Calibration of standards: foundation for understanding blood safety

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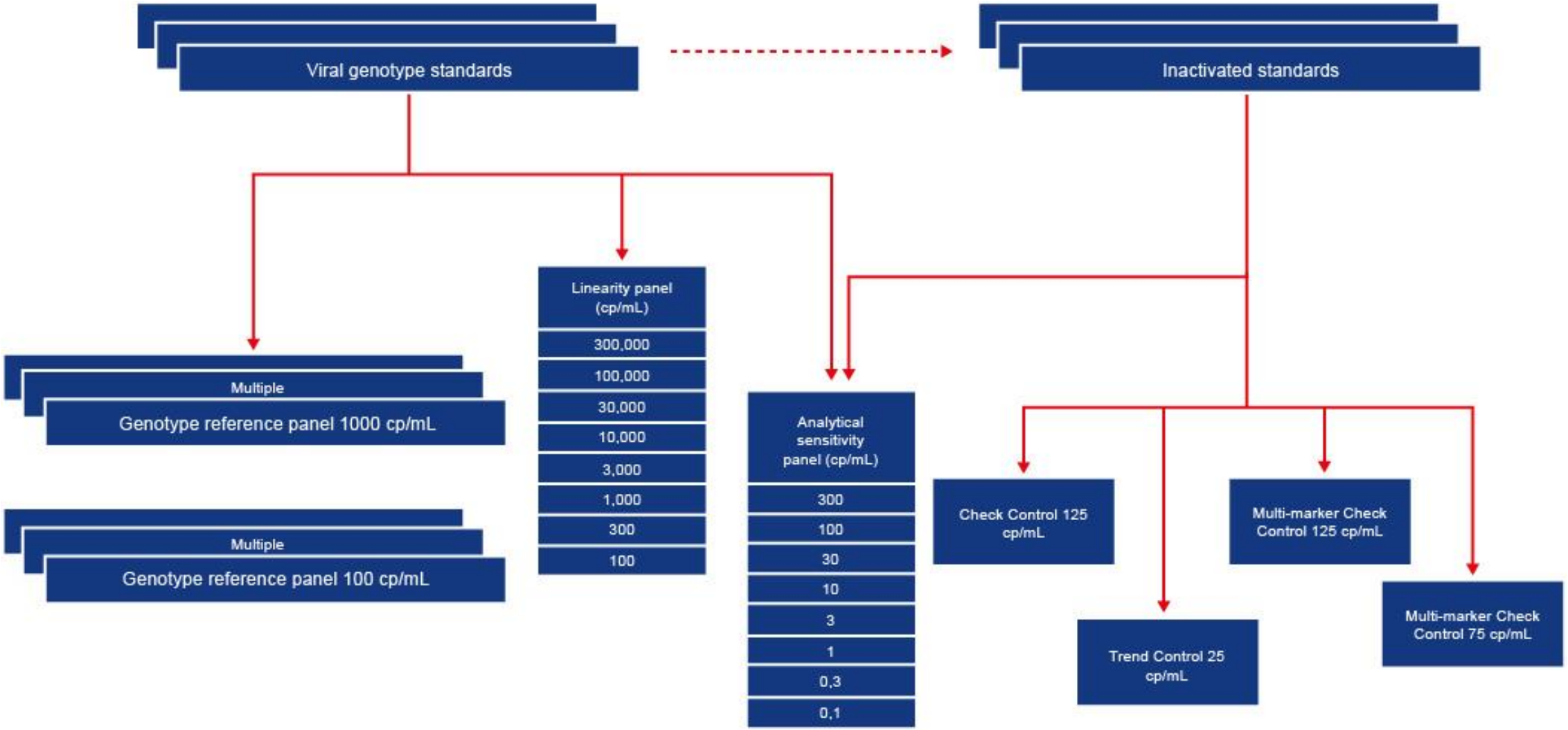
Bio QControl

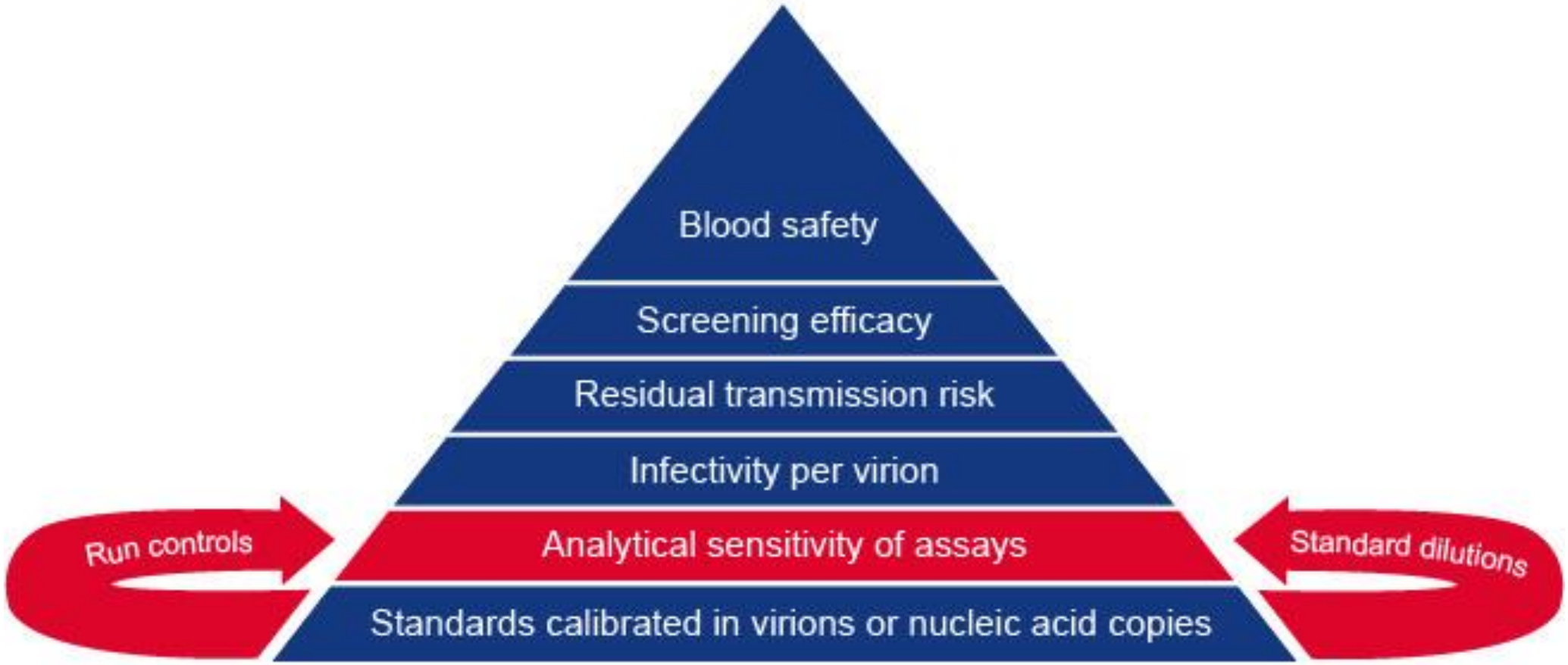
Preparation of panels and controls for NAT since 1992#



