Scientific validity of standards and controls for blood screening assays



Nico Lelie

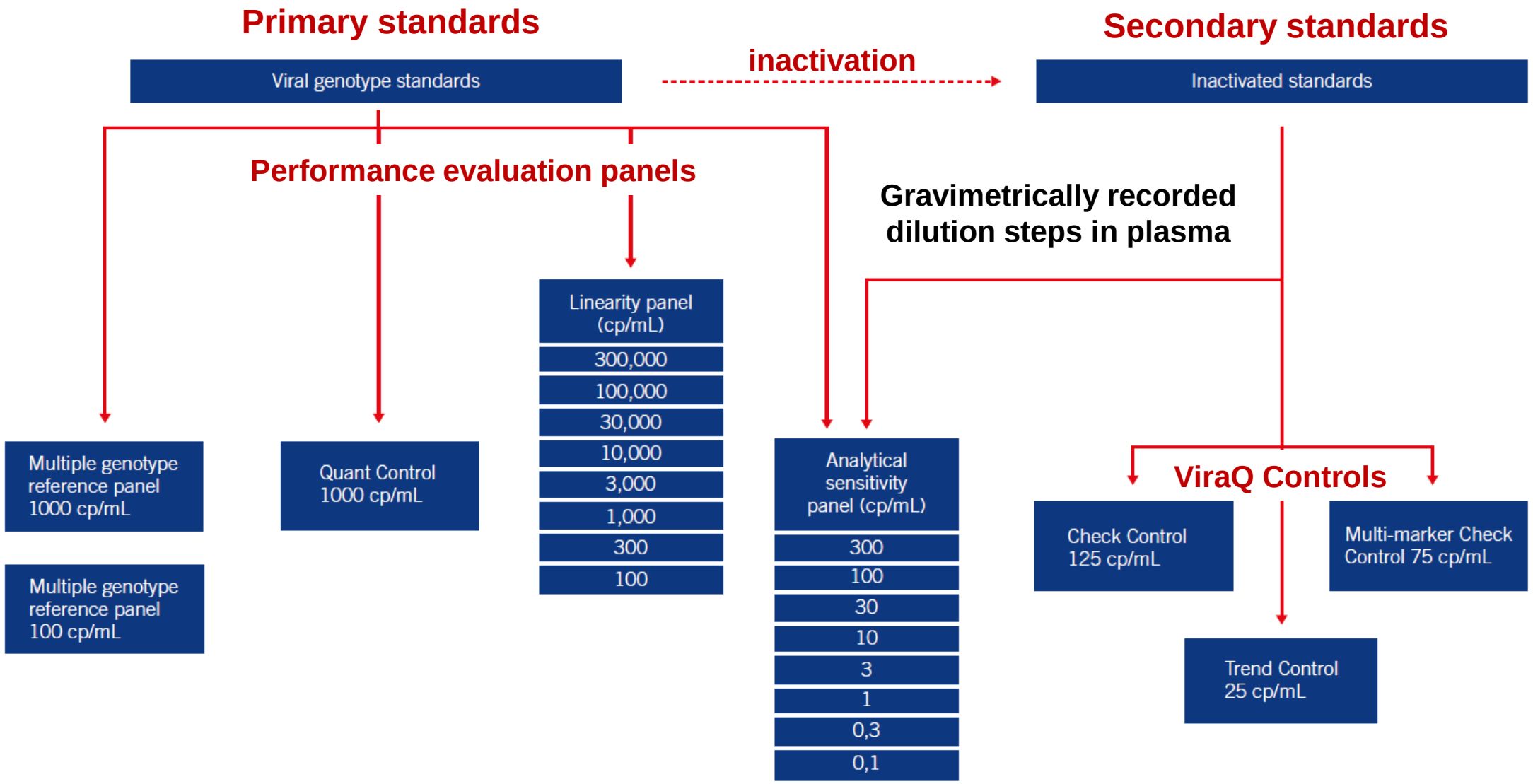
Biologicals Quality Control, De Droogmakerij 31h, Heiloo, The Netherlands

IPFA/PEI 30th International Workshop on Surveillance and Screening of Blood-borne Pathogens, Aarhus, Denmark, May 15-16

www. bioqcontrol.com

Bio QControl

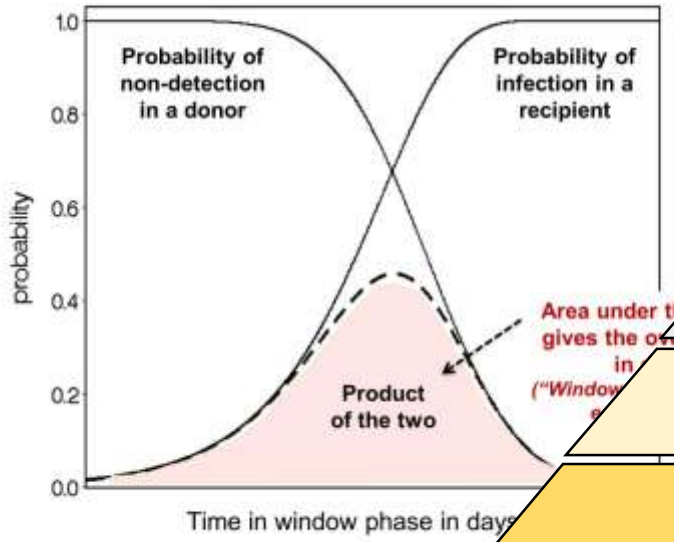
Preparation of panels and controls for NAT since 1992#



First international (Eurohep) proficiency study (Zaaijer et al. Lancet 1993;341:722-724)

Foundations for estimating TT-HXV risk reduction by NAT assays

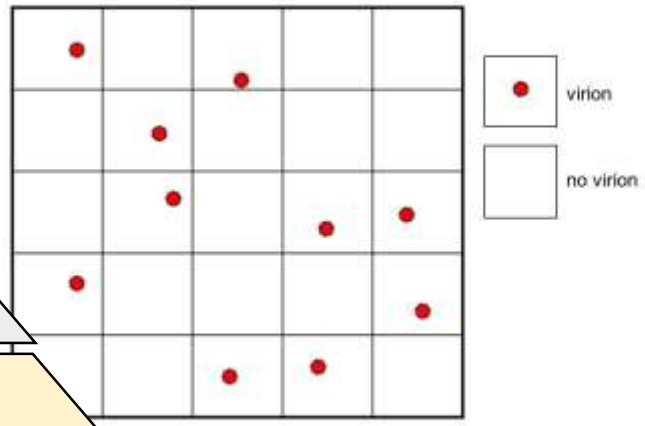
Probability of infectivity during the window period



Weusten J et al, Transfusion 2011;51:203-15

Poisson distribution

Probability of NAT detection and infectivity in WP or OBI



Screening efficacy (% risk avoided)

Residual risk (transmissions/million)

Infectivity in each infection stage (ID_{50})

Analytical sensitivity of assays (50%, 95% LOD)

Standardization in virions (nucleic acid copies)

Prevalence and incidence of viral markers (donor epidemiology)

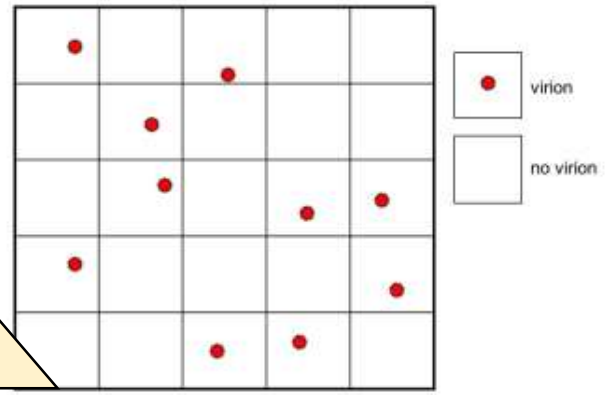
Confirmatory testing to exclude false positive results (NAT & serology)

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

Foundations for estimating TT-HXV risk reduction by NAT assays

Poisson distribution

Probability of NAT detection and infectivity in WP or OBI



Scientific validity of standards and controls

Screening efficacy (% risk avoided)

Residual risk (transmissions/million)

Infectivity in each infection stage (ID_{50})

Run controls

Analytical sensitivity of assays (50%, 95% LOD)

Standard dilutions

Standardization in virions (nucleic acid copies)

Prevalence and incidence of viral markers (donor epidemiology)

Confirmatory testing to exclude false positive results (NAT & serology)

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



Calibration of HBV-DNA standards

process step

calibration

HBV-DNA calibrator molecules in bDNA 3.0 assay

n=28

EUROHEP HBV-DNA genotype A standard 2.7 10^9 Eurohep Units/mL

1st WHO HBV-DNA 97/746 standard 500,000 IU/vial

5.33 (5.11-5.55) cp/IU

n=28

Primary S0011 VQC-Sanquin HBV-DNA genotype A standard

2nd WHO HBV-DNA 97/750 standard 500,000 IU/vial

5.20 (5.61-5.80) cp/IU

n=12

3rd WHO HBV-DNA 10/264 standard 425,000 IU/vial

- 1:100 dilution in PBS,
- 10 hours pasteurization at 65º
- centrifugation
- dilution of supernatant in plasma

n=12

84% recovery in bDNA 3.0 assay

P0065 ViraQ HBV Check Control 125 (83-187) cp/mL

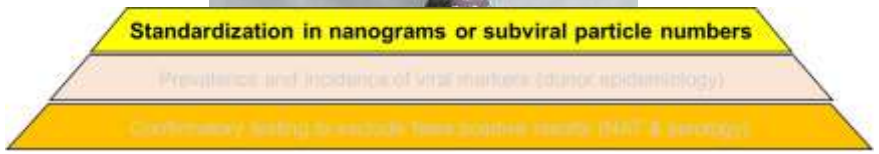
S0043 HBV-DNA genotype A inactivated standard

Bio QControl

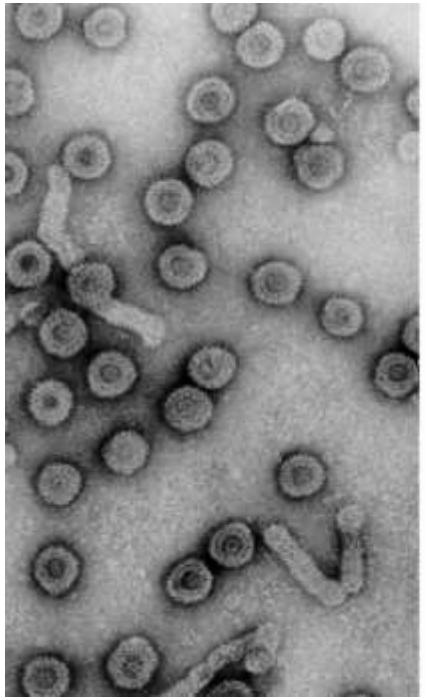
Ratio of HBV virions to subviral HBsAg particles

