External quality control ensuring sufficient analytical sensitivity of NAT assays

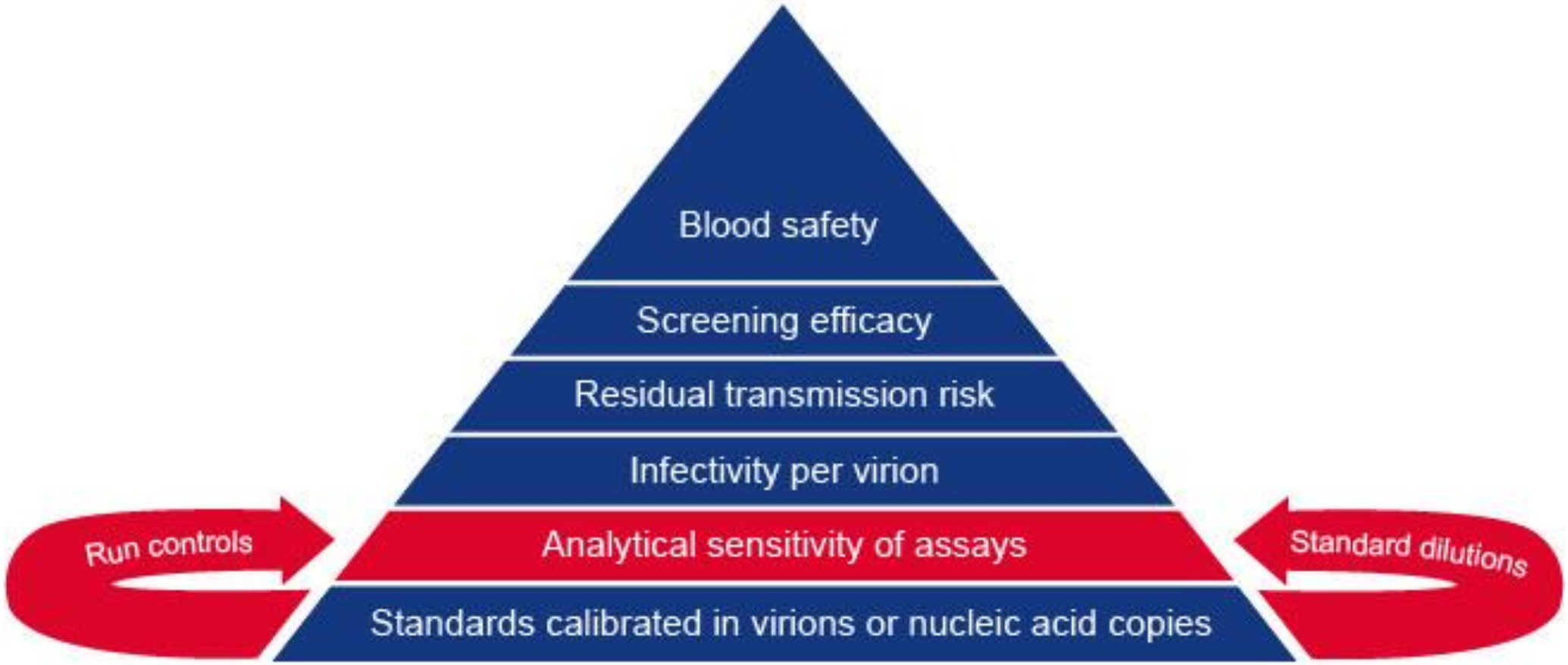
Nico Lelie

Bio Quality Control, Heiloo, Netherlands

Satellite Meeting before IPFA-PEI 25th Workshop Twenty-five Years Standardization and Quality Control of Nucleic Acid Amplification Technology for Detection of Blood Borne Viruses, May 15th 2018, Athens

Bio QControl

Foundations for evaluating blood safety



Bio QControl

Analytical sensitivity of Grifols Ultrio assay versions on native and inactivated HBV-DNA standards

