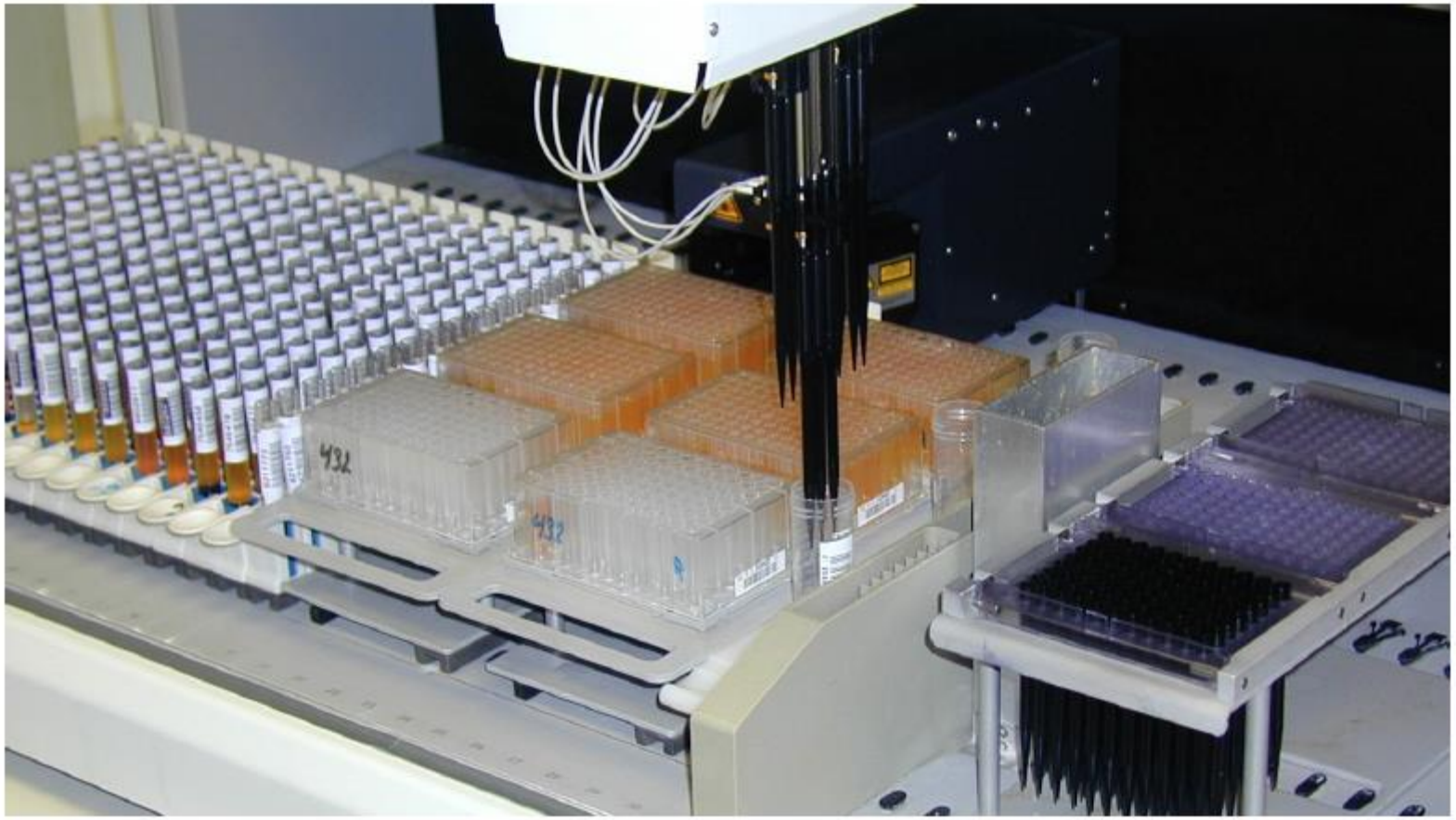
Residual risks of TTID 1993

Virus	Prevalence	Diag. window (days)	Estimated residual risk
HIV-1	2/100,000	45	1:500,000 – 1:1,000,000
HBV	40/100,000	45	1:50,000
HCV	100/100,000	90-120	<1:5,000

Kubanek et al. Infusionsth. Transfusionsmed. 1993;20:54-59

Introduction of MP-NAT into blood screening

- Pooling of 96 blood donations into one mini-pool for NAT testing



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Virus enrichment by centrifugation

- Virus enrichment by high speed centrifugation (1h/48,000xg)



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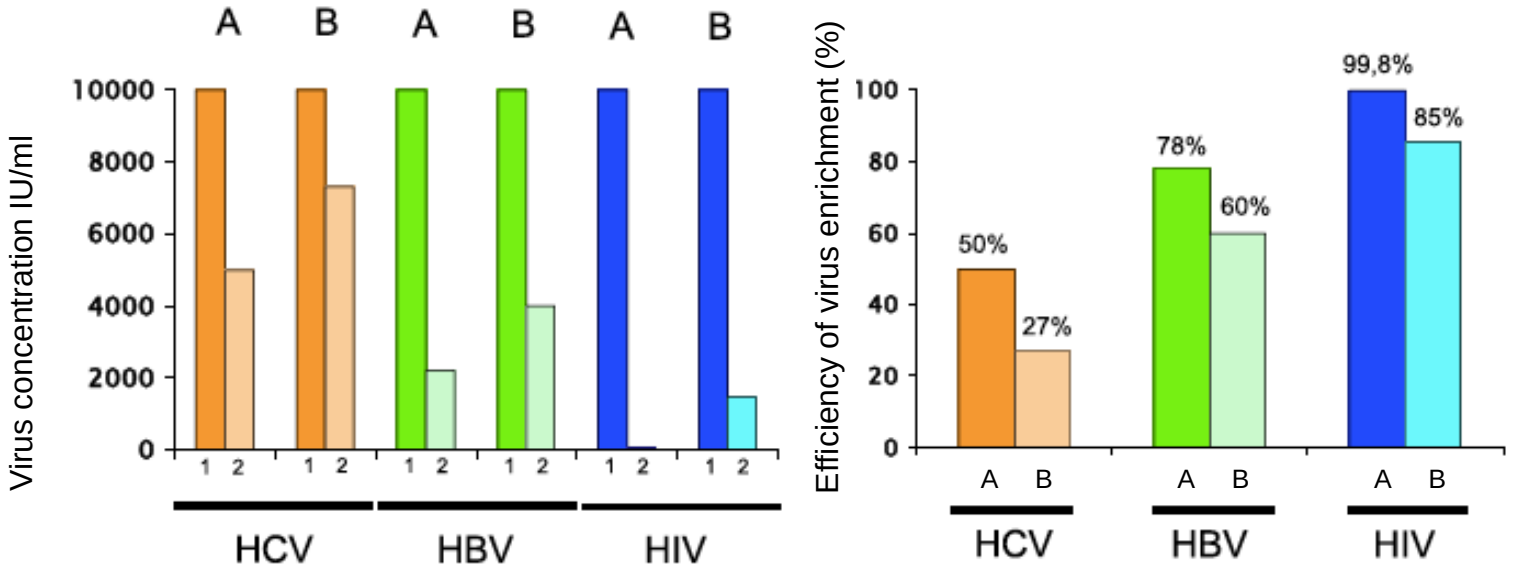
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Reduced efficiency in lipemic donations

- Reduced efficiency in lipemic blood donation especially for HCV

A: normal plasma
B: lipemic plasma

1: virus concentration before enrichment centrifugation
2: virus concentration after enrichment centrifugation