Prof W. Gerlich





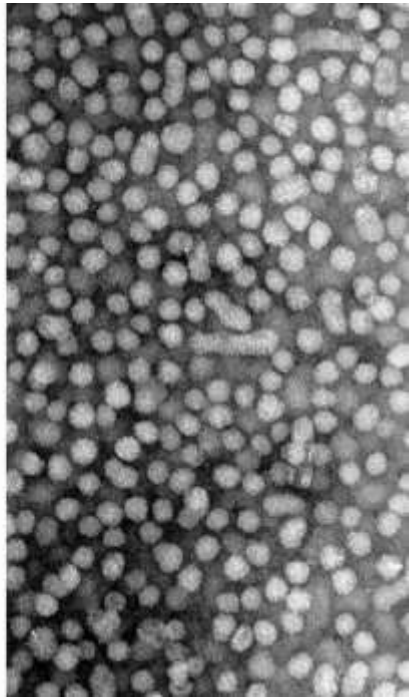
HBV virions



HBsAg filaments



HBsAg particles



HBV virion to HBsAg particle ratio:

- **Acute occult infection[1] < 1:20**
- **Acute ramp up phase[2,3] 1:1700 (500-3500)**
- **HBeAg positive HBsAg carriers[4] 1:2700 ± 1300**
- **Late acute phase[3] 1:3800 – 1:127,000**
- **HBeAg negative carriers[3] 1:10^9 (10^6 – 10^{12})**

1 nanogram (ng) HBsAg = 2.10^8 particles[4,5]
1 IU HBsAg = 0.67 nanogram HBsAg[6,7]
1 IU HBV-DNA = 5.3 (5.1-5.5) copies[9]

1. Bremer et al. Transfusion 2009;49:1621-29
2. Van Drimmelen et al. Viruses 2022:14:1942
3. El Ekiaby et al. J Viral Hepatitis: 2022:29:330-22:330-339
4. Gerlich et al. J Viral Hepatitis 2007:14 (Suppl 1):16-21
5. Gerlich et al. Biol Stand 1975;30:78-87
6. Chudy et al. J Clin Virol 2012:55:303-309
7. Chudy et al . J. Clin Virol 2013:58:47-53
8. Shuttler et al. J Clin Virol 2010, 47:238-242
9. Grabarczyk et al. Transfusion 2013;53:2512-2524

Calibration of chimpanzee plasma of known infectivity against HBV genotype A standard in bDNA 3.0 assay

Primary standard	Chimp infectivity plasma^	n S0011	n Chimp	copies/CID_{50} (range)
S0011 HBV gt A	C-246 (P-57) gt A	6	6	4.0 (1.3-12.6)

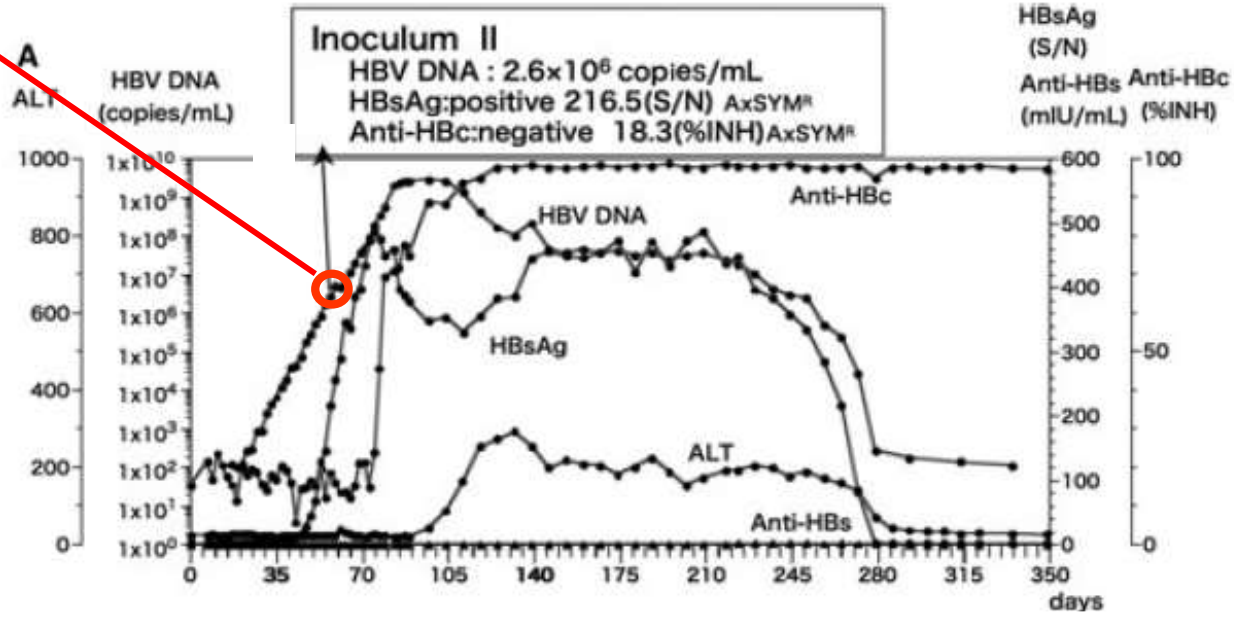
Prof Yoshizawa

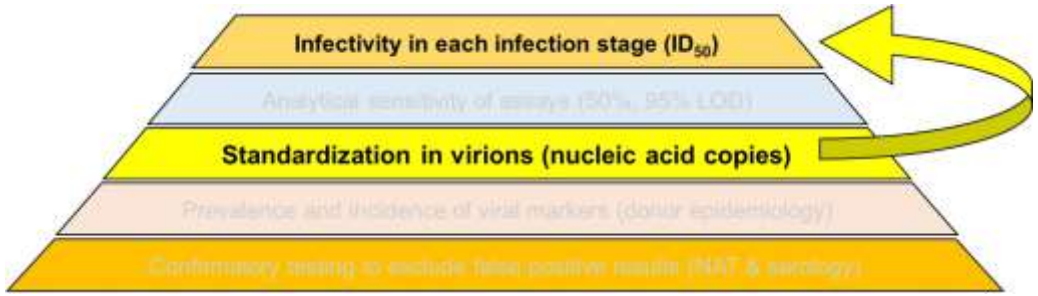


Prof Tanaka



^Komiya K et al. Transfusion 2008;48:286-9





Sample C-246 (P57) was kindly provided by Prof Yoshizawa and Prof Tanaka, Hiroshima University, Japan

Bio QControl

viruses

Viruses 2022, 14,1942

MDPI

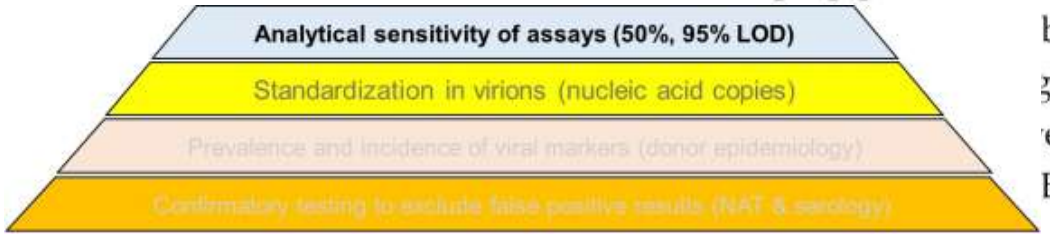
Article

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,*]

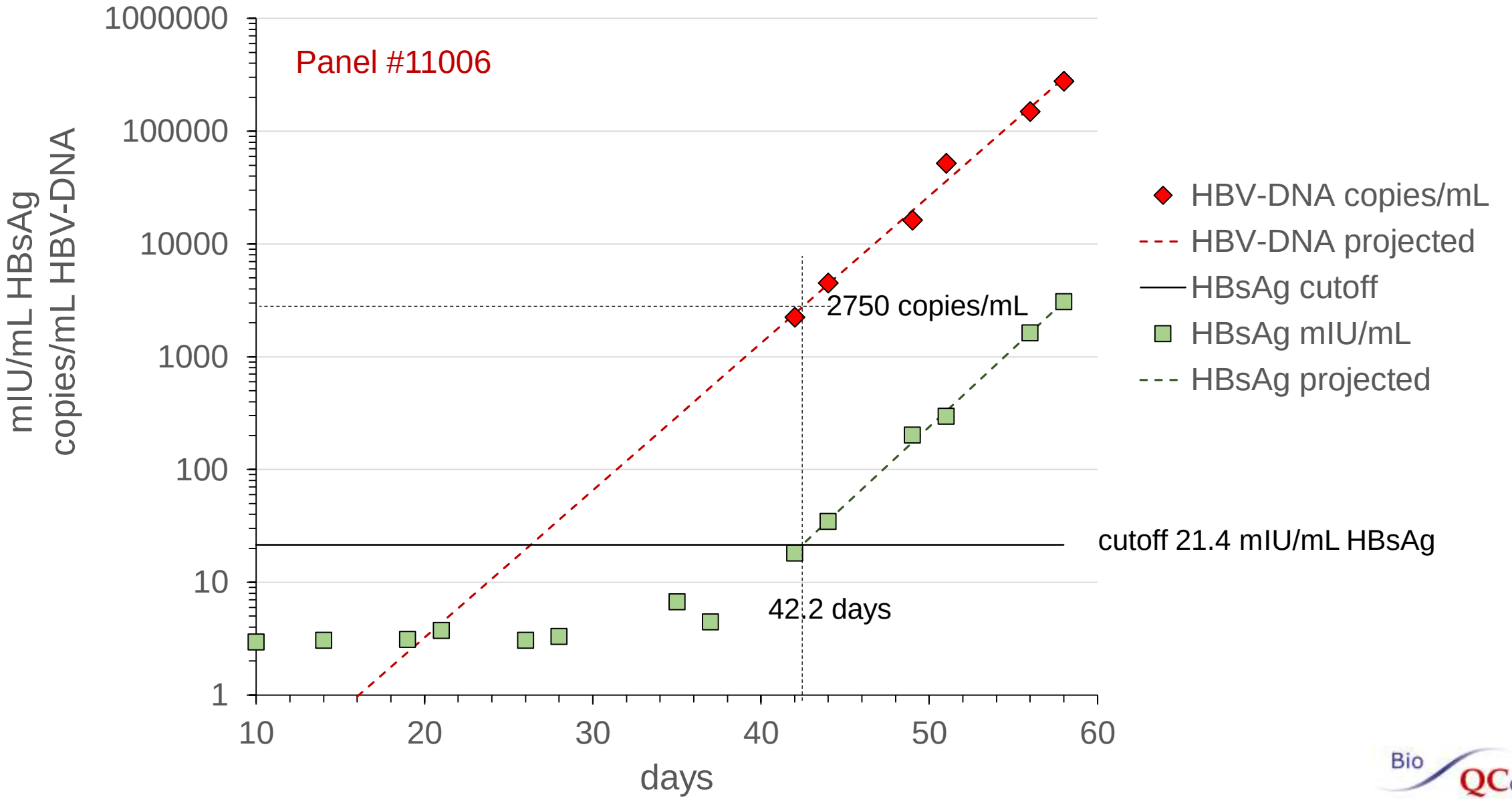
Can seroconversion panels be replaced by standard dilution panels?