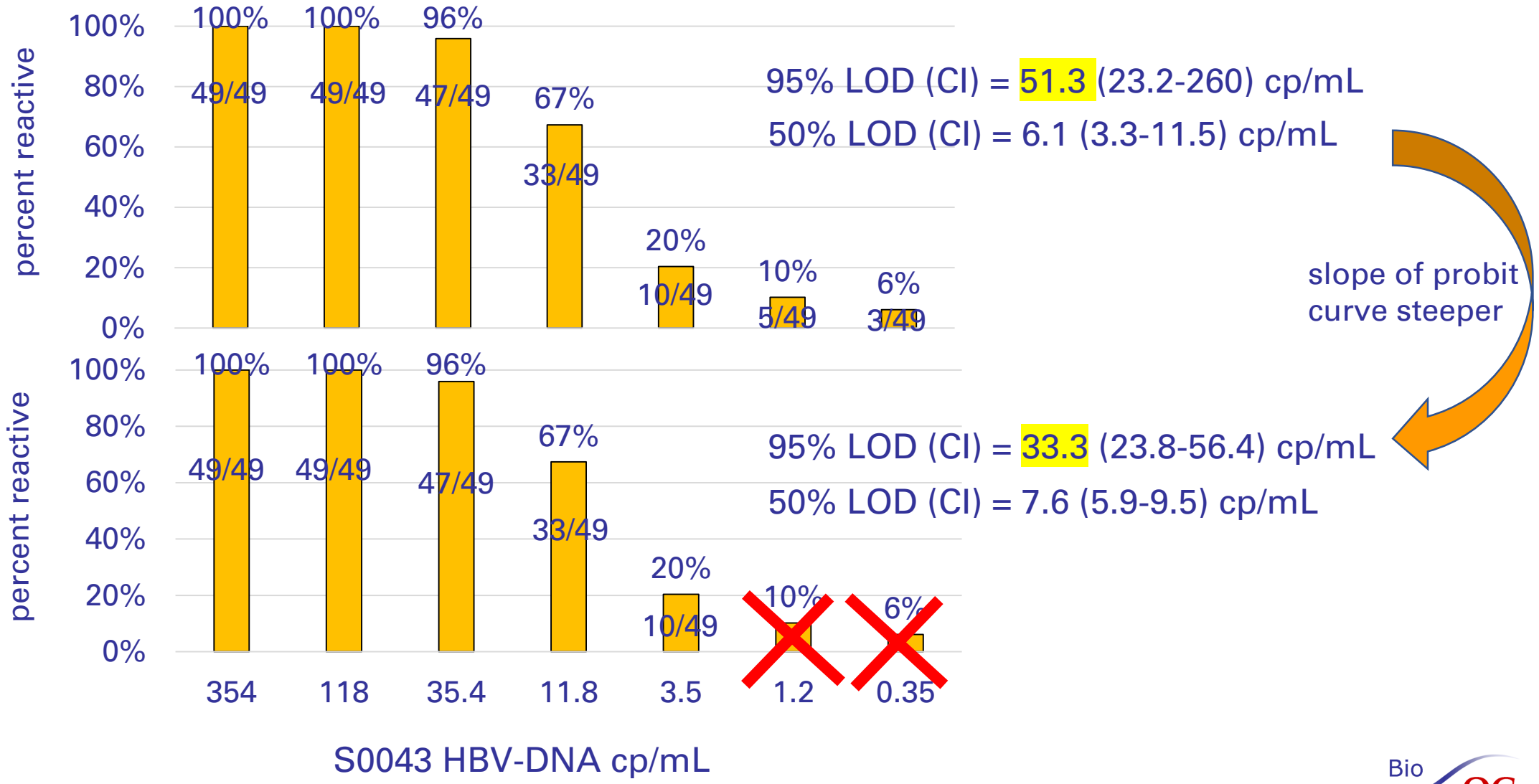
HBV genotype A standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0011 VQC-Sanquin	P0007	Ultrio	24	15.7 (7.0-33.9)	208 (77.6-2022)
S0043 BioQ inact.	P0031	Ultrio	58	56.5 (31.5-104)	715 (316-3046)
S0011 VQC-Sanquin	P0007	Ultrio Plus	48	4.8 (3.7-6.2)	38.8 (25.6-68.5)
S0043 BioQ A inact.	P0031	Ultrio Plus	24	6.6 (2.7-17.4)	64.2 (22.4-1099)
S0011 VQC-Sanquin	P0007	Ultrio Elite	74	3.4 (2.3-4.8)	43.2 (24.8-98.0)
S0043 BioQ inact.	P0031	Ultrio Elite	25	5.7 (4.0-8.2)	40.8 (24.3-91.7)

1 IU = 5.33 copies

Proportion reactive on P0154 ViraQ HBV Trend 50 Control batches in consecutive Ultrio Plus (UP) and Ultrio Elite (UE) batches.

TMA batch	ViraQ batch	p/n	reactivity	dellta (95% CI)
UP587882	B4154-003	102/103	99.00%	0.3 (0.1,0.5)%
UP592848	B4154-003	297/299	99.30%	0.6 (0.5,0.7)%
UP592848	B4154-004	66/67	98.50%	-0.2 (-0.6,0.1)%
UP601406	B4154-004	179/179	100.00%	1.2 (1.2,1.3)%
UP601923	B4154-004	203/204	99.50%	0.8 (0.7,0.8)%
UP612037	B4154-004	227/228	99.60%	0.8 (0.7,0.9)%
UP621787	B4154-004	90/90	100.00%	1.2 (1.2,1.3)%
UP626009	B4154-004	89/91	97.80%	-1.0 (-1.4,-0.5)%
UP All		**1253/1261**	**99.40%**	**0.6 (0.5,0.7)%**
UE616859	B4154-005	132/132	100.00%	1.2 (1.2,1.3)%
UE634122	B4154-005	345/355	97.20%	-1.6 (-1.9,-1.3)%
UE101801	B4154-005	85/87	97.70%	-1.1(-1.5,-0.6)%
UE101801	B4154-006	427/434	98.40%	-0.4(-0.5,-0.2)%
UE106862	B4154-006	138/141	97.90%	-0.9(-1.2,-0.5)%
UE All		**1127/1149**	**98.10%**	**-0.7(-0.8,-0.5)%**
UP, UE All		**2380/2410**	**98.80%**	**reference**

