DRK-Blutspendedienst
Baden-Württemberg | Hessen
gemeinnützige GmbH

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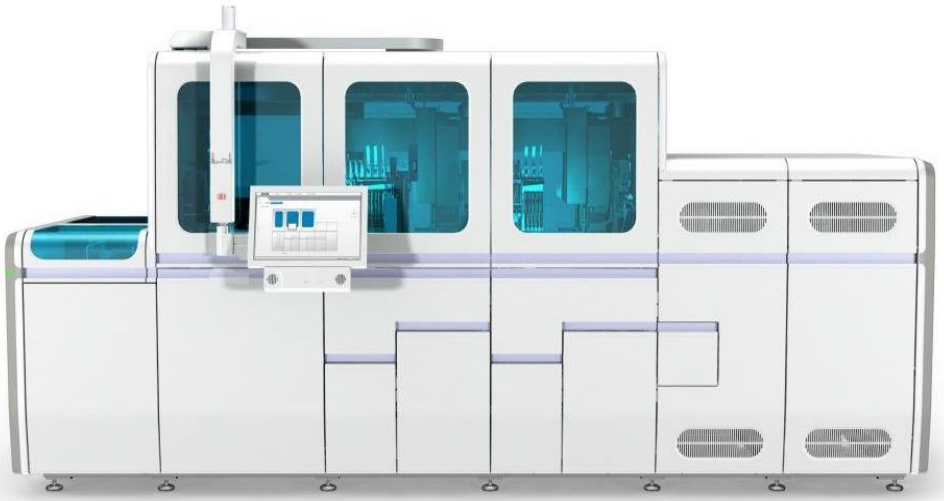
Wind of change - experience of the German Red Cross with NAT

Michael Schmidt



Content

1. The German Red Cross Blood donor service Baden-Württemberg - Hessen
2. Experience with blood donor screening by mini-pool NAT
3. Wind of change, implementation of the Roche Cobas 8800/ 6800 in blood donor screening
4. Implementation of new parameter into blood donor screening (WNV and HEV)
5. Summary

1| The German Red Cross Baden-Württemberg - Hessen

1. 84.4 million inhabitants
2. 16 districts
3. App. 3.5 million whole blood donations/year
4. Loacted in 6 districts (Schleswig-Holstein, Berlin, Brandenburg, Saxonia, Hesse, Baden-Württemberg)
5. Responsible for 1/3 of the blood supply of Germany
6. 7 Chairs (Frankfurt, Heidelberg, Mannheim, Ulm, Tübingen, Berlin, Dresden)
7. 2000 full time employees at our blood donor service

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Blood donor screening in Germany

Nr.	Pathogene	Serology (Antigen/Antibody)	NAT
1	Hepatitis A Virus		HAV/B19 Multiplex PCR
2	Hepatitis B Virus	HBsAg*, Anti-HBc* (2006)	HBV PCR CE*(2023)
3	Hepatitis C Virus	Anti-HCV*	HCV PCR CE* (1999)
4	HI-Virus 1	HIV combo*	HIV-1 PCR CE* (2004)
5	HI-Virus 2	HIV combo*	
6	Parvovirus B19		HAV/B19 Multiplex PCR
7	Treponema pallidum	ECLIA*	
8	Humanes Cytomegalie Virus (TKs)		HCMV
9	Bakterien in TKs		BAK-PCR CE
10	Hepatitis E Virus		HEV since 01.08.19**
11	West-Nil-Virus		WNV since 15.09.19*

- * = mandated
- ** = mandated since 01.01.2020 for red cells, platelets and plasma since 01.01.2021

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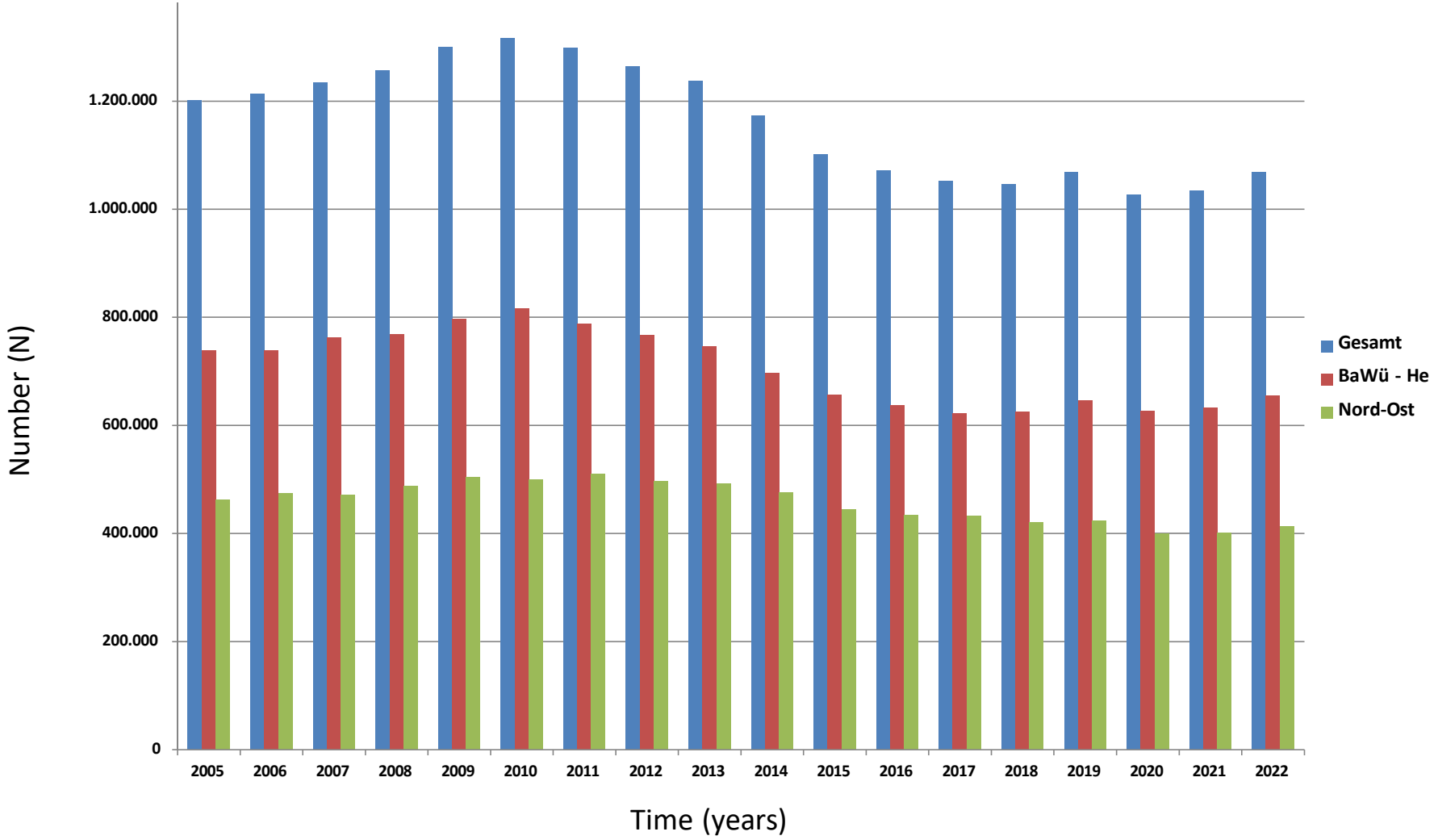
Blood donations per year at GRC BaWüHe