Abstract: The Common Specifications/EU 2017/746 regulation for market approval of class D in vitro diagnostic devices (IVDs) intended for detection of blood borne viruses requires testing of the International Standard and 10–30 seroconversion panels to demonstrate 'state of the art' assay performance. We examined whether these requirements for performance evaluation are reasonable for HBV-DNA and HBsAg assays. For this purpose, we quantified HBsAg and HBV-DNA (genotype A) in the ramp-up phase of five seroconversion panels and demonstrated a remarkably parallel increase both analytes over time. Testing of seroconversion panels by three nucleic gy (NAT) methods in multiple replicates and probit analysis with sufficient e panels taken together showed detection limits in copies/mL that were BV-DNA genotype A standard dilution panel. This indicates that the viral



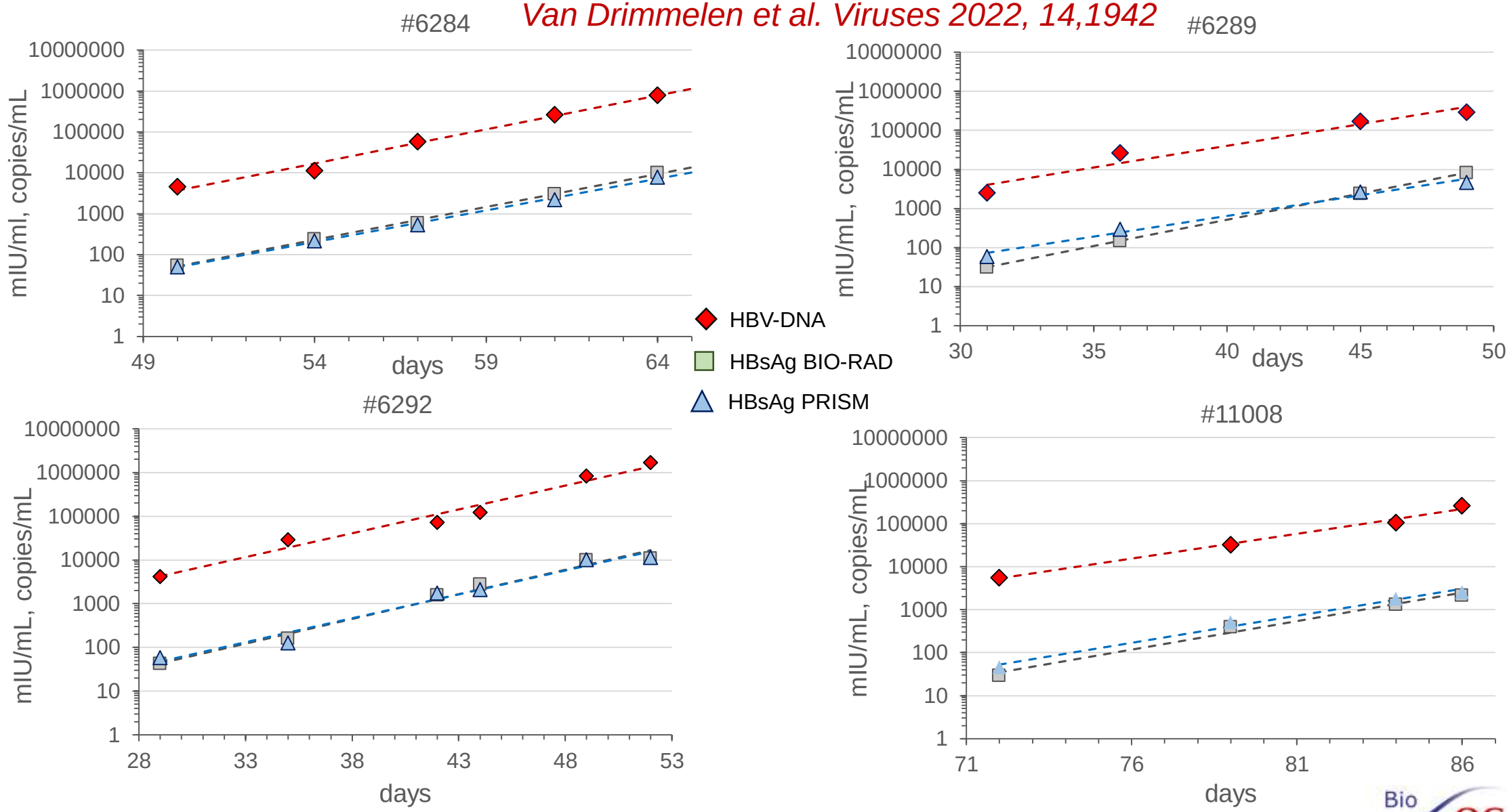
Course of HBV-DNA and HBsAg concentration in ramp-up phase of viremia

