Nucleic acid testing (NAT) in Polish blood donors - organization, methodology, results and significance for transfusion safety

Piotr Grabarczyk

Department of Virology

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Transfusion medicine in Poland

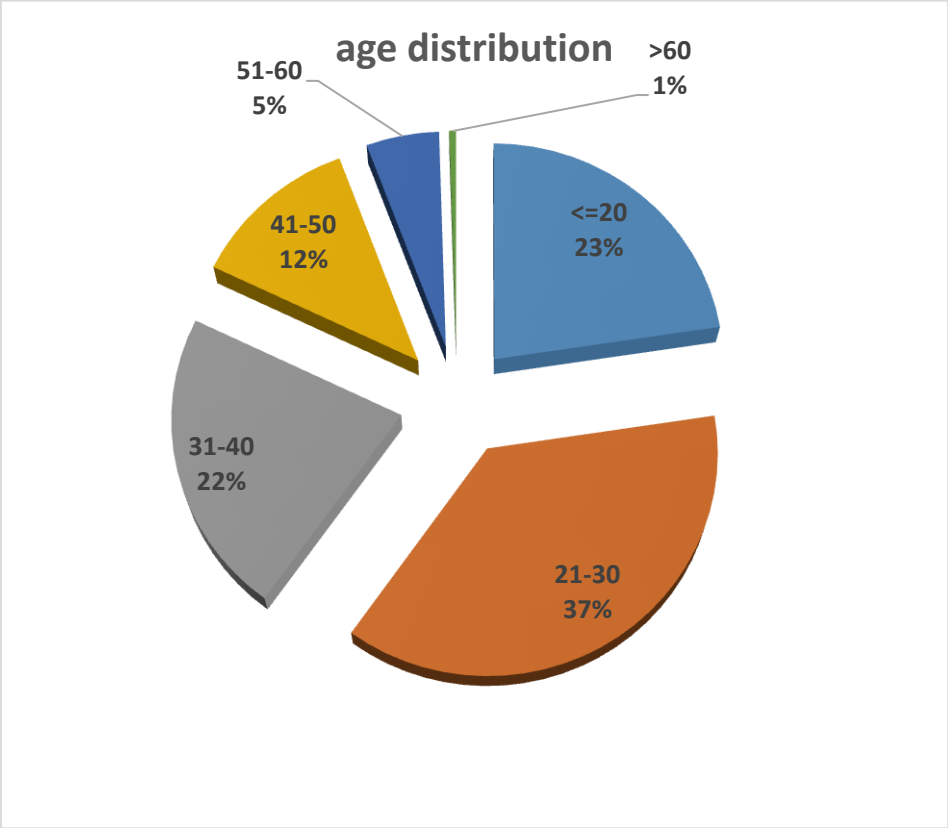
- Population in 2019: 38,5 mln
- Polish Blood Transfusion Act of 22 August 1997
- 23 blood transfusion centers
- 139 local collection sites
- 1,2 mln donations/year
- 600 hospitals

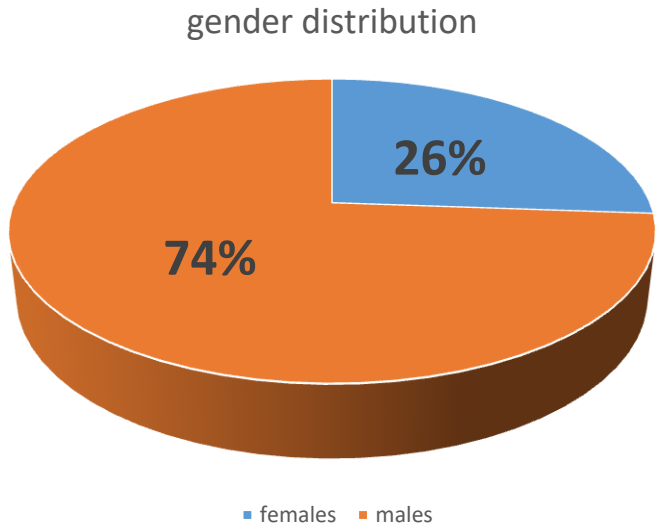
- *Rosiek A, Tomaszewska A, Lachert E, et al. Blood transfusion service in Poland in 2017. J Transfus Med 2018*
- *Stenholm E. Monitoring of blood transfusion operations in EU countries. Stockholm (Sweden): Karolinska University Hospital; 2015.*

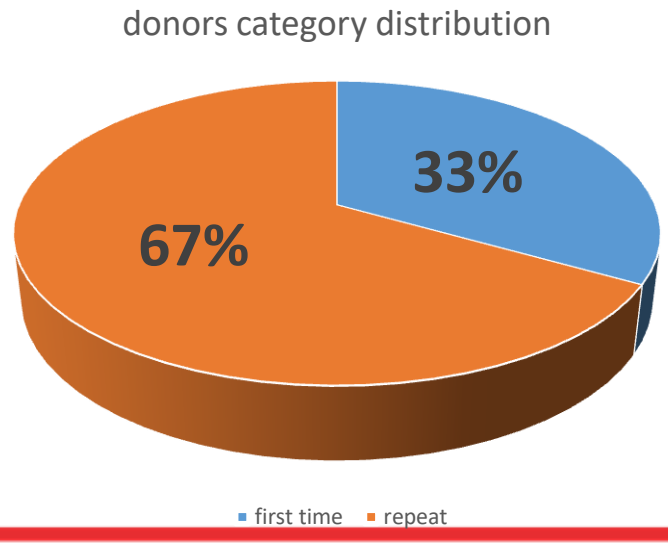
Characteristics of Polish blood donors

(data for 2005-2018)

- Voluntary and non-remunerated







TRANSFUSION 2004;44:1706-1710.

TRANSFUSION COMPLICATIONS

The hepatitis C virus genotype and subtype frequency in hepatitis C virus RNA-positive, hepatitis C virus antibody-negative blood donors identified in the nucleic acid test screening program in Poland

E. Brojer, A. Gronowska, J. Medyńska, P. Grabarczyk, M. Mikulska, M Łętowska, W. Kryczka, and A. Gietka

HEPATOLOGY 2006;44:1666-1674.

Characterization of HBV DNA$^+$/HBsAg$^-$ Blood Donors in Poland Identified by Triplex NAT

Ewa Brojer,[1] Piotr Grabarczyk,[1] Grzegorz Liszewski,[1] Maria Mikulska,[1] Jean-Pierre Allain,[2] Magdalena Letowska,[1] and the Polish Blood Transfusion Service Viral Study Group

ELSEVIER

Journal of Hepatology 49 (2008) 537–547

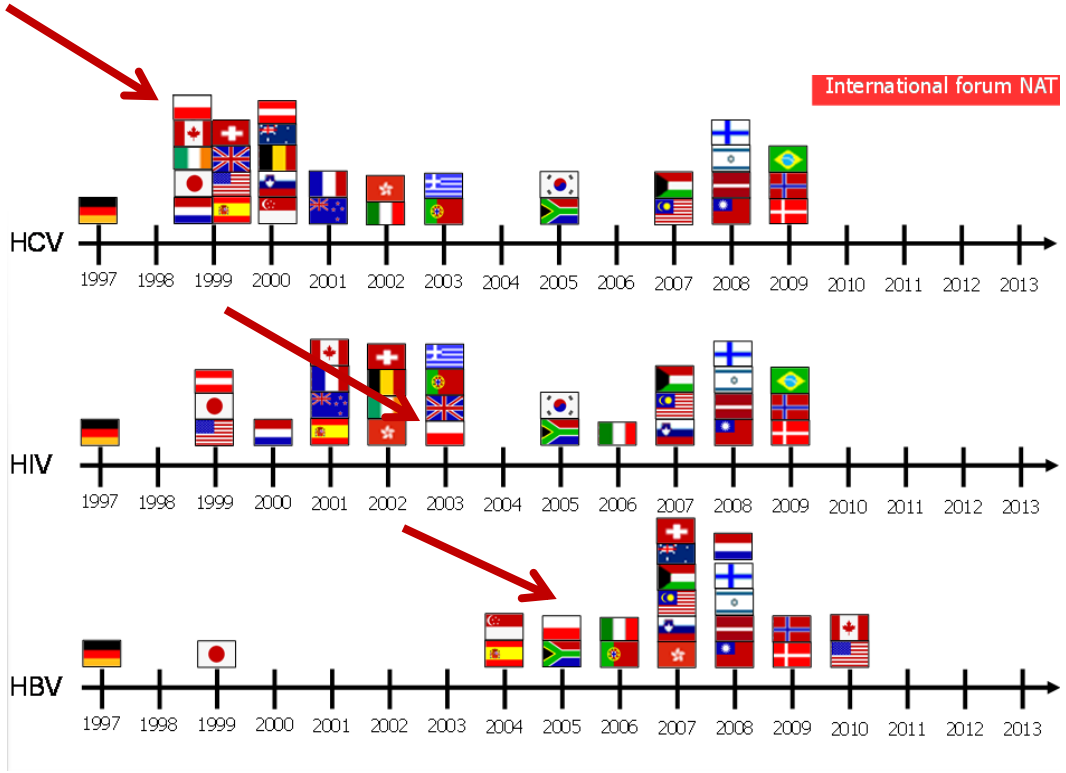
Journal of Hepatology

www.elsevier.com/locate/jhep

Characterization of occult hepatitis B virus from blood donors carrying genotype A2 or genotype D strains☆

Daniel Candotti[1], Piotr Grabarczyk[2], Paola Ghiazza[3], Roberto Roig[4], Natalia Casamitjana[5], Paola Iudicone[6], Michael Schmidt[7], Arthur Bird[8], Robert Crookes[9], Ewa Brojer[2], Michelina Miceli[6], Azin Amiri[10], Chengyao Li[1,11], Jean-Pierre Allain[10,*]

NAT



Roth WK, Busch MP, Schuller A, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox Sang 2012;102:82-90.