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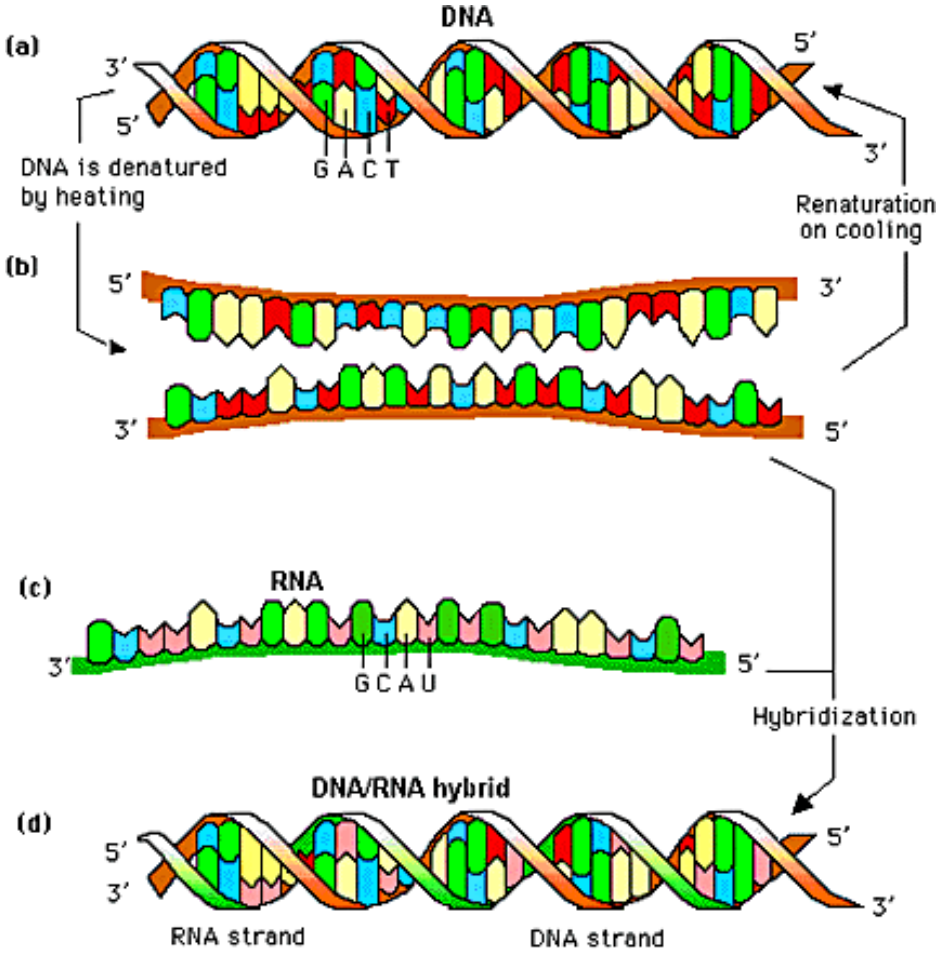
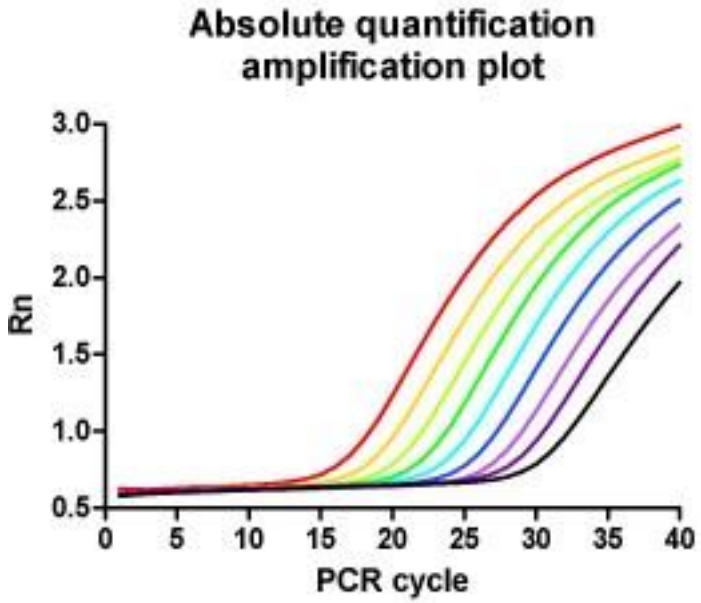
2| Experience by NAT at GRC





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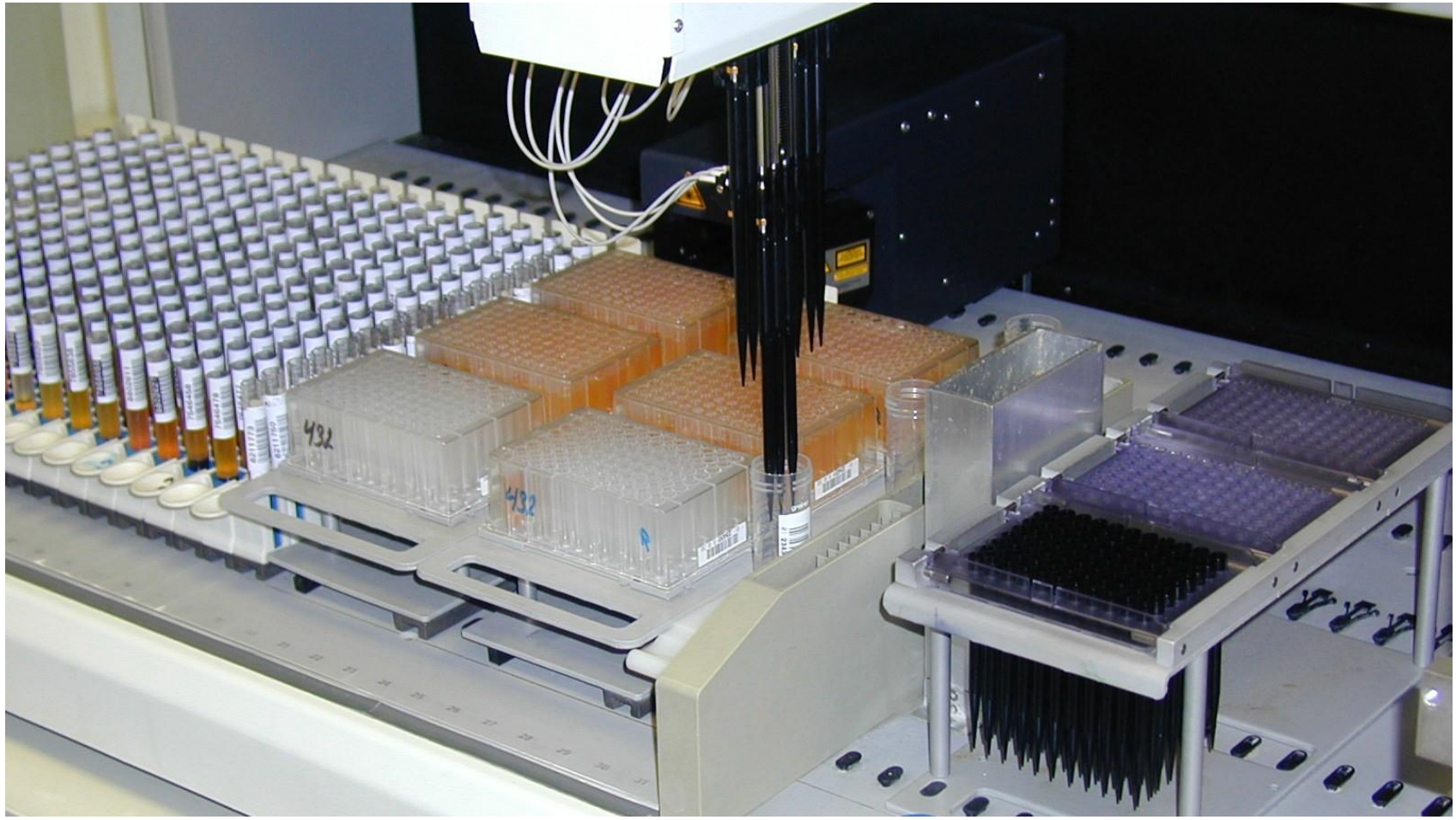
Overnight pooling in mini-pools of 96 samples/pool



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Virus enrichment by high speed centrifugation

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Manual extraction process by Qiagen columns

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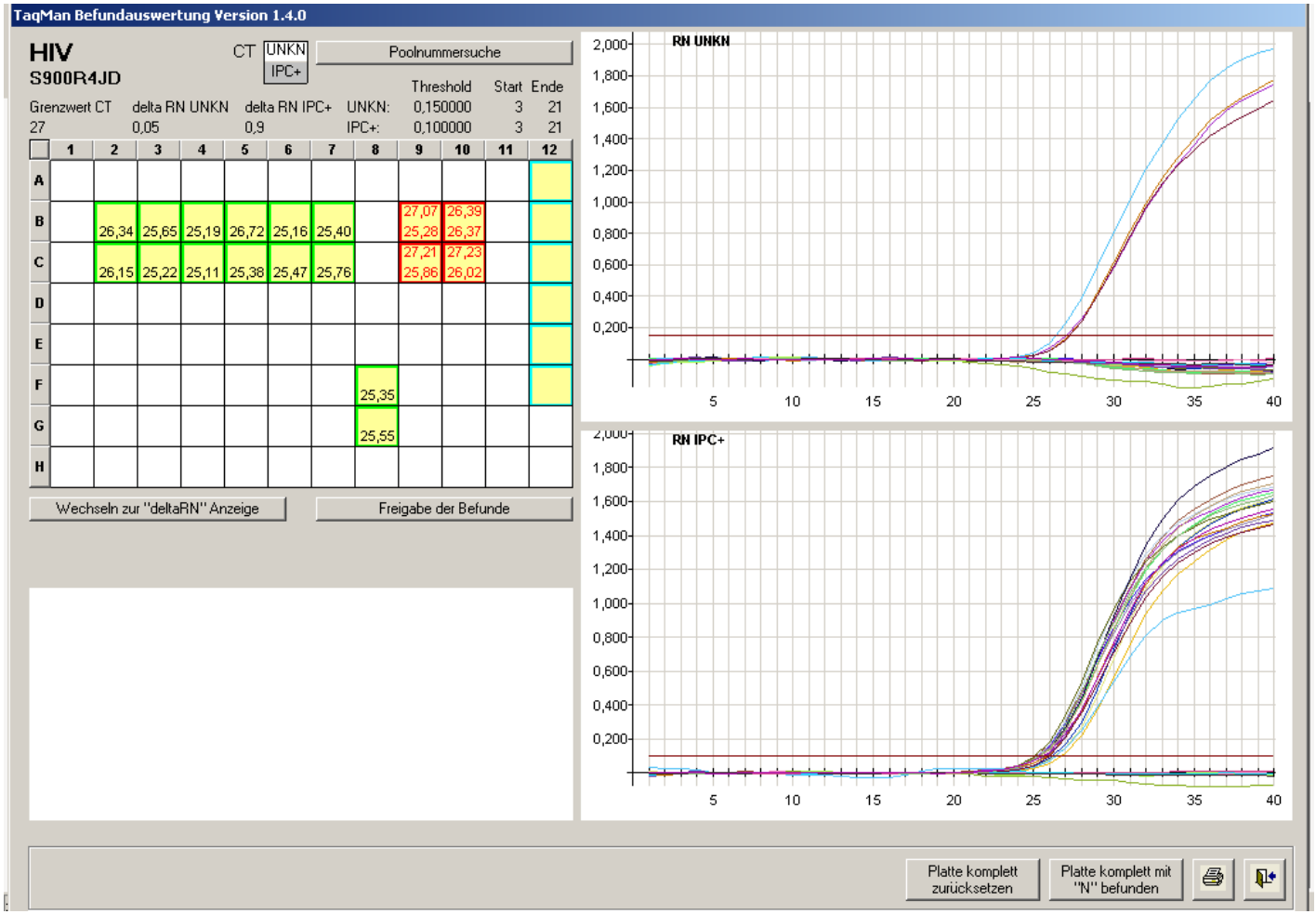
Development to realtime PCR