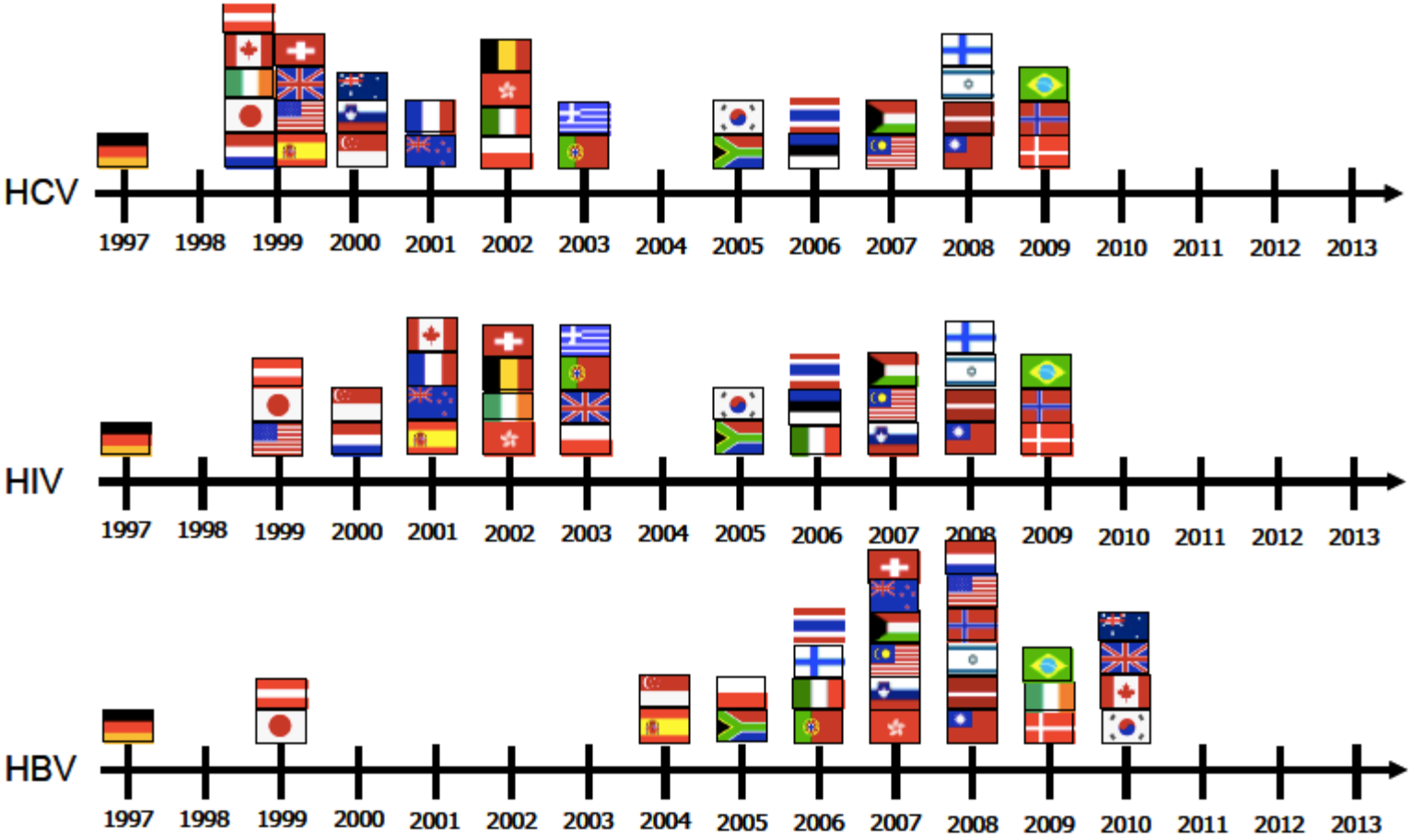
- Virus enrichment by centrifugation is not any longer state of the art since one decade!

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Source: publication under review

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Introduction of NAT worldwide



Internat. Forum Vox. Sanguinis 2012

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NAT only positive donations

Region/ country	Virus	Number of tested donations	NAT only positive	Rate/1.000.000 donations
	HIV-1	2,202,295	81	36.78
Africa	HCV	2,202,295	4	1.82
	HBV	2,202,295	76	34.51
	HIV-1	71,458,330	44	0.62
Asia/ Pacific	HCV	71,458,330	169	2.37
	HBV	50,679,100	1091	21.53
	HIV-1	110,860,111	73	0.66
Europe	HCV	139,474,595	206	1.48
	HBV	56,352,555	550	9.76
	HIV-1	87,652,586	45	0.51
North America	HCV	89,652,687	299	3.34
	HBV	5,062,264	11	2.17
	HIV-1	347,374	1	2.88
South America	HCV	408,167	2	4.90
	HBV	Not done	Not done	
	HIV-1	272,520,696	244	0.90
Total	HCV	303,196,074	680	2.24
	HBV	114,286,214	1,728	15.12

Internat. Forum Vox. Sanguinis 2012

NAT yield DRK NAT study

1997-2005

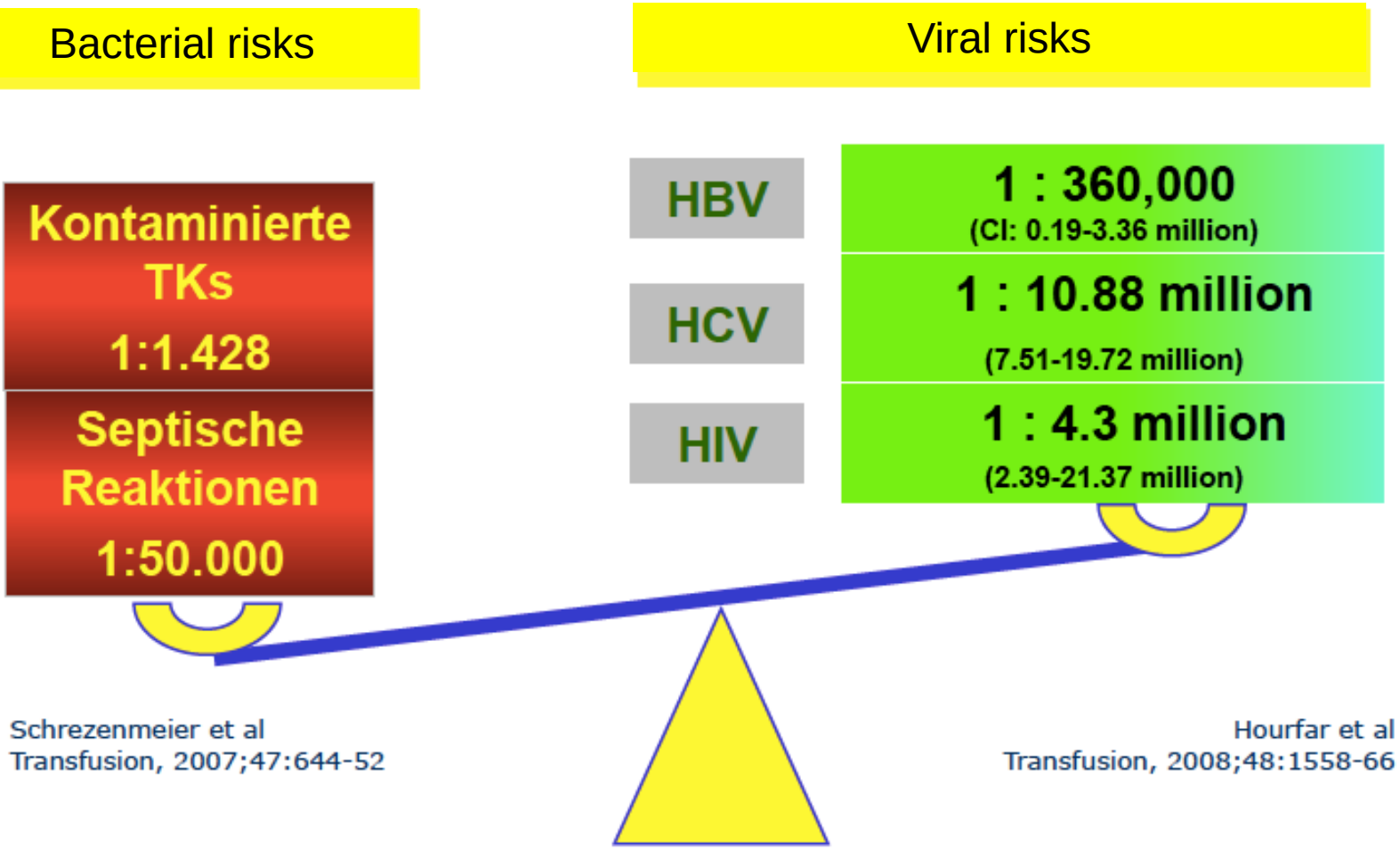
Virus	Mini-pool NAT only positive	Number donations (n)	Relation positive : total test number	Incidence per million
HBV	43	31,491,658	1: 732.364	1.4
HCV	22	31,491,658	1:1,431,439	0.7
HIV-1	8	31,491,658	1:3,936,457	0.25

relative residual risk without mini-pool NAT

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Residual risk based on DRK NAT study