Van Drimmelen et al. Viruses 2022, 14,1942

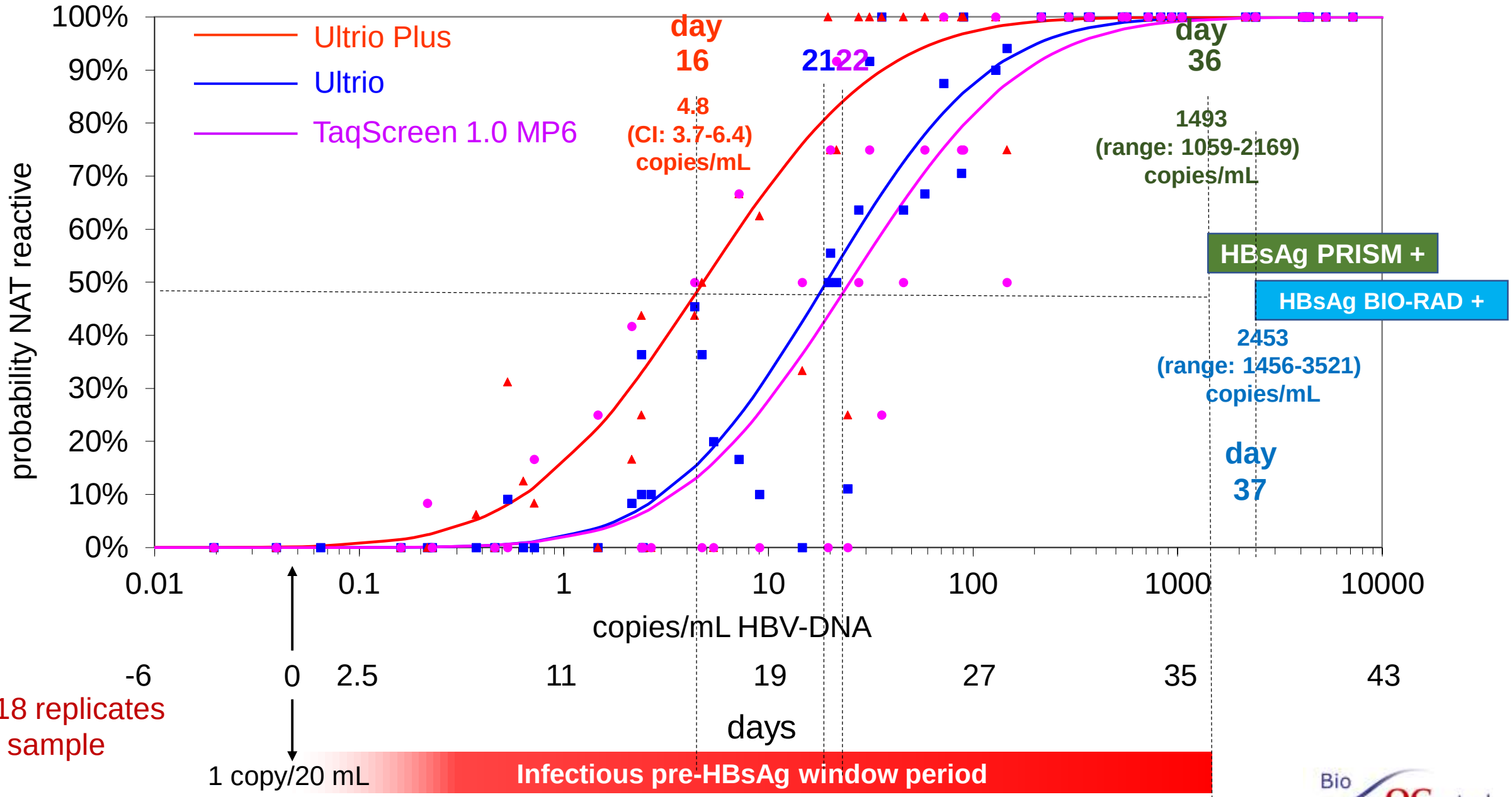


Parallel increase of HBV-DNA and HBsAg concentration in ramp-up phase of viremia

Van Drimmelen et al. Viruses 2022, 14,1942



NAT# and HBsAg conversion points in five seroconversion panels together

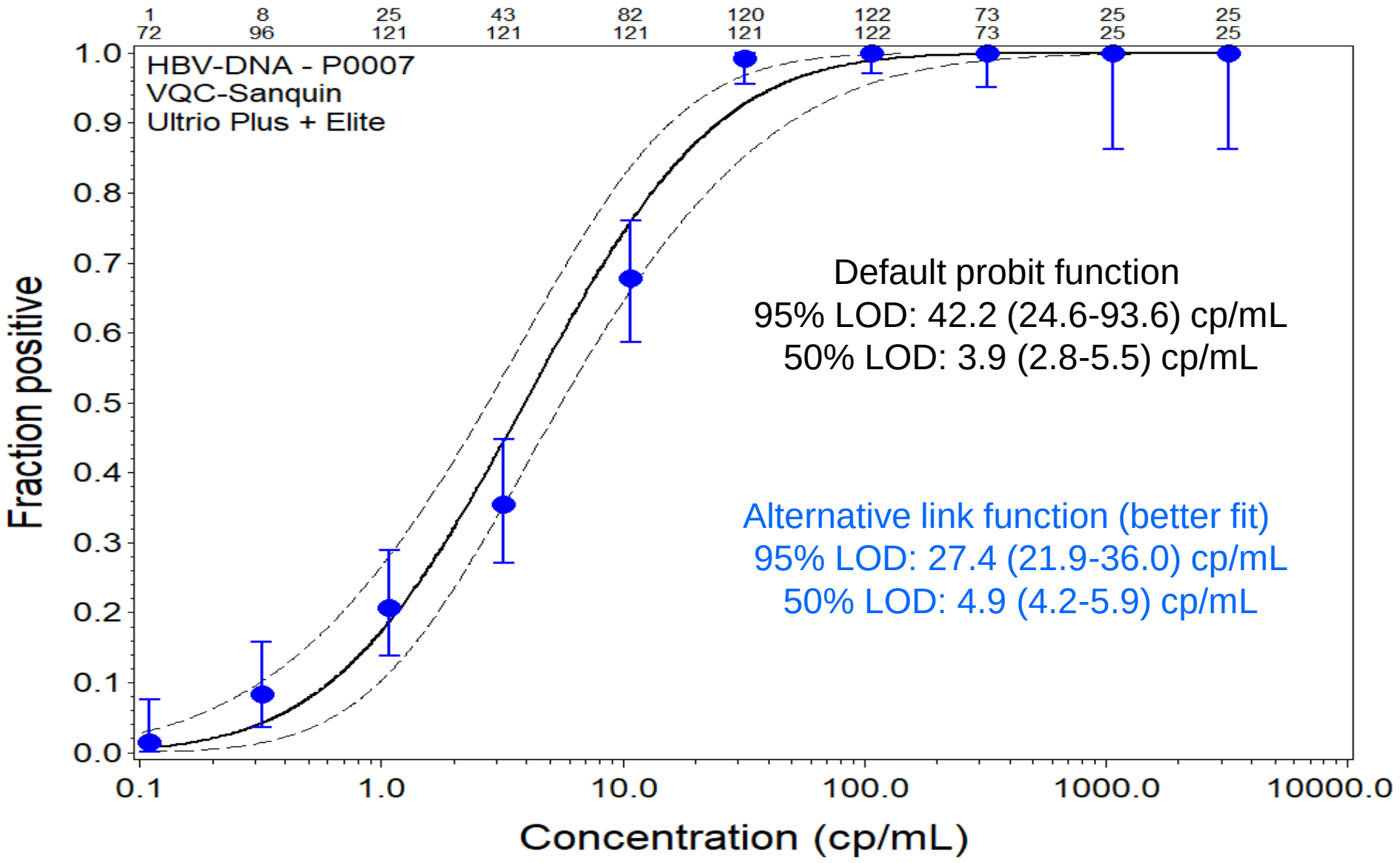


#4-18 replicates per sample

Van Drimmelen et al. Viruses 2022, 14,1942

Bio QControl

NAT detection limit on S0012 HBV-DNA standard dilutions



Bio QControl

NAT detection limits on primary HBV, HCV, and HIV-1 standards calibrated in copies/mL and IU/mL^

Panel no	Primary standard	marker and genotype	Assay	n	50% LOD (95% CI)# copies/mL	95% LOD (95% CI)# copies/mL
P0007	S0012 VQC-Sanquin	HBV-DNA gt A	Ultrio Plus/Elite	121	4.9 (4.2-5.9)	27.4 (21.9-36.0)
P0007	S0012 VQC-Sanquin	HBV-DNA gt A	cobas MPX	24	2.3 (1.6-3.3)	11.0 (7.3-20.5)
P0272	S0010 Eurohep	HBV-DNA gt A	cobas MPX	118	2.0 (1.7-2.3)	8.3 (7.0-10.3)
P0019	S0009 VQC-Sanquin	HCV-RNA gt 1	Ultrio Plus/Elite	160	2.1 (1.9-2.5)	8.9 (7.5-11.0)
P0019	S0009 VQC-Sanquin	HCV-RNA gt 1	cobas MPX	60	3.5 (2.7-4.4)	16.5 (12.5-23.8)
P0272	S0009 VQC-Sanquin	HCV-RNA gt 1	cobas MPX	118	3.7 (3.2-4.1)	14.4 (12.6-17.1)
P0025	S0012 VQC-Sanquin	HIV-1 RNA gt B	Ultrio Plus/Elite	78	2.2 (1.8-2.7)	10.1 (7.8-14.1)
P0272	S0012 VQC-Sanquin	HIV-1 RNA gt B	cobas MPX	166	1.6 (1.4-1.8)	6.8 (5.8-8.1)

^conversion factors according to calibration in bDNA 3.0 assay:
1 IU HBV-DNA = 5.33 copies (1st WHO standard)
1 IU HCV-RNA = 2.73 copies (1st and 2nd WHO standard)
1 IU HIV-1 RNA = 0.58 copies (2nd WHO standard)

LODs calculated by alternative link function for better fit of probit curves

Bio QControl

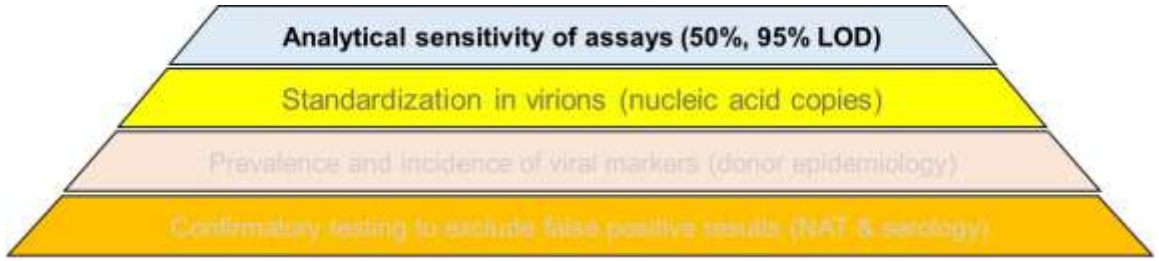
Conclusion

Early dynamics of viremia and antigenemia: implications for performance evaluation of blood screening assays

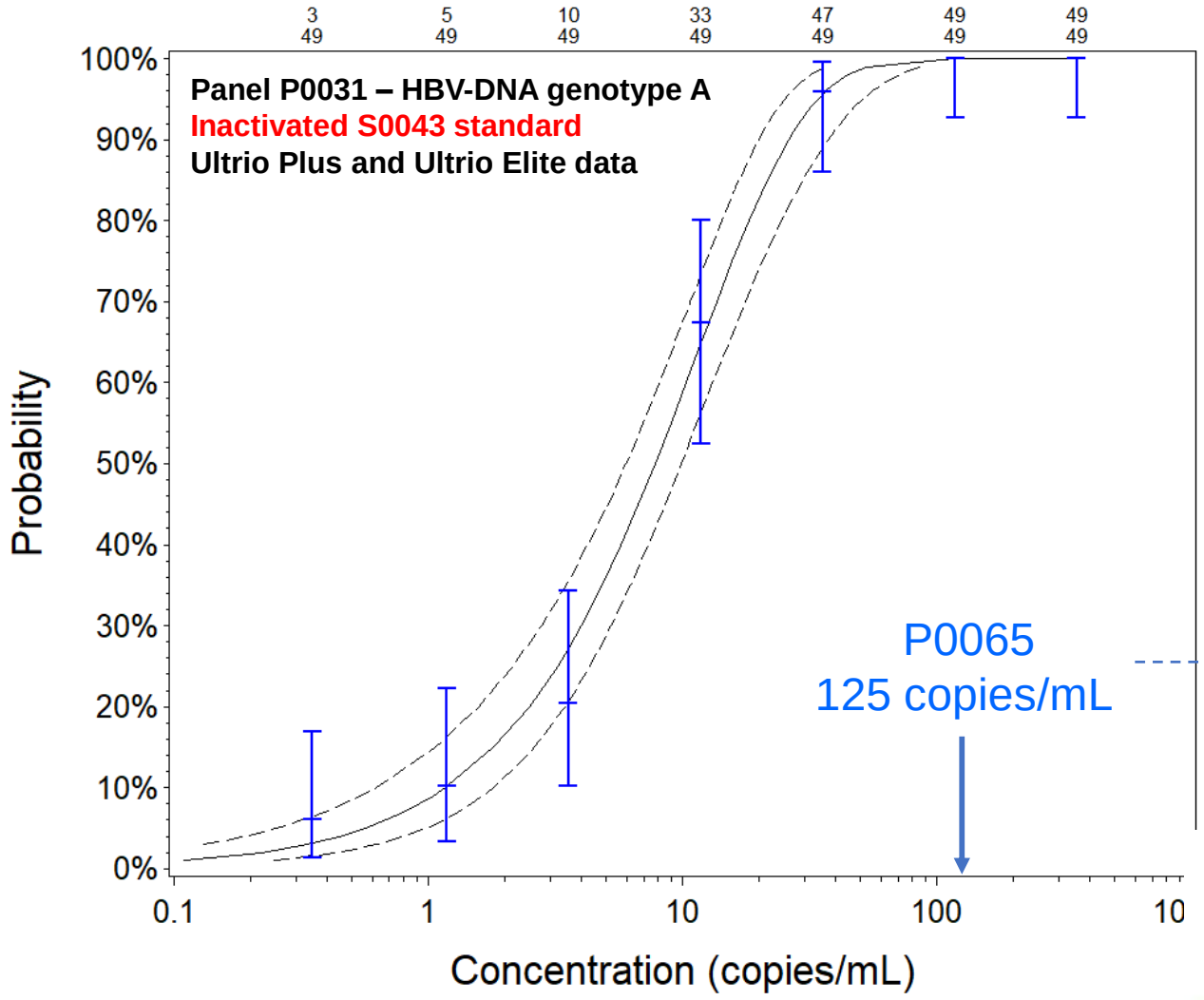
Van Drimmelen et al. Viruses 2022, 14,1942

- For performance evaluation of NAT and antigen assays standard dilution panels are functionally equivalent to seroconversion panels

- Limitations of seroconversion panels
  - Only 1-3 critical samples near seroconversion point
  - Genotypes originate mainly from US



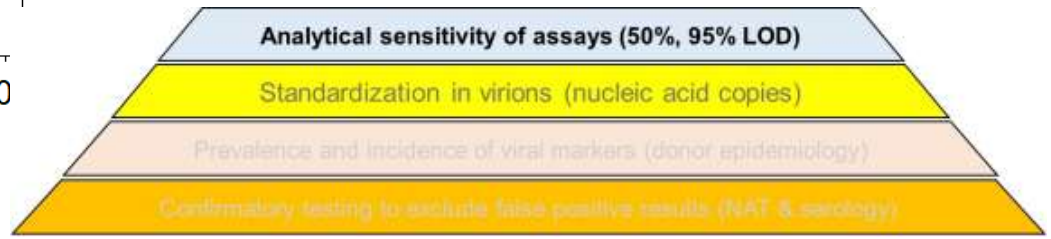
Positioning of ViraQ HBV Check 125 Control in Ultrio Plus and Ultrio Elite assay