NAT methods evolution in Poland

TMA

HCV/HIV/HBV — markers

Procleix HCV/HIV-1 | Procleix Ultrio Tigris | Procleix Ultrio Plus Tigris | Ultrio Elite Panther — system/test

PCR

HCV RNA, HIV RNA, HBV DNA

Amplicor | AmpliScreen | s201 MPX | s201 MPXv2.0 | cobas 6800 MPX — system/test

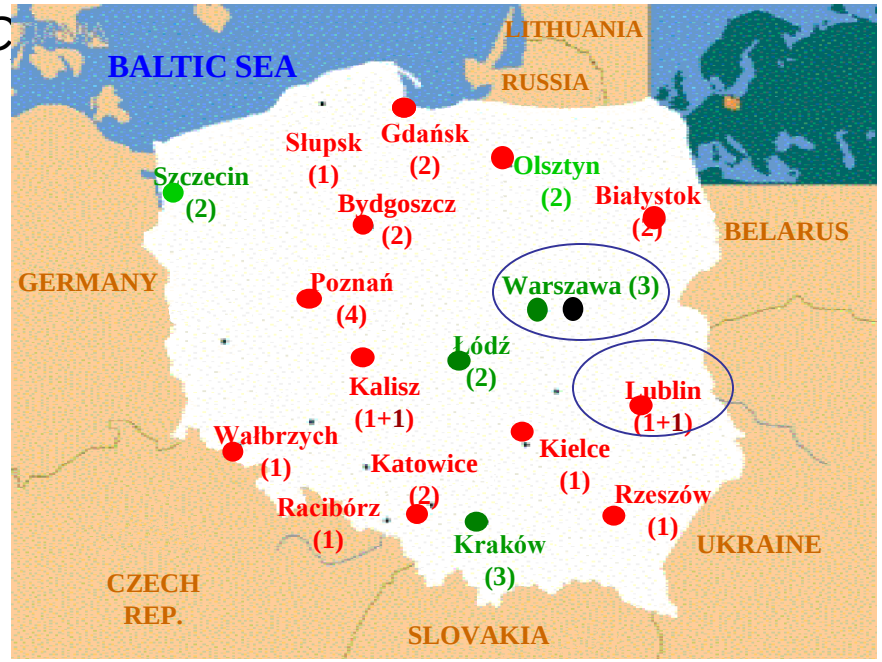
MP48 | MP24 | MP6 | MP6 — pool

2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019

NAT in Poland (2018)

SCREENING

Regional Blood Transfusion Centers (RBTC



Mini-pool (MP) testing

- **MP 6 -** cobas s201 MPX v2 (12-19)
  - cobas 6800 MPX (2-2)
- MP 4 – Ultrio Elite (1 - 2 Panthers)

Individual Donation Testing (IDT)

- Ultrio Elite (4 - 9 Panthers)

External Quality Control in each RBTC

- EDCNet : Every instrument's working-day from 2005- till now
- QCMD: 2/year

- February 2018: Evaluation BioQControl vs QConnect

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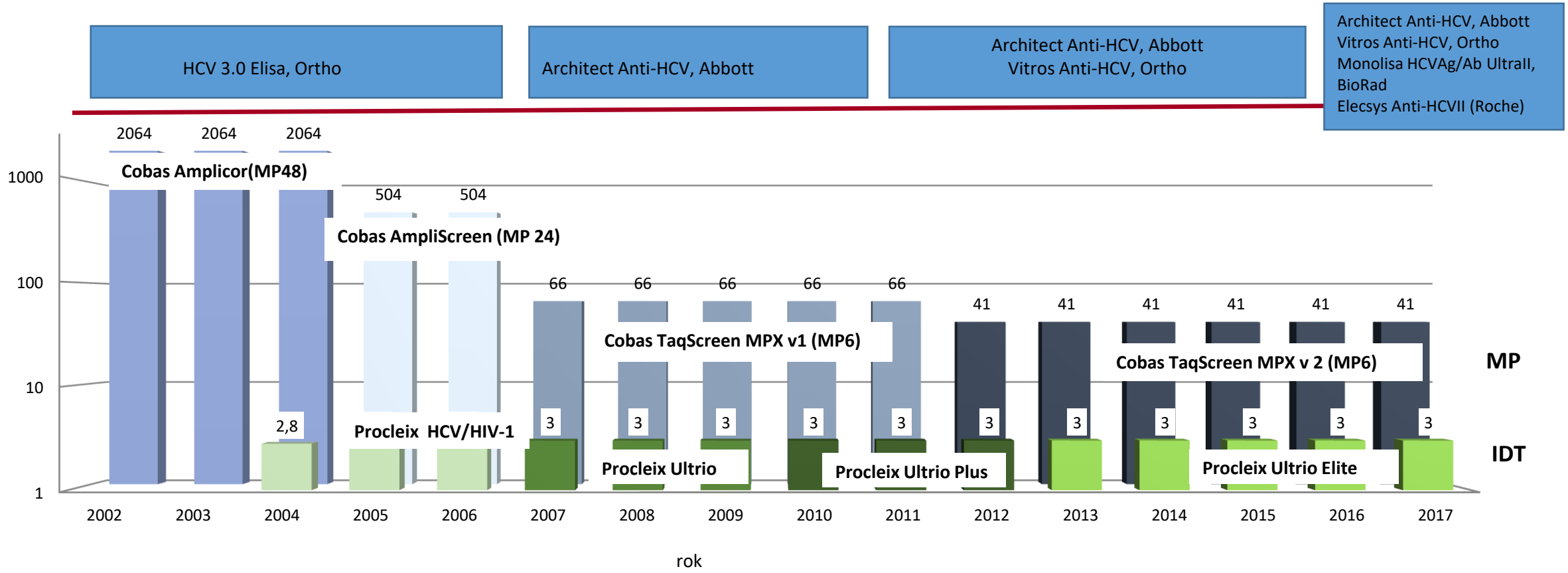
Analytical sensitivity of HCV RNA screening in Poland



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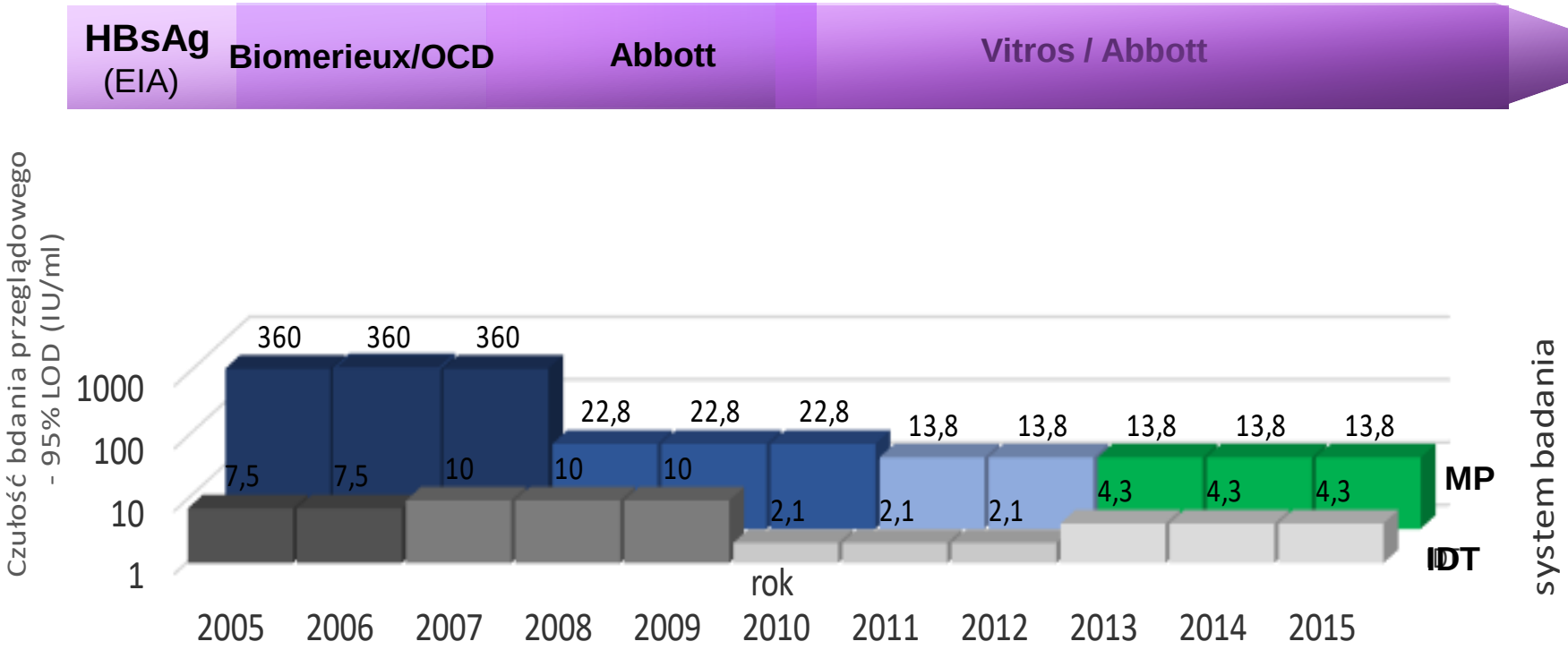
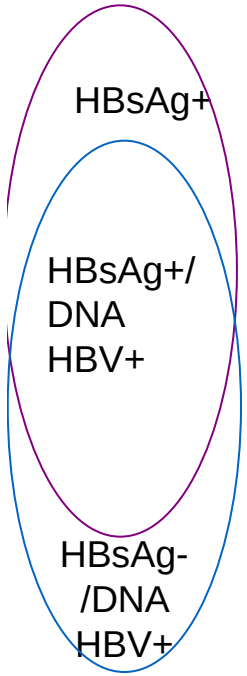
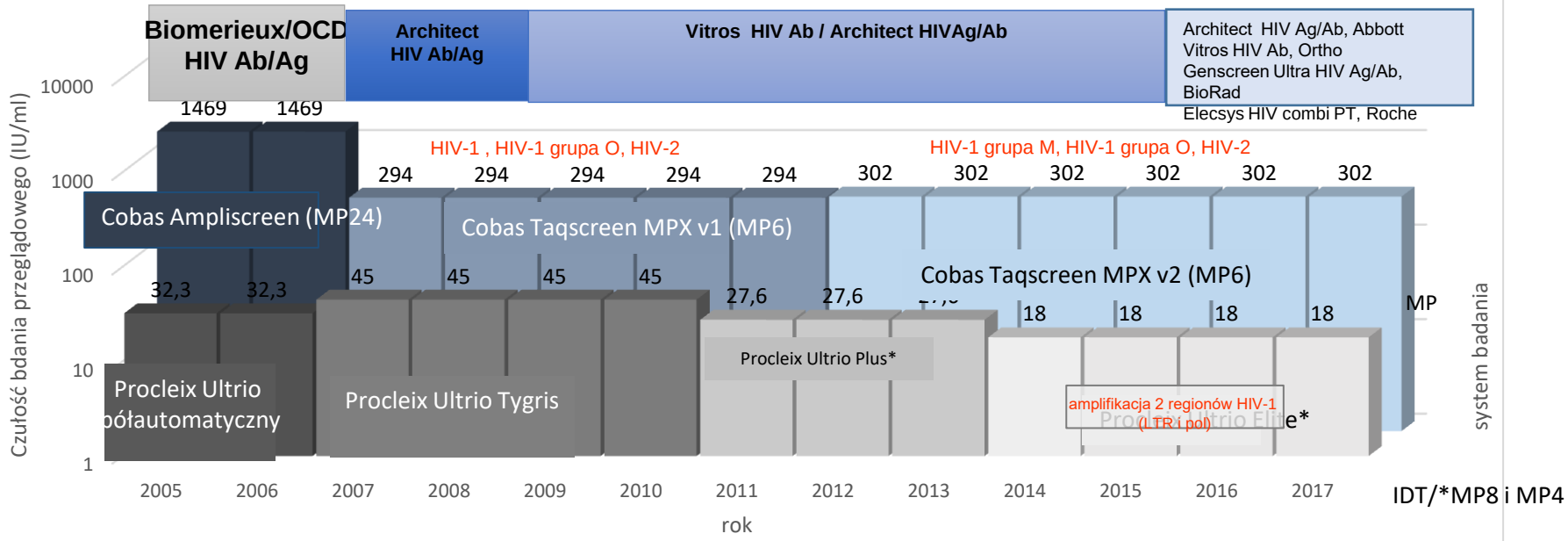
Analytical sensitivity of HBV DNA screening in Poland