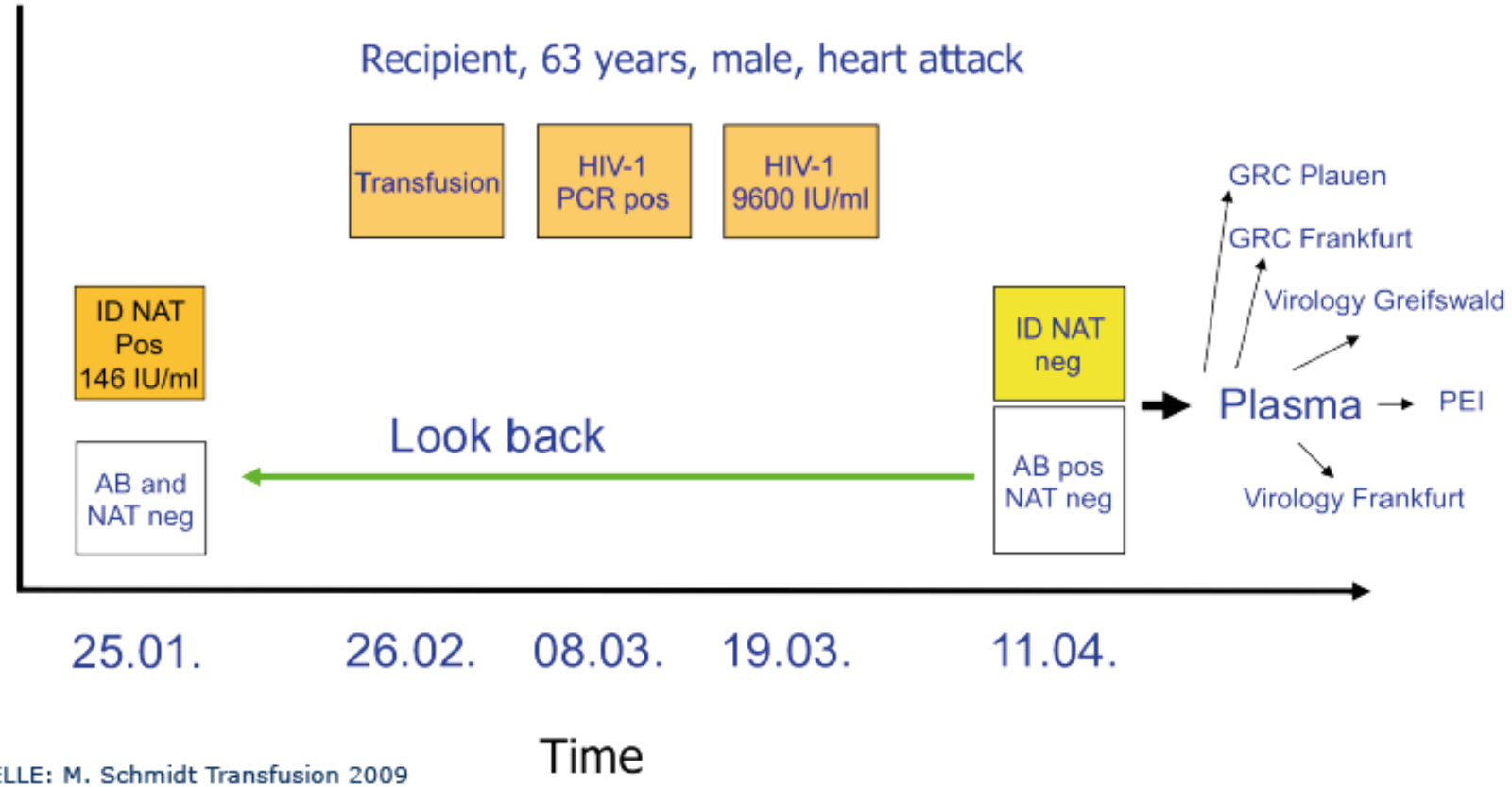


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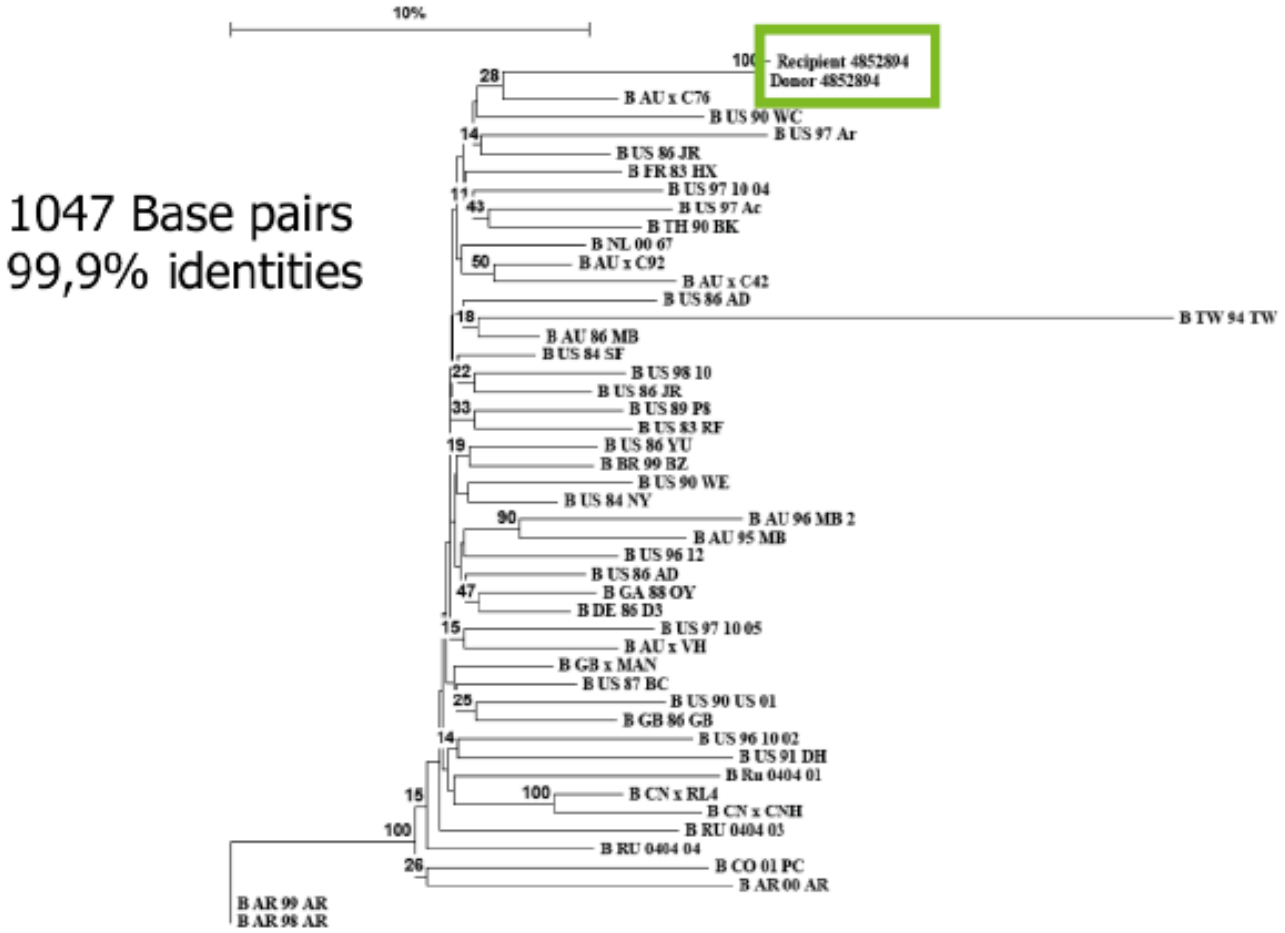
HIV-1 transmission by red cell product