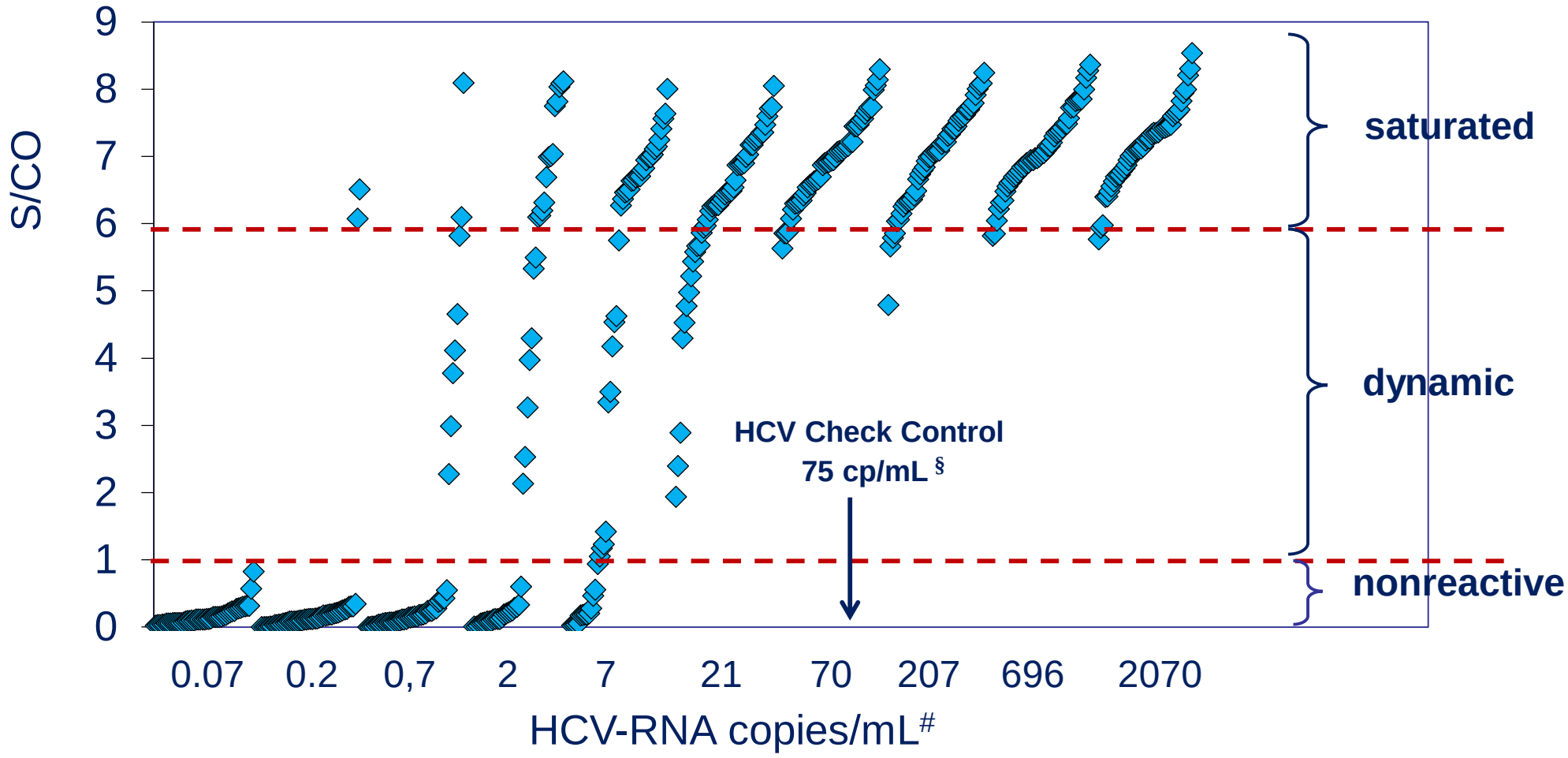
P0065
125 copies/mL

Alternative link function (n=49)
95% LOD: 33.9 (25.2-50.9) copies/mL
50% LOD: 7.8 (6.0-9.8) copies/mL

4 - 5 times 95% LOD



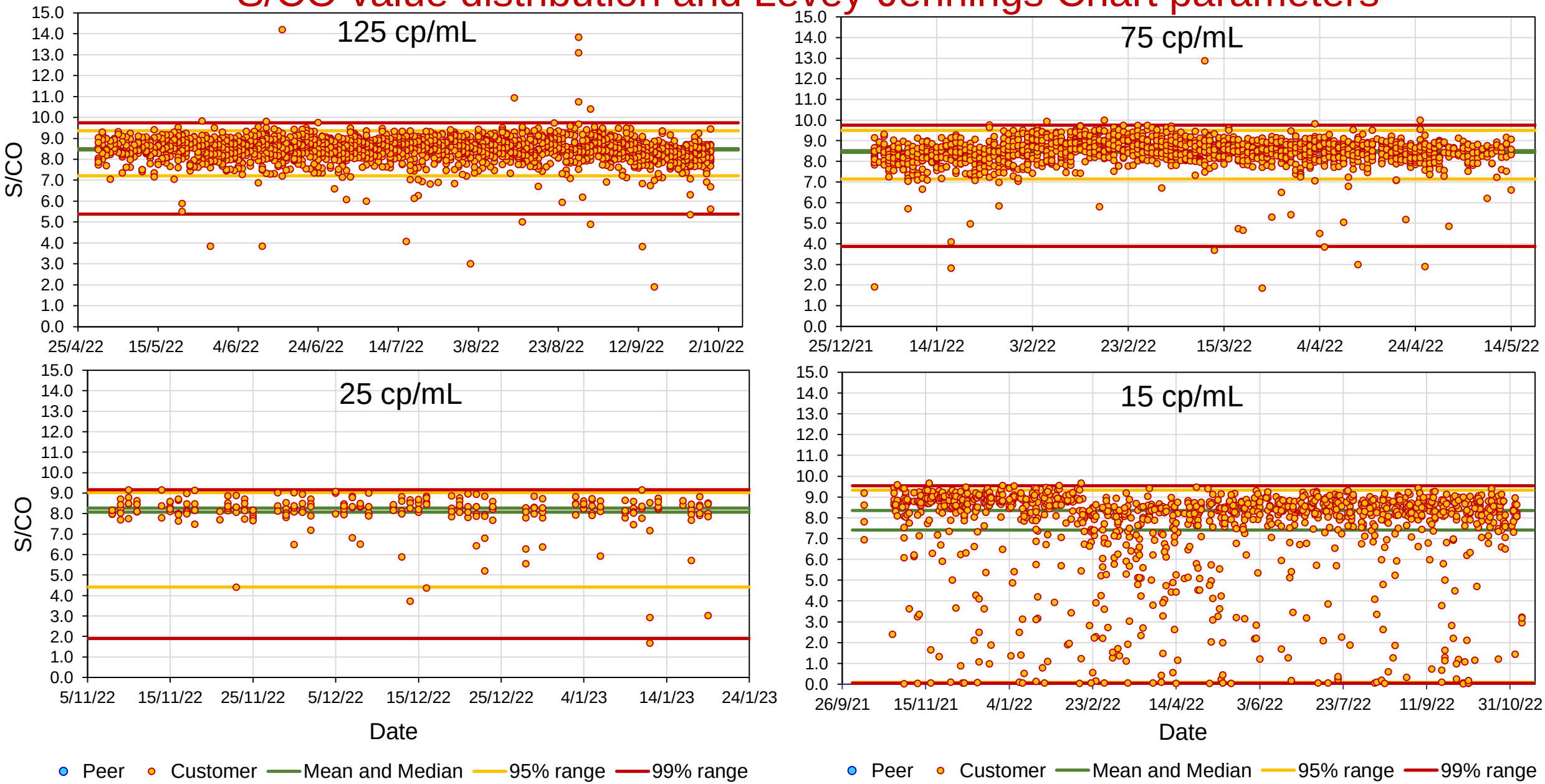
S/CO distribution of Ultrio assay on beta-propiolactone inactivated S0109 HCV-RNA genotype 3 standard dilutions and positioning of ViraQ Check Control



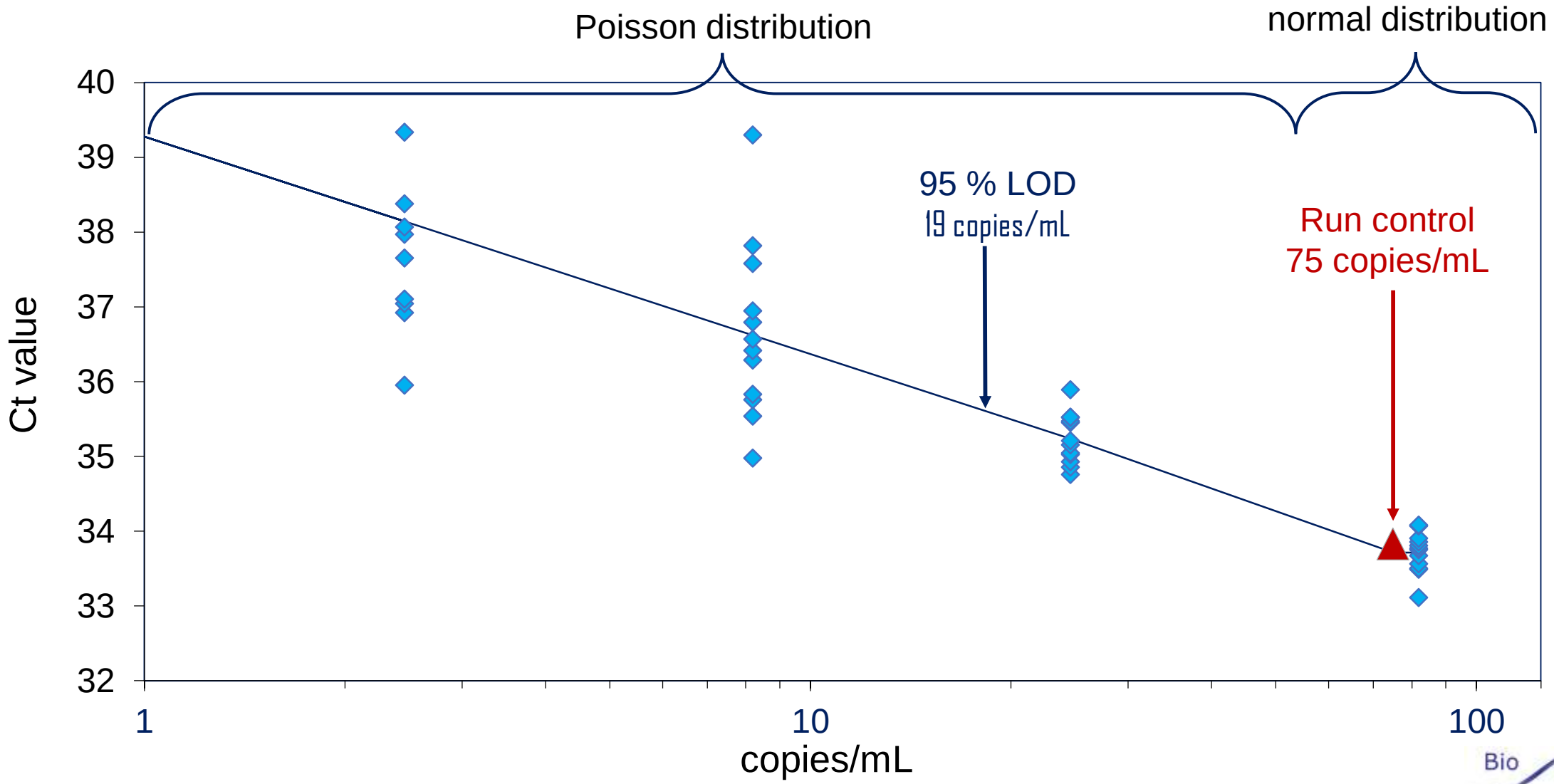
§ positioned at 4-5 times 95% LOD estimated at 17.2 (11.7-29.2) copies/mL by probit analysis (n=52)