Probit analysis using Ultrio Plus and Elite data on P0031 HBV-DNA gt A (inact.) standard dilutions



Comparison of Ultrio Elite reactivity rate on ViraQ Trend Controls of 50 and 25 cp/mL

Ultrio Elite batch	P0154 ViraQ HBV 50			P0069 ViraQ HBV 25		
	B4154-	p/n	%	B4064-	p/n	%
3	004	427/434	98.5%	001	56/60	93.3%
4	004	138/141	97.9%	001	123/133	92.5%
all	004	565/575	98.3%	001	179/193	92.7%

Bio QControl

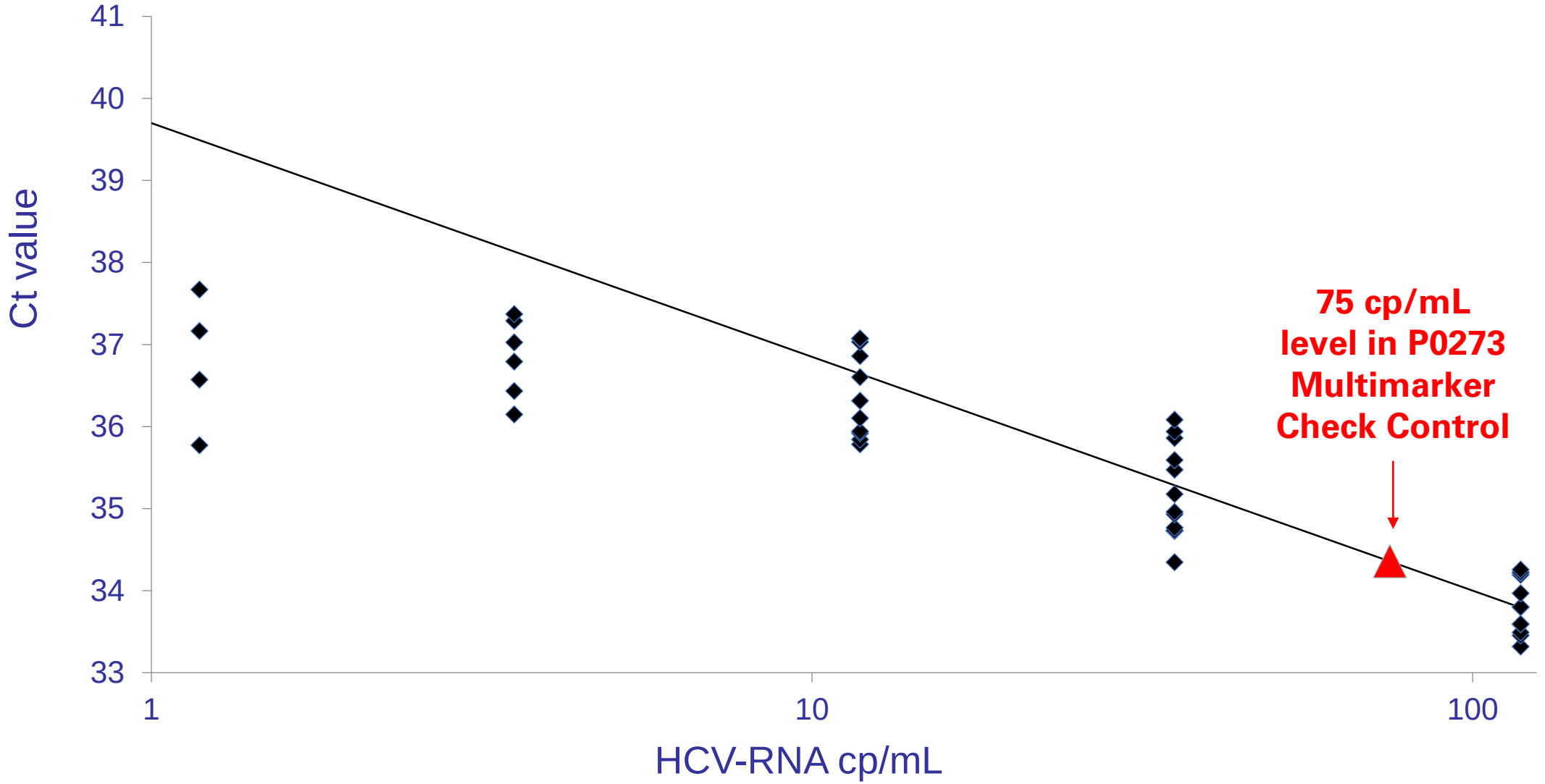
Probit analysis on inactivated viral standard dilutions and positioning of ViraQ Check and Trend Controls