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

Bio QControl

Foundations for evaluating blood safety



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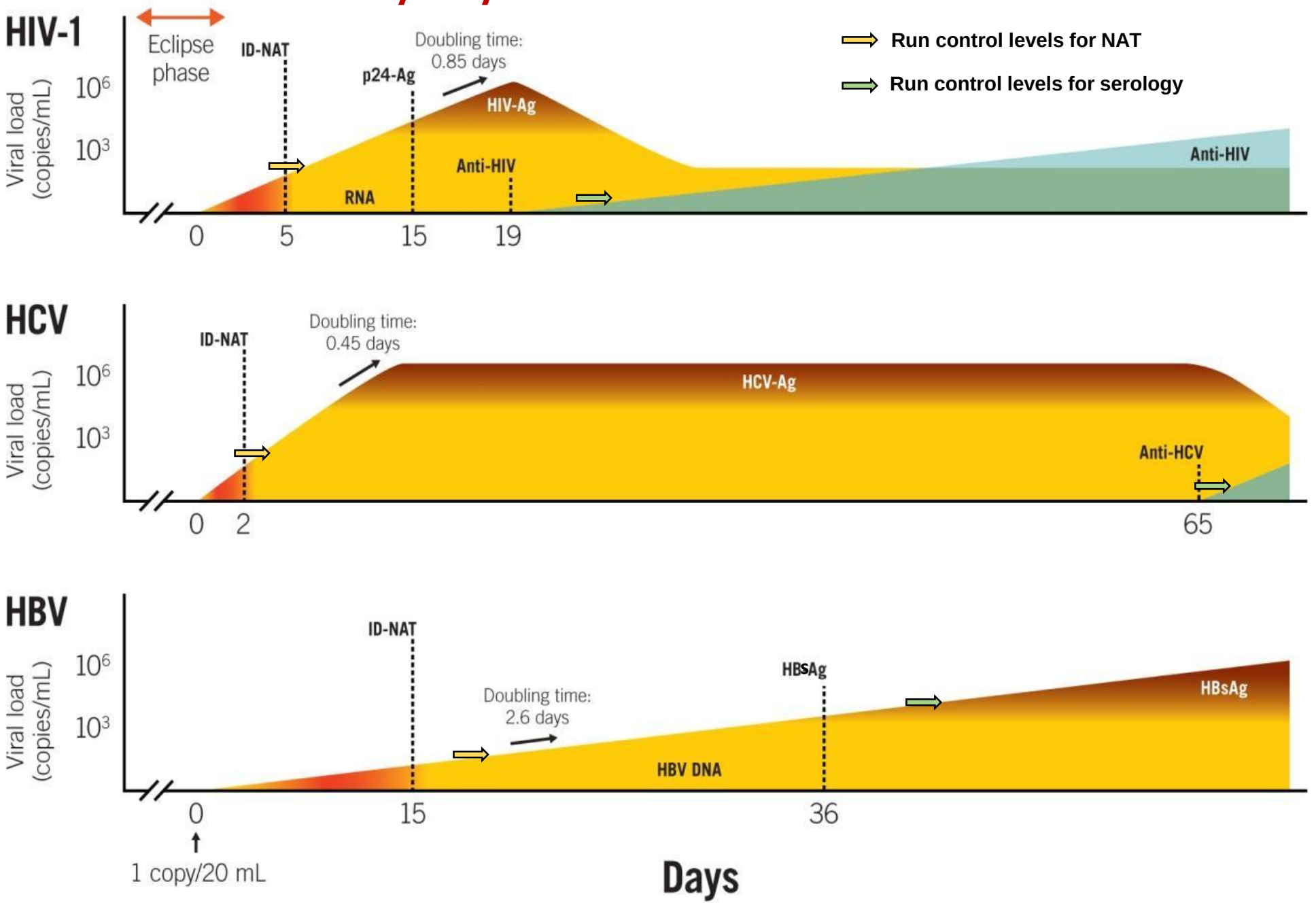
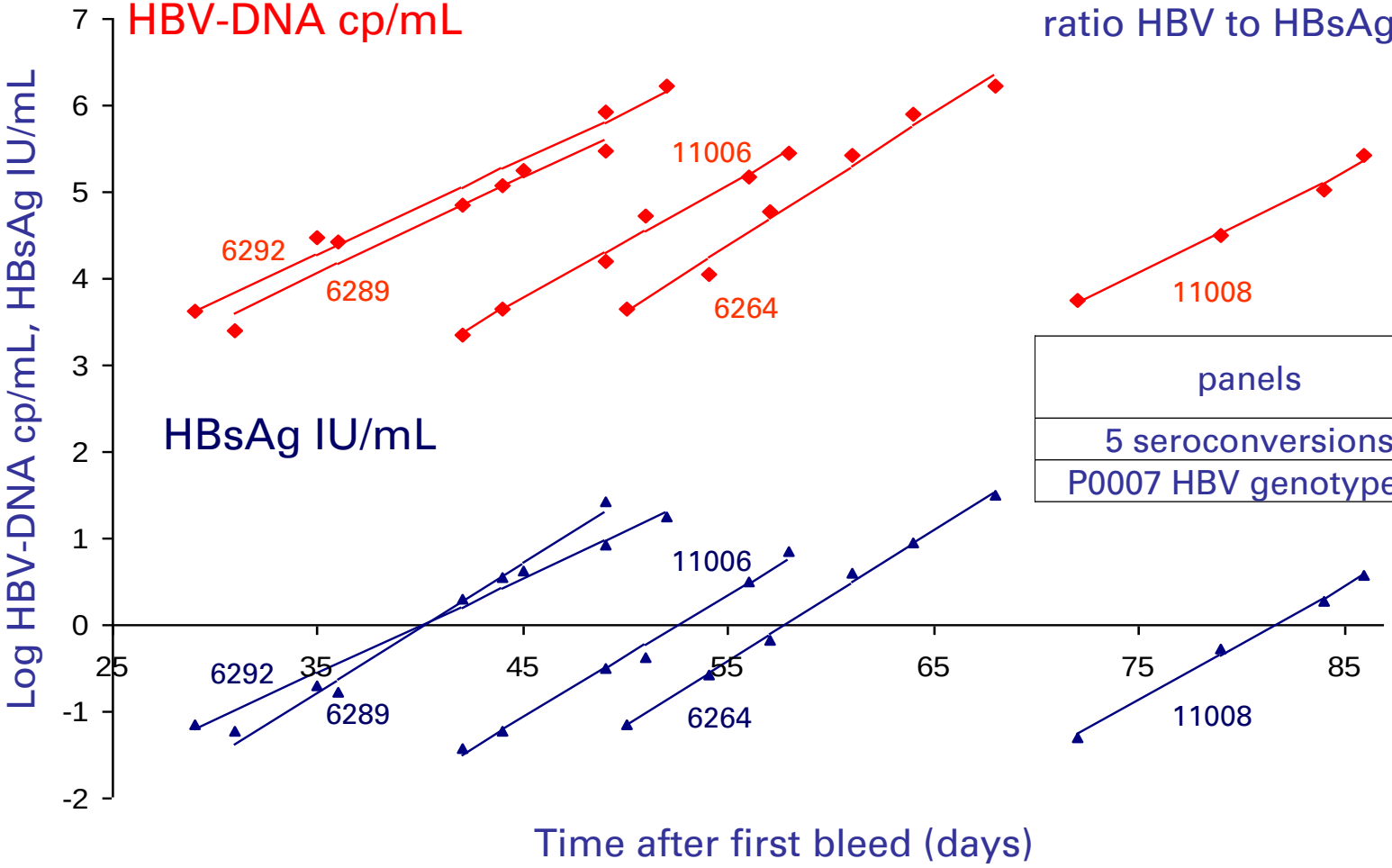
Early dynamics of viral markers