Bio QControl

Impact of HCV-RNA concentration in ViraQ Controls on Ultrio Elite S/CO value distribution and Levey-Jennings Chart parameters



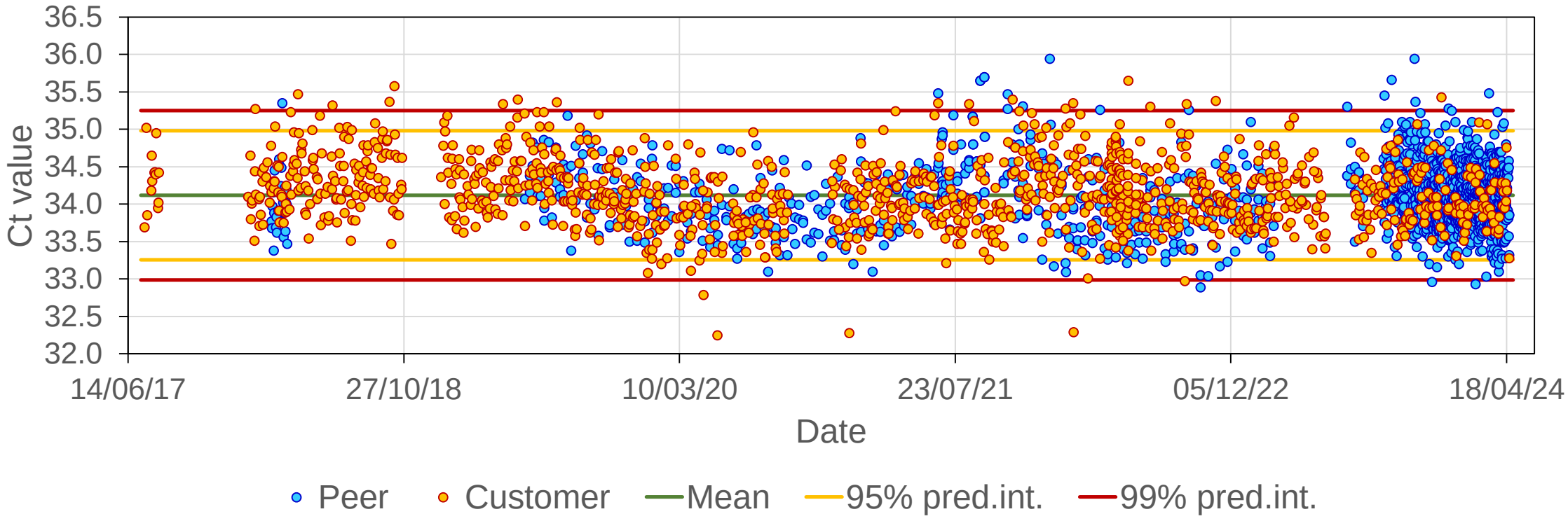
Ct values of Roche cobas MPX on HBV genotype A (inact.) standard dilution panel



§ positioned at 4-5 times 95% LOD estimated at 18.6 (9.1-75.9) copies/mL by probit analysis (n=12)

Bio QControl

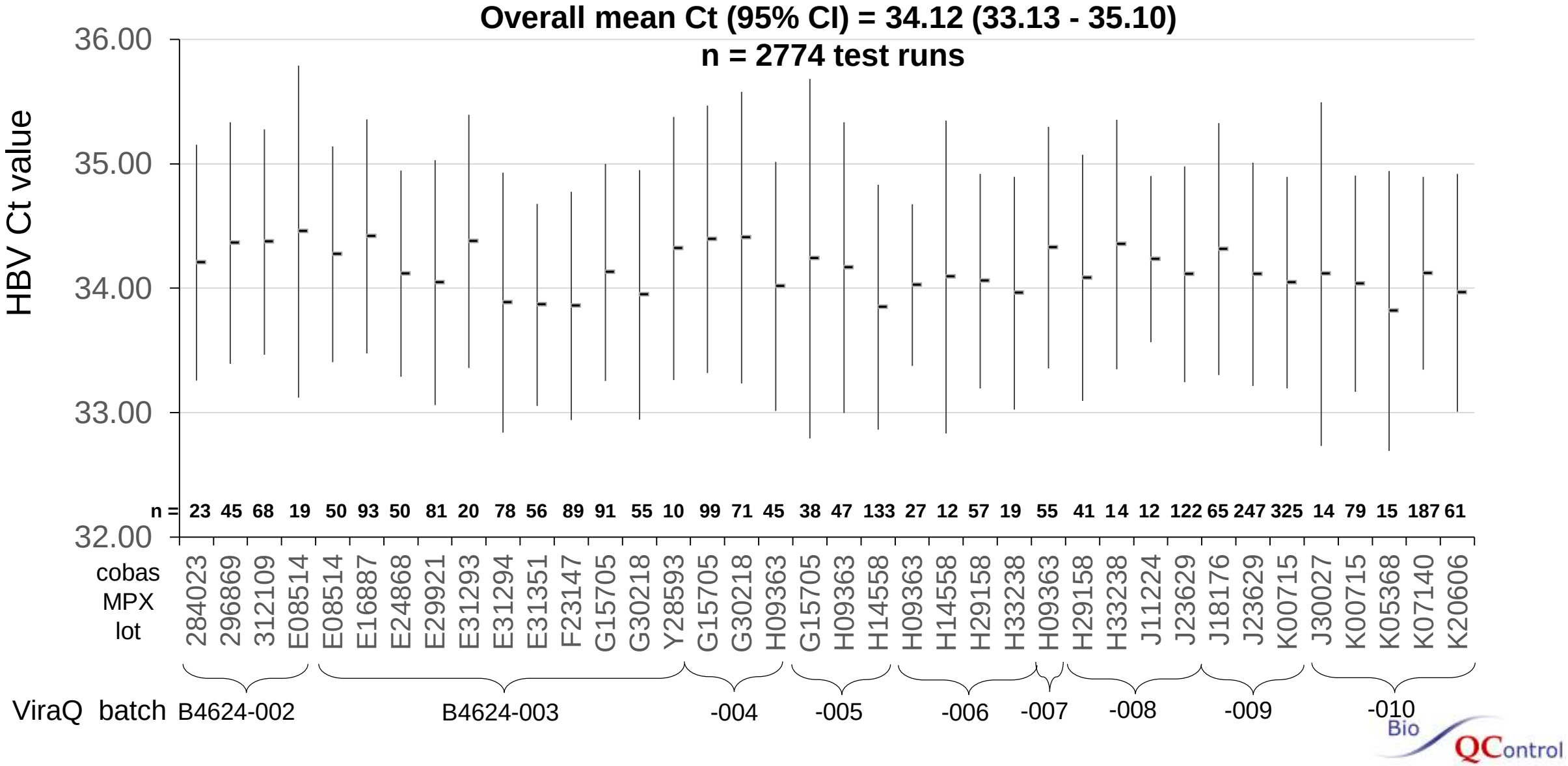
HBV-DNA Ct values in cobas MPX assay on P0273 ViraQ Multi-Marker Check Control (75 copies/mL)



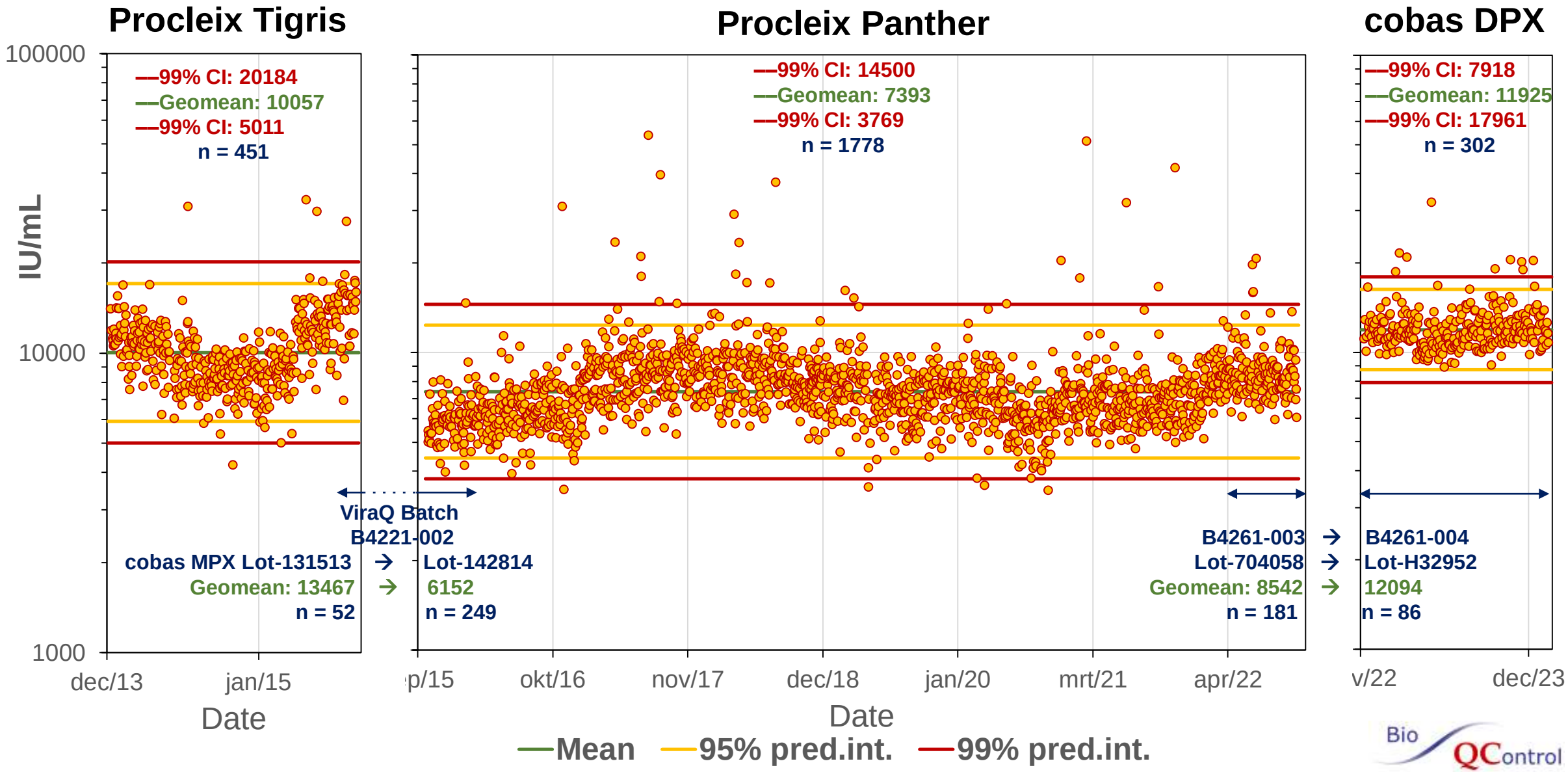
Summary statistics with intervals based on Normal distribution

	n	Mean	StDev	95% pred.int.		99% pred.int.	
Peer	1610	34,08	0,429	33,24	34,93	32,98	35,19
Customer	1164	34,16	0,450	33,28	35,05	33,00	35,32
All	2774	34,12	0,439	33,26	34,98	32,99	35,25