NAT assay	Marker	n	cp/mL at hit rate					hit rate at cp/mL		
			50%	95%	99%	99.5%	99.9%	Trend 25	Check 75	Check 125
cobas MPX	HBV-DNA	12	2.4	**18.6**	43.4	59.3	112		99.7%	
	HCV-RNA	10	2.5	**15.6**	33.3	44.0	78.0		99.9%	
	HIV-1 RNA	12	1.0	**5.8**	12.0	15.7	27.2		100%#	
TaqScreen 2.0	HBV-DNA	12	2.8	**23.8**	57.8	79.9	156			99.8%
	HCV-RNA	12	5.2	**35.2**	77.7	104	189			99.7%
	HIV-1 RNA	12	2.0	**7.6**	13.2	16.2	24.6			100%#
Ultrio Plus/ Elite	HBV-DNA	49	7.6	**33.3**	61.4	76.8	122	90.7%		99.9%
	HCV-RNA	52	3.7	**28.8**	67.4	92.0	175	93.7%		99.8%
	HIV-1 RNA	52	3.1	**20.2**	43.9	58.4	105	96.7%		99.9%

HIV-1 subtype B standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0012 VQC-Sanquin	PeliCheck	TaqScreen 1.0	12	4.2 (2.6-6.7)	22.8 (13.0-52.9)
WHO 97/650^	EFS	TaqScreen 1.0	24	4.5 (3.4-5.8)	27.6 (19.1-44.1)
S0041 BioQ inact.	P0251	TaqScreen 2.0	12	2.0 (1.3-2.8)	7.6 (4.9-21.4)
S0041 BioQ inact.	P0026	cobas MPX	12	1.0 (0.6-1.60)	5.8 (3.0-23.2)
WHO 97/650^	P0022	cobas MPX	12	2.7 (1.7-3.9)	5.8 (3.9-24.9)

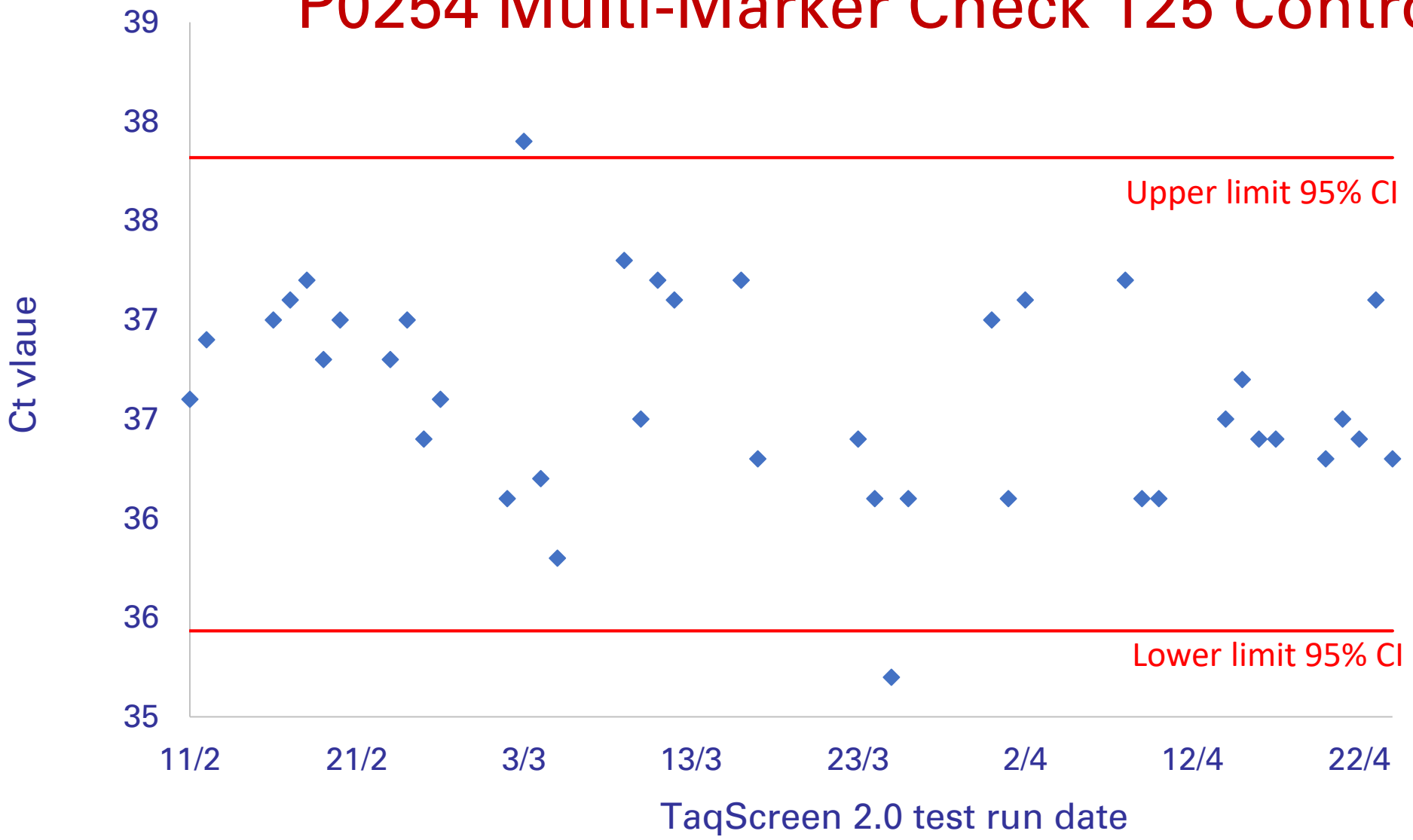
95% LOD for HIV in later cobas MPX versions improved more than expected in design phase of ViraQ Check Controls