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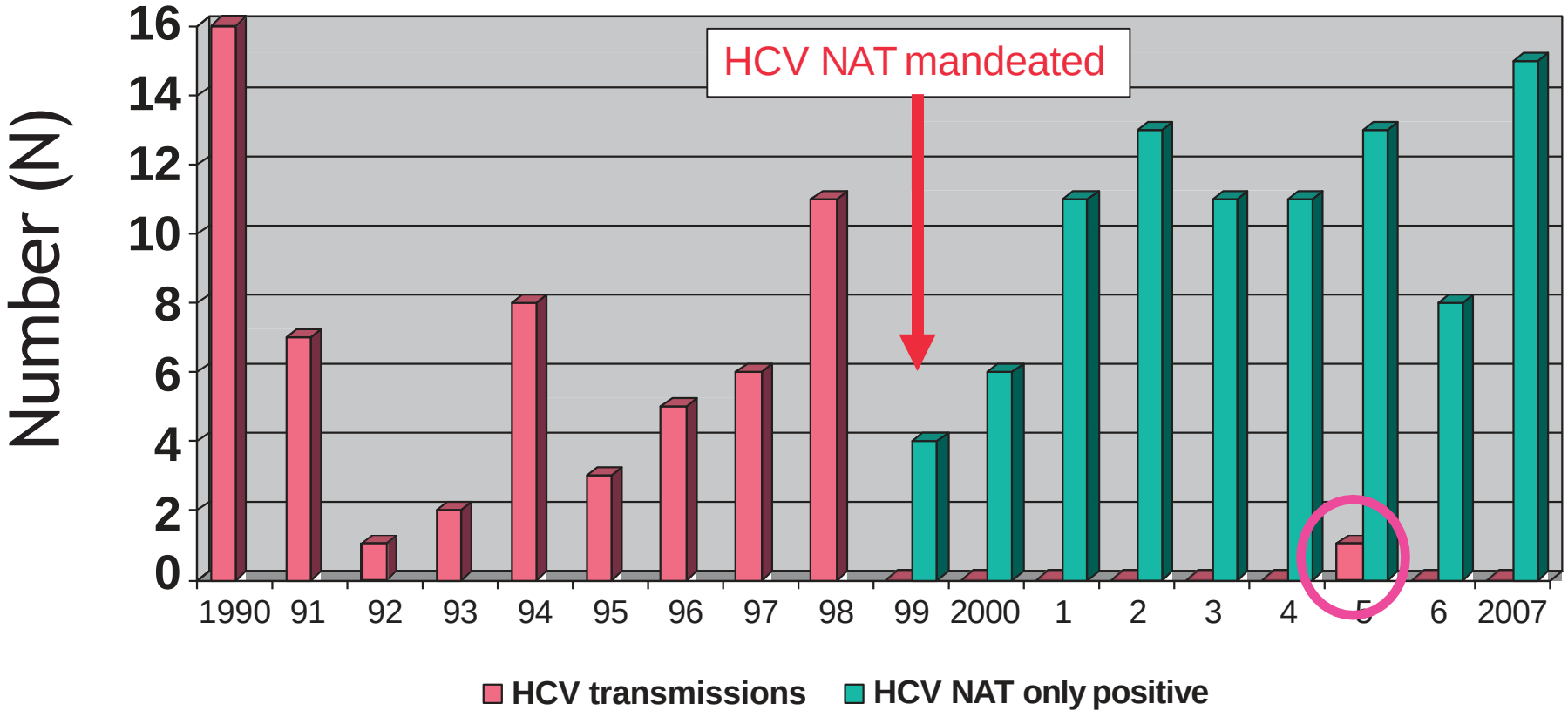
HCV transmissions by blood components



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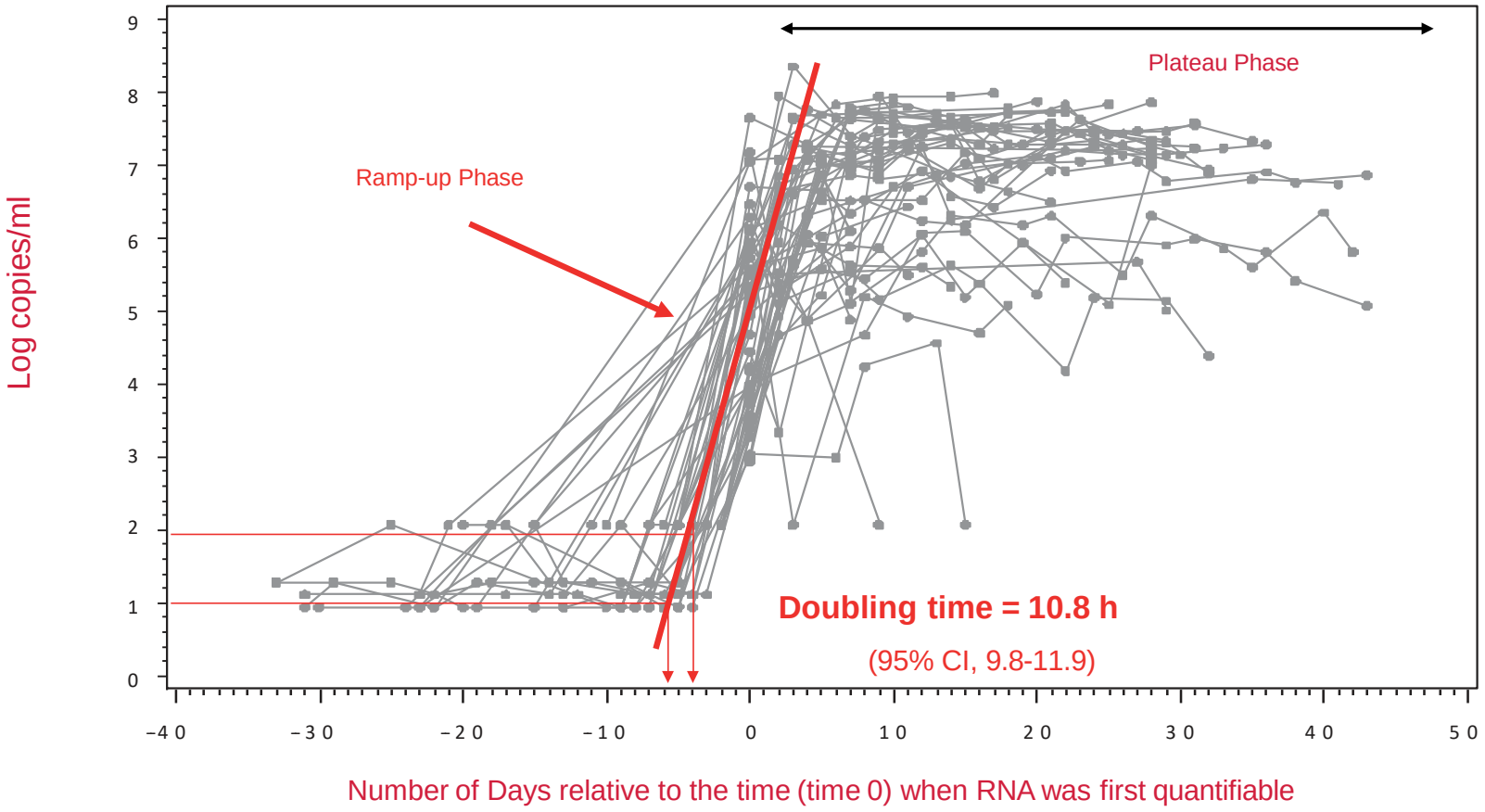
HCV doubling time (hours)