Average HBV-DNA Ct values (95% CI) in cobas MPX reagent lots on P0273 ViraQ Multi-Marker Check 75 Control batches

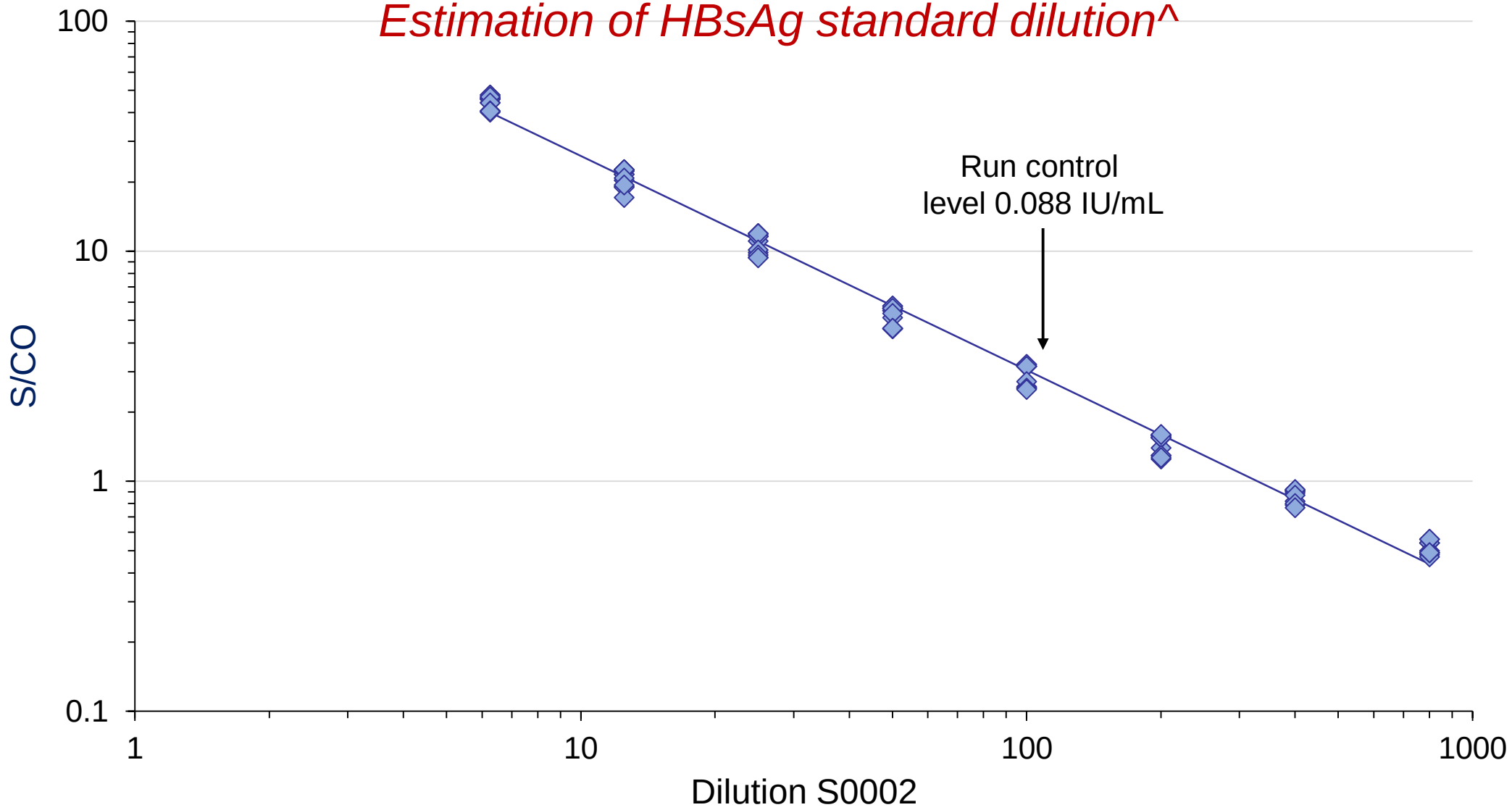


Ten-year Parvo B19V-DNA results on ViraQ Control (10,000 IU/mL) with three NAT assays reported by Finnish Red Cross



Design of P0386 SeraQ Alinity V4 Controls

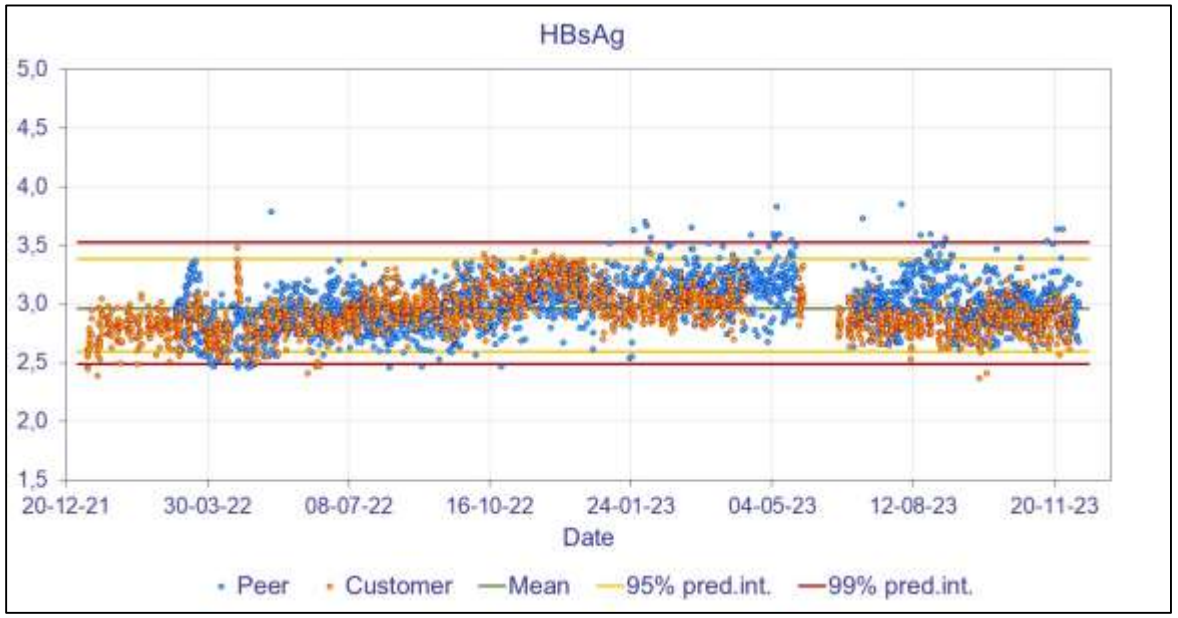
*Estimation of HBsAg standard dilution^*