Analytical sensitivity of HIV RNA screening in Poland



*2012-2013 4 CKIK (Procleix Ultrio Plus MP8)
*2017 1 CKIK (Procleix Ultrio Elite MP4)

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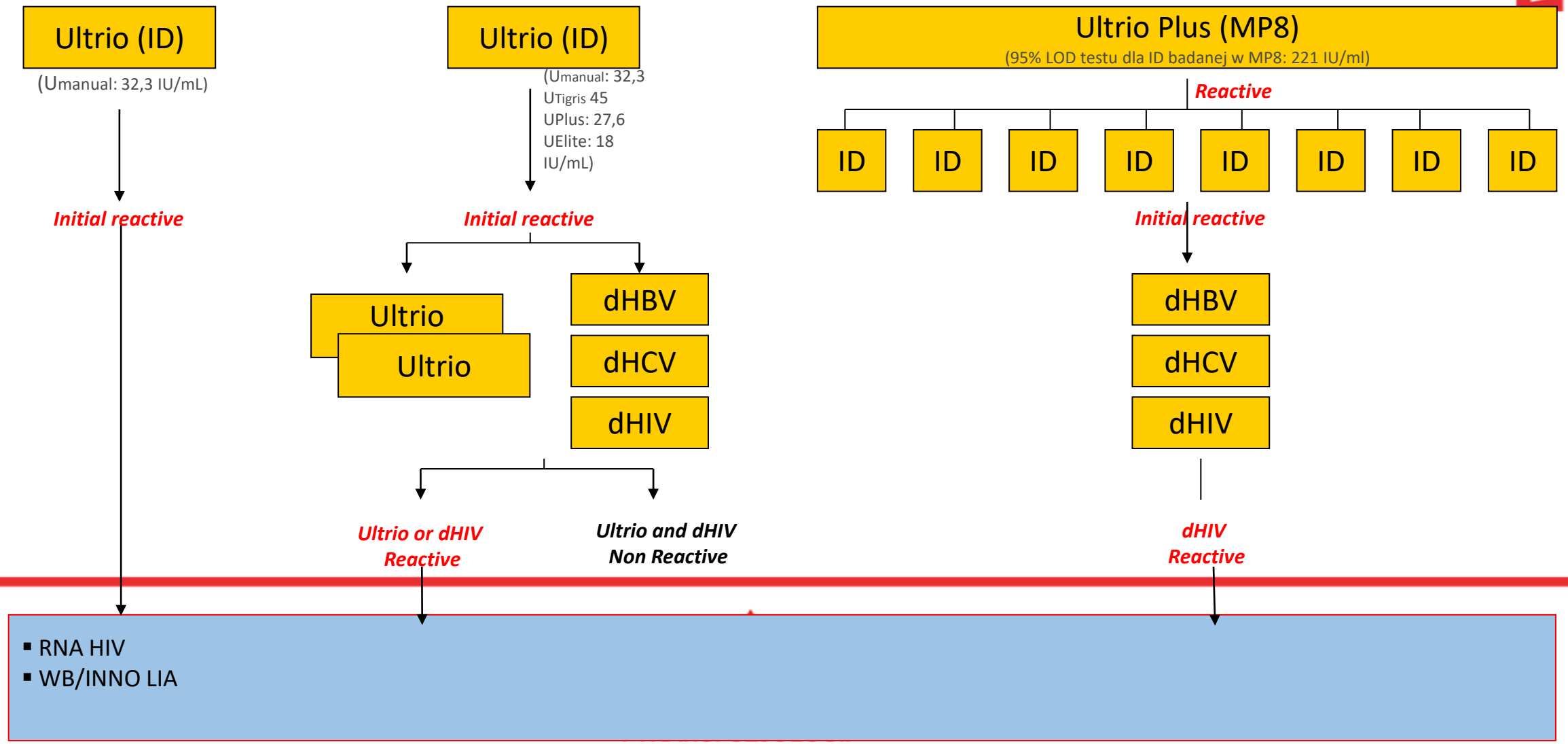
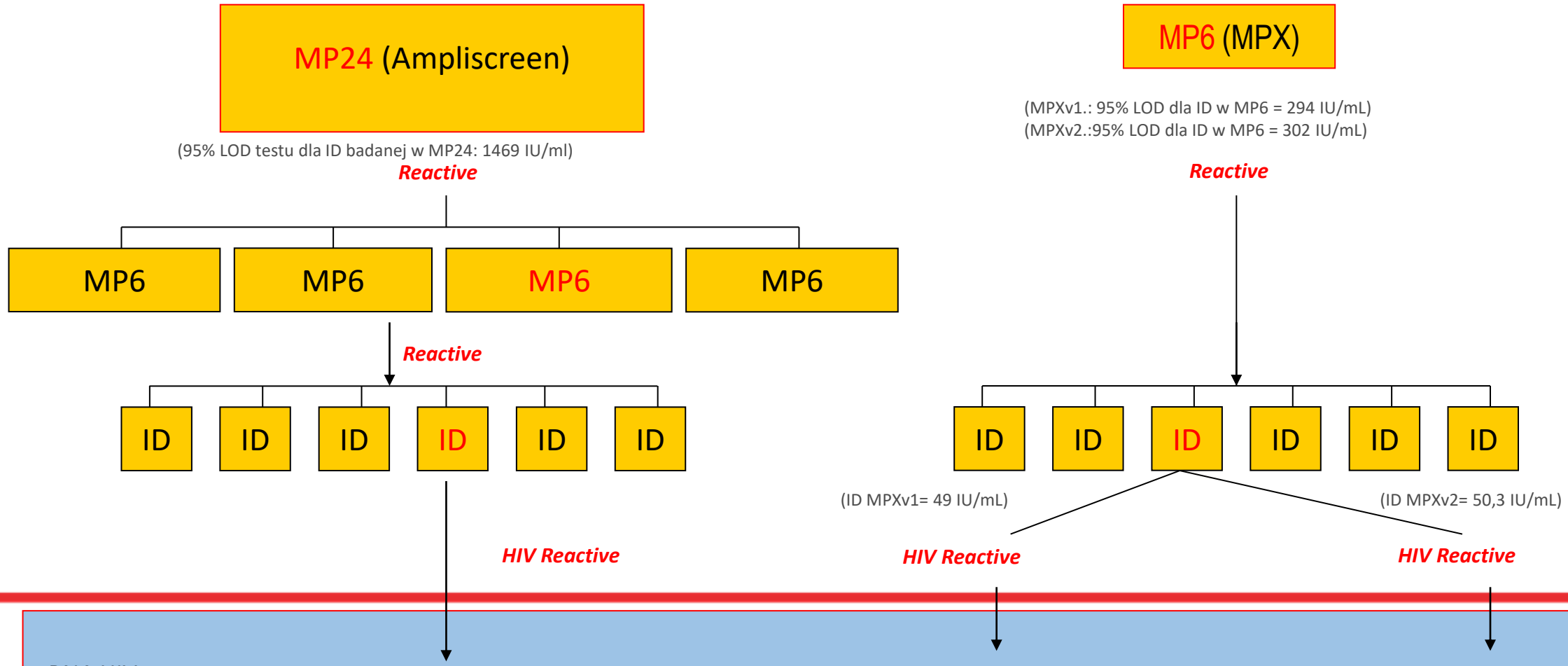
Screening and confirmatory testing algorithm - TMA

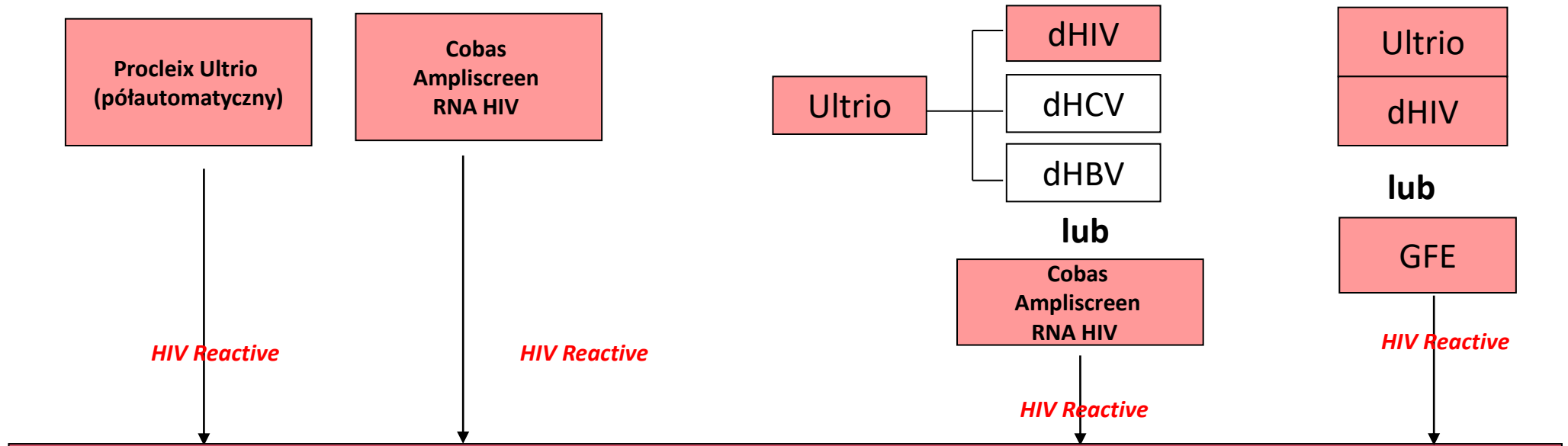


Screening and confirmatory testing algorithm - PCR



Screening and confirmatory testing algorithm:
HIV Ab -/HIV Ab/Ag -/NAT (HIV) Reactive

	Analytical sensitivity (95% LOD) IU/ml			
	2005 -	*2009 -*	*2012 -*	*2014 -*
Confirmatory testing	**Procleix Ultrio 32.3 IU/ml**	**Cobas Ampliscreen 61.2 IU/ml**	**Ultrio Elite 18 IU/ml** **Cobas Ampliscreen 61.2 IU/ml**	**Ultrio Elite 18 IU/ml** **GFE Blut v 1.1 89.5 IU/ml** **GFE Blut v 1.2 13.7 IU/ml**