Parallel kinetics of HBV-DNA and HBsAg in ramp up phase of 5 seroconversion panels

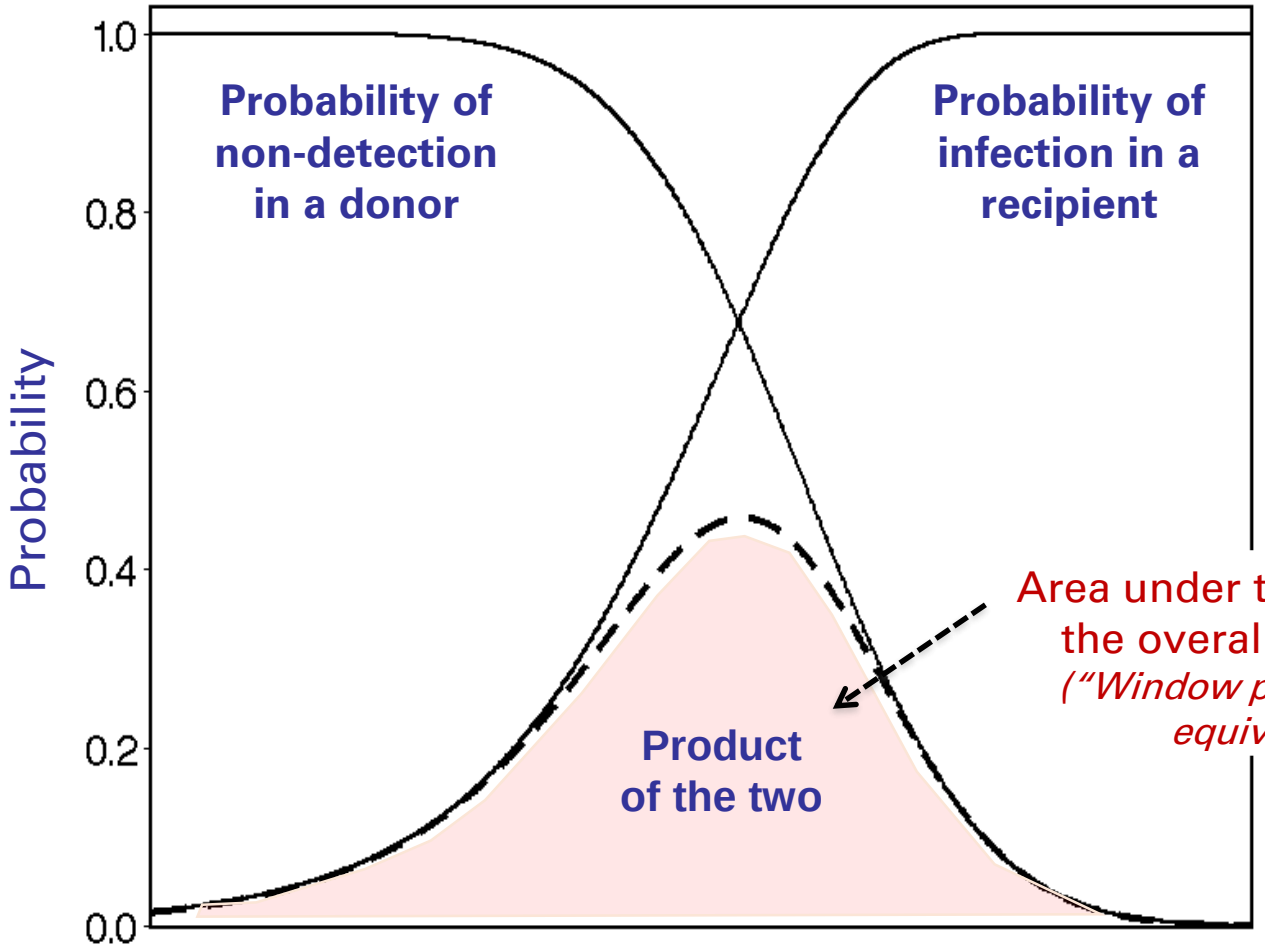


panels	n per sample	Ultrio Plus 50% LOD (CI)
5 seroconversions	5-18	4.6 (3.4-6.3)
P0007 HBV genotype A	122	4.3 (2.9-6.1)

Assal et al. Transfusion 2009; 49:301-310

Van Drimmelen et al, ISBT Berlin, 2010

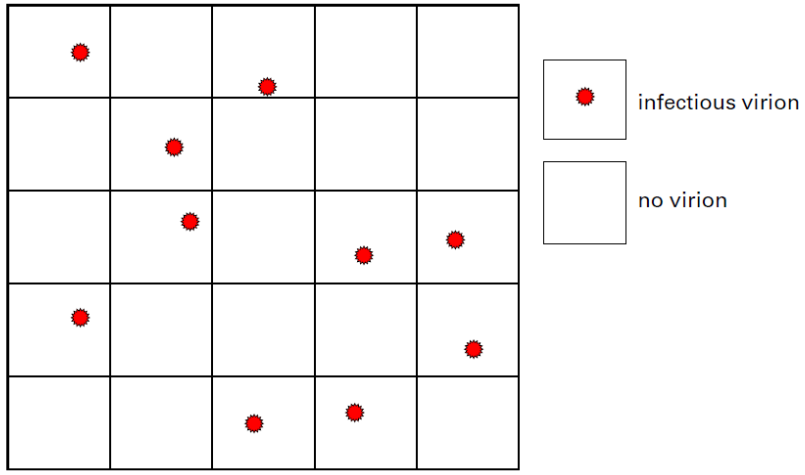
Probability of infectivity during the window period



Area under the curve gives the overall risk in days *("Window phase risk days equivalents")*

Poisson distribution

Probability of detection of virions in WP



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

Our view on metrological levels and traceability chain (ISO 17511:2003)^ for calibration of viral NAT standards in nucleic acid copies

Level	Traceable to SI unit	International reference measurement method	Calibrator material in measurement method	Reference standard
1	Yes	yes	NIST	NIST: P (Phosphate)
2	TBD	TBD: (Phosphate analysis, isotopic tracer, E260 and E280 extinction)#	P (Phospate)	bDNA 3.0 assay calibrators (Purified in vitro RNA transcripts or DNA plasmids)#
3	No*	TBD: Multiple replicate bDNA 3.0 assays over time	bDNA assay calibrators	VQC-Sanquin standards
4	No	TBD: Separate calibration per NAT method in WHO collaborative study	VQC-Sanquin standard	WHO IS

^The IVD Directive refers to the ISO 17511:2003 standard for traceability of standards for IVDs and provides guidance on metrological levels

Collins ML et al, Anal Biochem. 1995;226:120-9]
* Extraction efficiency unknown

Calibration of primary S0011 HBV genotype A standard in copies/mL by bDNA 3.0 assay as reference method^

Assay	n S0011	copies/mL (95% CI) in VQC-Sanquin standard
Chiron bDNA 1.0	17	3.22 (3.13-3.32) x 10^9
Siemens bDNA 3.0	**28**	**2.15 (2.11-2.20)** x 10^9
Roche Amplicor Monitor	198	2.11 (2.05-2.17) x 10^9
Roche Taqman	8	2.38 (1.01-5.61) x 10^9
Digene HCS	42	1.63 (1.57-1.69) x 10^9

^Calibration of nucleid acid copies in bDNA assay is based on three physico-chemical techniques [Collins ML et al, Anal Biochem. 1995;226:120-9].