Donor 44 years, male, multiple time donor, 52 donations



QUELLE: M. Schmidt Transfusion 2009

Phylogenetic analysis between donor and recipient



1047 Base pairs
99,9% identities

QUELLE: M. Schmidt/ L. Gürtler Transfusion 2009

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Mutations in the primer/probe binding region

Mismatches in the primer / probe binding region between the CAP/CTM HIV-1 Test and the detected HIV-1 virus subtype B.

Sense primer COBAS® Ampliprep / COBAS® TaqMan® HIV-1 Test: 1788 - 1819

CTM Primer: 5´ AGT GGG GGG ACA TCA AGC AGC CAT GCA AA 3´

Donor: 5´ AGT GGG GGG ACA TCA AGC AGC CAT GCA AA 3´

Probe COBAS® Ampliprep / COBAS® TaqMan® HIV-1 Test: 1821 - 1856

CTM Probe: 5´ TCT GCA GCT TCC TCA TTG ATG GT A TCT TTT AAC 3´

Donor: 5´ TCT GCA GCT TCC TCA TTG ATG GT T TCT TTT AAC 3´

Anti sense primer COBAS® Ampliprep / COBAS® TaqMan® HIV-1 Test: 1921 - 1950

CTM Primer: 5´ G G T ACT AGT AGT TCC TGC TAT GTC ACT T CC 3´

Donor: 5´ G T T ACT AGT AGT TCC TGC TAT GTC ACT A CC 3´

Positions of the nucleotides belong to the HXB2 genotype of the HIV-1 genome

Blood donations with HIV-1 with mutations

Case	Donor type, sex, age	Donation dates	Screening NAT	HIV Screening results	Viral load (IU/ml)	Donor Status	HIV-1 Trans-mission by
1	RD, male, 44	Jan 2007 Apr 2007	CAP CTM v1	Ab neg RNA neg **Ab pos** RNA neg	10,000 650	WP SC	RBC
2	RD, male, 26	Jul 2007 Oct 2007	CAP CTM v1	Ab neg RNA neg **Ab pos** RNA neg	0 80,000	0 SC	--
3	RD, male, 26	May 2009 Aug 2009 Jul 2010	CTS MPX CTS MPX VSPK v1.1	Ab neg RNA neg Ab neg RNA neg **Ab pos RNA pos**	0 20,000 260,000	-- WP SC	-- RBC
4	RD, male, 42	Mar 2010 Jun 2010 Oct 2010	VSPK v1.1	Ab neg RNA neg Ab neg RNA neg **Ab pos** RNA neg	0 0 200,000	-- -- SC	-- --
5	FTD, male, 18	Oct 2010	VSPK v1.1	**Ab pos** RNA neg	2,000	SC	

- Two transmissions by red cell concentrates

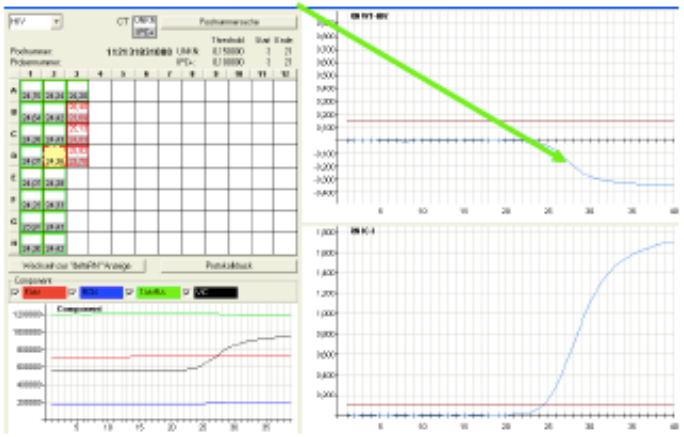
QUELLE: M. Chudy Transfusion 2012

Screening with dual target since 2010

- Since January 2015 blood donor screeing by NAT with dual targeting is mandated in Germany
- DRK Baden-Wuerttemberg – Hesse had implemented the dual target strategy in 2010 on a voluntary basis
- Between 2010 and 2014 three blood donations with mutations in the 5Ítr region were identified