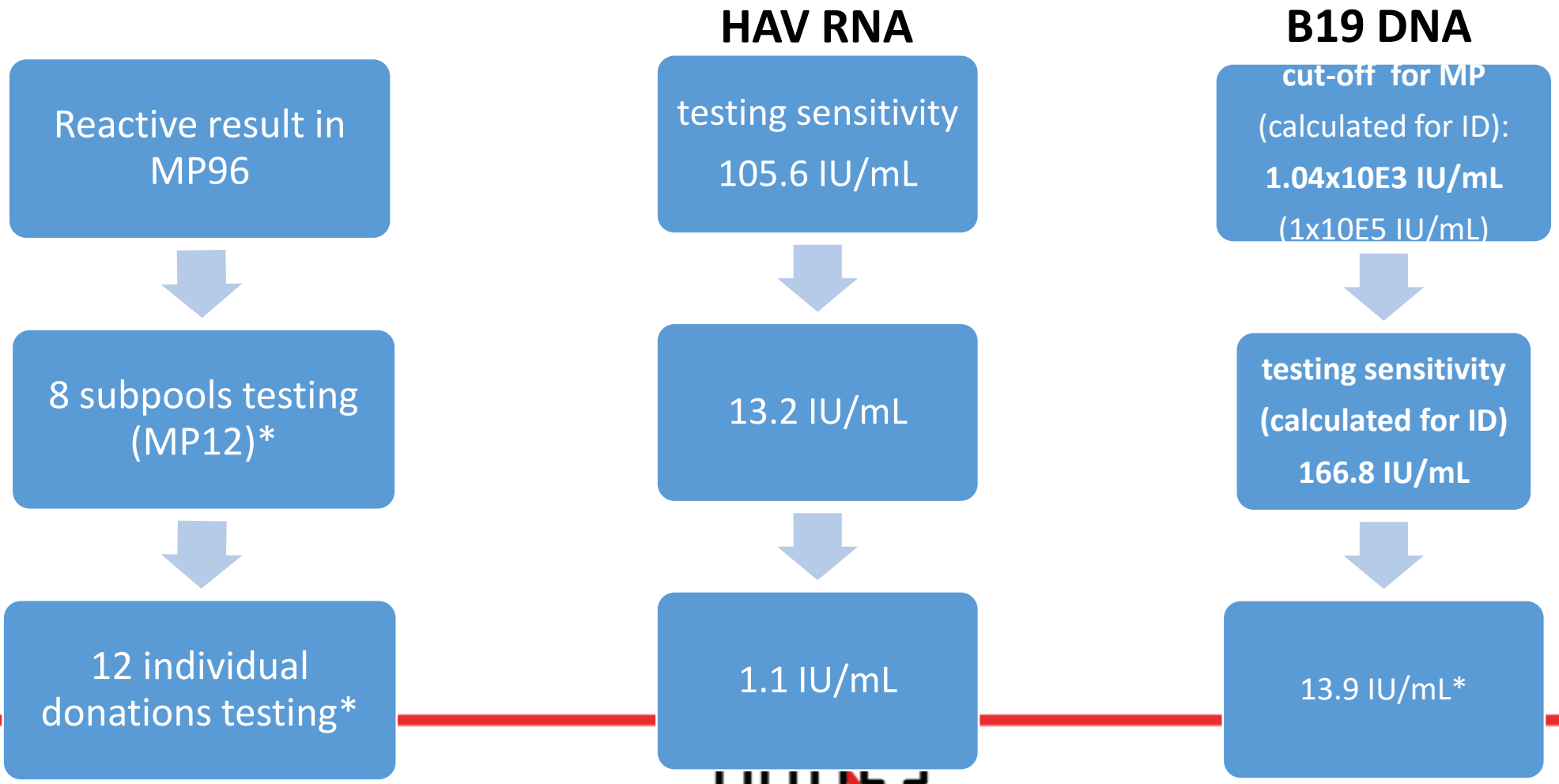
- WB / INNO LIA
- tesing of follow-up i look back samples
  - follow up: HIV Ab; WB or INNO LIA; RNA HIV
  - look back: RNA HIV

Non-enveloped viruses screening in Poland

	Requirements		Criteria for plasma discard	Time of screening	NAT results applied to qualification of cellular blood components
	domestic	Other			
HAV	not required	plasma for fractionation	confirmed infection	quality control before shipment to manufacturer	only if available
B19V	plasma dedicated for anti-D, and anti-HBs production, RBC for immunization	plasma for fractionation	VL> 1-1.6x10E5 IU/ml (ID)	quality control before shipment to manufacturer	only if available
HEV	not required	plasma dedicated for country with mandatory RNA HEV screening	confirmed infection	in paralel to RNA HCV, DNA HBV and RNA HIV screening	obligatory

Real-time PCR screening algorithm

LODs (cut-off) for screening and resolution of reactive MPs



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** Calculated for Cobas DPX based on data from instruction manual*

TMA screening algorithm

LODs (cut-off) for screening and resolution of reactive MPs

	HAV RNA	B19V DNA	HEV RNA
Reactive result in MP16	**HAV RNA 16.96 IU/mL**	**cut-off for MP** (in respect to ID): **1.0x10E4 IU/mL** (1.6x10E5 IU/mL)	**HEV RNA 126.24 IU/mL**
16 individual donation testing*	**1.06 IU/mL**	**325 IU/mL**	**7.89 IU/mL**

**LODs for Procleix Assays run on Procleix Panther based on instruction manual*

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Quality control for NAT in Poland

Preliminary evaluation of method (in IHTM prior to implementation)

- analytical sensitivity: multiple testing of six IS dilutions
- clinical sensitivity assessment (polymorphic forms, if available)
- proper identification of infected donations in MP testing (n=16-96) including high VL samples: risk of contamination and false results

Procedure validation (at RBTC, prior to implementation and every 12 months)

- several coded positive (including high VL) and negative samples tested in MPs
- several coded positive (including low VL) and negative samples tested individually

External quality control program (at RBTC, at least every 12 months)

e.g. LabQuality, EDQM, QCMD

IHTM -- Institute of Haematology and Transfusion Medicine, reference lab in Warsaw
RBTC – Regional Blood Transfusion Center, screening lab

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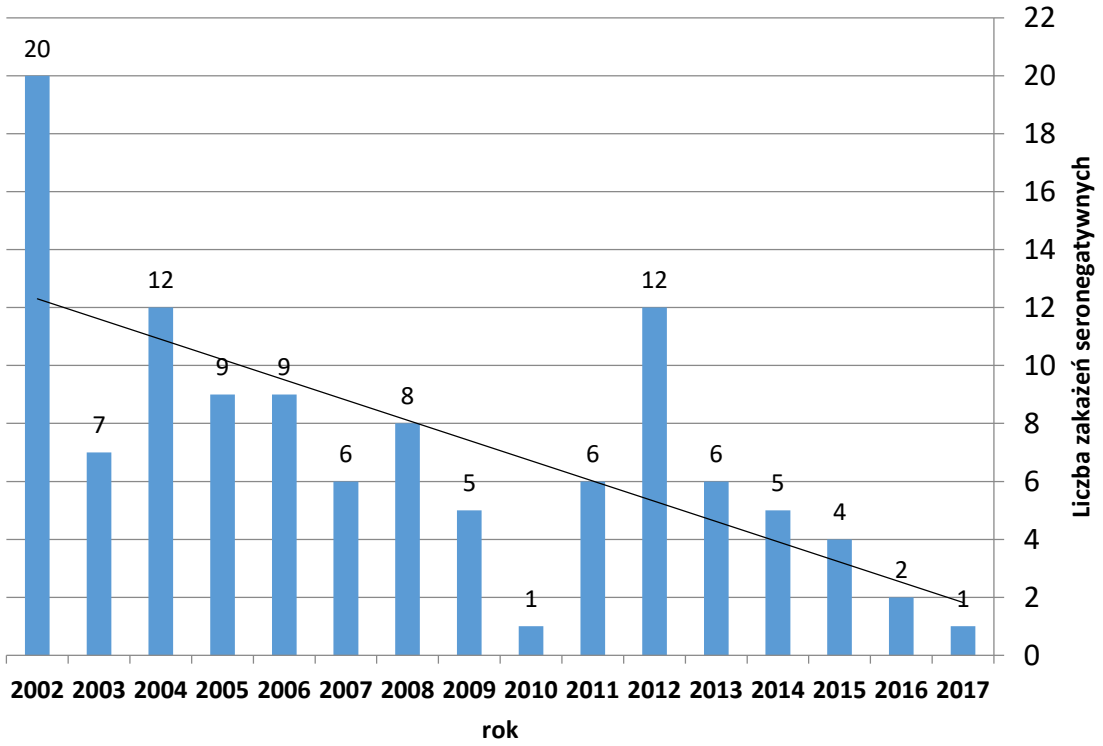
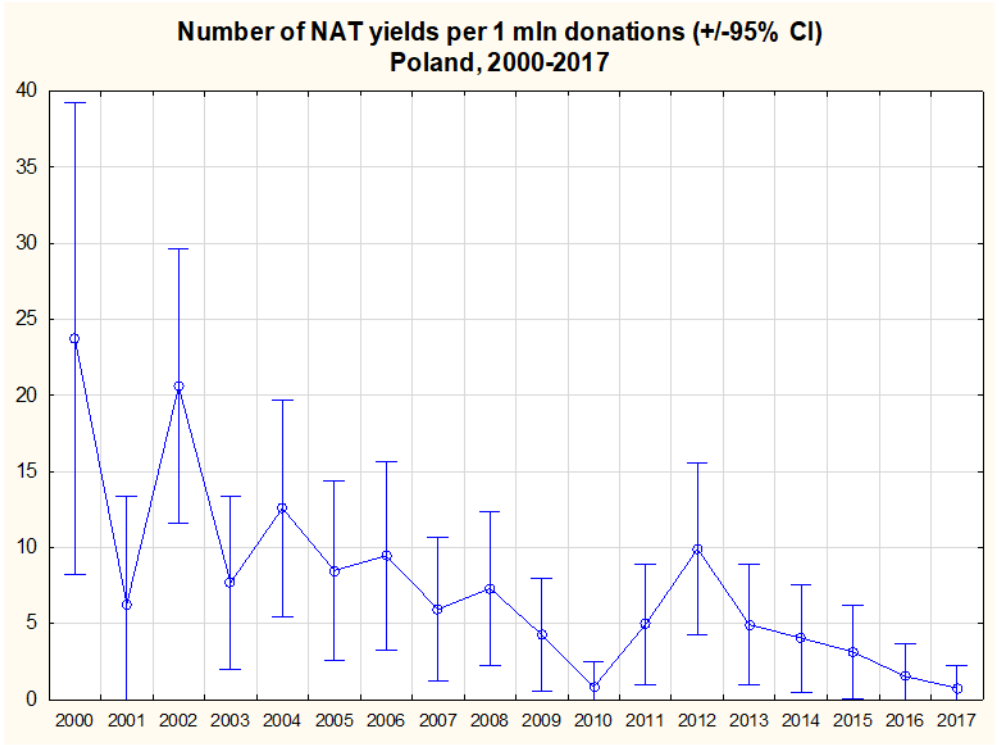
Identification of seronegative donations infected with HCV worldwide

Busch MP. 10 Years of NAT testing—what has been achieved and what needs to be done? Workshop on Surveillance and Screening of Blood Borne Pathogens Zagreb, Croatia, 2010, May 16-17

Region/kraj	donations number		
	tested	anti-HCV(-) /RNA HCV(+)	*NAT yields* /1 mln donations
USA	39.000.000	170	1:229.411.
Europe	50.000.000	50	1:1.000.000.
Poland(2008)	7.600.000	83	1:91.566.