^Equivalent to copy numbers in Eurohep standard [Heermann KH et al, J Clin Microbiol. 1999;37:68-73 and Van Drimmelen et al, VR4060, BioQControl product files for CE marking]

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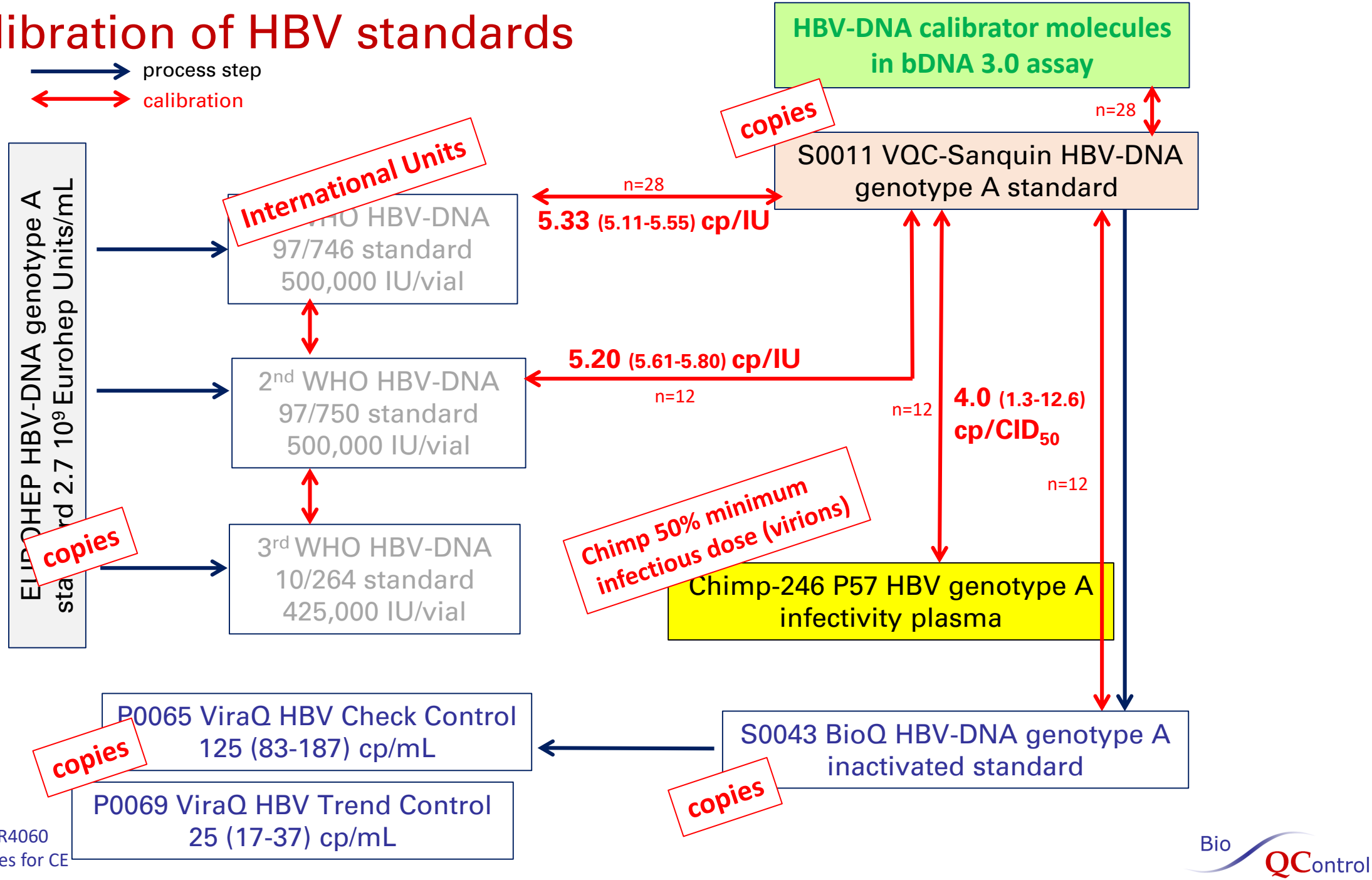
Calibration of HBV standards



Van Drimmelen et al, VR4060
BioQControl product files for CE registration

Bio QControl

Calibration of S0011 HBV genotype A standard against 1st and 2nd WHO standard in bDNA 3.0 assay^

International HBV Standard	VQC-Sanquin standard	n WHO	n S0011	copies/IU (95 %CI)
WHO 97/746	S0011 HBV gt A	12	16	5.33 (5.11-5.55)
WHO 97/750	S0011 HBV gt A	6	6	5.20 (4.61-5.80)