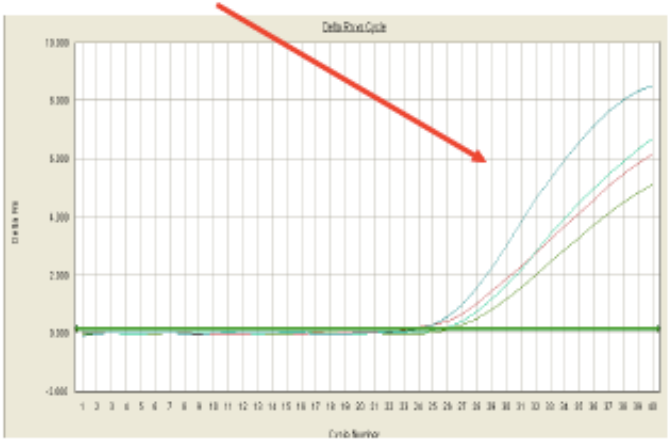
PCR 5´LTR-Region

Befund negativ



PCR gag-Region

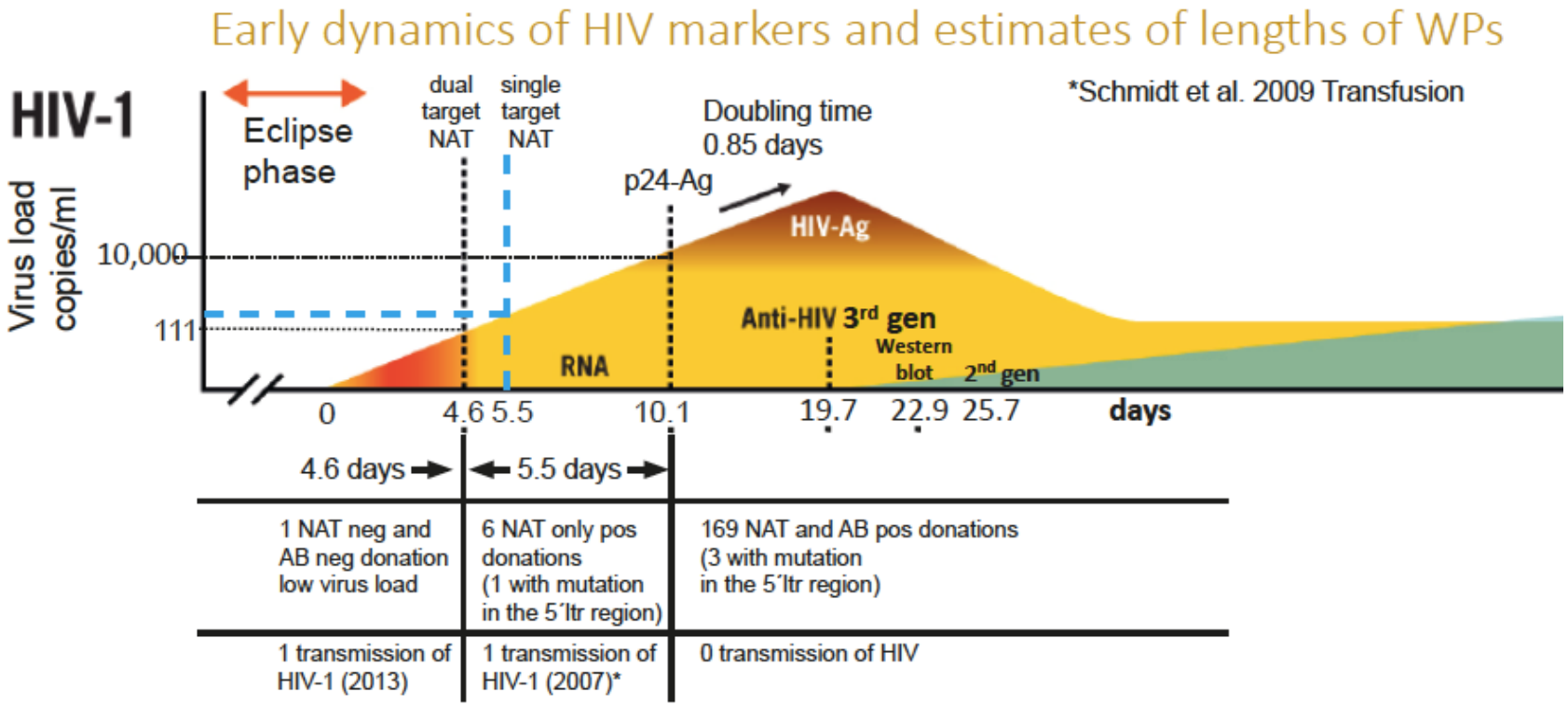
Befund positiv



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HIV diagnostic window Germany



Source: adopted from N. Lelie with data from the German Red Cross

New residula risk for HIV-1

DRK Blutspendedienste (1997-2005)	DRK BaWüHe/ Nord-Ost Single target	DRK BaWüHe/ Nord-Ost Dual target
1:4,3 million	1: 3,6 million	1:4,3 million
QALY gained 10,9 million €	QALY gained 10,0 million €	QALY gained 8,3 million €

- DRK NAT calculated without mutation risk
- „dual target“ reduce the residual risk about 19,4%
- Calculation was done in accordance to Weusten et al.

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Pathogen reduction methods

- Photochemical method: Intercept (S59 + UVA)
- Photodynamic method: Mirasol (Riboflavin + UVA)
- Irradiation only method: Theraflex (UVC + agitation)

Head-to-head comparison of pathogen reduction methods

	INTERCEPT	Mirasol	Theraflex
Viruses (enveloped)			
HIV-1, cell free	>6.2	5.9	1.4
HIV-1, cell-associated	>6.1	4.5	
HBV	>5.5	2.5a	≥2.8a
HCV	>4.5b	>4.1	≥5.0
HEV (genotype 3)		>3.0	
HTLV-I	4.7	n.t.c	
HTLV-II	5.1	n.t.c	
CMV, cell free		2.1d	ongoing
CMV, cell-associated	>5.9		ongoing
Bovine viral diarrhea virus	>6.0		2.7
WNV	>6.0	≥5.1	3.5-4.0
Chikungunya	>6.4	2.2	ongoing
Influenza A virus	>5.9	>5.0	≥5.3
SARS-CoV	>5.5		
Togavirus		3.2e	≥5.3e
Rabiesvirus		>6.3f	≥6.3[f]
Dengue-virus	>5.2		ongoing
La Crosse virus		>3.3	
Crimean-Congo hemorrhagic fever virus	2.9		
Viruses (non-enveloped)			
HAV	0	1.8	ongoing
Parvo B19	>6.2	>5.0g	5.0g
Blue tongue virus	6.1		
Human adenovirus	>5.9		
Calicivirus	1.7–2.4		
Picornavirus		3.2h	>4.0h
Bacteria gram-negative			
Escherichia coli	>6.4	4.4	>4.0
Enterobacter cloacae	5.9		>4.3
Klebsiella pneumonia	>5.6	2.8	4.8
Pseudomonas aeruginosa	4.5	>4.5	>4.9
Salmonella cholerasuis	>6.2		
Serratia marsescens	>6.7	4.0	≥4.9
Yersinia enterocolitica	>5.9	3.3	
Brucella neotomae		5.4	

	INTERCEPT	Mirasol	Theraflex
Bacteria gram-positive			
Bacillus cereus (incl. spores)	3.6		4.3
Bacillus cereus (vegetative)	>6.0	2.6	4.3
Bifidobacterium adolescentis	>6.5		
Clostridium perfringens (vegetative)	>6.7		>4.7
Corynebacterium minutissimum	>6.3		
Listeria monocytogenes	>6.3		
Proprionobacterium acnes	>6.2	>2.8	4.5
Staphylococcus aureus	6.6	4.8	≥4.8
Staphylococus epidermidis	>6.6	4.6	≥4.9
Streptococcus pyogenes	>6.8	2.6	
Lactobacillus species	>6.9		
Spirochaetes			
Borrelia burgdorferi	>6.8		
Treponema pallidum	>6.8	ongoing[i]	
Parasites			
Babesia microti	>5.3	>4.0	
Babesia divergens			≥5.0
Leishmania major	>4.3	>4.0	
Leishmania mexicana	>5.0		
Orientia tsutsugamushi		>5.0	
Plasmodium falciparum	>6.0	>3.2	≥4.9
Plasmodium yoelli		>4.4[j]	
Trypanozoma cruzi	>5.3	>5.0	2.8 to 4.2

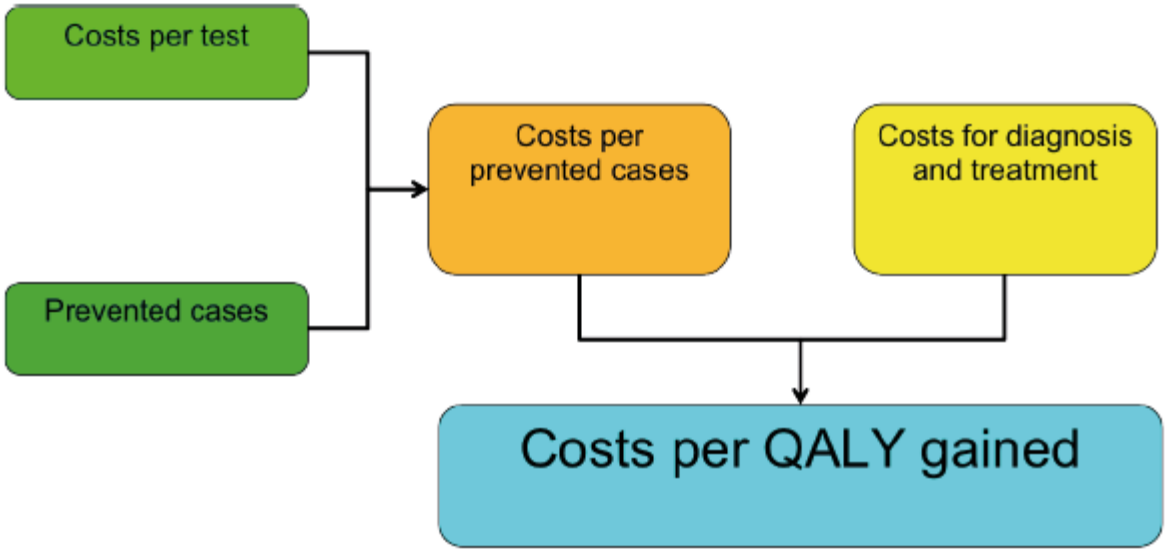
[a]Pseudorabies virus (suides herpes virus). [b]Chimpanzee transfusion studies. [c]HIV is used with some restrictions as model virus also for other retroviruses such as the oncogenic HTLV-I/II. [d]Bovine rhinotrachitis virus, in addition mouse CMV in vivo mouse transfusion model. [e]Sindbis virus, HCV model. [f]Vesicular stomatitis virus. [g]Porcine parvovirus which suitability as model virus for human Parvo B19 remains questionable. [h]Porcine encephalomyocarditis virus, HAV model. [i]In rabbits. [j]In vivo mouse malaria model.

