Viral standards and assay performance: the foundations for estimating transmission risk of NAT screened blood

Nico Lelie

Biologicals Quality Control, De Droogmakerij 31h, Heiloo, The Netherlands

Satellite Meeting before IPFA-PEI 30th Workshop on Surveillance and Screening of Blood Borne Pathogens on Tuesday, May 9th 2023, 14.30-17.45 hours in Hotel NH Bologna de la Gare, Piazza XX Settembre 2, 40121 Bologna BO, Italy

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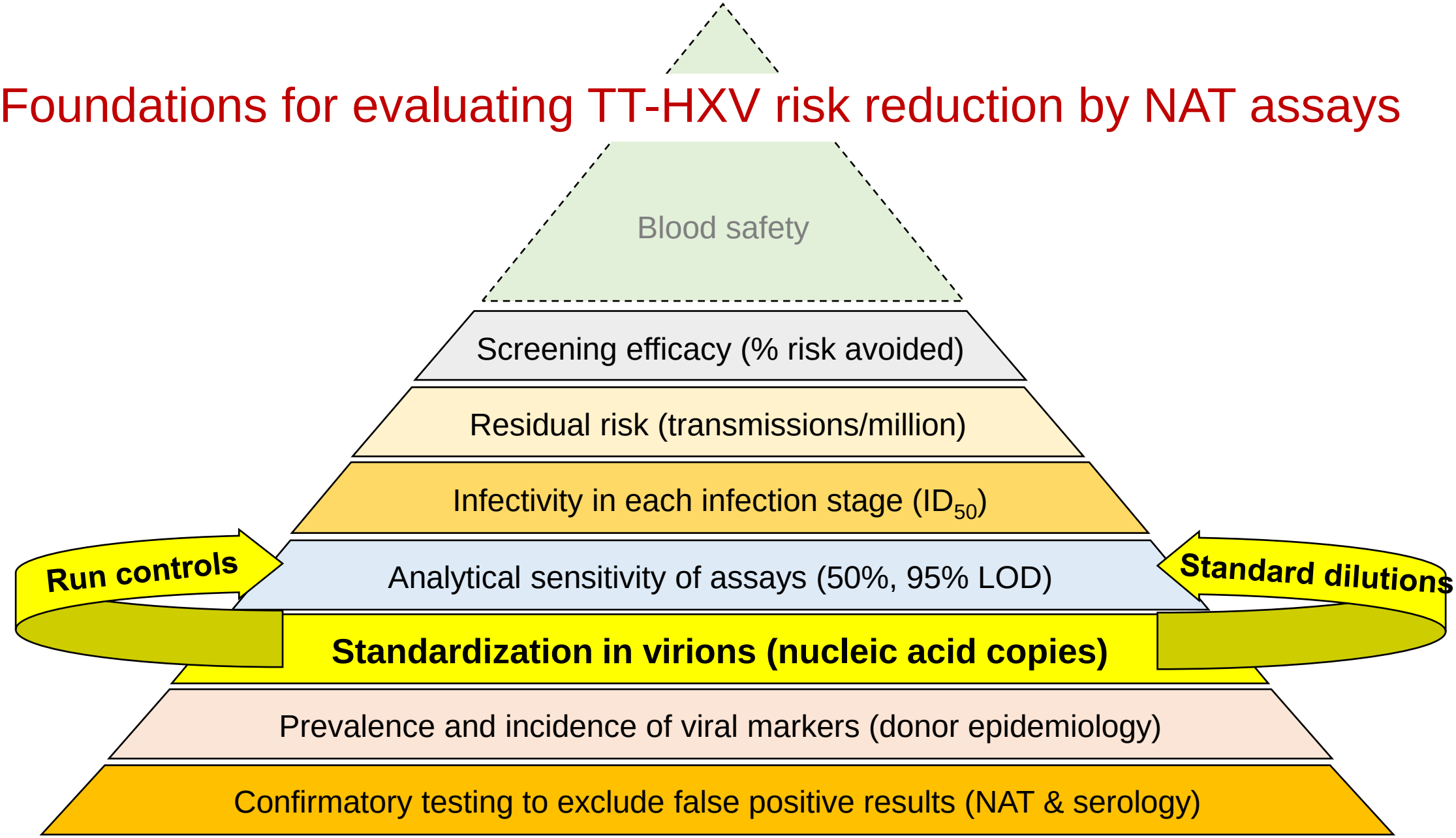
Foundations for evaluating TT-HXV risk reduction by NAT assays



Bruhn et al, Transfusion 2013:53:2399-2412, Bruhn et al Transfusion 2015;55:1195-205, Lelie et al, Viruses 2022, 14, 2263

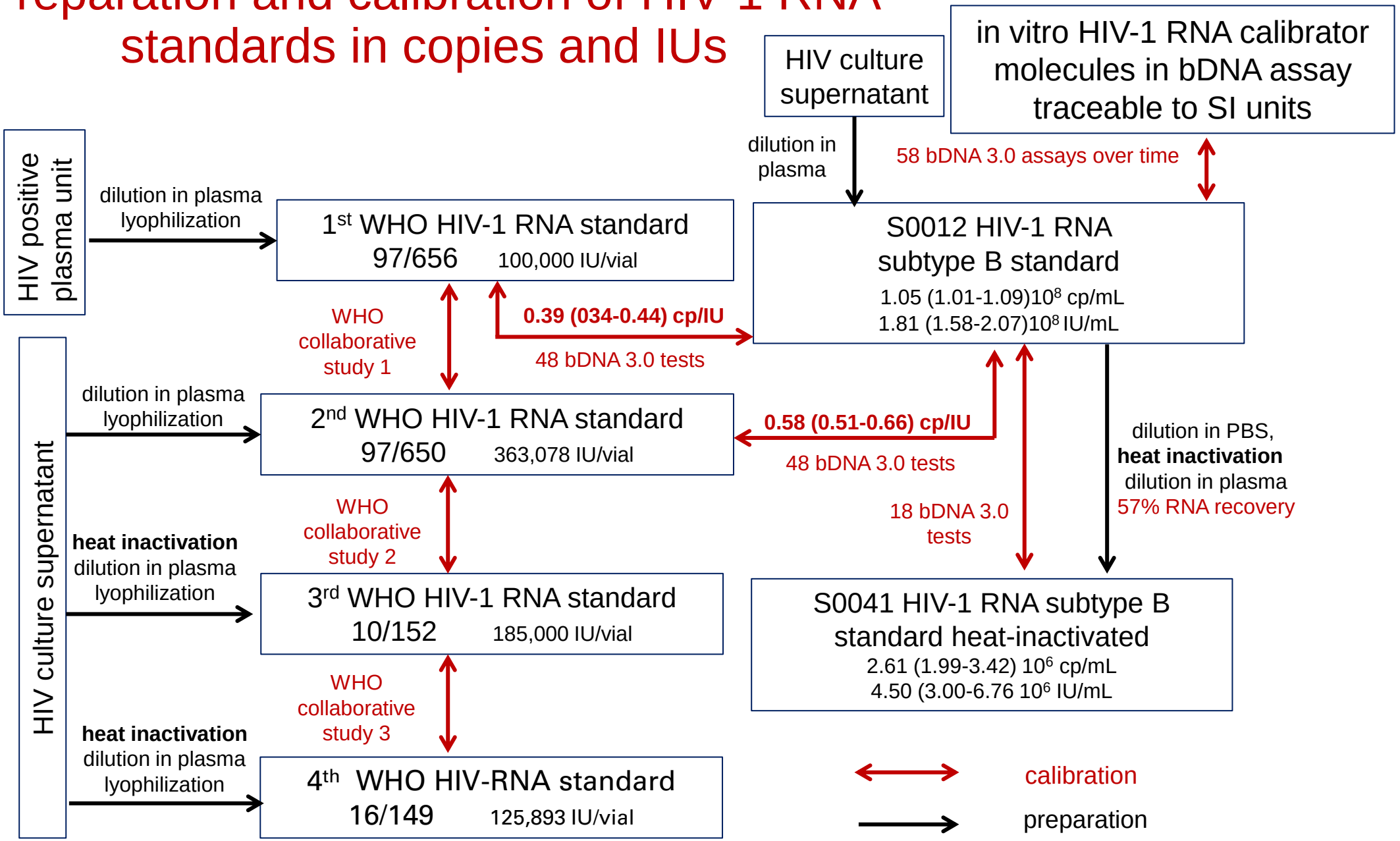
New EU IVD regulation 2017/746

- more emphasis on post-market performance follow up

Common Specifications

- Analytical sensitivity – WHO standard
- Diagnostic sensitivity – Seroconversion panels (NAT: n ≥10, immunoassay: n≥30)
  - Acceptance Criterion – ‘State of the Art’
- No sensitivity requirements for detection of different genotypes