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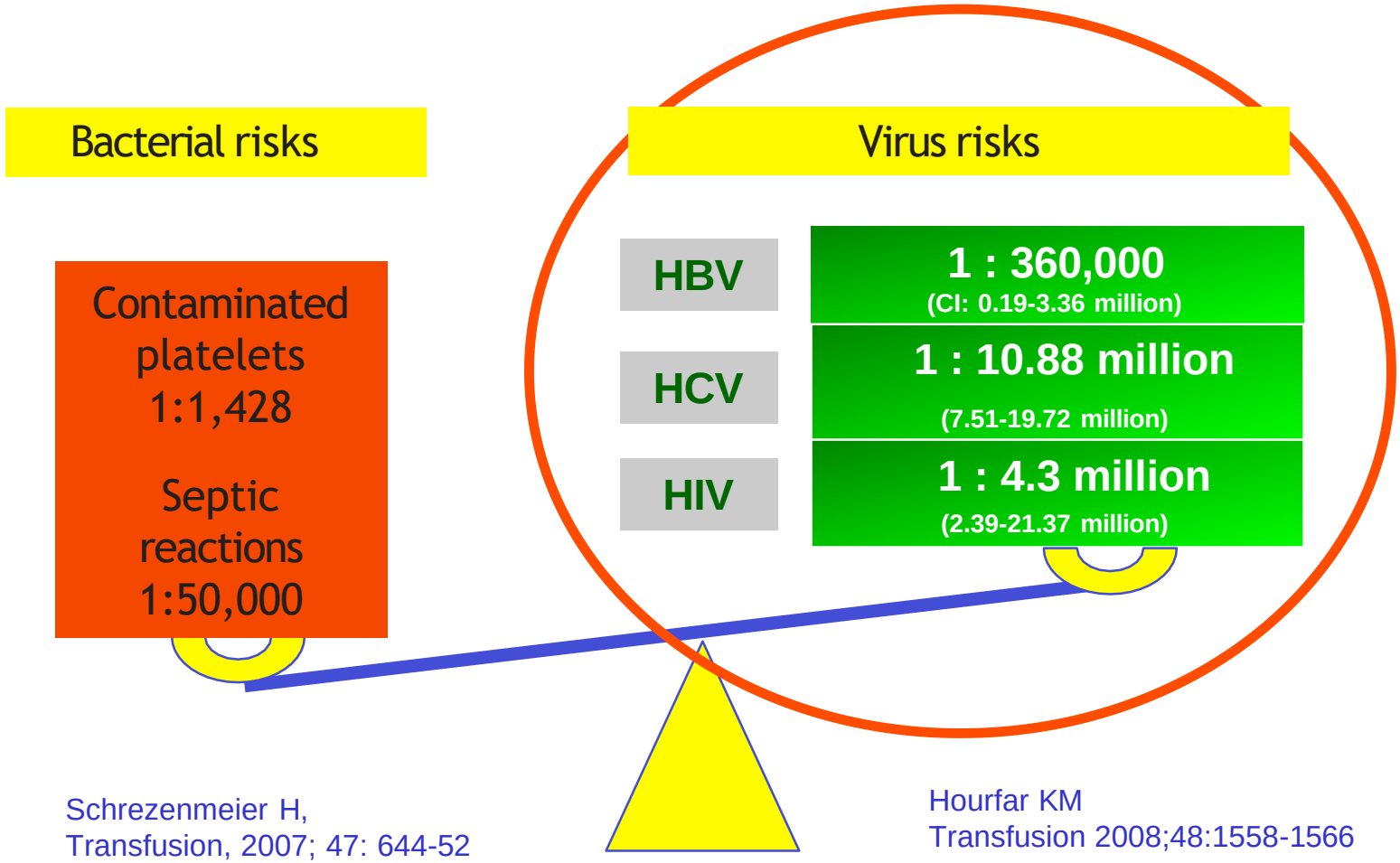
Residual risk for viruses and bacteria



Schrezenmeier H,
Transfusion, 2007; 47: 644-52

Hourfar KM
Transfusion 2008;48:1558-1566

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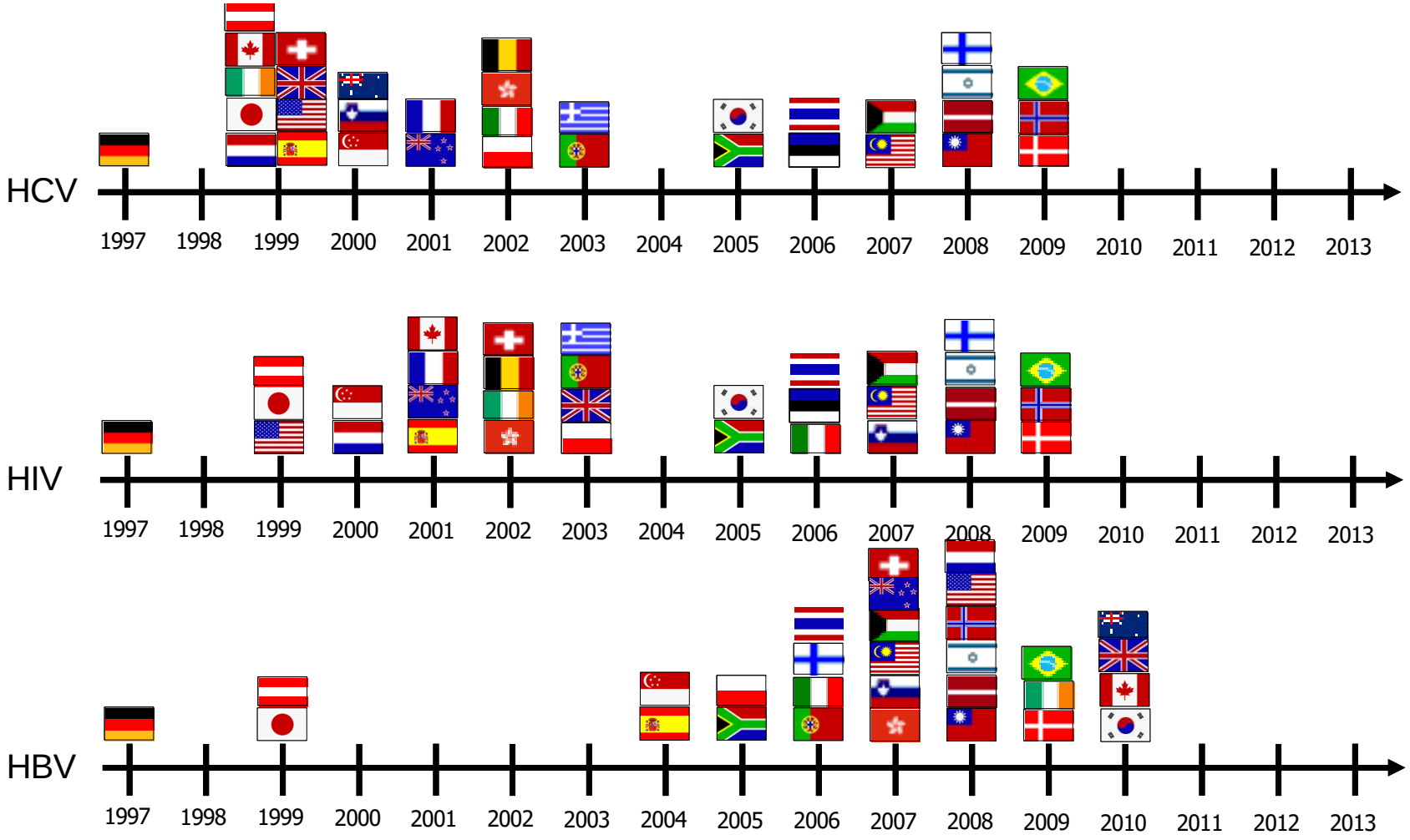
NAT screening world wide



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NAT only reactive samples

	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

New risks by mutations for HIV-1

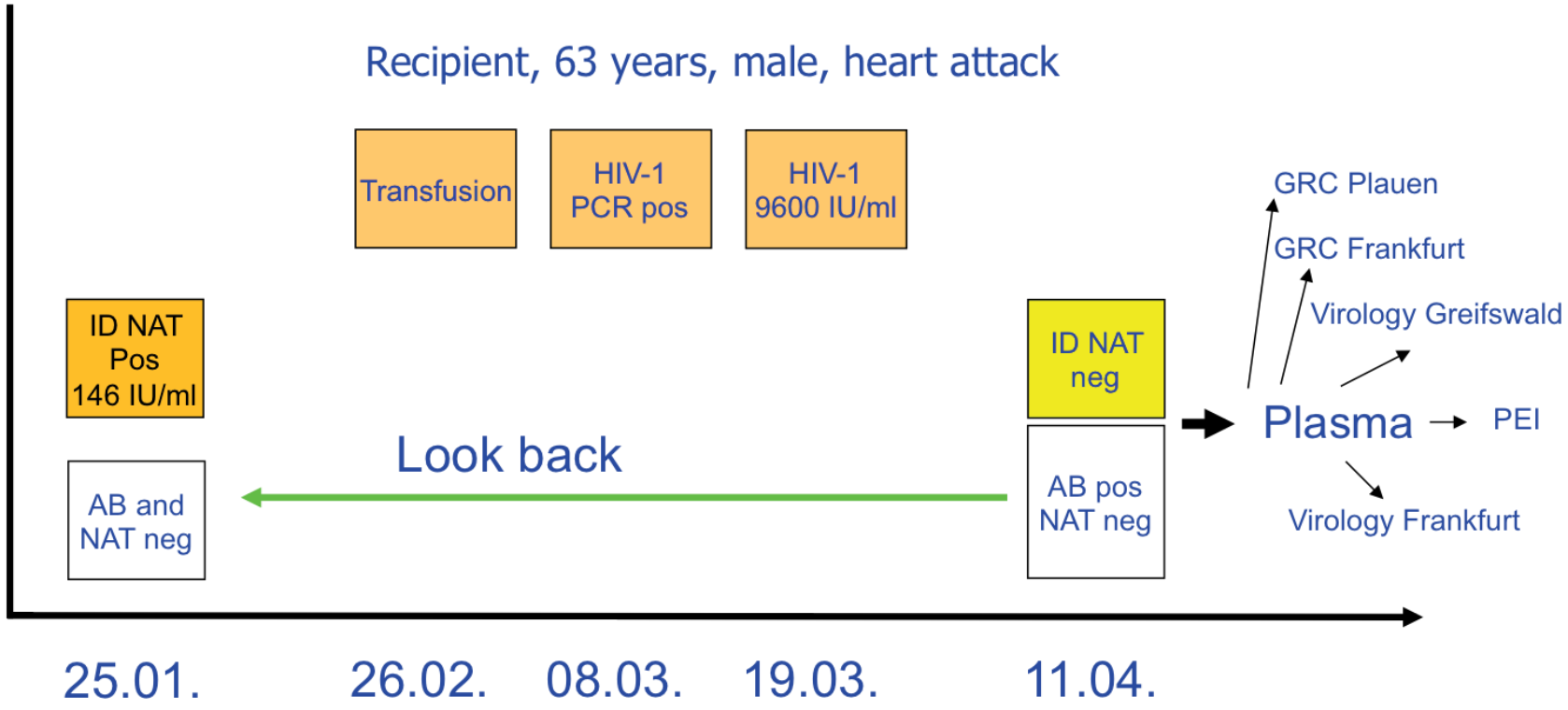
Case	Donor type, sex, age	Donation dates	Screening NAT	HIV Screening results	Viral load (IU/ml)	Donor Status	HIV-1 Trans-mission by
1	RD, male, 44	Jan 2007 Apr 2007	CAP CTM v1	Ab neg RNA neg **Ab pos** RNA neg	10,000 650	WP SC	RBC
2	RD, male, 26	Jul 2007 Oct 2007	CAP CTM v1	Ab neg RNA neg **Ab pos** 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 transfusion transmitted infection by blood components caused by mutation in the primer/ probe binding region