Source: Schlenke 2014 Transfus. Med. Hemother.

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Quality adjusted life years (QALY)



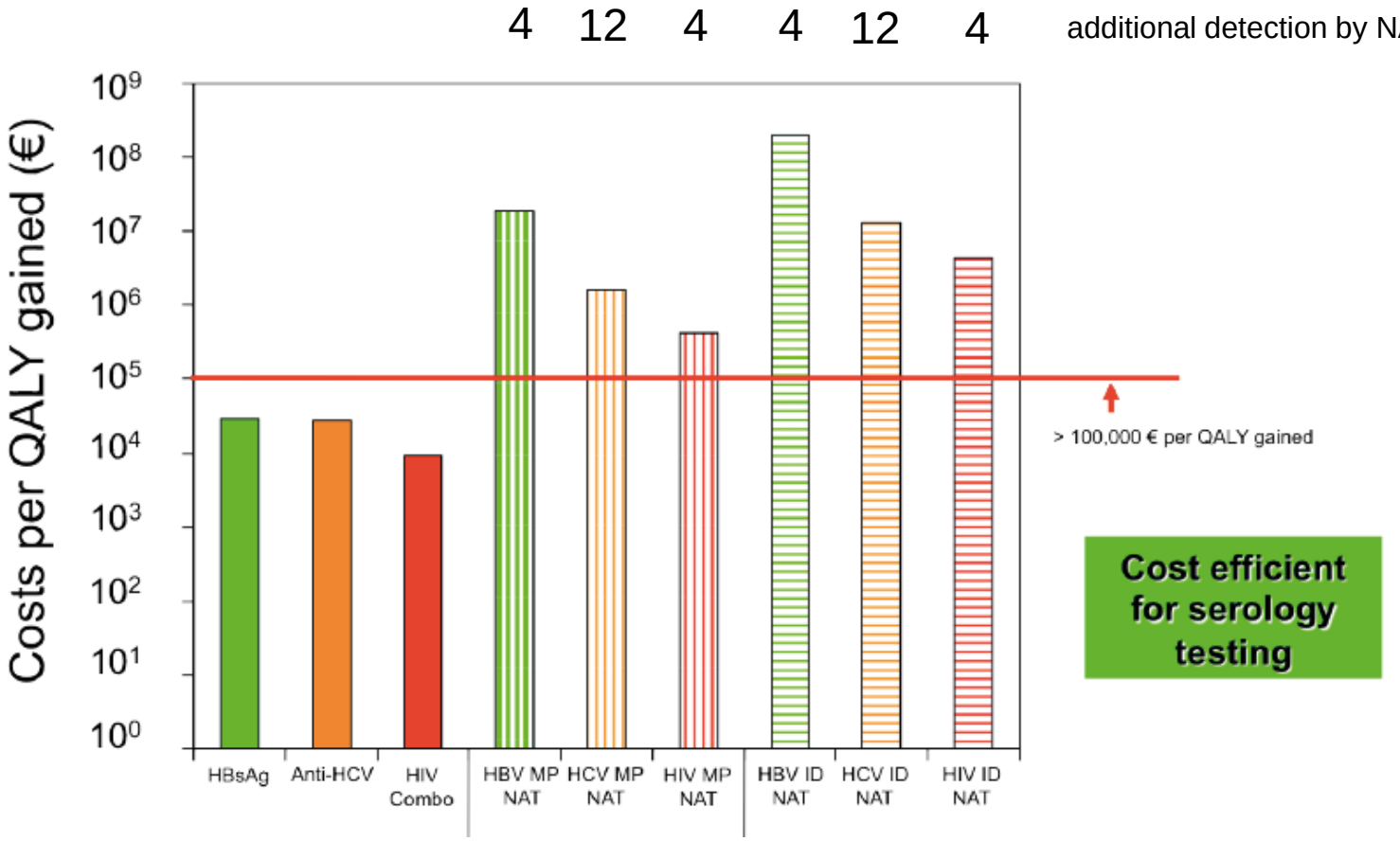
$$\text{Formula:} \quad \frac{\text{Cost per test (€)}}{\text{Number of prevented cases (n)}} = \text{costs per prevented cases}$$

Costs per prevented cases x virus factor (F) = QALY (€) gained

F for HIV = 7.1
F for HCV = 0.6
F for HBV = 0.16

QALY classic calculation (time between 2008 – 2015)