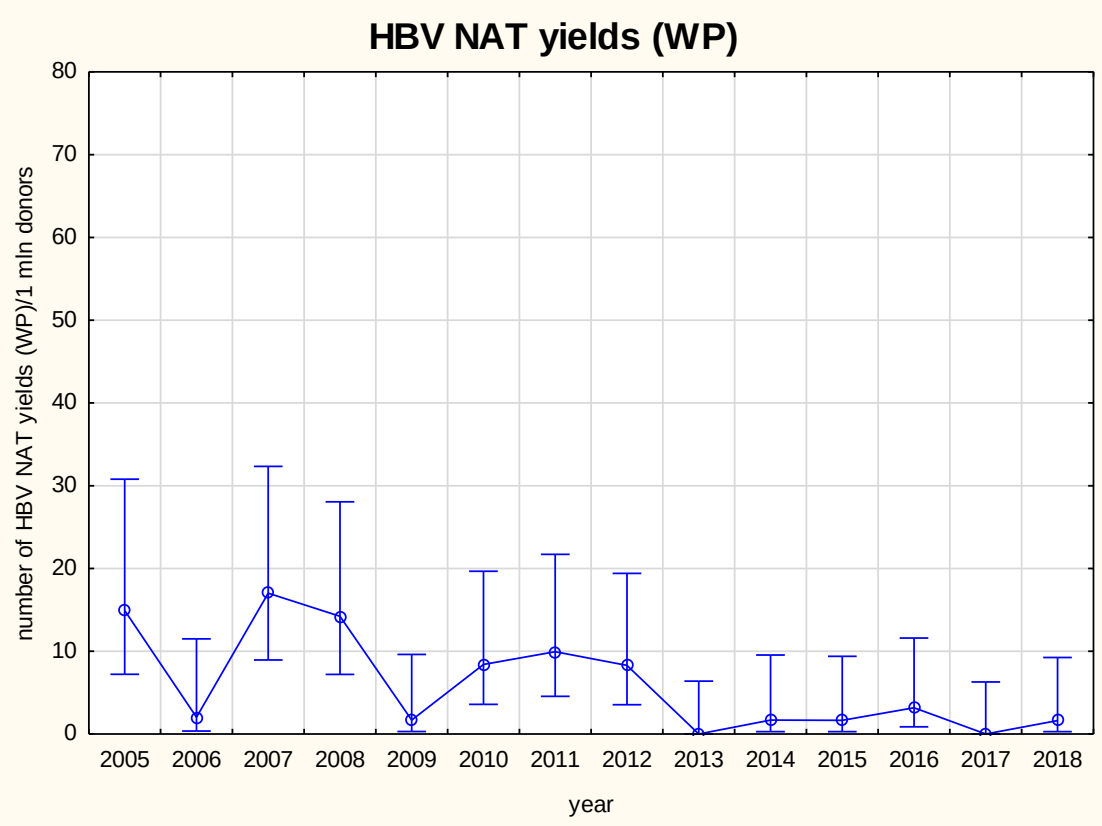
Detection of early HCV infections (*window period*) in blood donors in Poland

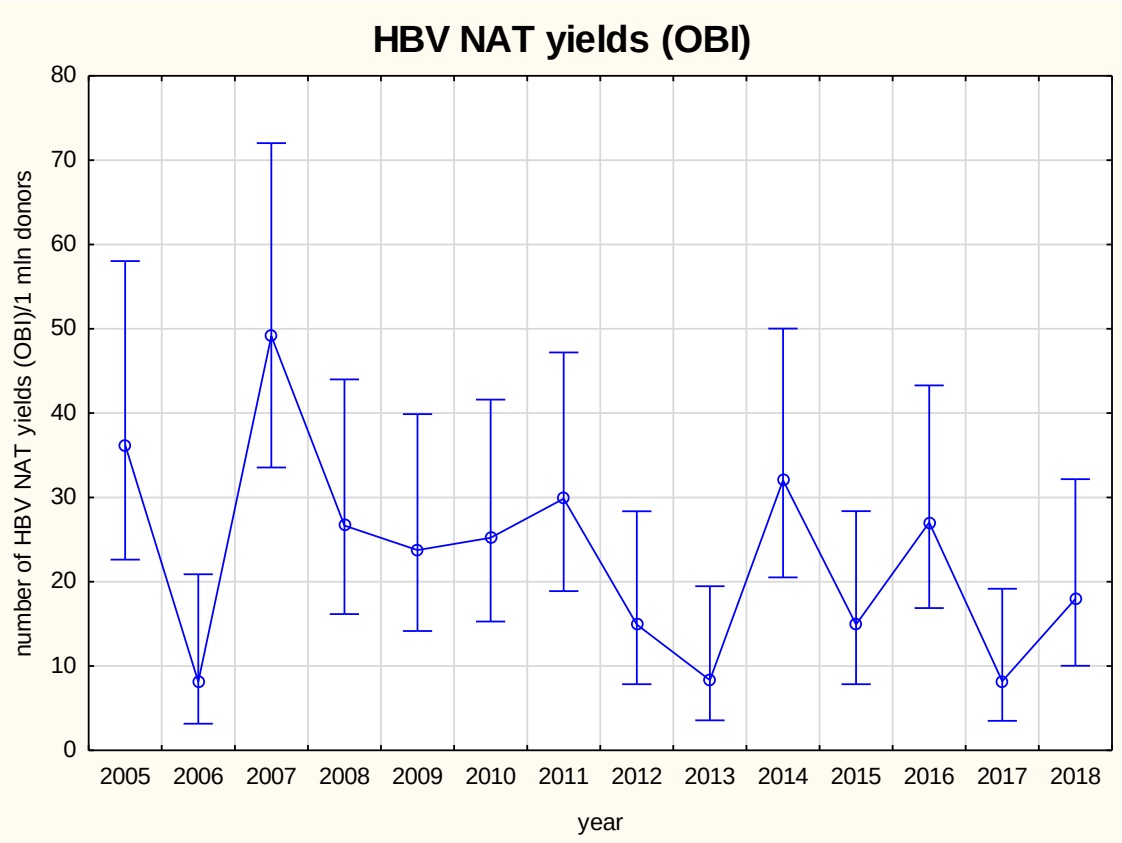




	Number of HCV infected blood donors			frequency/1 mln donors (+/-95% CI)
	In total	min (/rok)	max (/rok)	
HCV WP	75	1	12	9.3 (7.4-11.6)

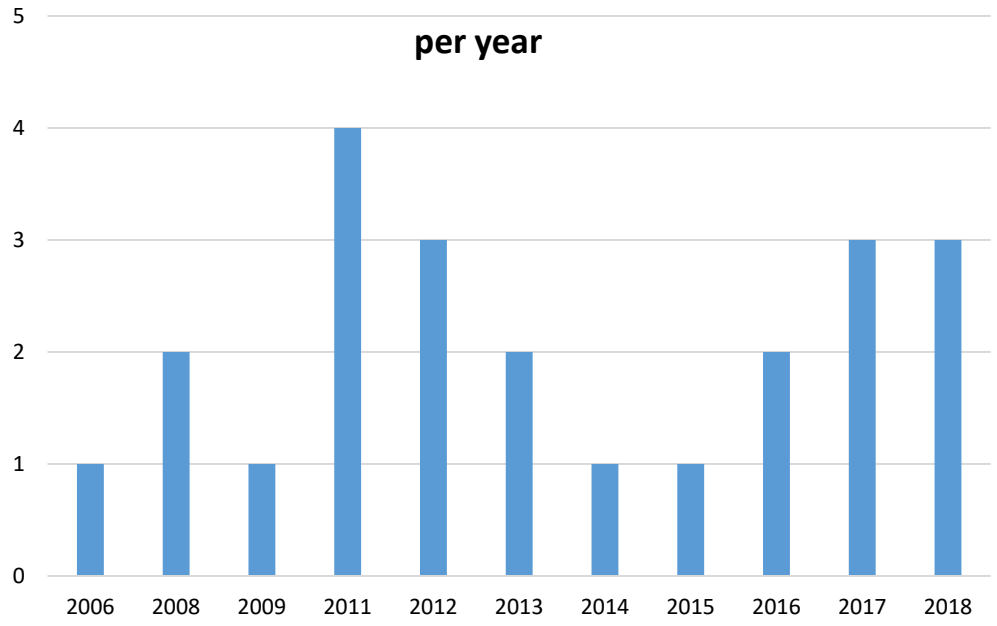
Detection of seronegative HBV infections (occult and *window period*) in blood donors in Poland

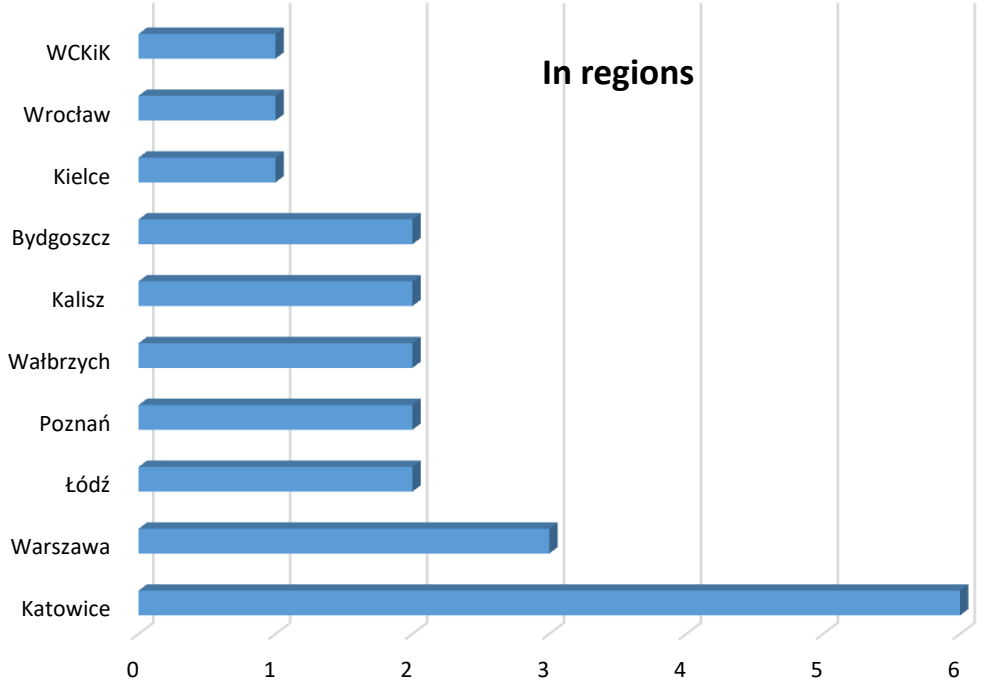




	Number of HBV seronegative infections			frequency /1 mln donors (+/-95% CI)
	In total	**Min** (/rok)	**Max** (/rok)	
HBV OBI	184	4	26	22.7 (19.7-26.3)
HBV WP	47	0	9	5.8 (4.4-7.7)