Source |M. Chudy Transfusion 2012

HIV-1 transmission 2007 at GRC

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



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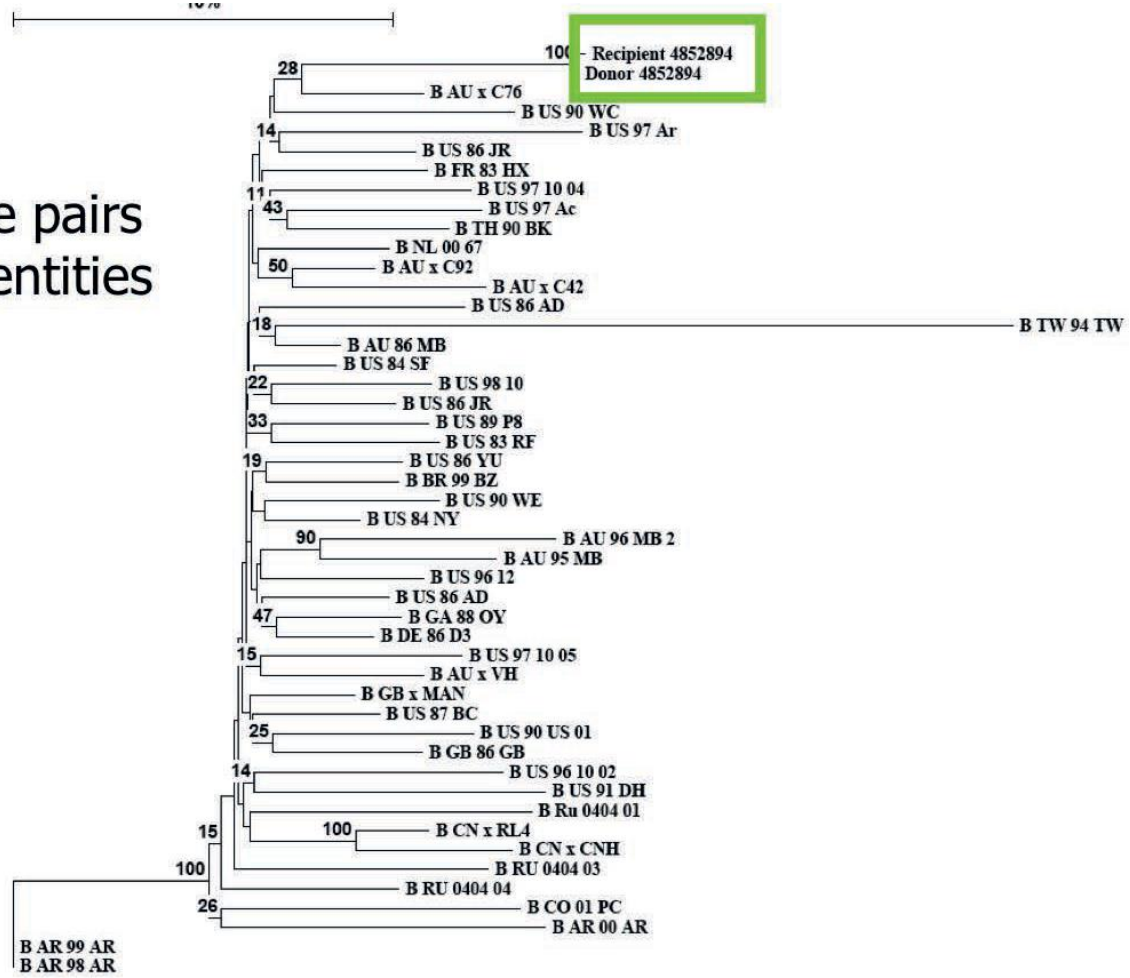
Phylogenetic analysis

1047 Base pairs
99,9% identities



QUELLE: M. Schmidt/ L. Gürtler Transfusion 2009

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Mutation 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

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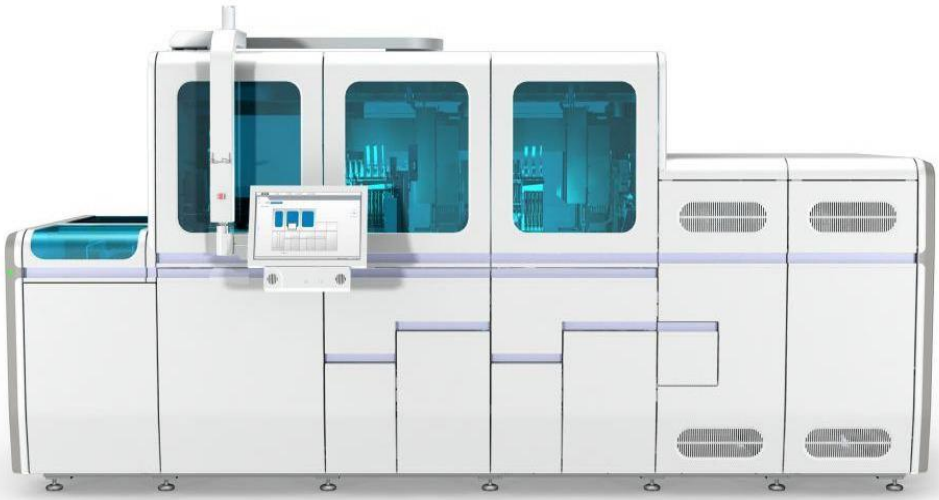
3| Wind of change - implementation of the Roche Cobas 8800



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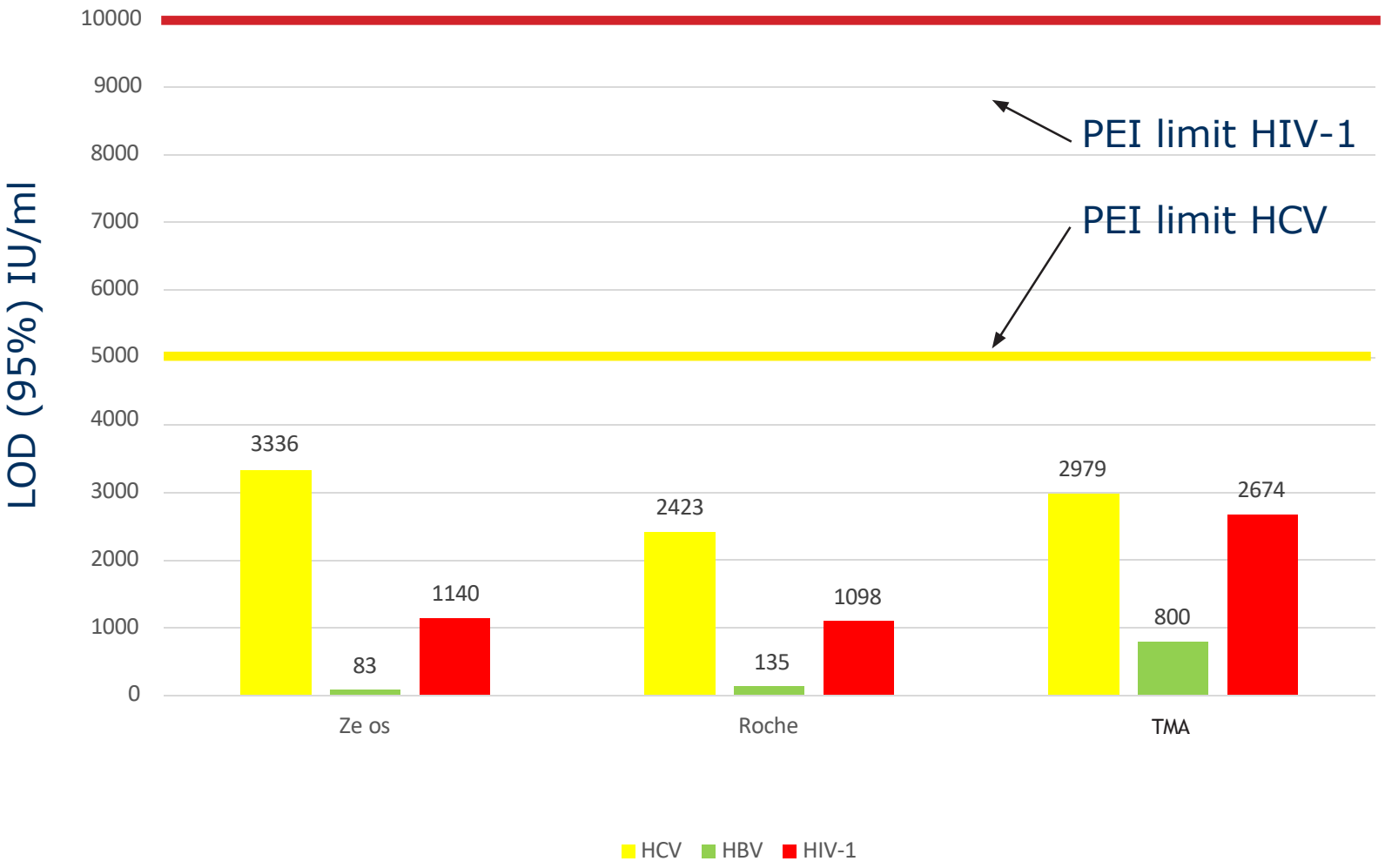
Head-to-head-to-head study