Analysis of 10.9 million blood donations

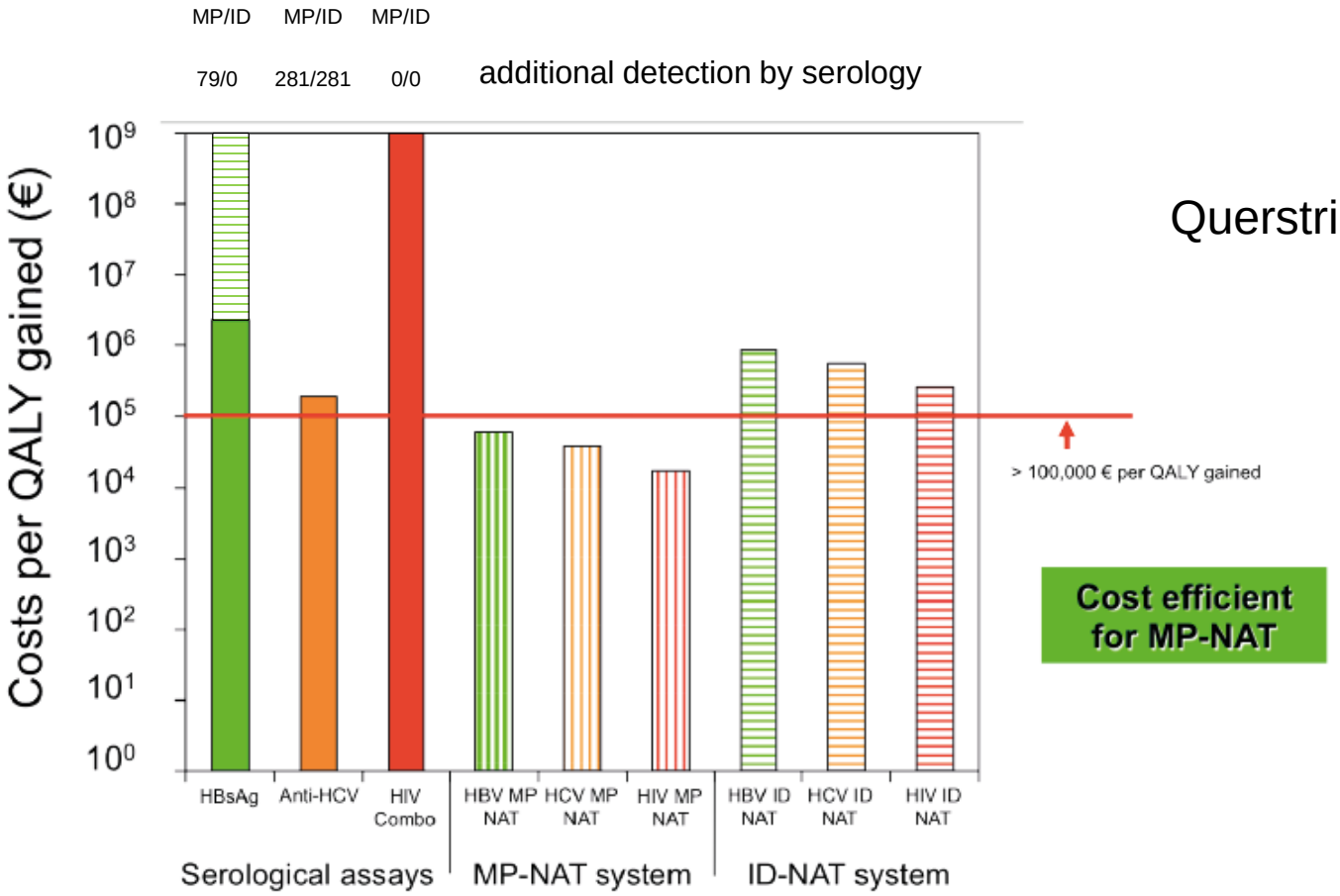


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QALY WP calculation (time between 2008 – 2015)

Analysis of 10.9 million blood donations



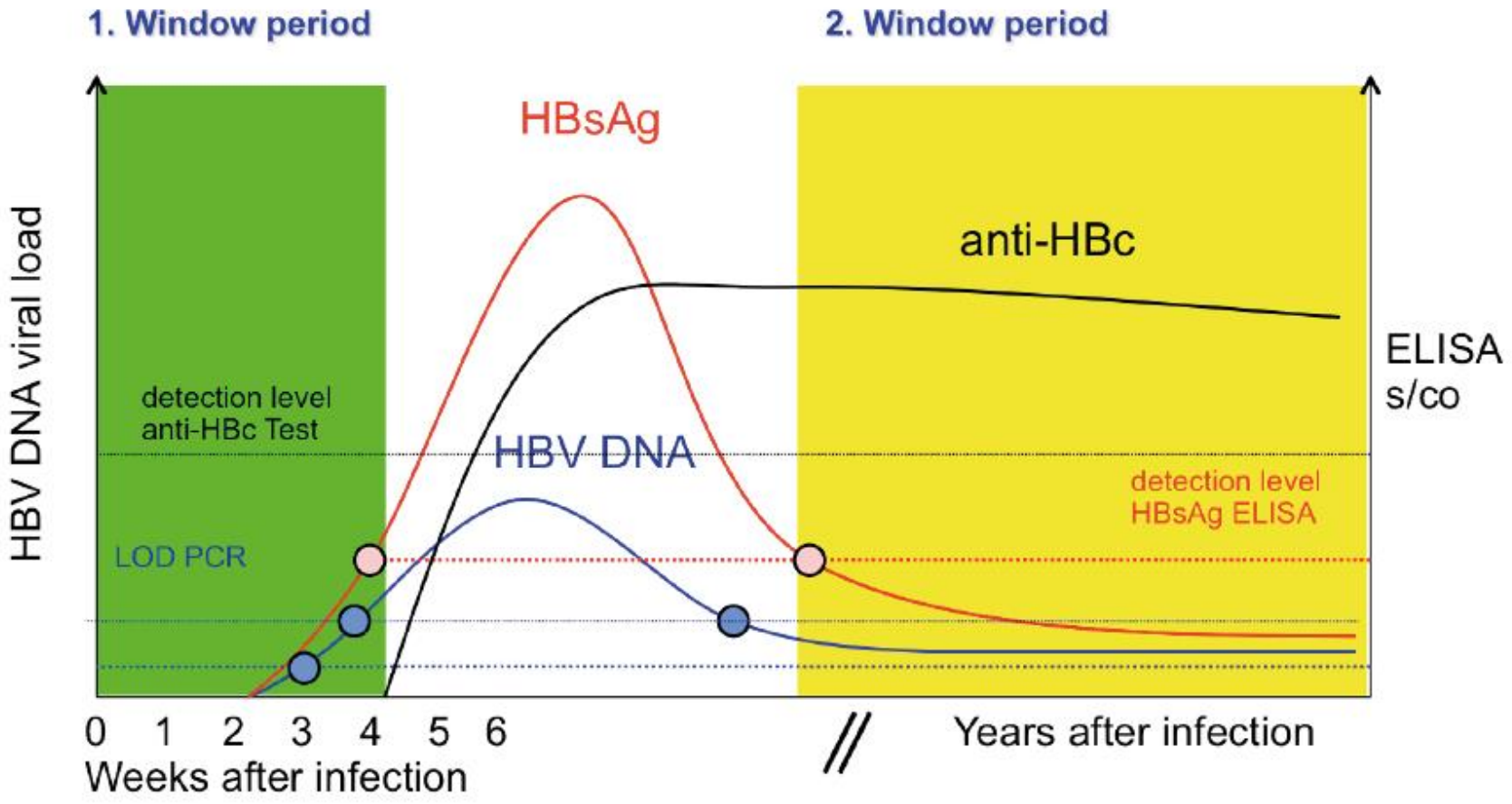
Querstriche hoch

Cost efficient for MP-NAT

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Hepatitis B virus infections



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HBV diagnostic versus pathogen reduction

Diagnostic	Pathogen reduction (PR)	
HBsAg (WP 38 days)	Intercept	>5.5 logs
Anti-HBc (WP 55 days)	Mirasol	2.5 logs
MP-NAT (WP 30 days)	Theraflex	>2.8 logs
ID-NAT (WP 22 days)		

Strategy	Residual risk	Cost per QALY gained
HBV MP NAT only	1:360,000	27,545 €
HBsAg	1:200,000	27,700 €
HBsAg + MP NAT	1:360,000	55,245 €
HBsAg + ID NAT	1:450,000	2.8 million €
PR*	1:1,000,000	1.38 million €
PR* + HBsAG	1:1,500,000	1.41 million €
PR* + MP-NAT (384 pool)	1:1,500,000	1.38 million €

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* PR in the future is feasible for pathogen reduction in whole blood

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HBV

- HBsAg can probably replaced by HBV MP-NAT + Anti-HBc, but HBsAg only reactive donation are detected, further investigations are necessary if HBsAg only reactive donation are infectious
- Blood donor screening by PR only still have the risk on infectious blood donations with virus loads higher than 10^6 IU/ml
- Cost per QALY will be dramatically increased by the implementation of a PR method, because a PR for whole blood is currently not available
- Blood donor screening by small mini-pools or ID-NAT is a good alternative to blood donor screening by anti-HBc
- Highest safety level will be achieved by PR + MP-NAT

HCV diagnostic versus pathogen reduction

Diagnostic	Pathogen reduction (PR)	
Anti-HCV (WP 59 days)	Intercept	>4.5 logs
MP-NAT (WP 6 days)	Mirasol	>4.1 logs
ID-NAT (WP 4 days)	Theraflex	>5.0 logs

Strategy	Residual risk	Cost per QALY gained
HCV MP NAT only	1:10,880,000	22,200 €
Anti-HCV	1:1,960,000	23,200 €
Anti-HCV + MP NAT	1:10,880,000	45,400 €
Anti-HCV + ID NAT	1:10,880,000	2.29 million €
PR*	1:8,430,000	1.13 million €
PR* + Anti-HCV	1:8,430,000	1.16 million €
PR* + MP-NAT (384 pool)	1:10,880,000	1.14 million €

* PR in the future is feasible for pathogen reduction in whole blood

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HCV

- Blood donor screening by anti-HCV can probably be replaced by MP-NAT or ID-NAT
- Blood donor screening by PR only still have the risk on infectious blood donation with virus loads higher than 10^6 IU/ml
- Cost per QALY will be dramatically increased by the implementation of a PR method, because a PR for whole blood is currently not available
- Highest safety level will be achieved by PR + MP-NAT