Detection of seronegative HIV infections (*window period*) in blood donors in Poland



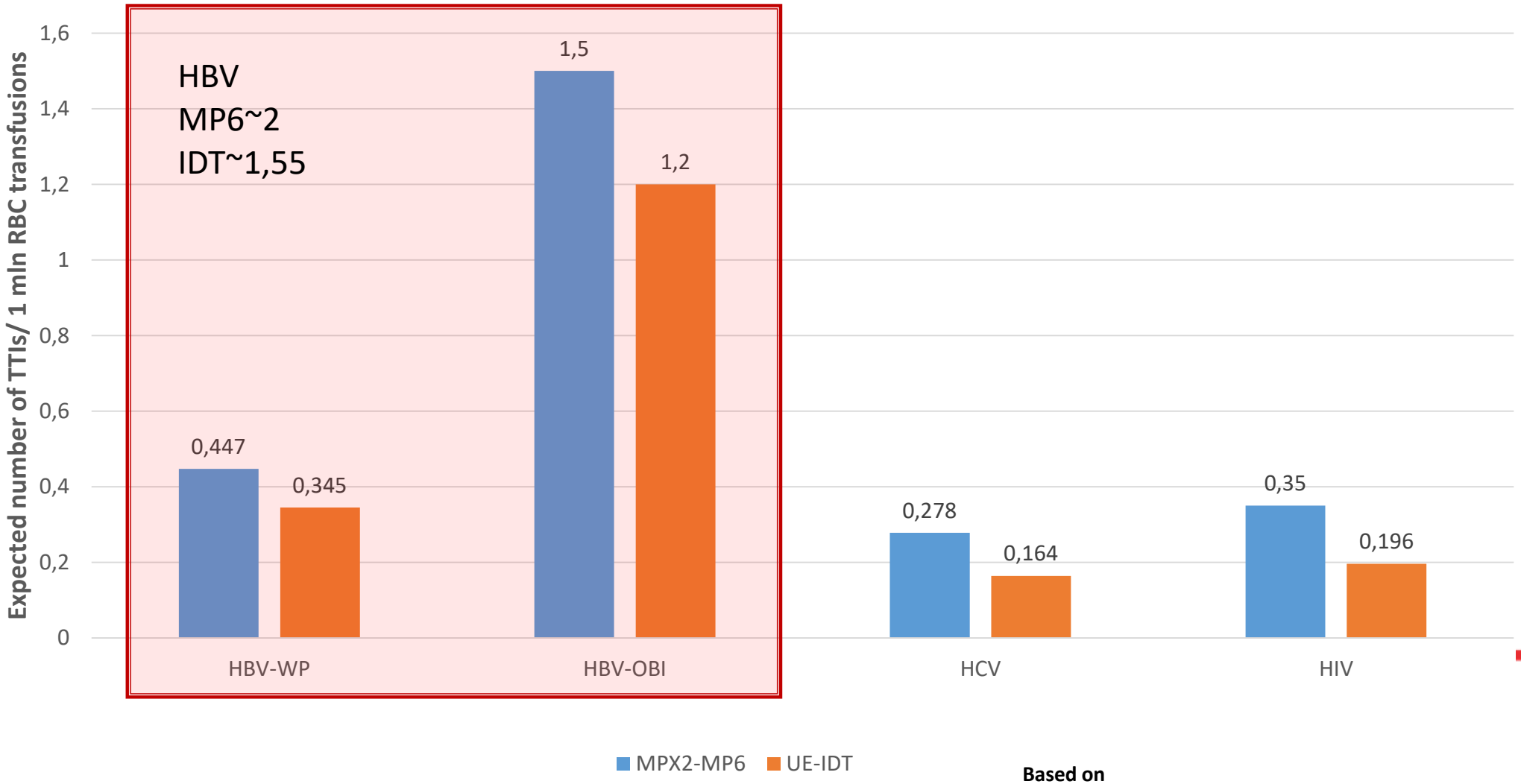


	number of seronegative HIV infections			frequency /1 mln donors (+/-95% CI)
	In total	min (/year)	max (/year)	
HIV WP	**23**	**0**	**4**	**2.8 (1.9-4.3)**

Residual risk for HBV, HCV, HIV

- for RBC: MP6 and cobas MPX v2; IDT and UE

cumulated data for period 2005-2015, repeat blood donors; WP ID_{50}=3.16, OBI ID_{50}=316,