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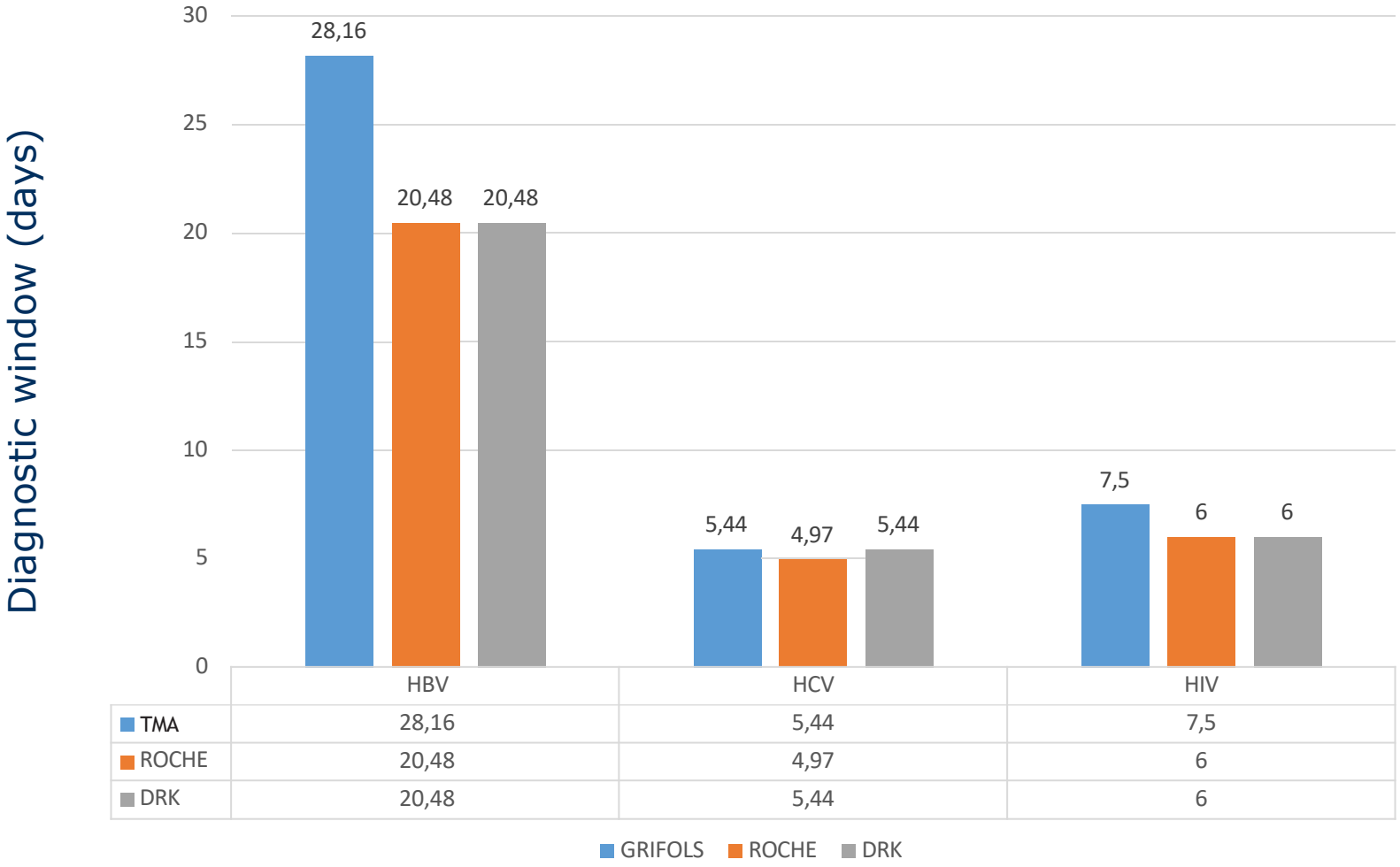
Diagnostic window period



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Advantages (10 reasons for Roche Cobas 8800/6800)

1. Complete barcoded process
2. Close system, one room approach
3. High sensitivity for HBV, HCV and HIV (1+2)
4. Flexibility in blood donor testing from ID-NAT to MP-NAT
5. Multi color analysis
6. On board assays, system always in stand by
7. Reduced hands on time and elevated work alone time
8. Open chanel for new parameters possible
9. High throughput NAT system (> 1,000 samples in a 8h working shift)
10. No discriminatory assays necessary (multi color, multiplex analysis)

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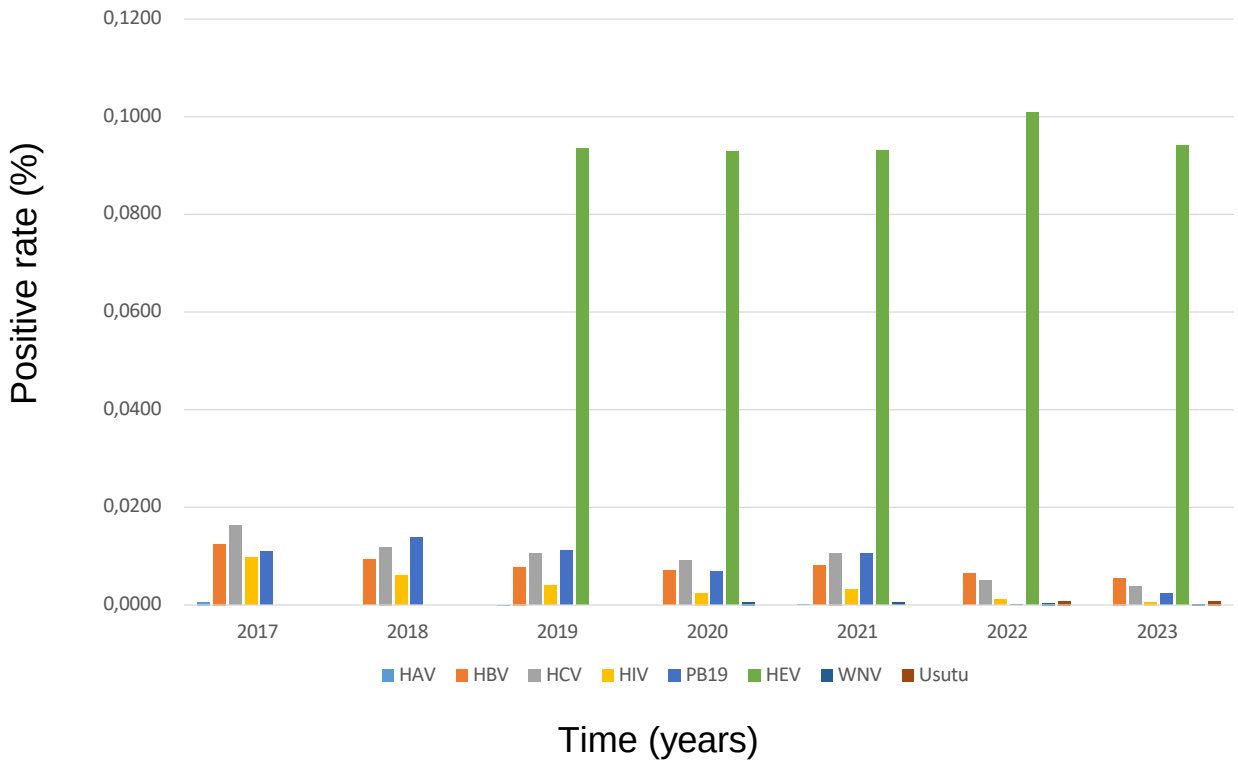
Routine quality data NAT with Cobas 8800