Preparation and calibration of HIV-1 RNA standards in copies and IUs



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J Med Virology;92:3246-3253

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Accuracy of quantitative HIV-1 RNA test methods at 1000 copies/mL and the potential impact of differences in assay calibration on therapy monitoring of patients

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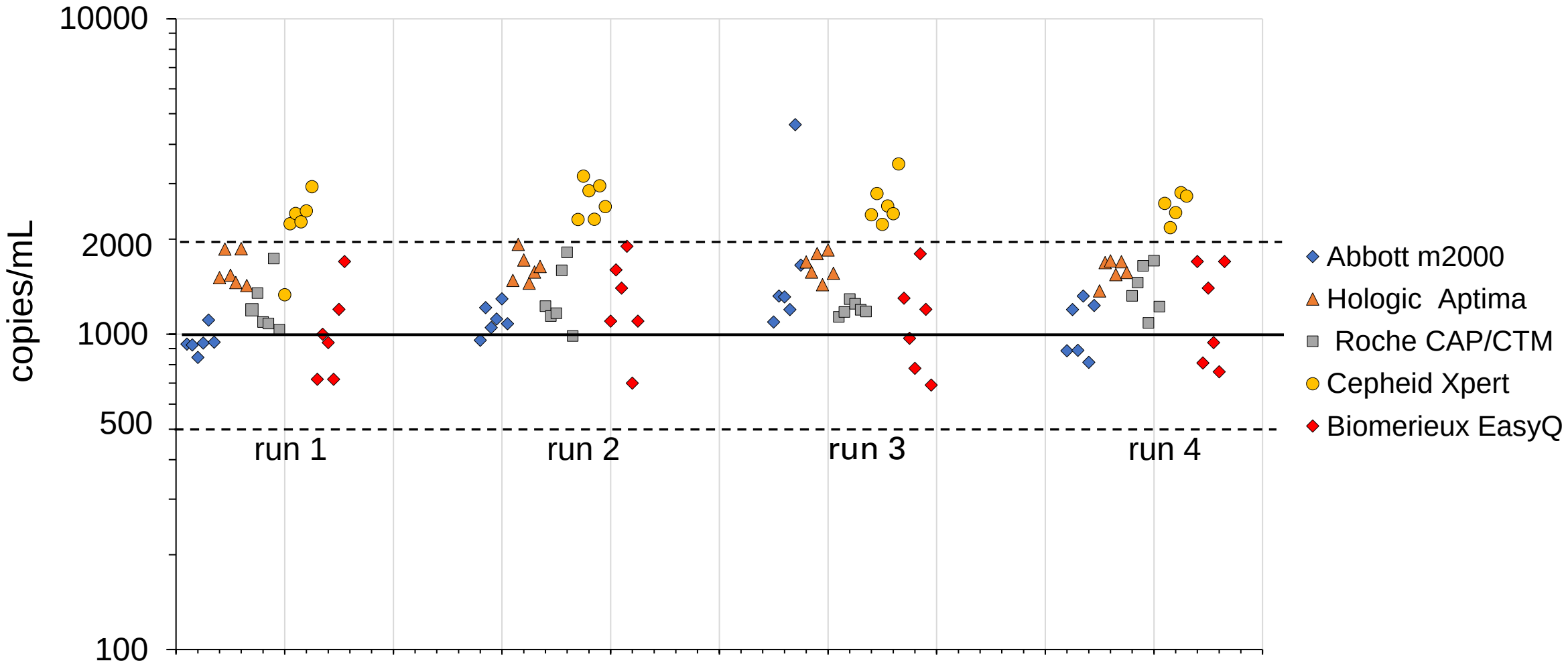
Abstract

The World Health Organization (WHO) recommends the clinical use of a human immunodeficiency virus 1 (HIV-1) viral load (VL) threshold level of 1000 copies (cp)/mL in patients on antiretroviral therapy (ART) to distinguish between viral control (VL < 1000 cp/mL) and viral failure or poor adherence (VL > 1000 cp/mL). The accuracy of five quantitative HIV-1 RNA assays at this level was compared by

Quantification of P0327 ViraQ HIV-1 Quant 1000 Control by five viral load assays

1000 copies/mL of S0012 VQC-Sanquin HIV-1 subtype B standard



Lelie and van Drimmelen. J Med Virol 2020;92:3246-3253

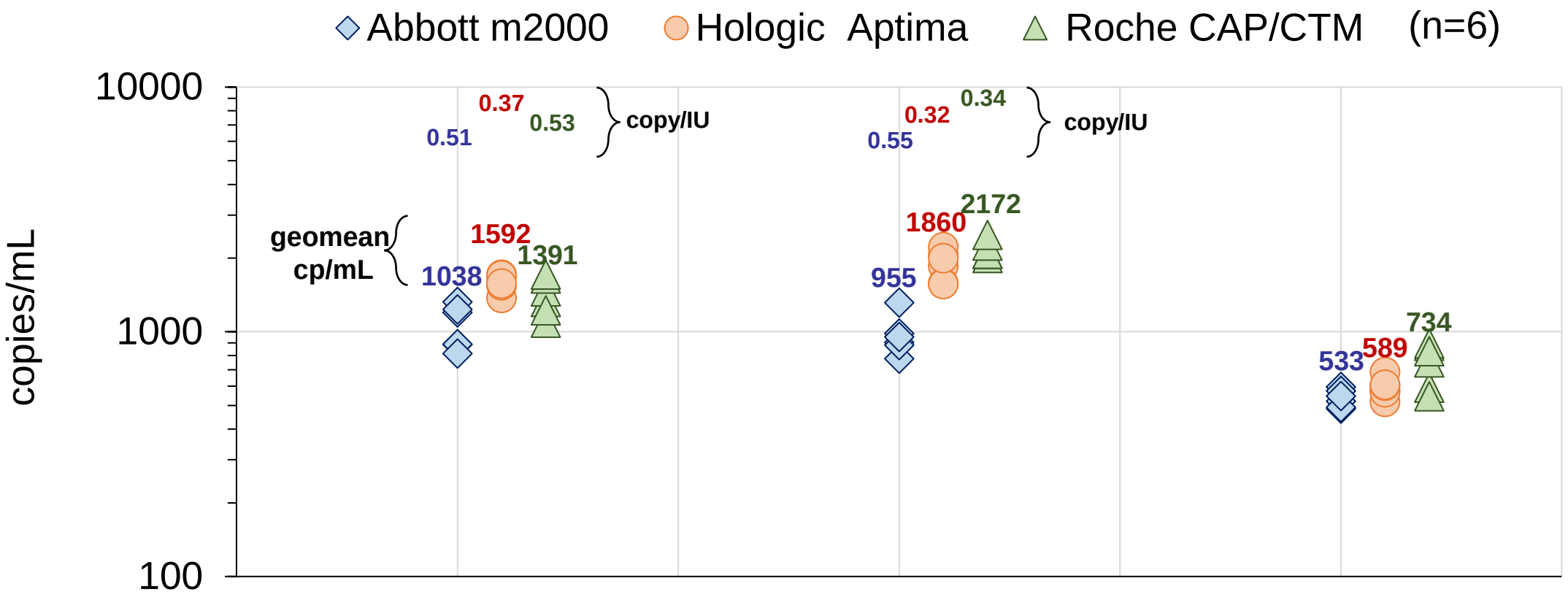
Accuracy and precision of five viral load assays on P0327 ViraQ HIV-1 Quant 1000 Control