Ct values of cobas MPX assay on P0020 HCV (inactivated) standard dilution panel



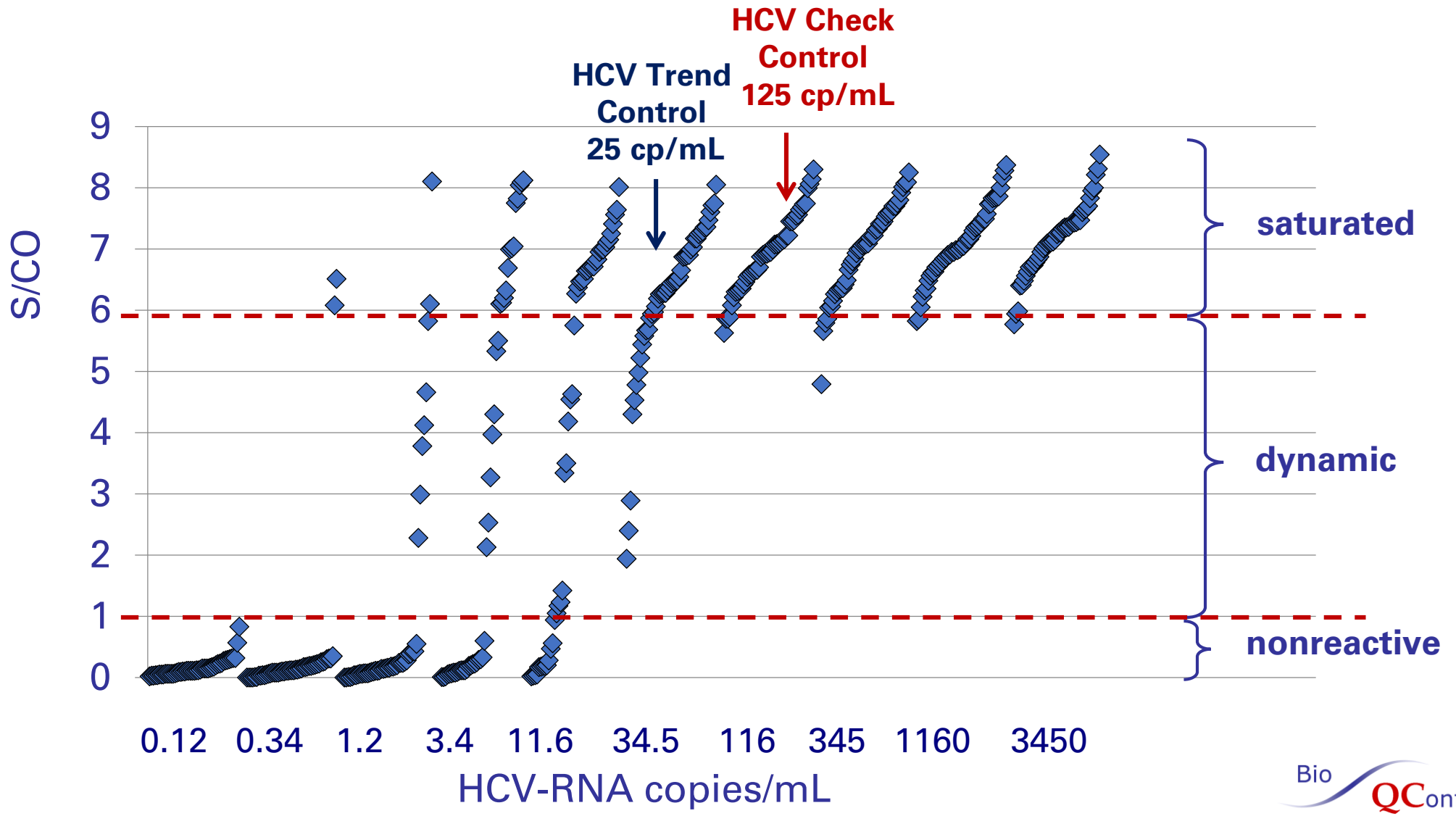
Bio QControl

HCV Ct values of cobas MPX TaqScreen 2.0 assay on P0254 Multi-Marker Check 125 Control