^Van Drimmelen et al, VR4060 BioQControl product files for CE registration

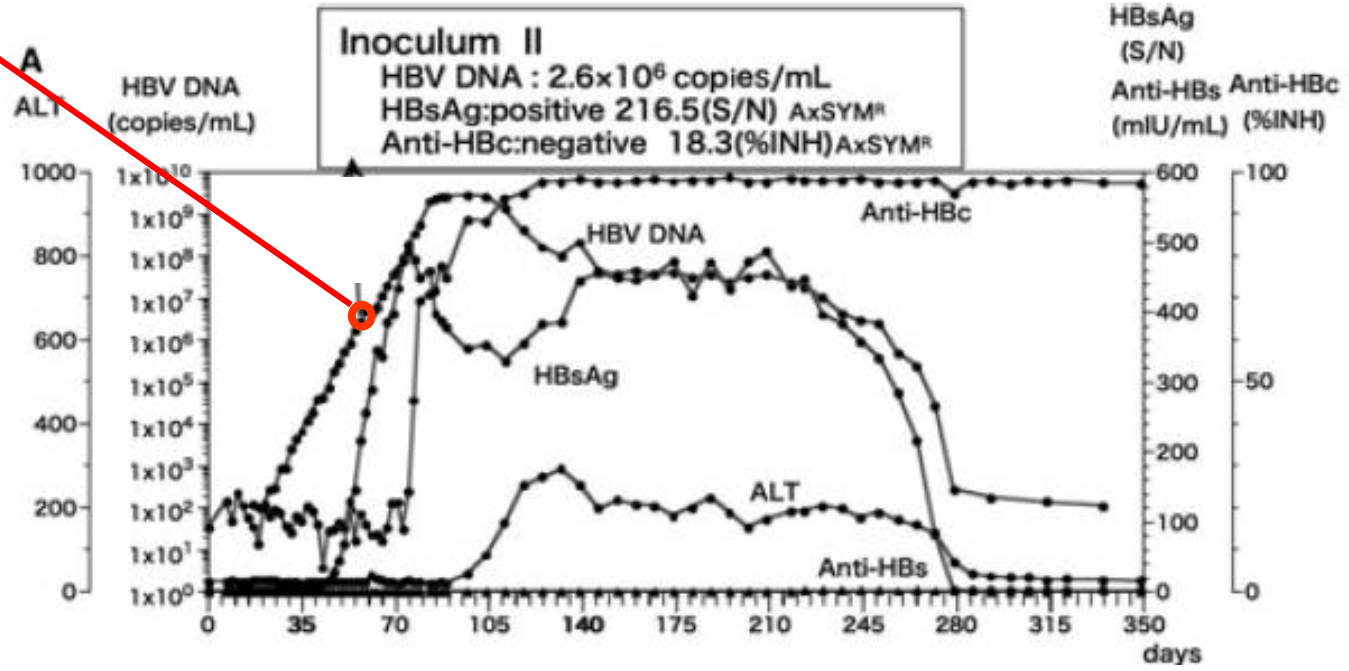
Bio QControl

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

VQC-Sanquin 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)

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

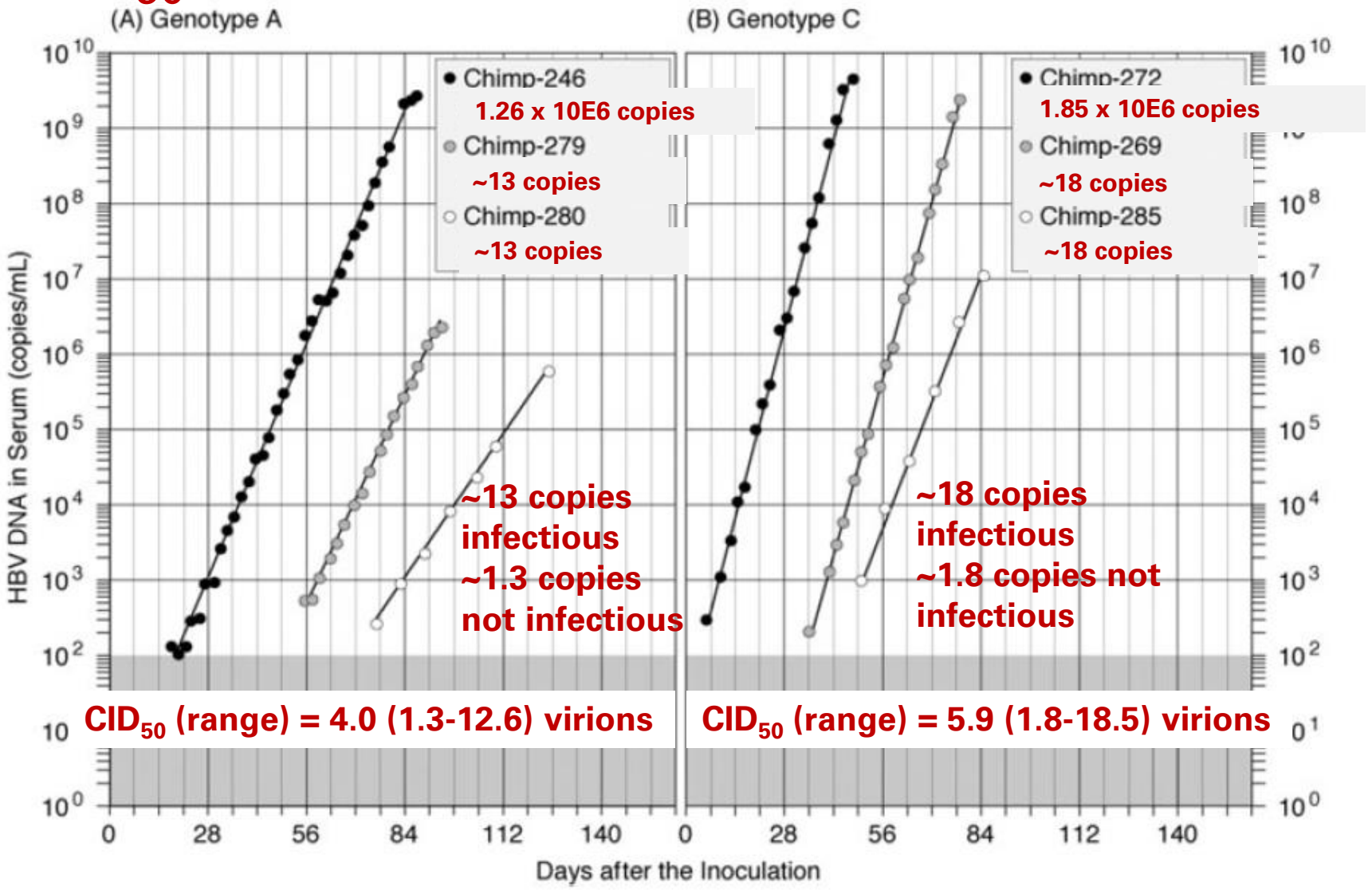
Van Drimmelen et al, VR4060
(BioQControl CE files)



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

Bio QControl

Estimation of 50% chimpanzee minimum infectious dose (CID_{50}) of HBV after recalibration of inocula