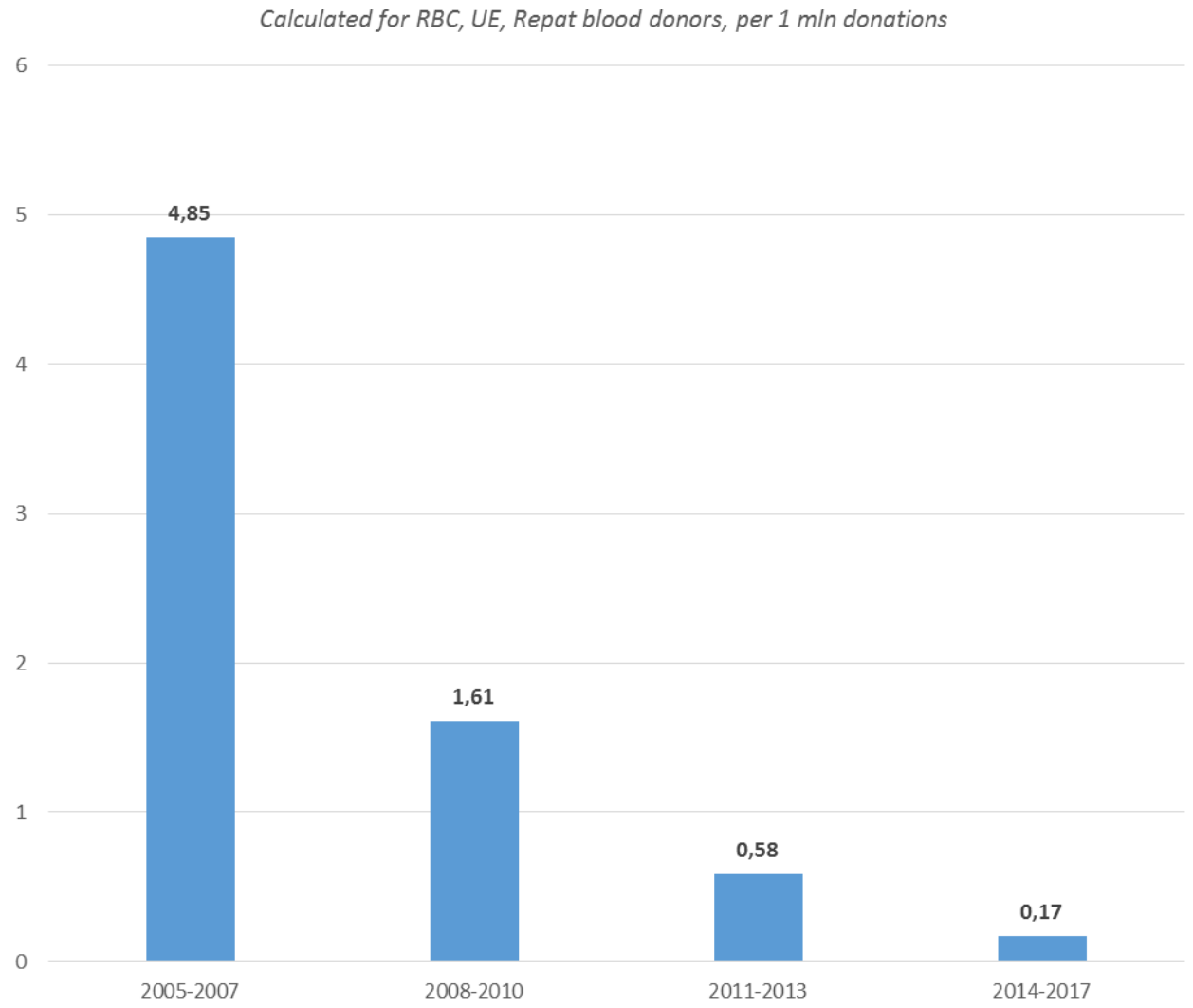


Based on

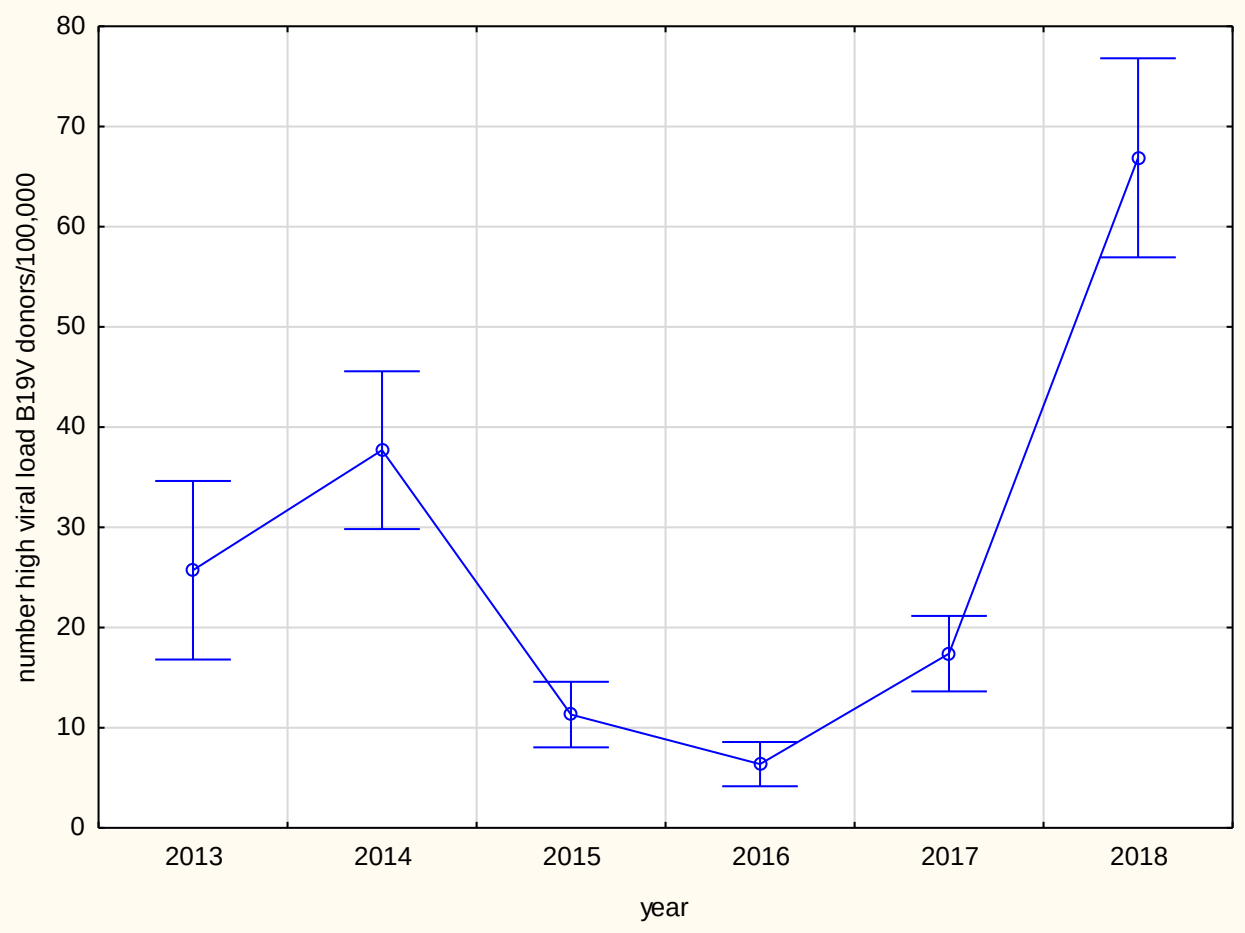
Weusten J, et al., Transfusion 2017 Mar;57(3pt2):841-849.

Weusten J et al., Transfusion 2011 Jan;51(1):203-15.

Residual risk for HBV (WP)



Frequency of high viral load parvovirus B19 infections in Polish blood donors, 2013-2018



relative risk for 2016 vs 2018 = 10,5 (95% CI: 7.2-15.3), p<<<0.05

	donors number	
period	**tested**	**high VL B19V+**
2013-18	1.998.451	454
2013	124.425	32
2014	233.452	88
2015	406.673	46
2016	502.513	32
2017	471.536	82
2018	260.164	174

Hepatitis A virus incidence in Polish blood donors 2013-2018

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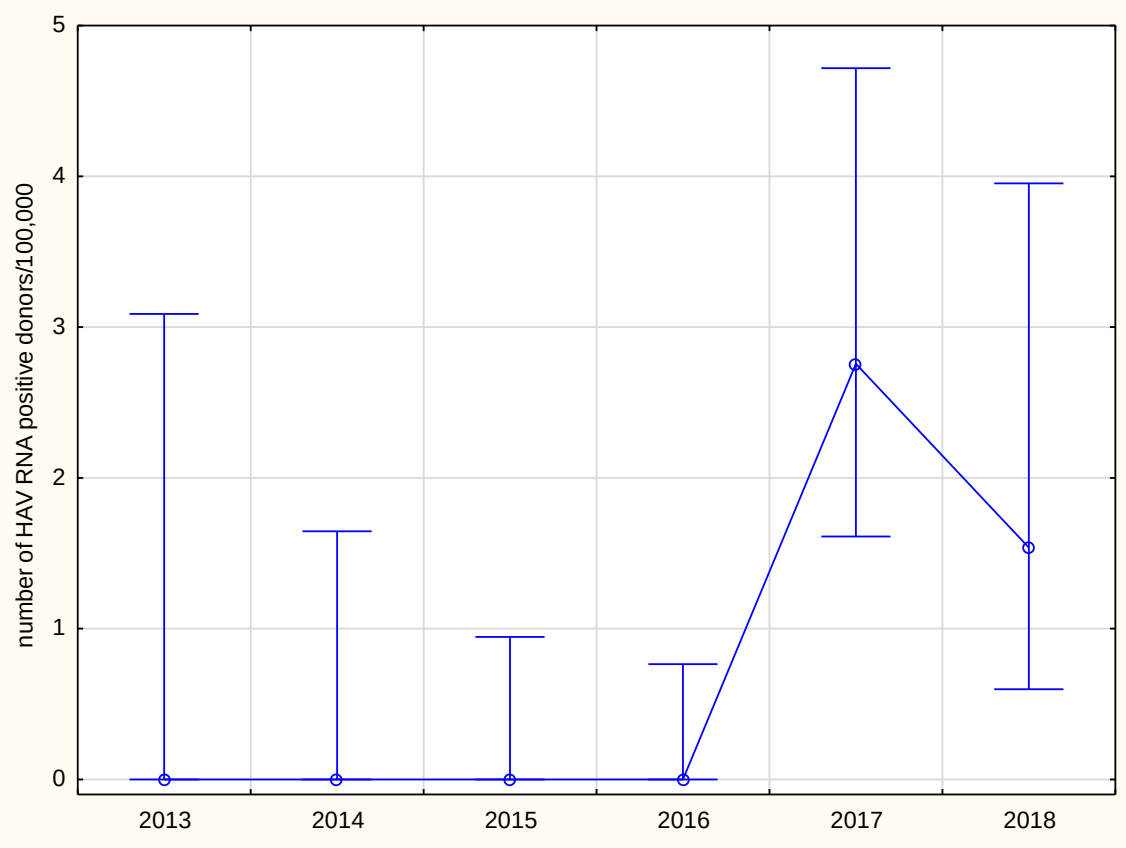
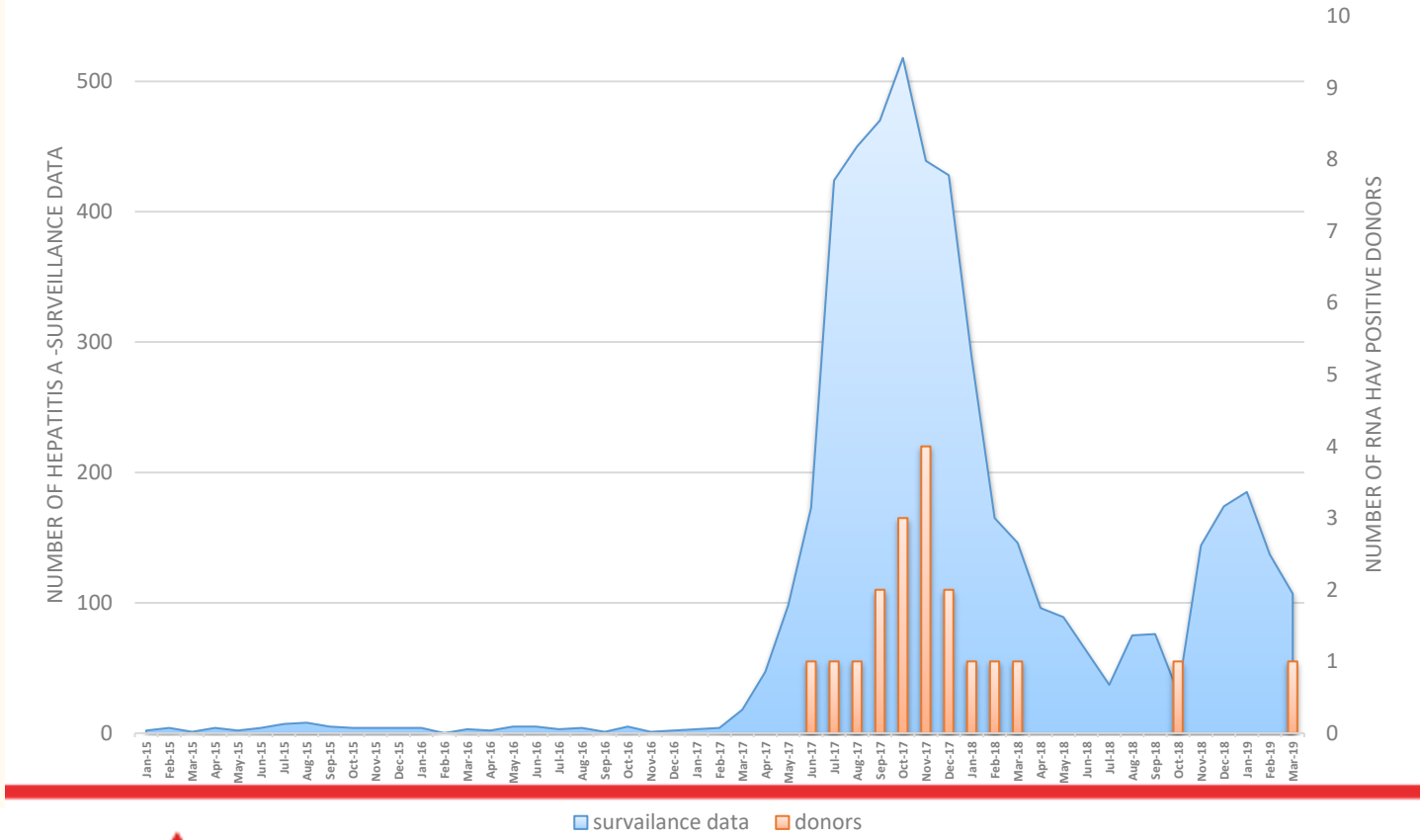
frequency (95% CI) in blood donors*



number of infected blood donors vs surveillance data**



data reported for donors tested until the end of 2018
***infected donors and surveillance data reported until the end of April 2019*