1000 copies/mL of S0012 VQC-Sanquin HIV-1 subtype B standard

Assay	n	geomean cp/mL	(95% CI) cp/mL
Abbott m2000 RealTime Assay m2000	24	1084	(784-1572)
Hologic Aptima	24	1616	(1324-1973)
Roche CAP/CTM	24	1277	(892-1828)
Cepheid GeneXpert	24	2502	(1333-3465)
BioMerieux NucliSens EasyQ	24	1110	(690-1900)

Lelie and van Drimmelen. J Med Virol 2020;92:3246-3253

Assay dependent quantification of HIV-1 subtype B standards



according to calibration in multiple replicate bDNA 3.0 tests (n=18 - 48)

Calibration of VQC HIV-1 standard against three WHO replacement standards in Abbott RealTime Assay

HIV-1 Standard	Nominal value of standard^	n^	geomean (95% CI) copies/mL^	Potency (95% CI) = copy/IU (95% CI)
S0012 VQC	1000 cp/mL	18	944 (698-1276)	1.0 (reference)§
2nd WHO 97/650	1000 IU/mL	6	392 (266-577)	0.42 (0.27-0.63)
3rd WHO 10/152	1000 IU/mL	18	291 (220-577)	0.31 (0.23-0.61)
4th WHO 16/149	1000 IU/mL	18	236 (156-356)	0.25 (0.15-0.41)

^ Concentrations of 1000, 3000 and 10,000 copies/mL or IU/mL were prepared and tested in 6 replicate Abbott RealTime assays. Of 2nd WHO 97/650 standard only 1000 IU/mL concentration was available for testing.

§ 0.58 (0.51-0.66) copies/IU according to calibration in bDNA 3.0 assay against 2nd WHO 97/650 standard (n=48)

±

Conclusion

- Differences in calibration of VL assays in copies/mL
- Drift in copies/IU in heat-inactivated WHO replacement standards
- S0012 HIV-1 subtype B standard is stable and in use since 1996
  - Directly traceable to 1st and 2nd WHO standard
  - Can serve as alternative to 3rd and 4th WHO replacement standards

J Med Virology;92:3246-3253

viruses Viruses 2022, 14,1942 MDPI

Article

Early Dynamics of Hepatitis B Virus (HBV)-DNA and Surface Antigen (HBsAg) in Ramp-Up Phase of Viremia: Implications for Performance Evaluation of Blood Screening Assays

Harry van Drimmelen [1] and Nico Lelie [1,2,*]

Citation: van Drimmelen, H.; Lelie, N. Early Dynamics of Hepatitis B Virus (HBV)-DNA and Surface Antigen (HBsAg) in Ramp-Up Phase of Viremia: Implications for Performance Evaluation of Blood Screening Assays. *Viruses* **2022**, *14*, 1942. https://doi.org/10.3390/v14091942

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Abstract: The Common Sp
in vitro diagnostic devices (
the International Standard a
performance. We examined w
HBV-DNA and HBsAg assay
in the ramp-up phase of five
in the Log concentration of b
acid amplification technology
critical samples from all five
comparable to those on a HB

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ORIGINAL ARTICLE

J Viral Hepat. 2022;29:330-339

JOURNAL OF VIRAL HEPATITIS JVH WILEY

Infectivity of hepatitis B virus (HBV) surface antigen (HBsAg) positive plasma with undetectable HBV-DNA: Can HBsAg screening be discontinued in Egyptian blood donors?

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[3]Biologicals Quality Control, Heiloo, The Netherlands
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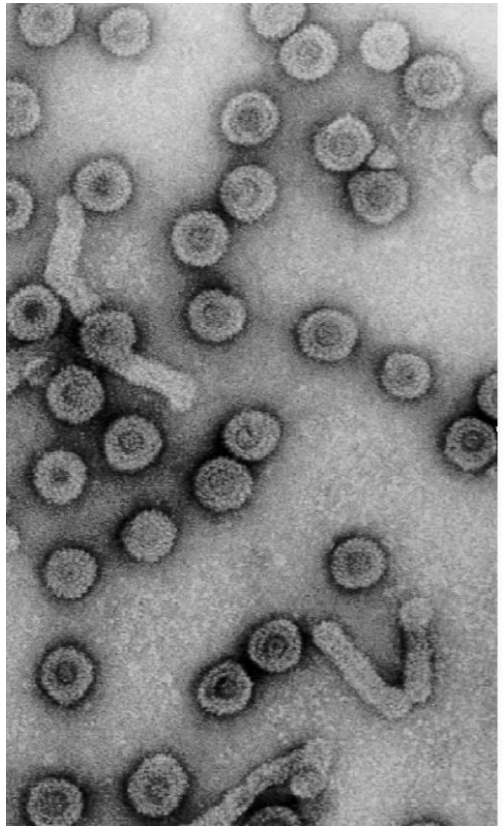
[5]Lelie Research, Alkmaar, The Netherlands

Abstract

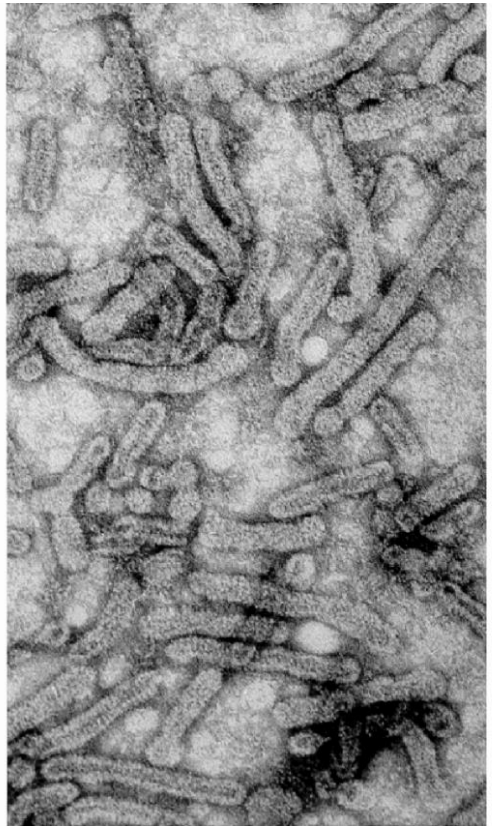
HBV infectivity data were reviewed and the 50% infectious dose (ID_{50}) was reassessed in different HBsAg positive infection stages enabling modelling of transfusion-transmitted (TT)-HBV infection risk if HBsAg donor screening was replaced by individual donation nucleic acid amplification technology (ID-NAT). Quantitative

Ratio of HBV virions to subviral HBsAg particles

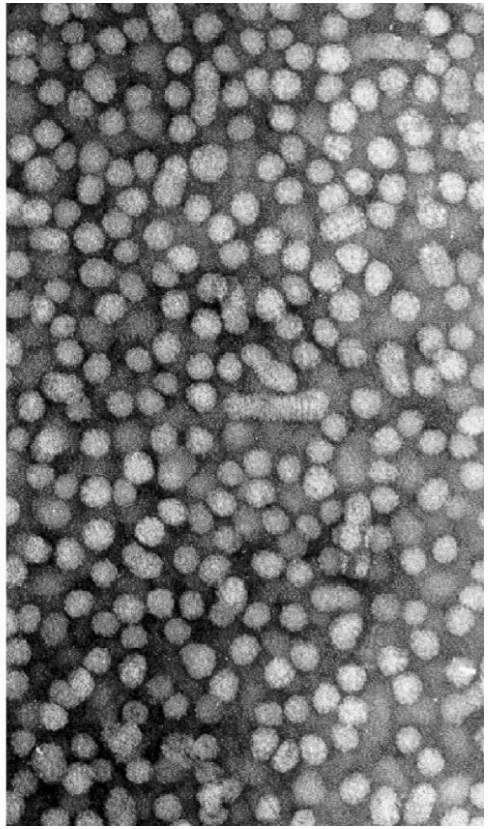
HBV virions



HBsAg filaments



20 nm HBsAg particles



Prof W. Gerlich

HBV virion to HBsAg particle ratio:

- **HBeAg positive HBsAg carriers[1,2] 1:2700 ± 1300**
- **Acute occult infection[3] < 1:20**

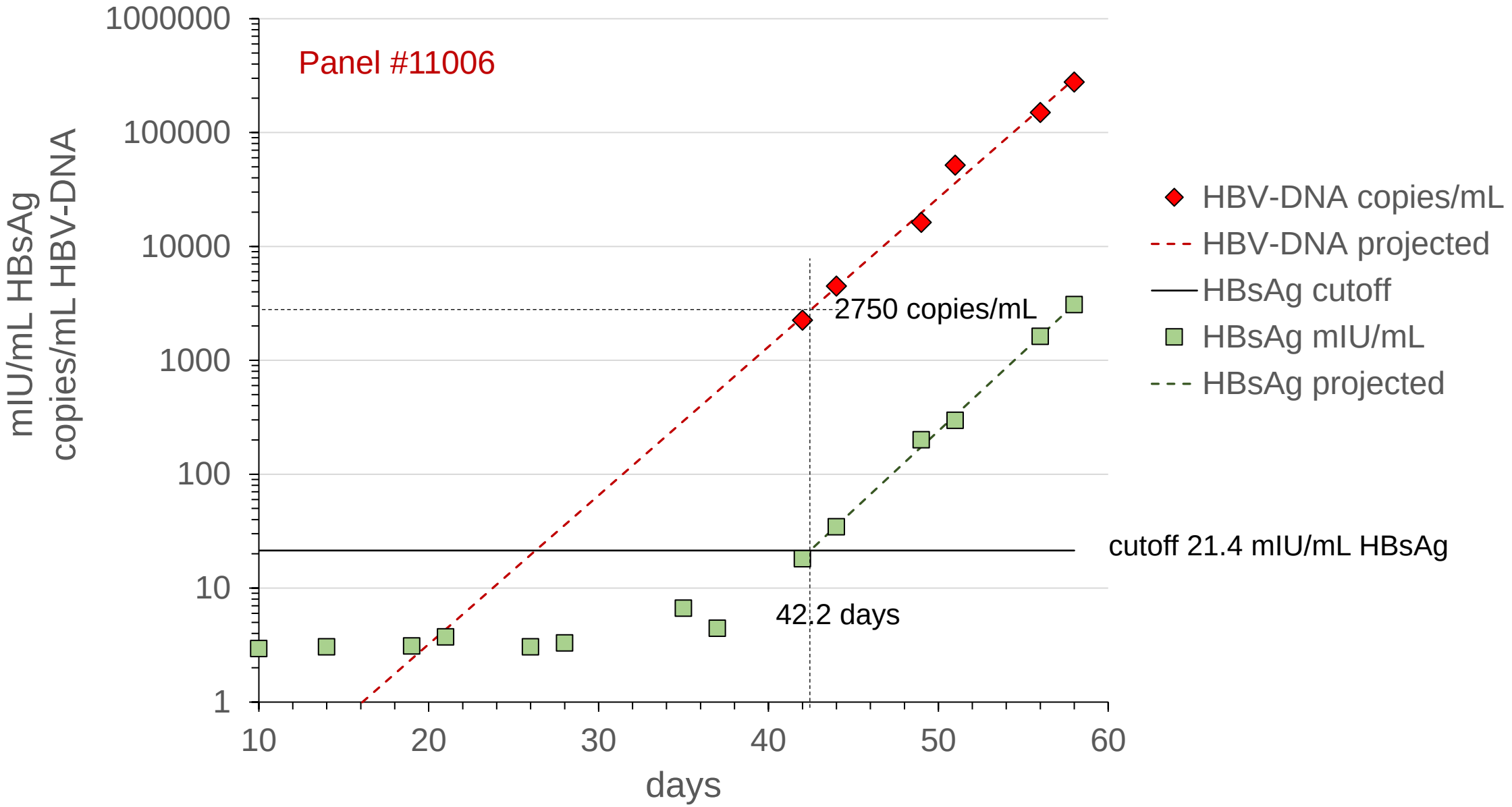
1. Gerlich et al. J Viral Hepatitis 2007:14 (Suppl 1):16-21
2. Chudy et al. J Clin Virol 2012:55:303-309
3. Bremer et al. Transfusion 2009;49:1621-29

1 nanogram (ng) HBsAg = 2.10^8 particles[4]
1 IU HBsAg = 0.67 nanogram HBsAg[5,6]
1 IU HBV-DNA = 5.3 (5.1-5.5) copies[7]

4. Gerlich et al. Biol Stand 1975;30:78-87
5. Chudy et al . J. Clin Virol 2013:58:47-53
6. Shuttler et al. J Clin Virol 2010, 47:238-242
7. Grabarczyk et al. Transfusion 2013;53:2512-2524

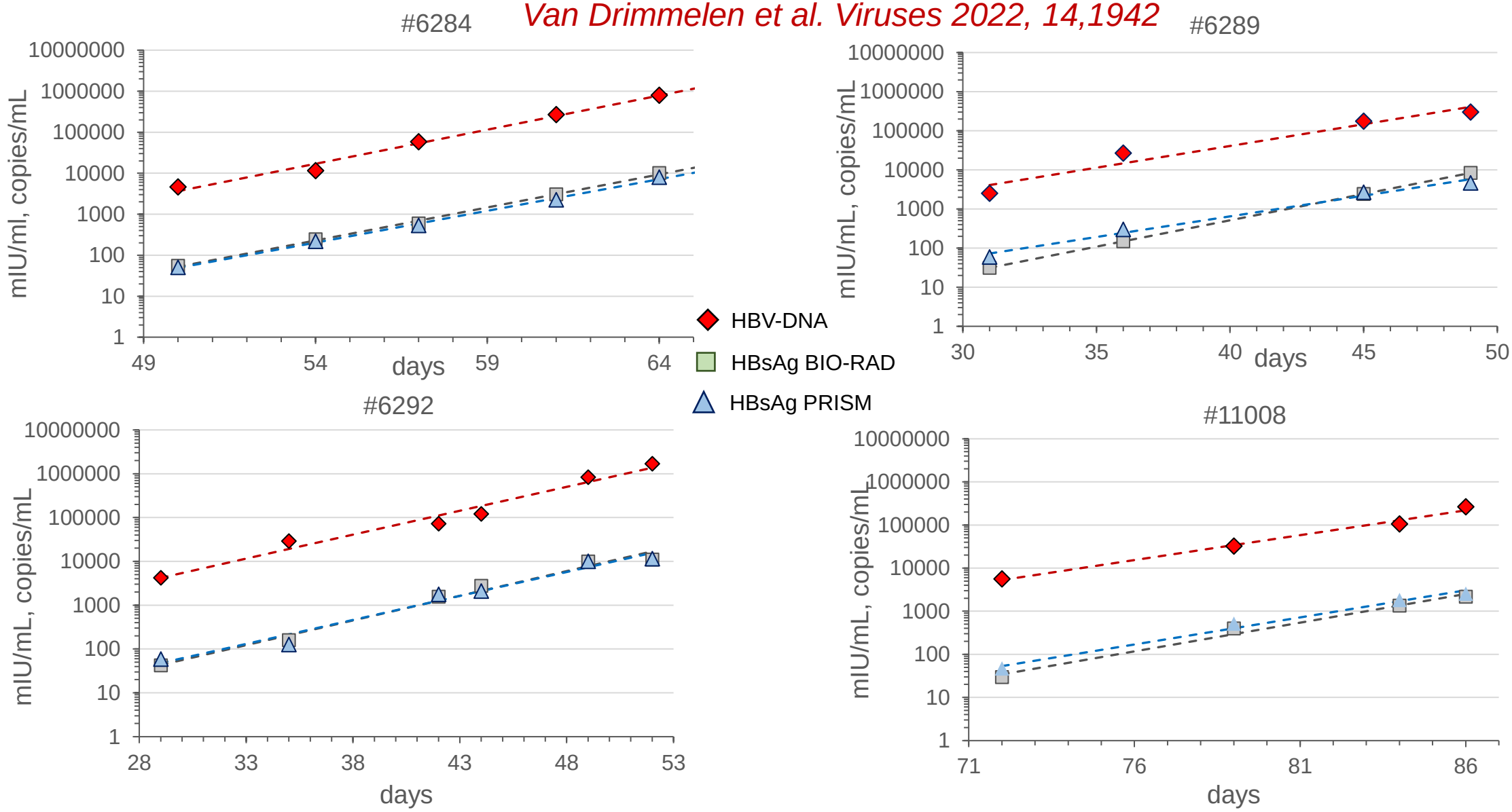
Course of HBV-DNA and HBsAg concentration in ramp-up phase of viremia