Bio QControl

50% chimpanzee minimum infectious dose (CID_{50}) of HCV after recalibration of inocula in bDNA 3.0 assay

VQC-Sanquin standard	Chimp infectivity plasma^	n C-210	n S0009	copies/ CID_{50} (range)
S0009 HCV gt 1	C-210 (wk-7) gt 1	6	6	**8.1 (2.6-25.6)**

^Katayama K et al. Intervirology 2004;47:57-64

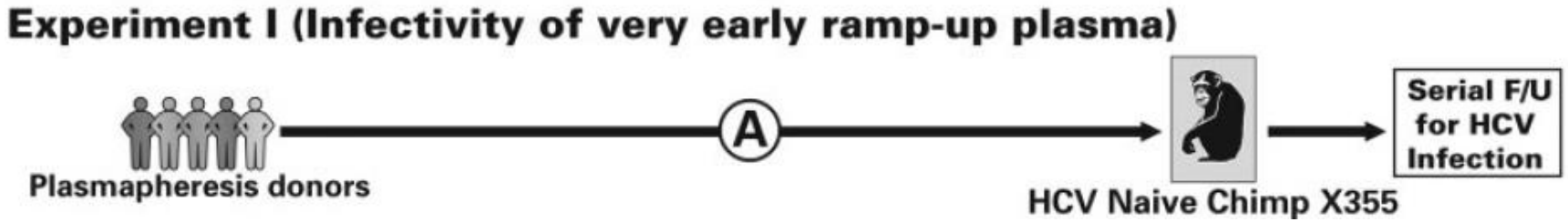
Van Drimmelen et al, VR4060 (BioQControl CE files)

Sample C-210 (wk-7) was kindly provided by Prof Yoshizawa and Prof Tanaka, Hiroshima University, Japan

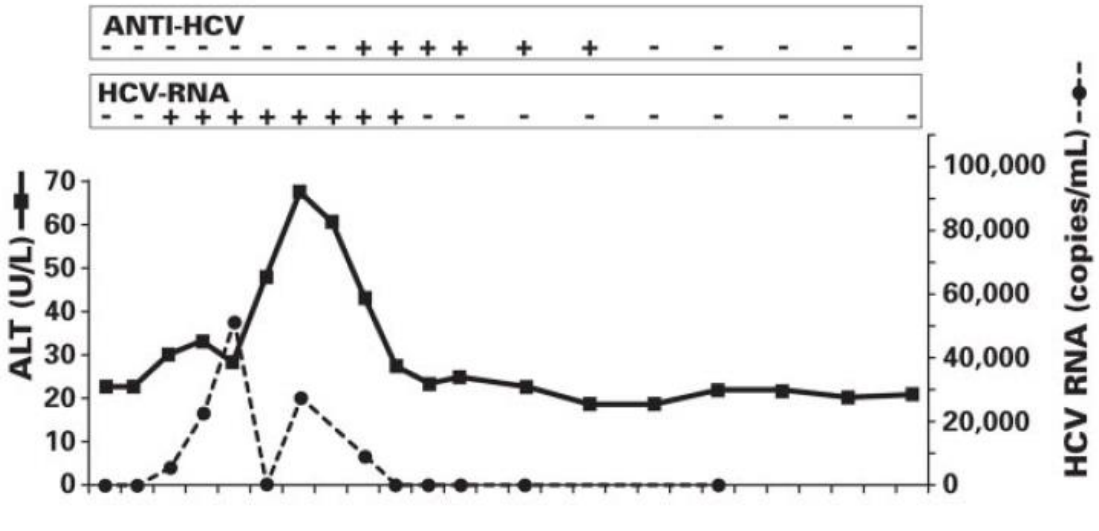
Bio QControl

Re-estimation of infectious dose of HCV in very early ramp-up plasma

Busch et al. Blood 2012;119(26):6326-6334



HCV-RNA estimation in 50 mL inoculum of infectious donor by probit analysis **(2/27 TMA reactive)**	
according to Busch et al [Blood 2012:119:6326]	on S0009 VQC-Sanquin standard dilutions
1.2 (0.5-1.9) cp/mL 60 (25-95) copies	0.3 (0.1-0.6) cp/mL 14 (5-30) copies



CID_{50} (range) could be as low as 4.4 (1.4-14) virions

For worst case risk modelling according to Weusten et al [Transfusion 2011;51:203-15] a MID_{50} (range) of 3.2 (1-10) copies or virions was proposed by Kleinman et al [Transfusion 2009:49:2454-89]

Current NAT efficiencies^ estimated on primary Eurohep and VQC-Sanquin standards calibrated in copies/mL

Standard	Assay	n	% efficiency (CI)
S0011 VQC-Sanquin HBV-DNA genotype A2	Ultrio Plus	48	27 (20-36)%
	Ultrio Elite	74	35 (23-53)%
S0010 Eurohep HBV-DNA genotype A2	Ultrio Plus	96	34 (27-43)%
	cobas MPX	48	42 (25-68)%
S0009 VQC-Sanquin HCV-RNA genotype 1	Ultrio Plus	48	74 (53-99)%
	Ultrio Elite	112	81 (67-97)%
	cobas MPX	60	24 (18-30)%
S0012 VQC-Sanquin HIV-1 RNA subtype B	Ultrio Plus	48	75 (54-101)%
	Ultrio Elite	24	71 (47-100)%
	cobas MPX	48	55 (42-73)%

^calculated from 63% LOD (CI) in cp/mL on standard dilution panels
0.5 mL plasma input in Ultrio versions
1.0 mL plasma input in cobas MPX.