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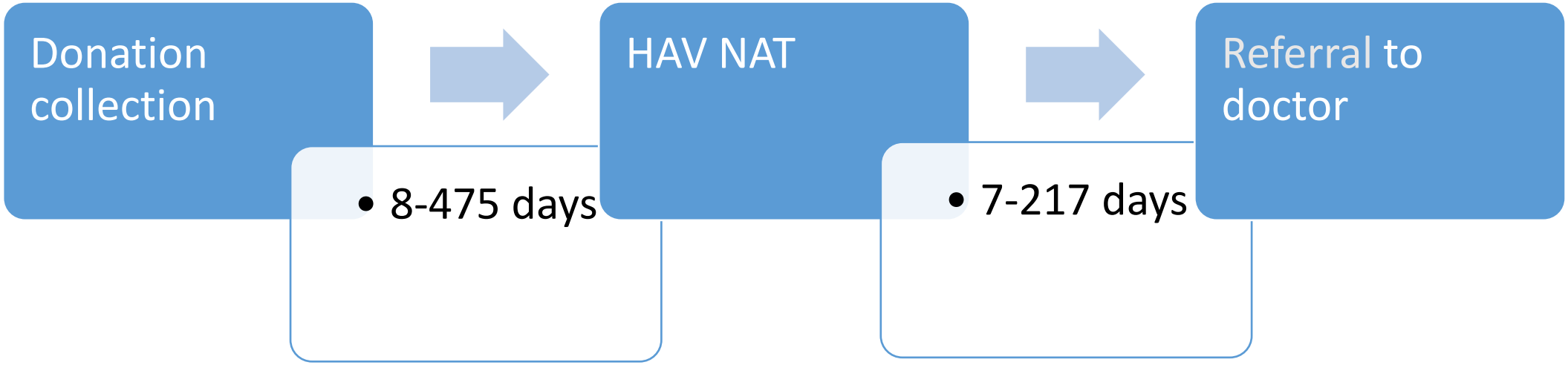
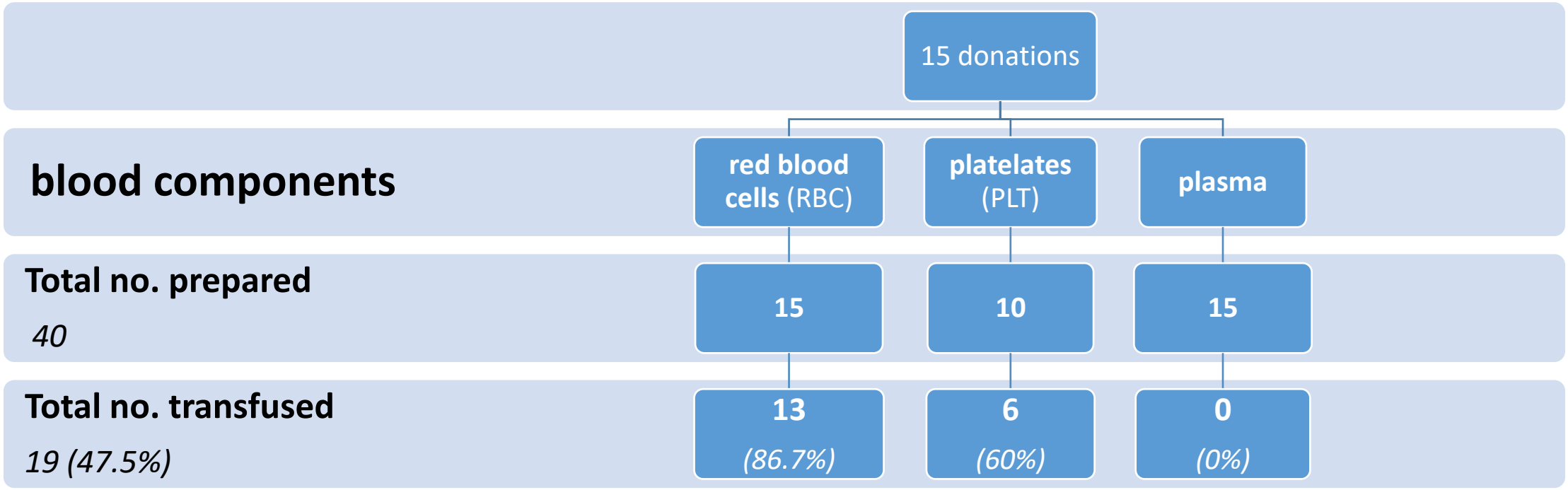
Source: PZH-NIZP IHTM

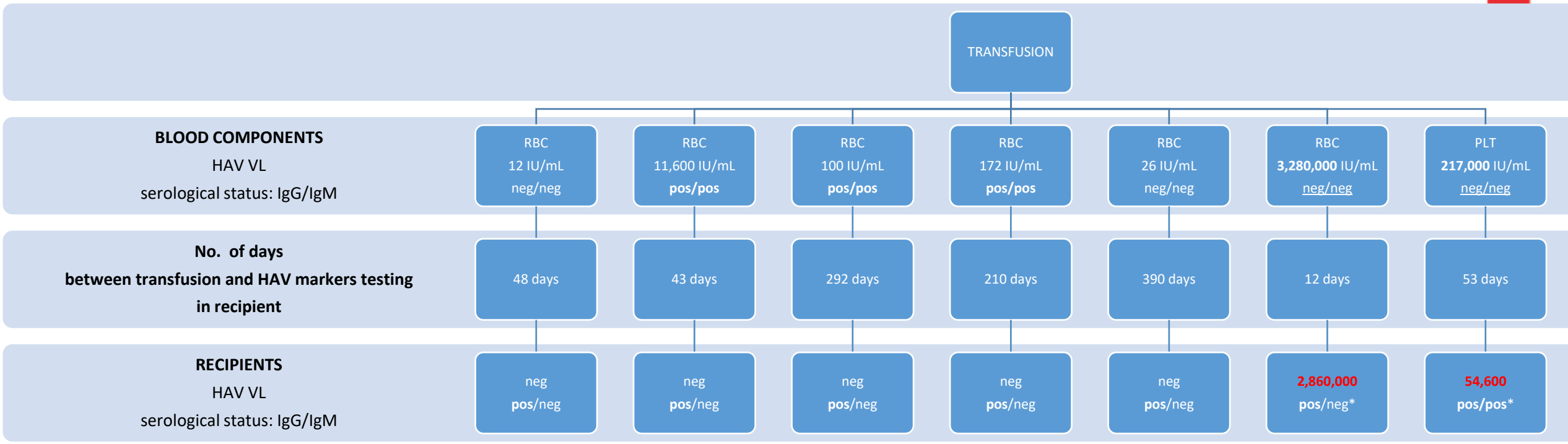
RNA HAV positive blood components follow-up (1)



RNA HAV positive blood components follow-up (2)



RNA HAV positive blood components follow-up (3)



*64 year-old patient /neurological ward, no symptoms of hepatitis A after transfusion

**30 year-old oncological patient with bile duct cancer, died due to exacerbation of basic illness, assessment of clinical significance of HAV infection in process

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HEV infections in Polish blood donors

TRANSFUSION 2018;58;1245–1253

DONOR INFECTIOUS DISEASE TESTING

Year

study: **2015**
publication: 2018

Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland

TABLE 3. Distribution of HEV infection seroprevalence among Polish blood donors by sex, age, and geographic region

Group	No. of donations tested for IgG and IgM	Anti-HEV IgM-positive results		Anti-HEV IgG-positive results	
		No.	Frequency (95% CI), %	No.	Frequency (95% CI), %
All	3079	39	1.27 (0.93-1.73)	1340	43.52 (41.78-45.28)
Males	1986	30	1.51 (1.06-2.15)	923	46.48 (44.29-48.67)
Females	1093	9	0.82 (0.43-1.56)	417	38.15 (35.32-41.07)
Age group, y					
18-27	778	3	0.39 (0.13-1.13)	177	22.75 (19.94-25.83)
28-37	780	15	1.92 (1.17-3.15)	333	42.69 (39.27-46.19)
38-47	747	9	1.20 (0.64-2.27)	369	49.40 (45.82-52.98)
48-57	574	10	1.74 (0.95-3.18)	349	60.80 (56.75-64.71)
58-70	200	2	1.00 (0.27-3.57)	112	56.00 (49.07-62.70)

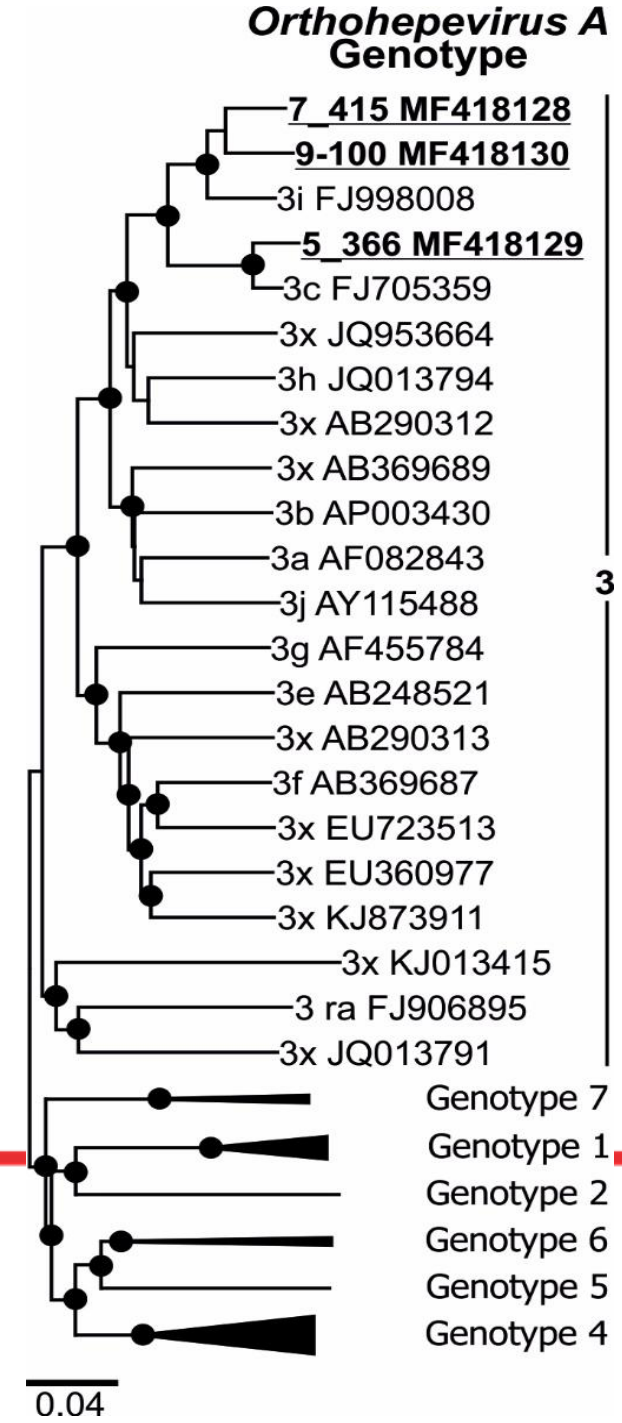
Phylogenetic analysis of HEV isolates from Polish blood donors – identification of **genotype 3** (subtypes **3c** and **3i**)

DONOR INFECTIOUS DISEASE TESTING

TRANSFUSION 2018;58;1245–1253

Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland

Piotr Grabarczyk,[1] Ewa Sulkowska,[1] Jolanta Gdowska,[2] Aneta Kopacz,[1] Grzegorz Liszewski,[1] Dorota Kubicka-Russel,[1] Sally A. Baylis,[3] Victor M. Corman,[4,5] Ewa Noceń,[1] Dariusz Piotrowski,[2] Jolanta Antoniewicz-Papis,[1] and Magdalena Łętowska[1]



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NAT perspectives

Application

- HEV
- HAV i B19V
- *Emerging pathogens*

Further improvements

- Widening tests panel in multiplex (+HEV; +HAV)
- Increasing HBV DNA testing sensitivity (?)

Screening centralization