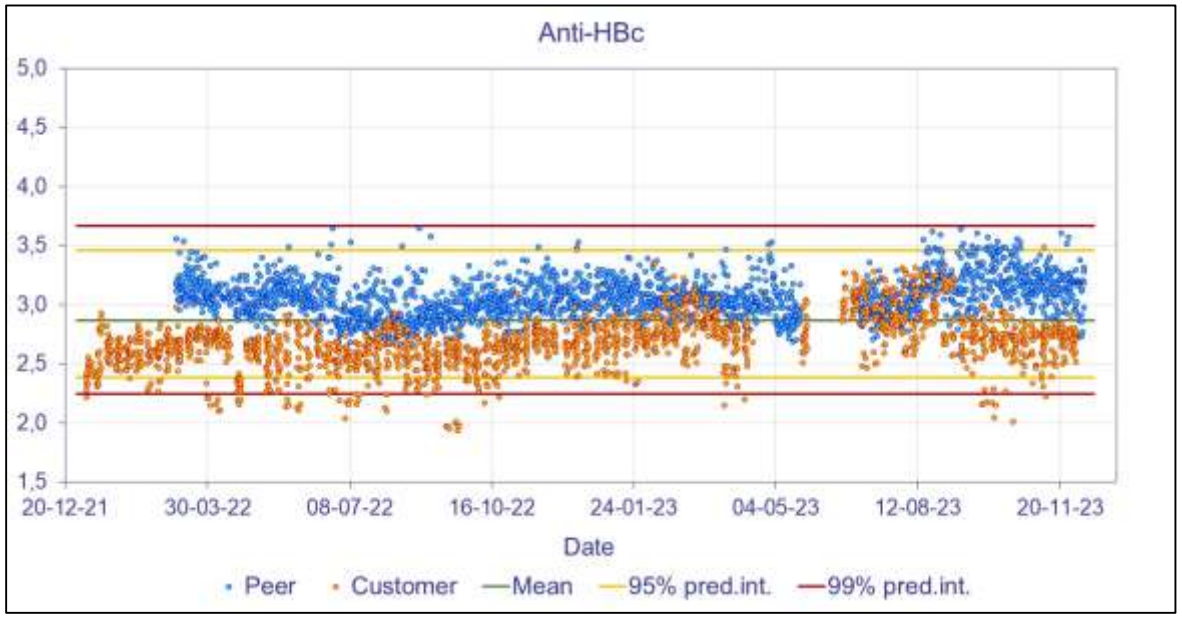


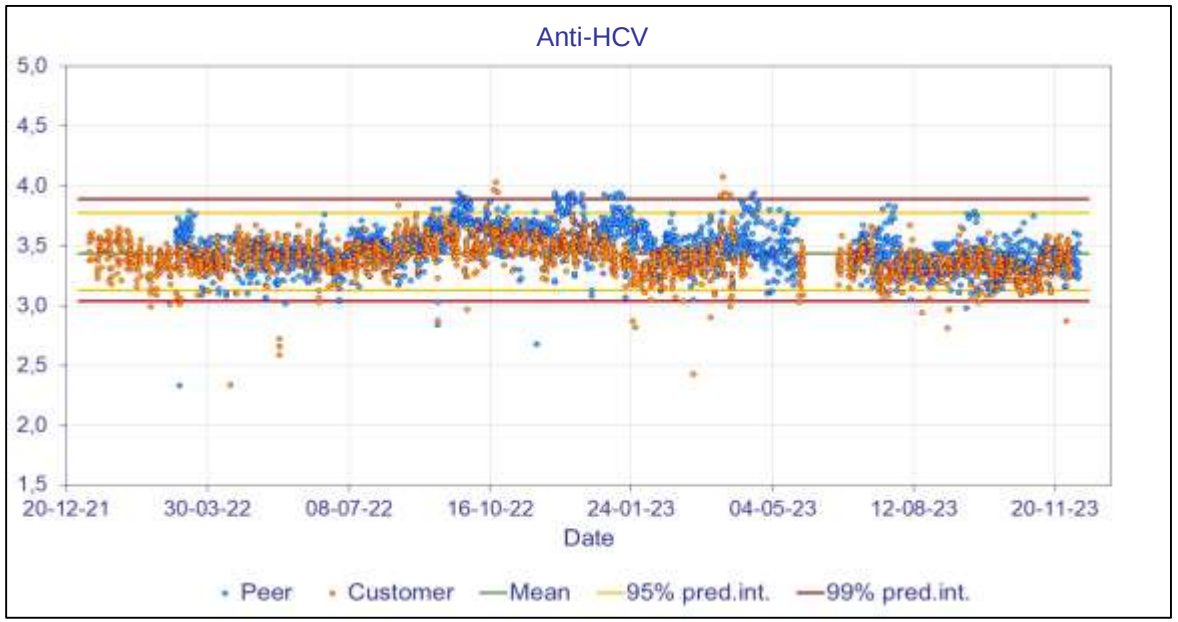
^same standard as used for preparation of 2nd WHO 00/588 standard
(Shuttler et al. J Clin Virol 2010, 47:238-242)

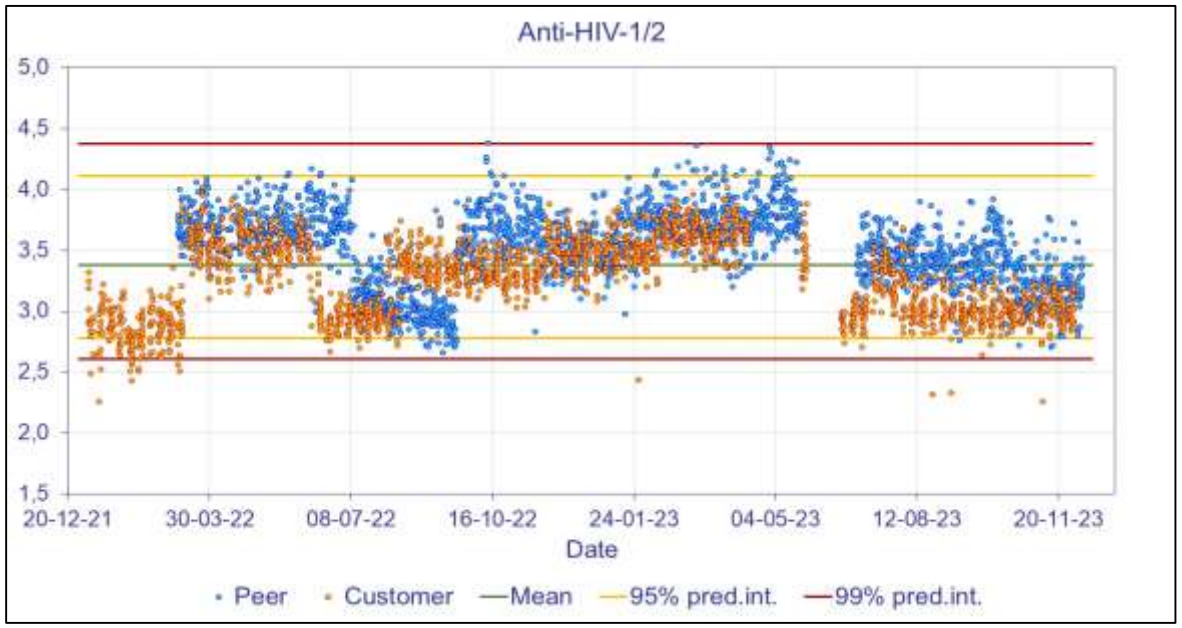
Bio QControl

Performance evaluation of P0386 SeraQ Alinity V4 Control









Post-Market Performance Follow-up (PMPF) system

IVD NAT test system



ViraQ
Control



Inactivated virus standards

test run reports

www. bioqcontrol.com
DataQ
Analytics

statistical report

Serologic IVD test system

SeraQ
Control



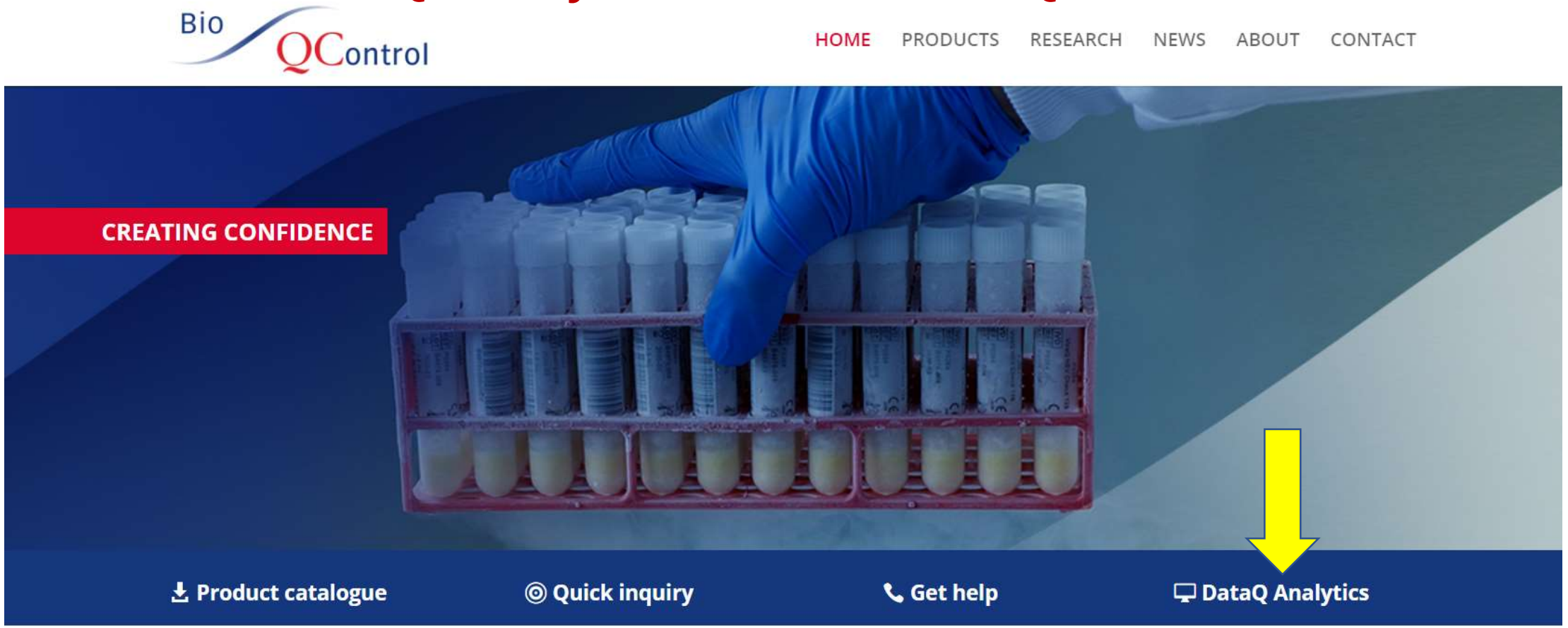
Inactivated antibody or antigen standards



ViraQ and SeraQ Controls are IVD test system specific and ensure sufficient analytical sensitivity

Bio QControl

To DataQ Analytics on www.BioQControl.com



YOUR PARTNER IN QUALITY CONTROL

BioQControl provides consistent validation and quality control products for infectious disease tests. For more than two decades our reference panels and run controls have been used to evaluate blood safety testing internationally.

Bio QControl

Summary slides

Summary of standardization and design of run controls I

- Matrix mimics plasma or serum in clinical donor samples.
- Target standard dilutions are ensured by gravimetrically recorded dilution steps.
- Primary standards are metrologically traceable to:
  - nucleic acid copies in bDNA 3.0 assay
  - nanograms or number of subviral HBsAg particles
  - IUs in 1st and 2nd native WHO standards
  - CID_{50} in chimpanzee infectivity studies
- For performance evaluation of NAT and antigen assays analytical sensitivity panels are functionally equivalent to seroconversion panels.
- Several publications on window period reduction and virus transmission risk by blood transfusion are based on our primary standards.
- Secondary inactivated standards are traceable to primary standards but calibration results (recoveries after inactivation) are assay dependent.
- To ensure biological safety of run controls inactivation of secondary standards is performed by methods of known viridical capacity validated in the plasma fractionation industry.
  - More than 6 logs overkill for HBV and HIV and 2-3 logs for HCV

Bio QControl

Summary of standardization and design of run controls II

- Design of SeraQ and ViraQ products is based on the dose response curves of the secondary standards used for manufacturing of these run controls.
  - SeraQ Controls S/CO values between 2.0 – 4.0
  - ViraQ Controls concentration at 4-5 (3-10) times the 95% LOD
- Different platform specific run control variants have been designed to ensure early recognition of poor test performance.
- Shelf life of run controls is based on long-term real-time (frozen state) and in-use (liquid state) stability studies.
  - Two year shelf life of ViraQ Controls at –30ºC to ensure <20% degradation of viral RNA
  - One week in-use shelf life of SeraQ Controls at 2-8ºC to ensure <10% degradation of HBsAg
- Scientific validity of using ViraQ and SeraQ controls is based 30 years standardization work which has contributed to blood safety research.
- CE-marking application under the IVDR has taken place.
  - as class D device
  - as a quantified control with claims on the concentration and stability of the analytes and the expected performance data in the Instructions for Use.

Bio QControl

Summary of design of DataQ Analytics system

- Easy-to-use data entry and reporting functions
- Data protection by two-factor authentication
- Laboratory and IVD test system-specific data entry screens
  - Editable until 60 days back
  - Multiple runs per instrument per day possible
- Reagent lot and run control batch numbers remain registered until lot change.
- Assay-specific statistical report
  - Levey-Jennings chart parameters based on distribution of assay response values (normal, log-normal or distribution-free)
  - Identification of deviating results using Nelson rules
  - Comparison of run control batches, test reagent lots and instruments
  - Comparison of your data with aggregated data of peers
- Option to enter donation testing data for specificity and robustness analysis (false positive rate, invalid rate)^
- Database structure also suitable for reference panels (e.g., proficiency panels)

^statistical report in development

Bio QControl