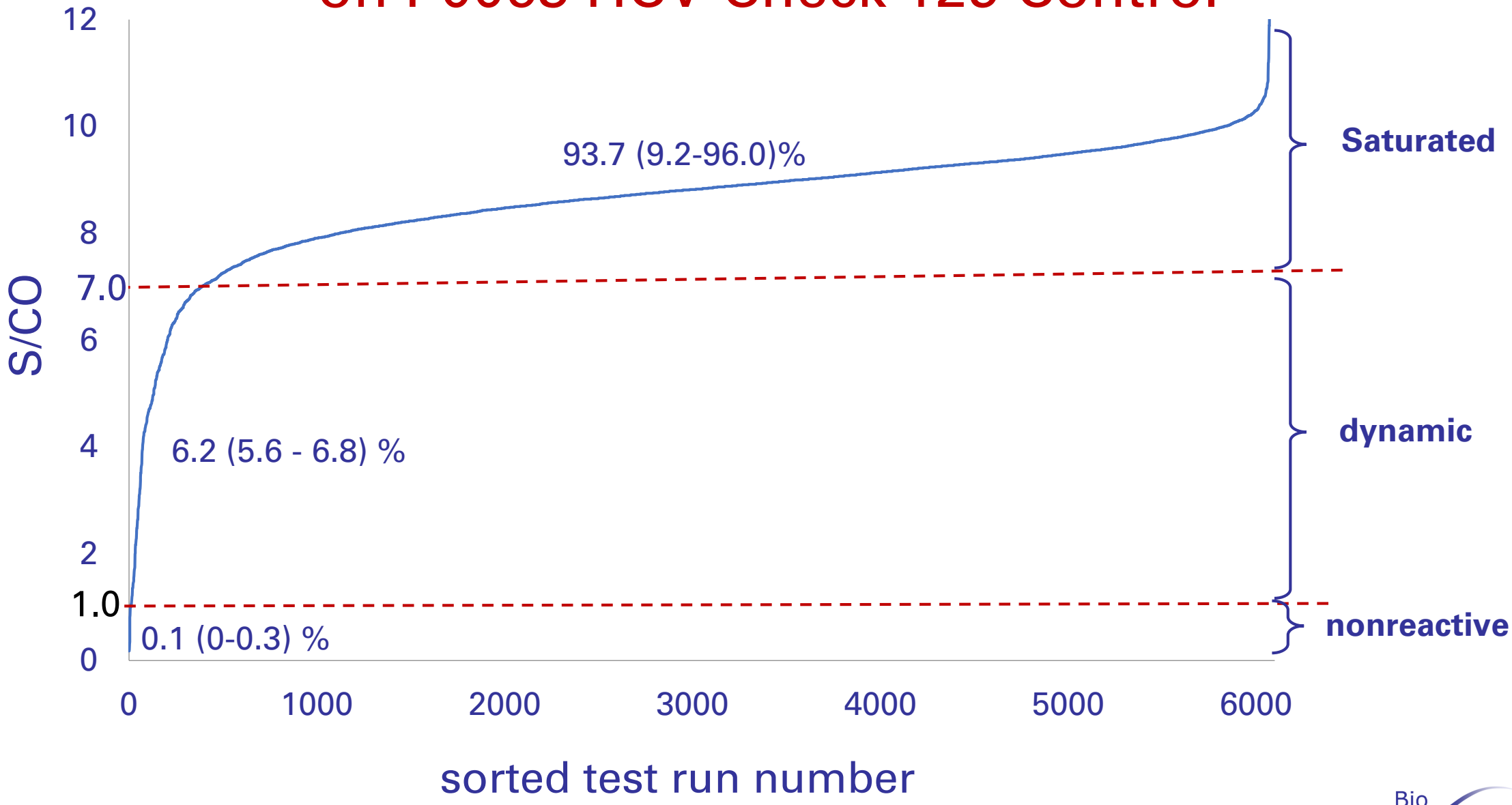
Bio QControl

S/CO ratios of Ultrio Plus on P0020 HCV genotype 3 (inactivated) standard dilution panel