- The Cobas 8800 NAT system is very robust and feasible for blood donor screening
- No transfusion transmitted infections since 2016

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Danger through re-emerging viruses

Year	Pathogen
1981/´82	HTLV III (= HIV-1) / AIDS
1986	HIV-2
1988	Hepatitis E (Caliciviridae)
1989	Hepatitis C (Flaviviridae)
1992	Vibrio O 139
1992	Bartonella hensellae
1993	Sin Nombre Virus
1995	Hepatitis G (Flaviviridae)

Year	Pathogen
1995	HHV 8[1]
1996	Variant CJD (vCJD) / Prions
1997	Avian Influenza Virus A (H5N1)
1999	West Nile Virus (WNV; Flaviviridae) in USA
2003	SARS (Coronaviridae)
2003	Monkeypox Virus
2004	Metapneumo Virus
2005	Chikungunya Virus

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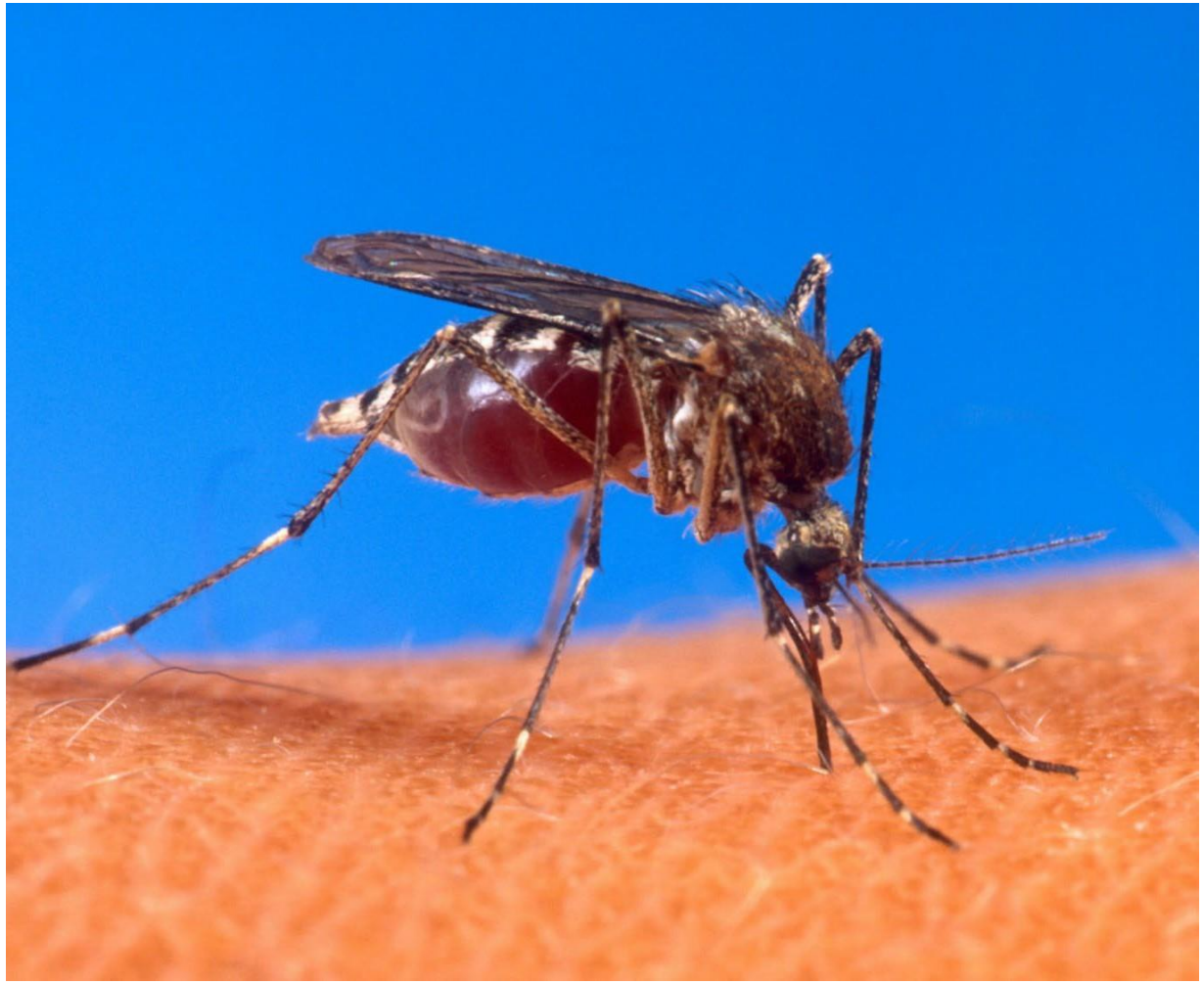
Tiger mosquito as a viral vector



Vector:
Aedes aegyptii

Host:
Birds, horses
Humans are mishosts

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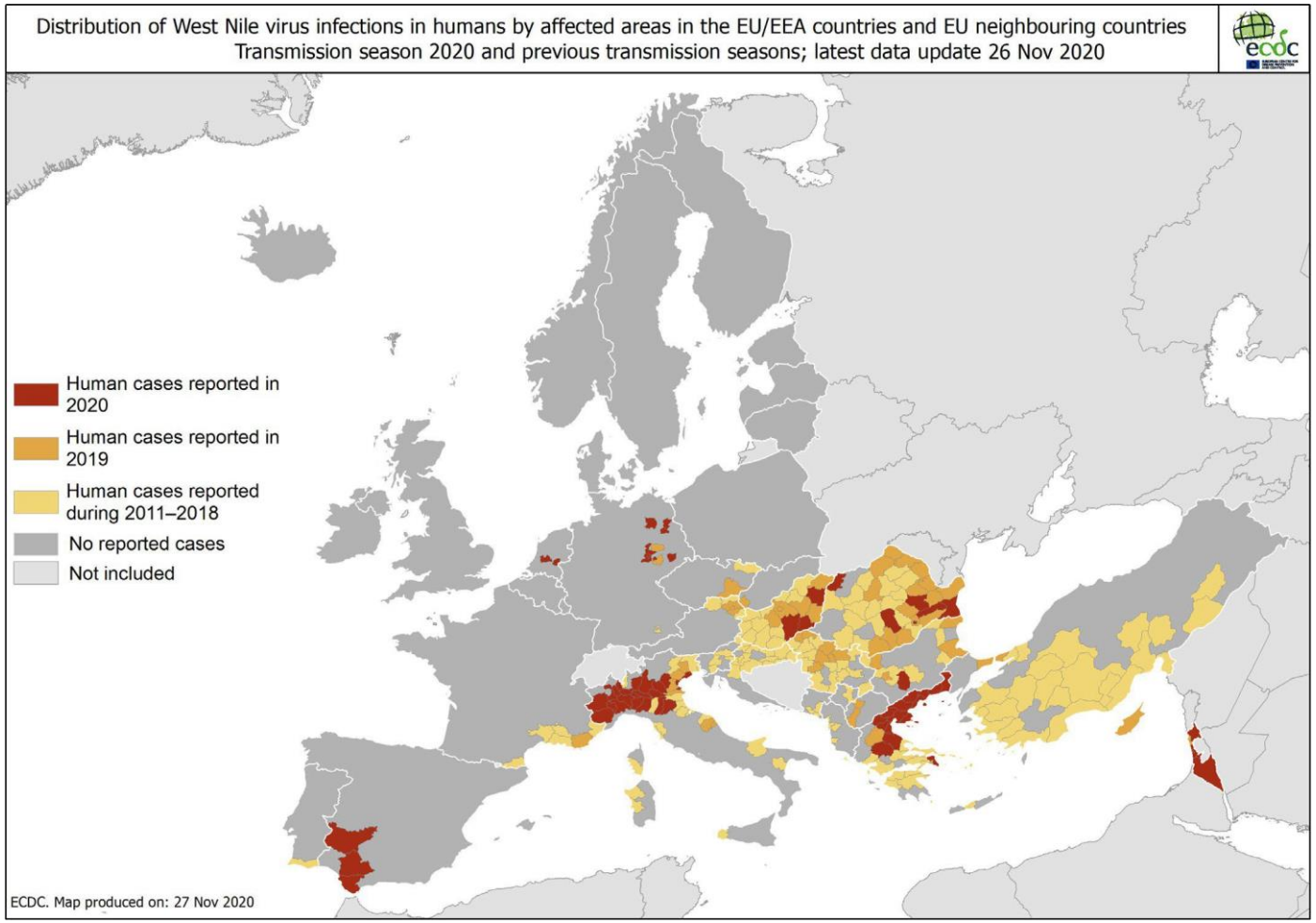
ECDC WNV data 2011 - 2020