Calibration of VQC-Sanquin HIV-RNA subtype B standard on 1st and 2nd IS in WHO collaborative study

Assay	N assays			VQC cp/IU on 1st WHO (97/656) standard		VQC cp/IU on 2nd WHO (97/650) standard	
	1st WHO	2nd WHO	VQC	mean	(95%CI)	mean	(95%CI)
Abbott LCx	14	15	14	0.76	(0.60-0.96)	0.69	(0.56-0.86)
Roche Amplicor Monitor	125	134	112	0.70	(0.60-0.81)	0.93	(0.80-1.08)
Siemens bDNA 3.0	**64**	**69**	**48**	**0.39**	**(0.34-0.44)**	**0.58**	**(0.51-0.66)**
Organon Tekika NucliSens^	46	51	36	0.80	(0.69-0.92)	0.43	(0.36-0.50)
Roche Amplicor Mon UltraSens	16	15	11	0.51	(0.27-0.95)	0.86	(0.49-1.51)

calculated from raw data reported by the laboratories participating in the first WHO collaborative study (Holmes H et al, J. Virological Methods 2001, 92; 141-150)
^ primer mismatch

Comparable NAT detection limits on primary and secondary HBV standards

HBV genotype A standard	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO 97/750	Ultrio Plus	303	4.4 (3.3-5.9)	28.4 (18.0-57.7)
S0011 VQC-Sanquin	Ultrio Plus	48	4.8 (3.7-6.2)	38.8 (25.6-68.5)
S0010 Eurohep	Ultrio Plus	96	3.6 (2.9-4.4)	40.4 (29.2-60.2)
WHO 97/750	Ultrio Elite	252	4.4 (3.6-5.4)	30.9 (22.4-47.4)
S0011 VQC-Sanquin	Ultrio Elite	74	3.4 (2.3-4.8)	43.2 (24.8-98.0)
S0010 Eurohep	Ultrio Elite	24	7.9 (5.5-11.2)	49.1 (29.4-116)
WHO 97/750	cobas MPX	12	1.8 (0.93-2.8)	8.0 (4.4-37.4)
S0011 VQC-Sanquin	cobas MPX	24	1.9 (1.3-2.7)	13.0 (7.7-29.6)
S0010 Eurohep	cobas MPX	48	1.7 (1.0-2.4)	10.3 (6.2-28.8)

I IU = 5.33 (5.11-5.55) copies

Comparable NAT detection limits on primary and secondary HIV-1 standards

HIV-1 subtype B standard	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO 97/650	Ultrio Plus	288	2.4 (2.2-2.6)	13.4 (11.4-16.3)
S0012 VQC-Sanquin	Ultrio Plus	48	1.7 (1.3-2.2)	15.1 (9.9-26.9)
WHO 97/650	Ultrio Elite	229	2.2 (1.4-3.2)	17.2 (10.3-40.1)
S0012 VQC-Sanquin	Ultrio Elite	24	2.1 (1.5-2.9)	9.0 (5.8-19.5)
WHO 97/650	cobas MPX	12	2.7 (1.7-3.9)	5.8 (3.9-24.9)
S0012 VQC-Sanquin	cobas MPX	48	1.3 (1.0-1.6)	7.3 (5.3-11.8)

I IU = 0.58 (0.51-0.66) copies

Comparable NAT detection limits on primary and secondary HCV standards

HCV genotype 1 standard	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO 96/798	Ultrio	24	2.6 (1.9-3.4)	20.5 (13.9-33.6)
WHO 06/100	Ultrio	32	2.5 (1.8-3.4)	18.9 (11.7-39.0)
S0009 VQC-Sanquin	Ultrio	36	2.9 (2.1-3.9)	23.9 (15.0-46.7)
WHO 06/100	Ultrio Plus	288	2.9 (2.0-4.2)	20.7 (12.2-50.3)
S0009 VQC-Sanquin	Ultrio Plus	48	1.8 (1.3-2.3)	15.1 (9.9-26.6)
WHO 06/100	Ultrio Elite	244	3.4 (2.0-5.4)§	26.8 (14.2-89.4)§
S0009 VQC-Sanquin	Ultrio Elite	112	1.7 (1.5-2.0)§	10.0 (7.7-13.8)§
S0009 VQC-Sanquin	cobas MPX	60	2.9 (2.3-3.6)	20.4 (14.3-33.3)

§ $p<0.05$

I IU = 2.73 (1.4-4.8) copies

50% LOD (CI) **IU/mL**	95% LOD (CI) **IU/mL**
0.27 (0.73 -1.98)§	9.82 (5.20-32.7)§
0.37 (0.55-0.73)§	3.66 (2.82-5.05)§

S0009 VQC-Sanquin HCV standard is currently used as alternative to WHO 06/100 and 06/102 standards in analytical sensitivity studies

Conclusions

- Calibration of VQC-Sanquin HBV, HCV and HIV-1 standards in nucleic acid copy (or virion) numbers enabled estimating lengths of diagnostic window periods and residual transfusion transmission risk.
- The likelihood of nearly accurate calibration of primary VQC-Sanquin standards in copies/mL with bDNA 3.0 reference assay has been supported by our studies with chimpanzee plasmas of known infectivity and by limiting dilution NAT efficiency studies.
- Viral standard dilution panels calibrated in copies/mL can replace seroconversion panels for evaluating sensitivity of NAT (and antigen) assays and estimating diagnostic window periods.
- VQC-Sanquin HBV, HCV, HIV-1, HAV, Parvo B19V standards are directly traceable to 1st and 2nd WHO standards and can be used as an alternative to WHO standards in analytical sensitivity studies of NAT methods.
- The amount of HIV-1 copies per IU increased approximately 1.5 fold by replacing the 1st by the 2nd WHO IS (because of variable detection efficiency of NAT methods for the two International Standards in the WHO collaborative study).