Van Drimmelen et al. Viruses 2022, 14,1942



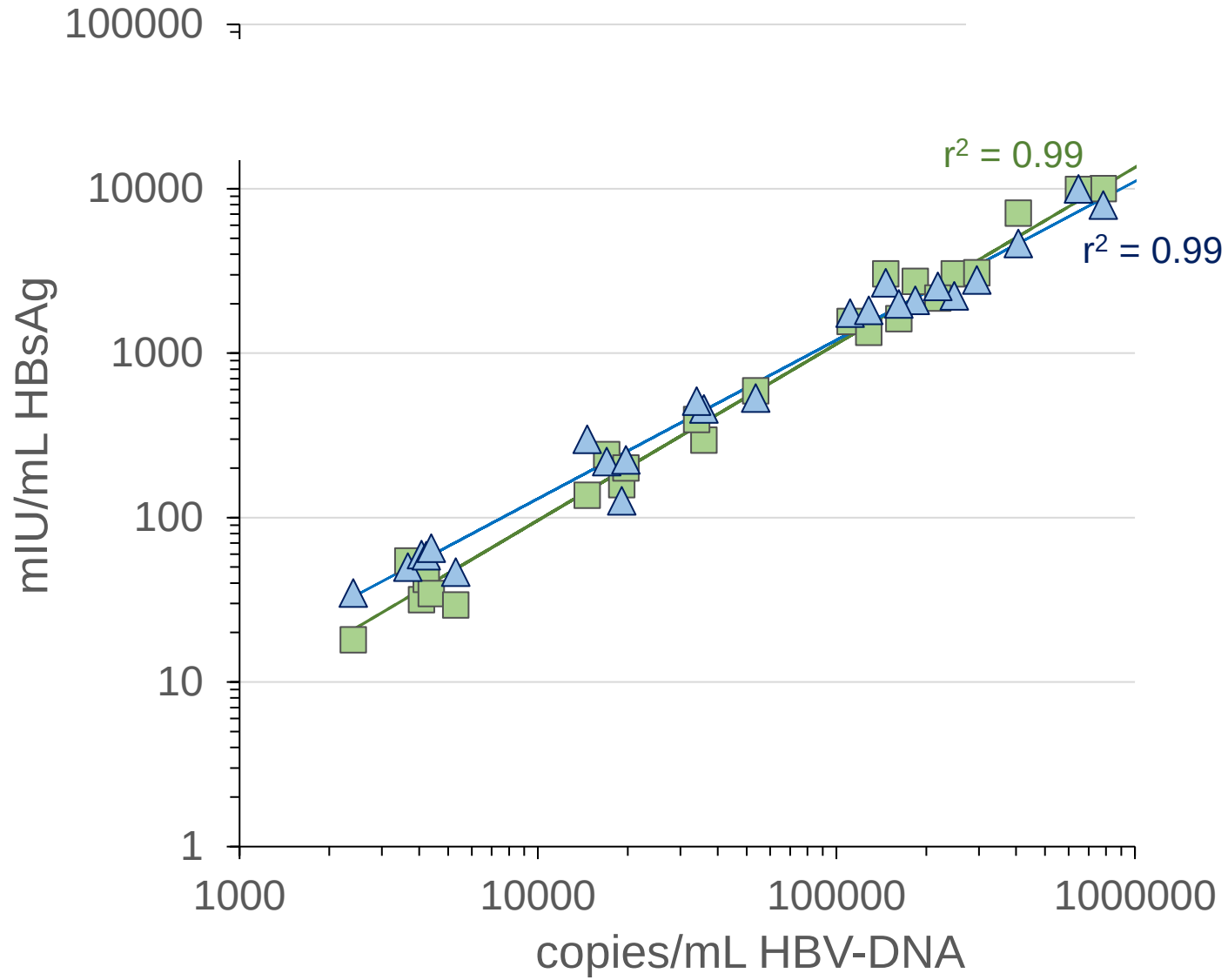
Parallel increase of HBV-DNA and HBsAg concentration in ramp-up phase of viremia