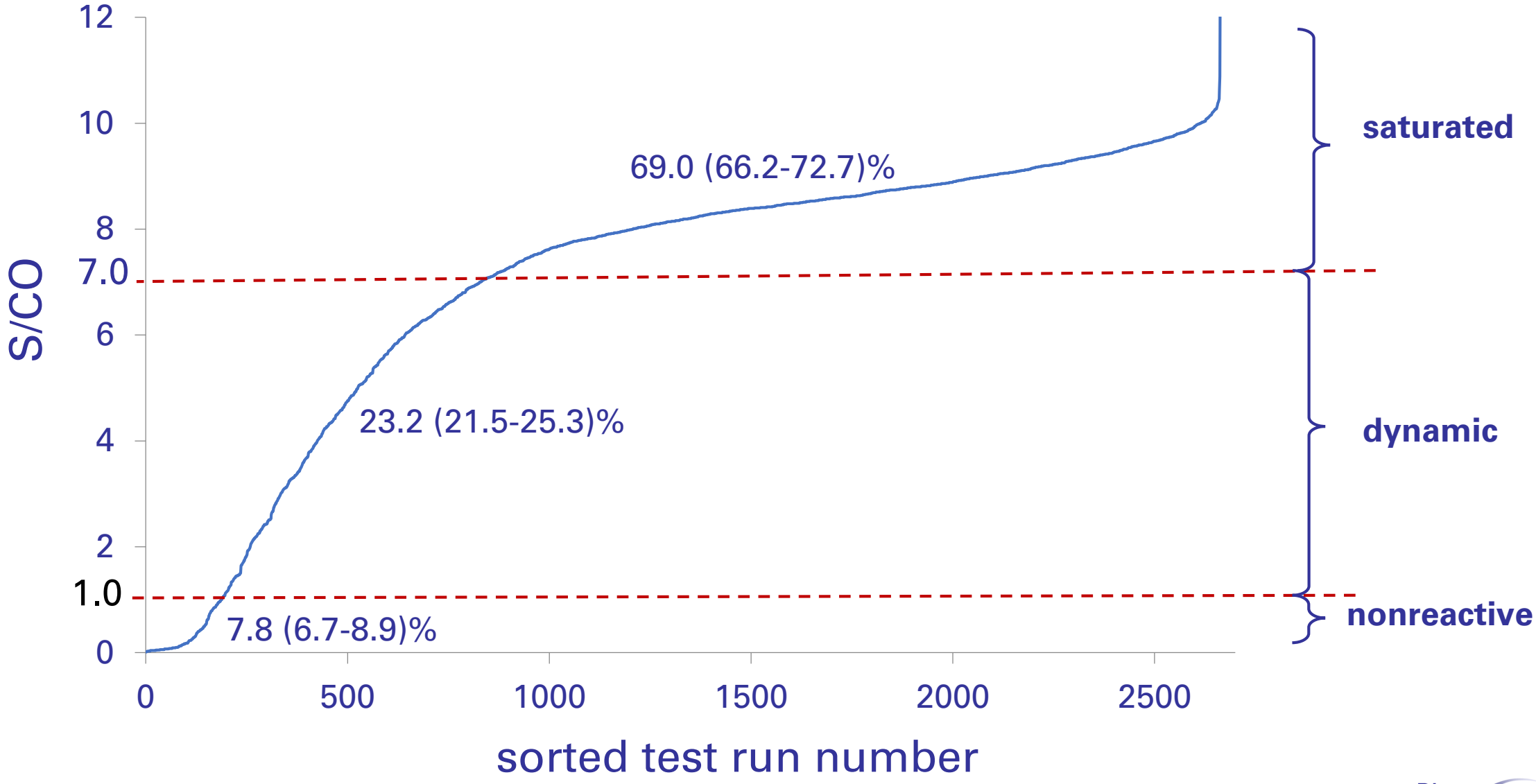
Bio QControl

Cumulative distribution of S/CO ratios in Ultrio Plus on P0063 HCV Check 125 Control