HIV diagnostic versus pathogen reduction

Diagnostic	Pathogen reduction (PR)	
HIV combo	Intercept	>6.2 logs
HIV MP-NAT	Mirasol	5.9 logs
HIV ID-NAT	Theraflex	1.4 logs

Strategy	Residual risk	Cost per QALY gained
HIV MP NAT only	1:4,300,000	8,550 €
HIV combo	1:2,610,000	9,150 €
HIV combo + MP NAT	1:4,300,000	17,700 €
HIV combo + ID NAT	1:4,300,000	894,000 €
PR*	1:4,300,000	442,700 €
PR* + HIV combo	1:4,300,000	451,600 €
PR* + MP-NAT (384 pool)	1:5,100,000	444,900 €

* PR in the future is feasible for pathogen reduction in whole blood

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HIV

- Blood donor screening by HIV combo can probably be replaced by MP-NAT or ID-NAT
- Blood donor screening by PR only still have the risk on infectious blood donation with virus loads higher than 10^6 IU/ml
- Cost per QALY will be dramatically increased by the implementation of a PR method, because a PR for whole blood is currently not available
- Highest safety level will be achieved by PR + MP-NAT

Virus induced cancer

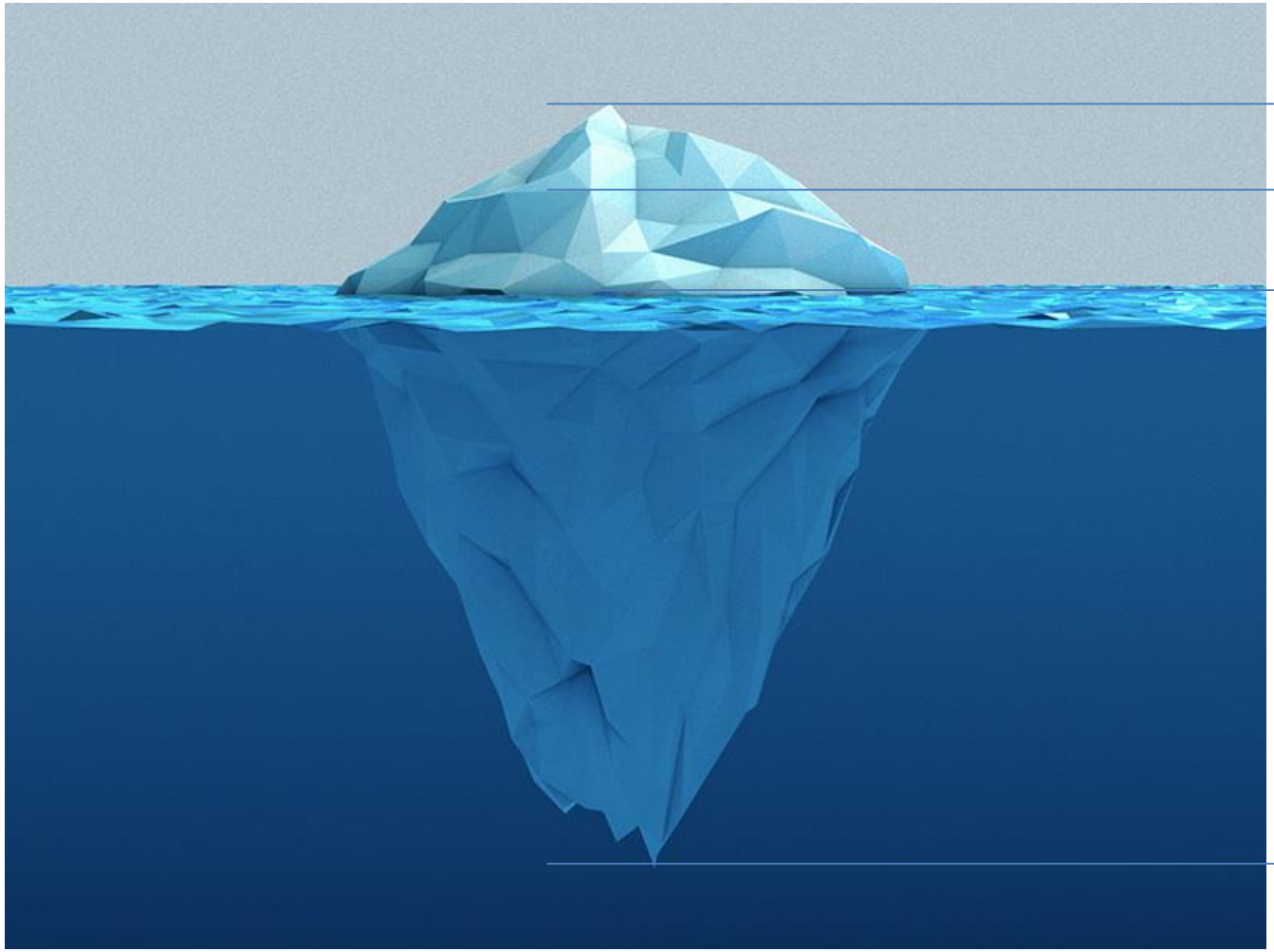
Virus	Cancer	Publication
HSV-2	Metastasis of lung cancer by mircoRNA expression	Tumor Biol 2017
HBV, HCV	Liver cell carcinoma	Cancer 2017
HPV	Cervical cancer	Oncotarget 2017
HHV-8	Intraepithelial neoplasia	Biomed Res Int 2015
FLVI1 (friend leukemia virus integration 1)	Small cell lung cancer	Oncotarget 2017
EBV	T- and NK-cell neoplasms	Plos One 2017

- PR will prevent virus induced cancer

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Iceberg screening strategy



Donor selection (1-2 logs)
10% blood safety

Donor sreening (1-2 logs)
10% blood safety

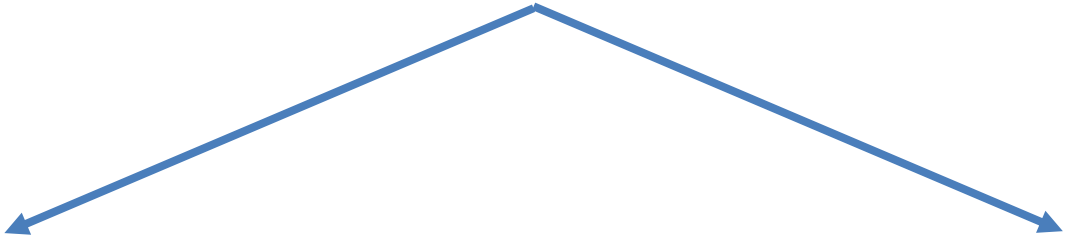
Pathogen reduction (6 logs)
80% blood safety

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Future blood safety strategy

- General pathogen reduction method for whole blood with reduction of 5-6 logs



- MP-NAT (pool size 384) or 4th generation serology tests

- PR + MP-NAT will achieve highest safety level and best cost efficiency, especially if more parameter like HEV, HAV, Dengue virus, Malaria, HTLV, bacteria, Chikungunya virus were added to the calculation

Summary

- The additional impact on blood safety for Anti-HCV and HIV combo is very low in the presence of blood donor screening by NAT, both parameters can probably abolished
- Pathogen reduction methods have currently the disadvantage, that no system is feasible for whole blood before separation to blood components
- MP-NAT is needed even in case of PR. This is specificly true for HIV and the use of the Theraflex system
- If a pathogen reduction method will be available in the near future for whole blood a combination with MP-NAT (pool size 384) will achieve highest safety level and best cost efficiency. Blood donor screening by serology can be stopped by this strategy
- New emerging pathogens (e.g. Chikungunya virus, Dengue virus, Malaria etc.) will increase the cost efficience for pathogen reduction methods