Van Drimmelen et al. Viruses 2022, 14,1942



Correlation of HBV-DNA and HBsAg concentration in ramp-up phase of viremia

Van Drimmelen et al Viruses 2022, 14,1942



HBsAg BIO-RAD

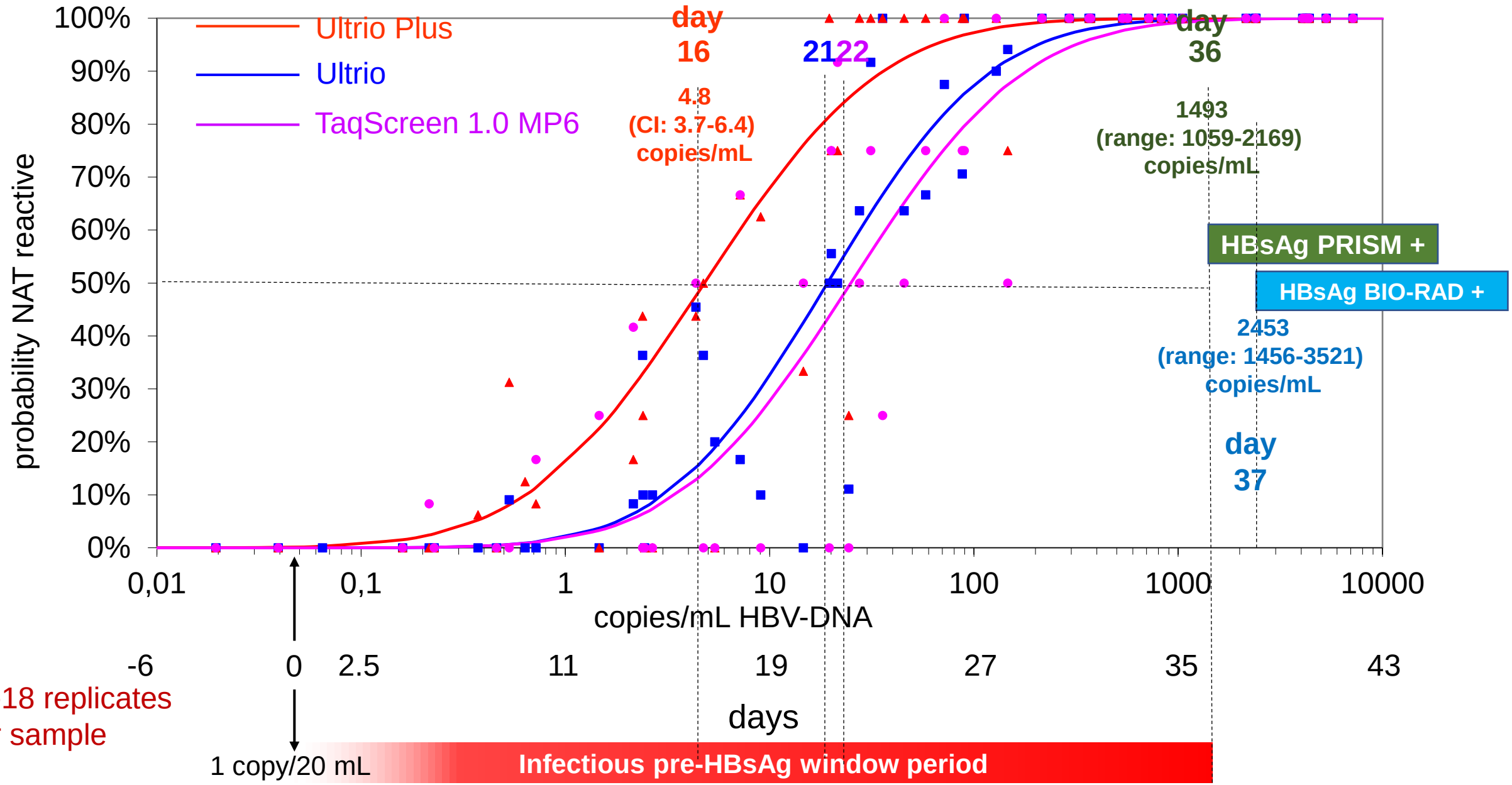
HBsAg PRISM

1 mIU = 0.67 pg HBsAg
1 pg = 2.10^5 HBsAg particles
1 IU = 5.33 copies HBV-DNA

HBV to HBsAg particle ratio (95%CI):

PRISM – 1 : 1650 (960 – 2830)
BIO-RAD – 1 : 1450 (770 – 2740)

NAT# and HBsAg conversion points in five seroconversion panels together



#4-18 replicates per sample

Van Drimmelen et al. Viruses 2022, 14,1942

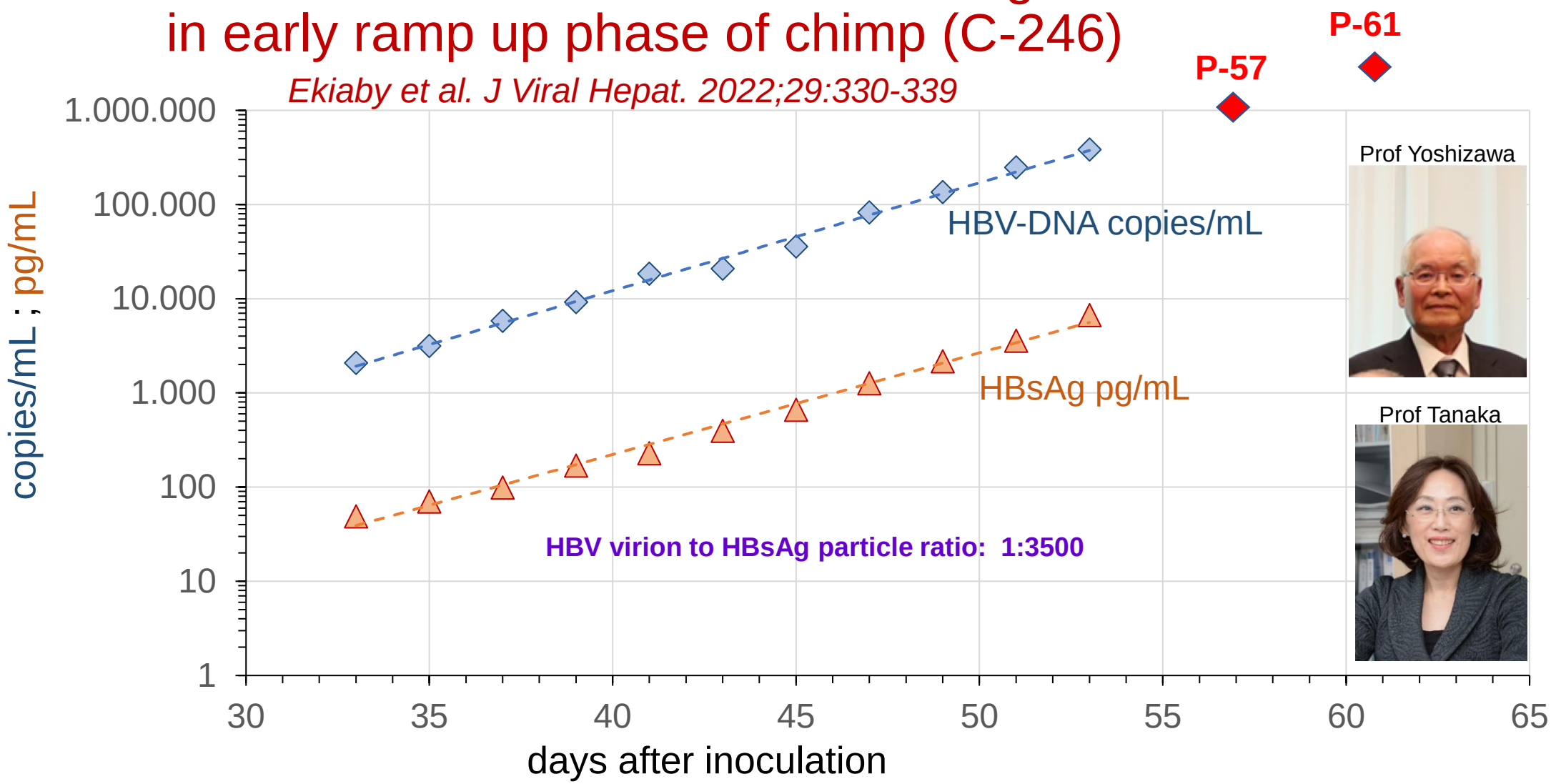
Conclusion

Van Drimmelen et al. Viruses 2022, 14,1942

- For performance evaluation of NAT assays (and antigen tests) standard dilution panels are functionally equivalent to seroconversion panels
- Limitations of seroconversion panels
  - Only 1-2 critical samples near seroconversion point
  - Genotypes originate mainly from US
  - No acceptance criteria defined in EU-IVDR-CS

Kinetics of HBV-DNA and HBsAg in early ramp up phase of chimp (C-246)

Ekiaby et al. J Viral Hepat. 2022;29:330-339



P-57 and P61 were inoculated in human liver chimeric mice with ID_{50} of ~3 (1-10) HBV-DNA copies

Kinetics of HBV-DNA and HBsAg in late declining viremic phase of chimp (C246)