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WNV blood donor screening

2020

GRC	WNV	Usutu
BaWü-He	0	4
Nord-Ost	3	2

2021

GRC	WNV	Usutu
BaWü-He	0	0
Nord-Ost	3	1

2022

GRC	WNV	Usutu
BaWü-He	0	3
Nord-Ost	2	1

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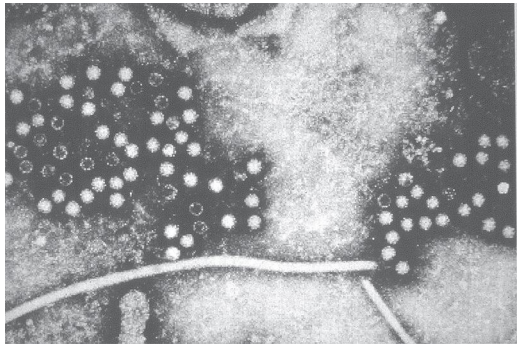
Hepatitis E Virus

Virus information:

- Virus family: Caliciviridae or Hepeviridae (Emerson et al. 2004)
- single strand RNA virus, diameter 32-34nm

Clinical symptoms:

- like HAV,
- incubation time 30-40 days,
- death rate between 0.5% and 4%,
- during pregnancy app 25%,
- vaccination in development



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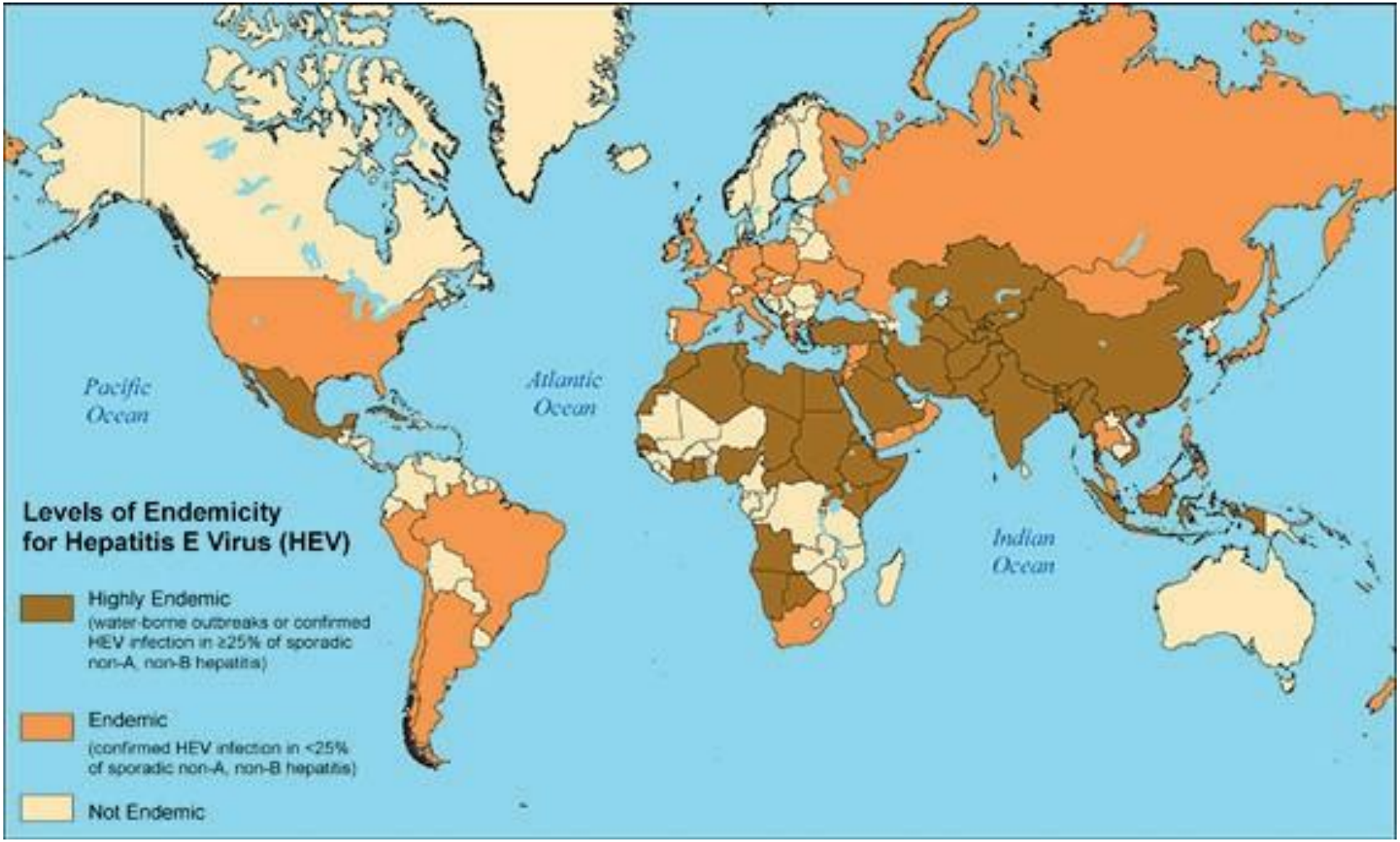
Hepatitis E Virus distribution world wide



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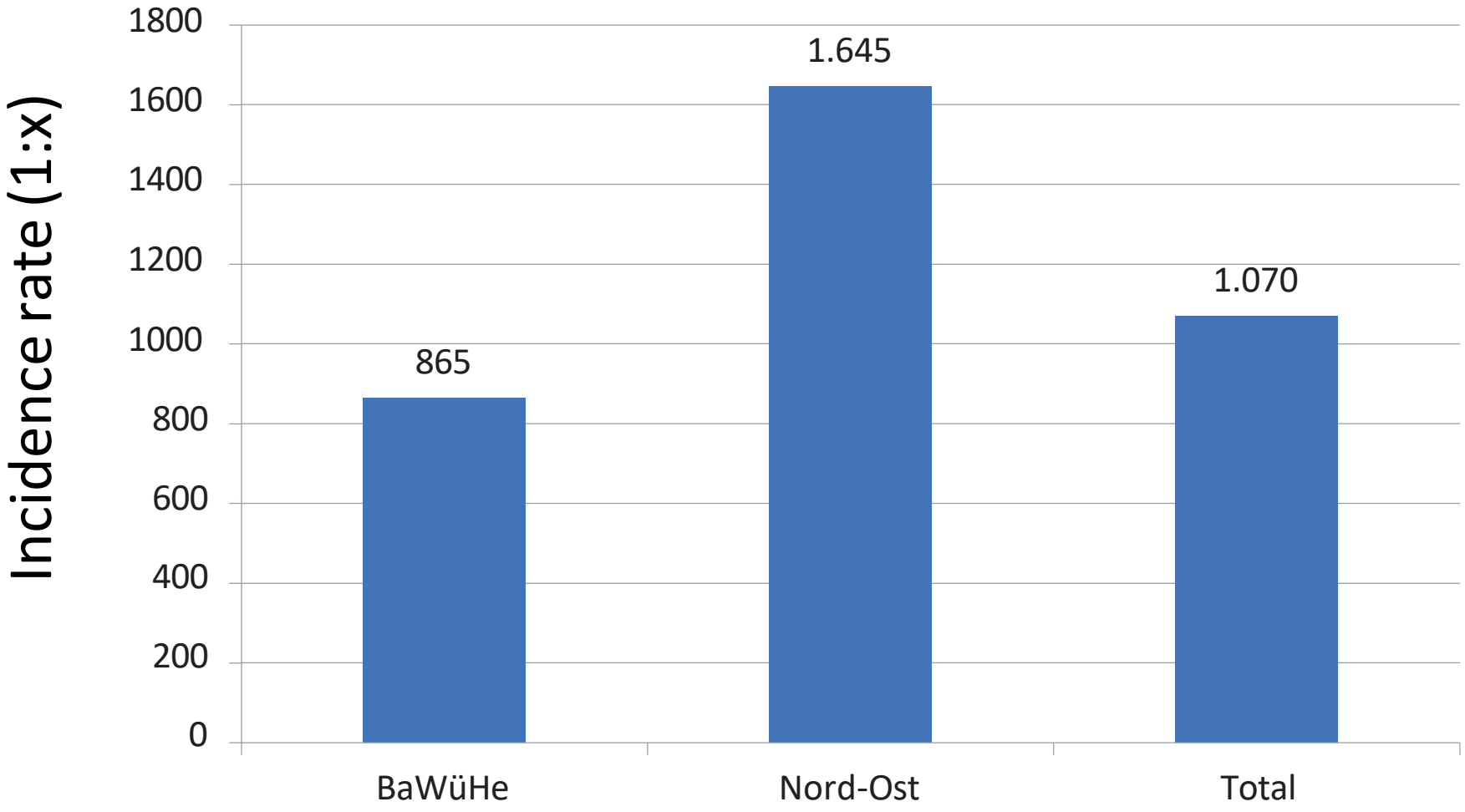
Hepatitis E Virus incidence



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Summary - experience of the GRC by NAT

- Blood donor screening by NAT is the most important procedure to reduce the diagnostic window period to a minimum
- Change to a commercial NAT system reduced test time and improved robustness of the system
- Blood donor screening is currently done for HAV, HBV, HCV, HIV-1, HIV-2, B19 HEV, WNV, CMV
- Utility channel enable developing of inhouse PCR for emerging pathogens
- Roche Cobas 6800/ 8800 enable the integration into a track system in the future

- NAT reduces the diagnostic window period to a minimum, and restores the population‘s lost trust in blood safety

- NAT is the most powerful technology to implement new parameter if necessary in a sort time period (e.g. WNV and HEV)

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Acknowledgements

1. Prof. Dr. Torsten Tonn
2. Dr. Kai Hourfar