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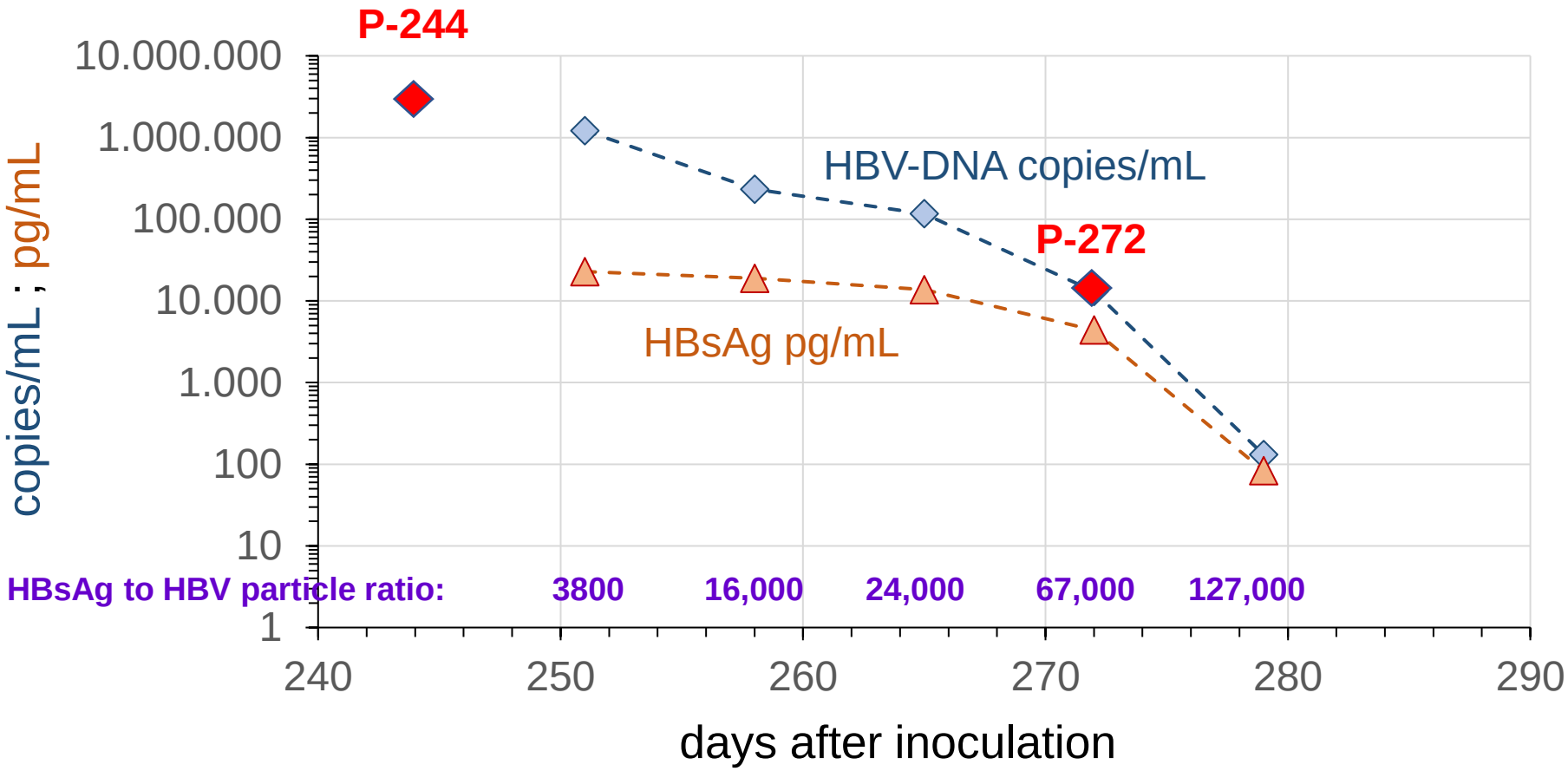

Prof Yoshizawa

Prof Tanaka

P-244 and P272 were inoculated in human liver chimeric mice with ID_{50} of ~300 (100-1000) HBV-DNA copies

HBsAg to HBV particle ratios in Egyptian donor samples

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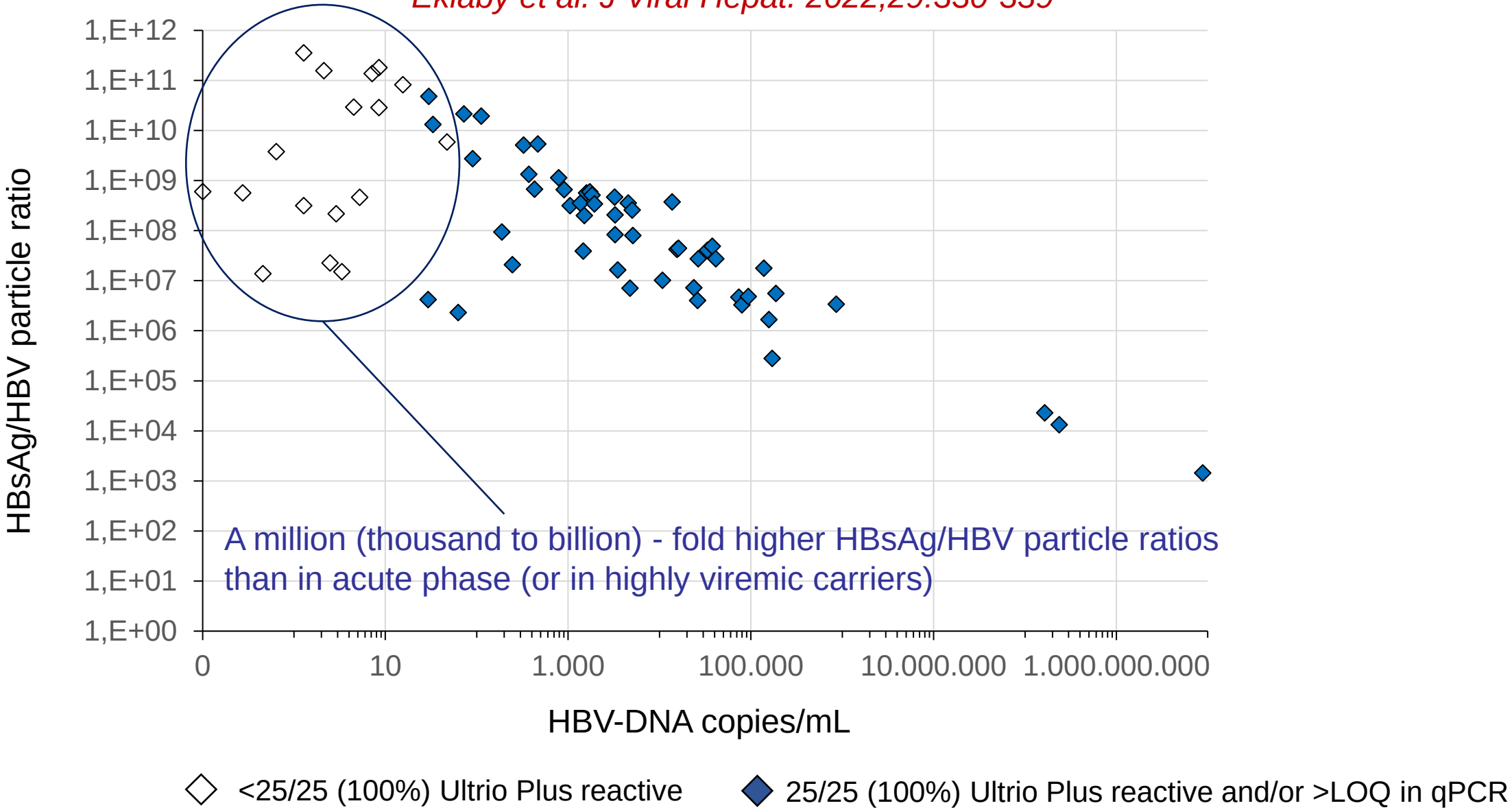

Data in 17 HBsAg+ Egyptian donor samples with lowest viral load (<25/25 (100%) reactivity in Ultrio Plus assay)

unit number	HBV-DNA cp/mL	HBsAg ng/mL	HBsAg/HBV particle ratio	Ultrio Plus r/n (%)	TaqScreen r/n
1	0.1*	0.3	6.03E+08	0/25 (0%)	0/2
2	0.3	0.9	5.67E+08	1/25 (4%)	1/2
3	0.5	0.03	1.38E+07	2/25 (8%)	0/2
4	0.6	12.2	3.79E+09	3/25 (12%)	0/2
5	1.3	2047	3.57E+11	6/25 (24%)	2/2
6	1.3	1.8	3.16E+08	6/25 (24%)	0/2
7	2.1	1379	1.57E+11	9/25 (36%)	1/2
8	2.5	0.23	2.28E+07	10/25 (40%)	1/2
9	2.9	2.47	2.18E+08	11/25 (44%)	1/2
10	3.3	0.19	1.51E+07	12/25 (48%)	2/2
11	4.5	419	2.94E+10	14/25 (56%)	1/1
12	5.2	8.6	4.63E+08	15/25 (60%)	2/2
13	7.2	3357	1.38E+11	17/25 (68%)	1/2
14	8.5	807	2.87E+10	18/25 (72%)	2/2
15	8.5	5079	1.81E+11	18/25 (72%)	2/2
16	15.5	3839	8.22E+10	21/25 (84%)	1/2
17	35.0	696	5.64E+09	24/25 (96%)	2/2

*imputed

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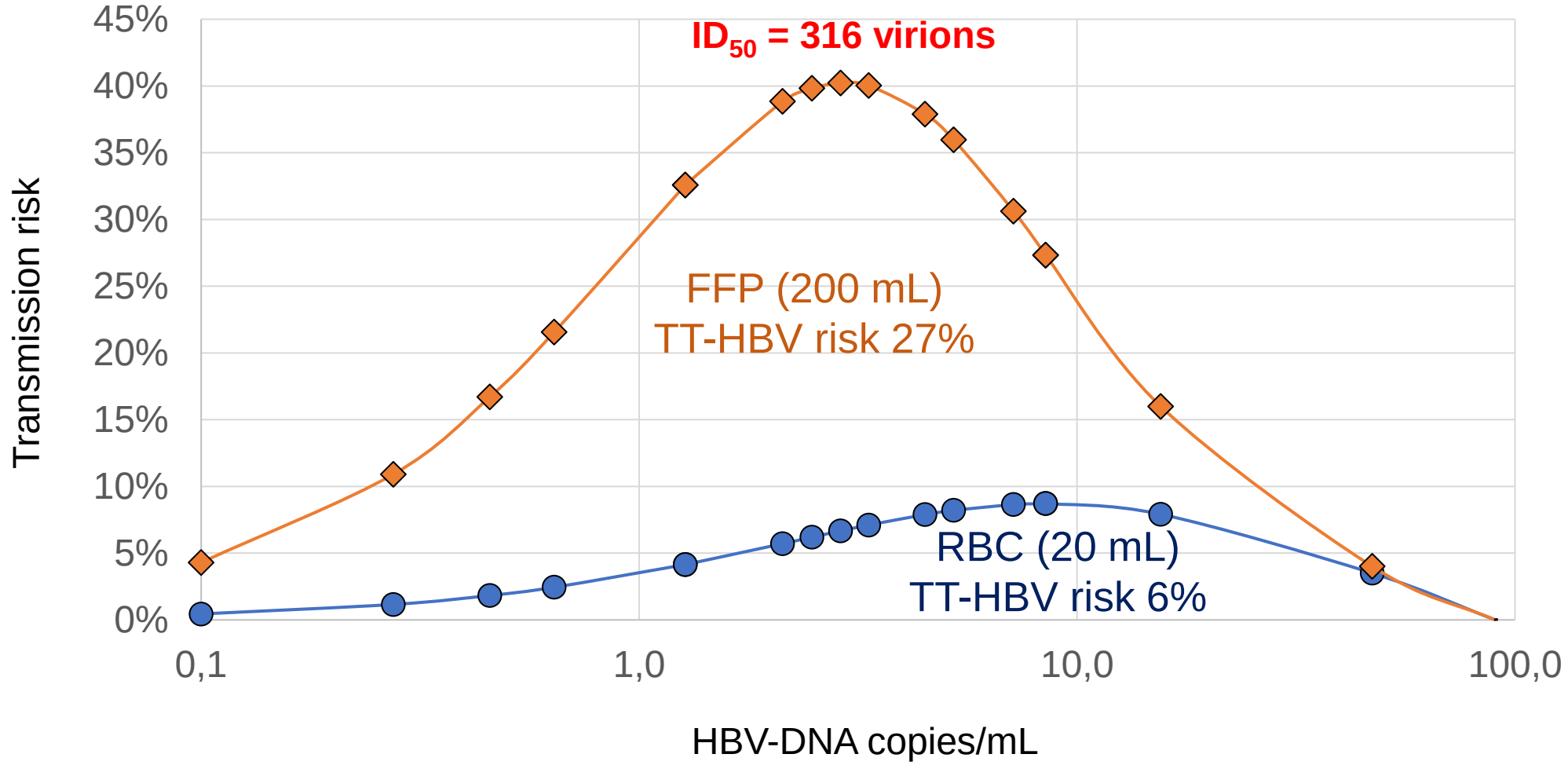
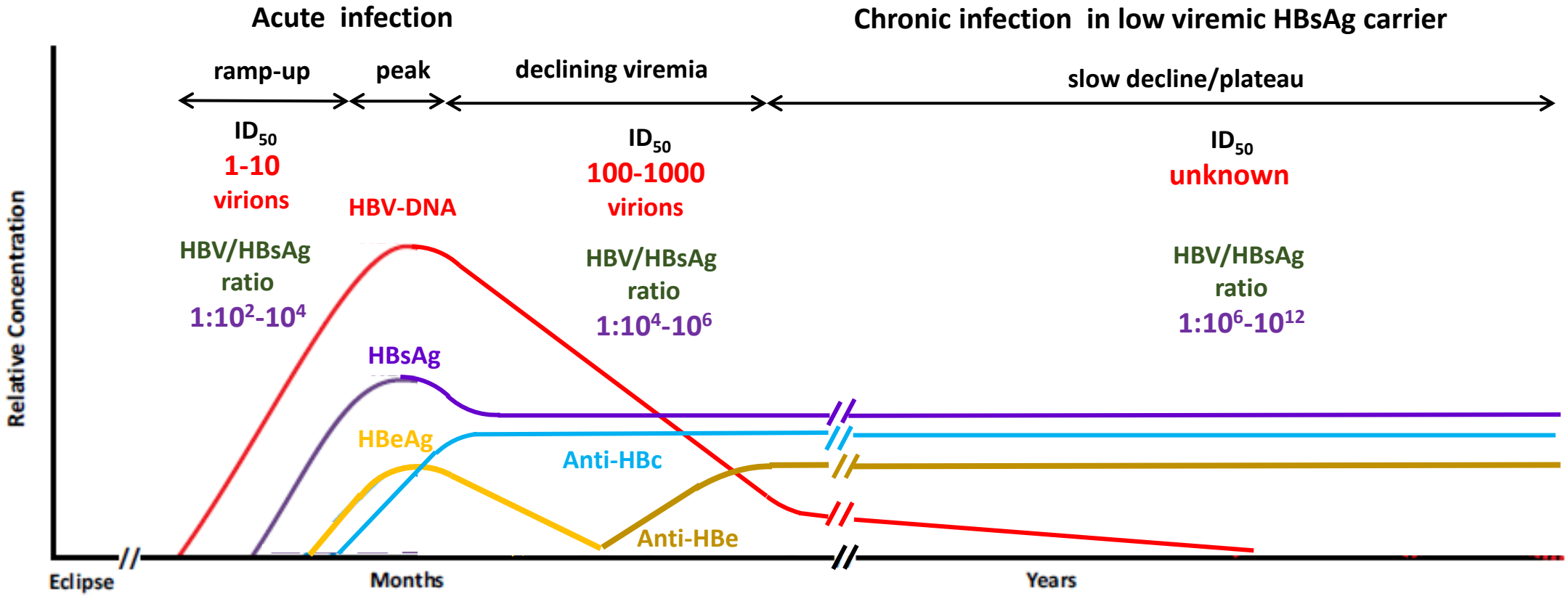
Probability of HBV transmission in 17 HBsAg+ Egyptian donor samples with less than 25/25 (100%) ID-NAT (Ultrio Plus) reactivity

Ekiaby et al. J Viral Hepat. 2022;29:330-339



1. Weusten et al Transfusion 2011;51:203-15 (WP)
2. Weusten et al. Transfusion 2017;57:841-849 (OBI).

Conclusion and discussion



- HBsAg positive blood without detectable HBV-DNA seems not safe and may be infectious in ~6% of RBC and ~27% of FFP transfusions.
- Infectivity of HBV in anti-HBe+ HBsAg carriers without detectable HBV-DNA is unknown (possible neutralization by anti-PreS 1 antibodies).

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