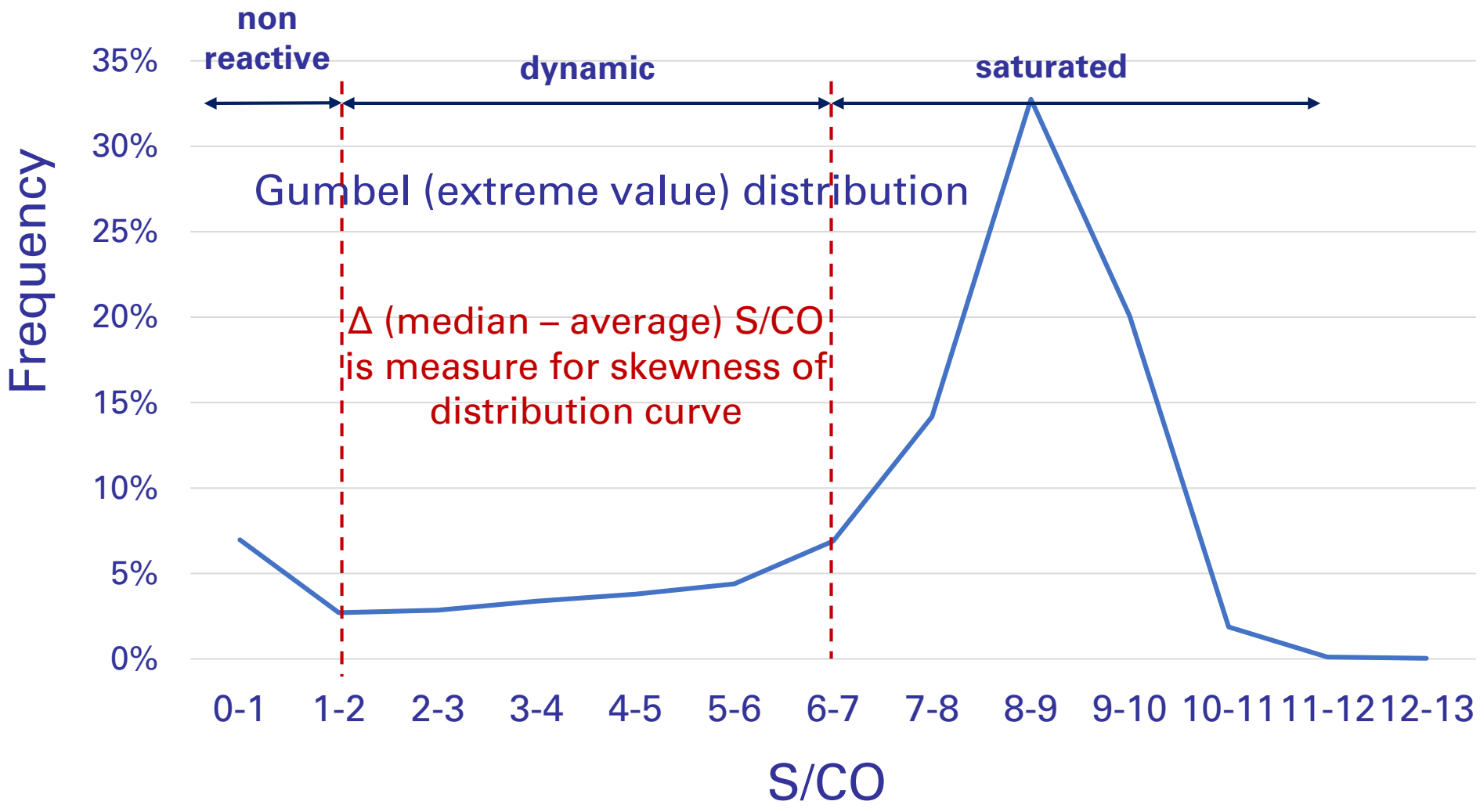
Bio QControl

Cumulative distribution of S/CO ratios in Ultrio Plus on P0067 HCV Trend 25 Control



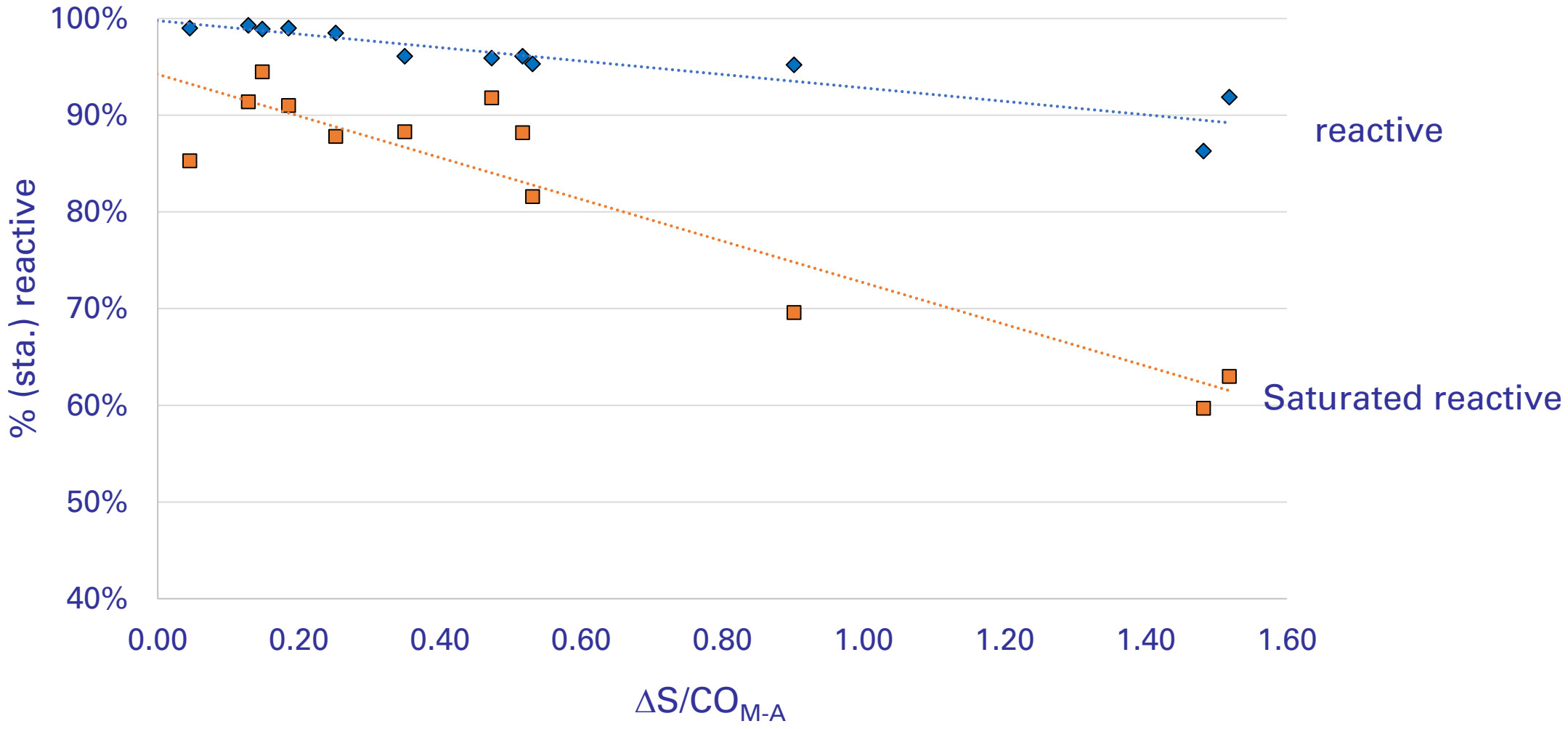
Bio QControl

Distribution of S/CO ratios in Ultrio Plus/Elite on P0063 ViraQ HCV Trend Control of 25 cp/mL



Bio QControl

Correlation between Δ (median – average) S/CO and proportion (saturated) reactive on P0068 ViraQ HIV-1 Trend 25 Control in different reagent lot combinations

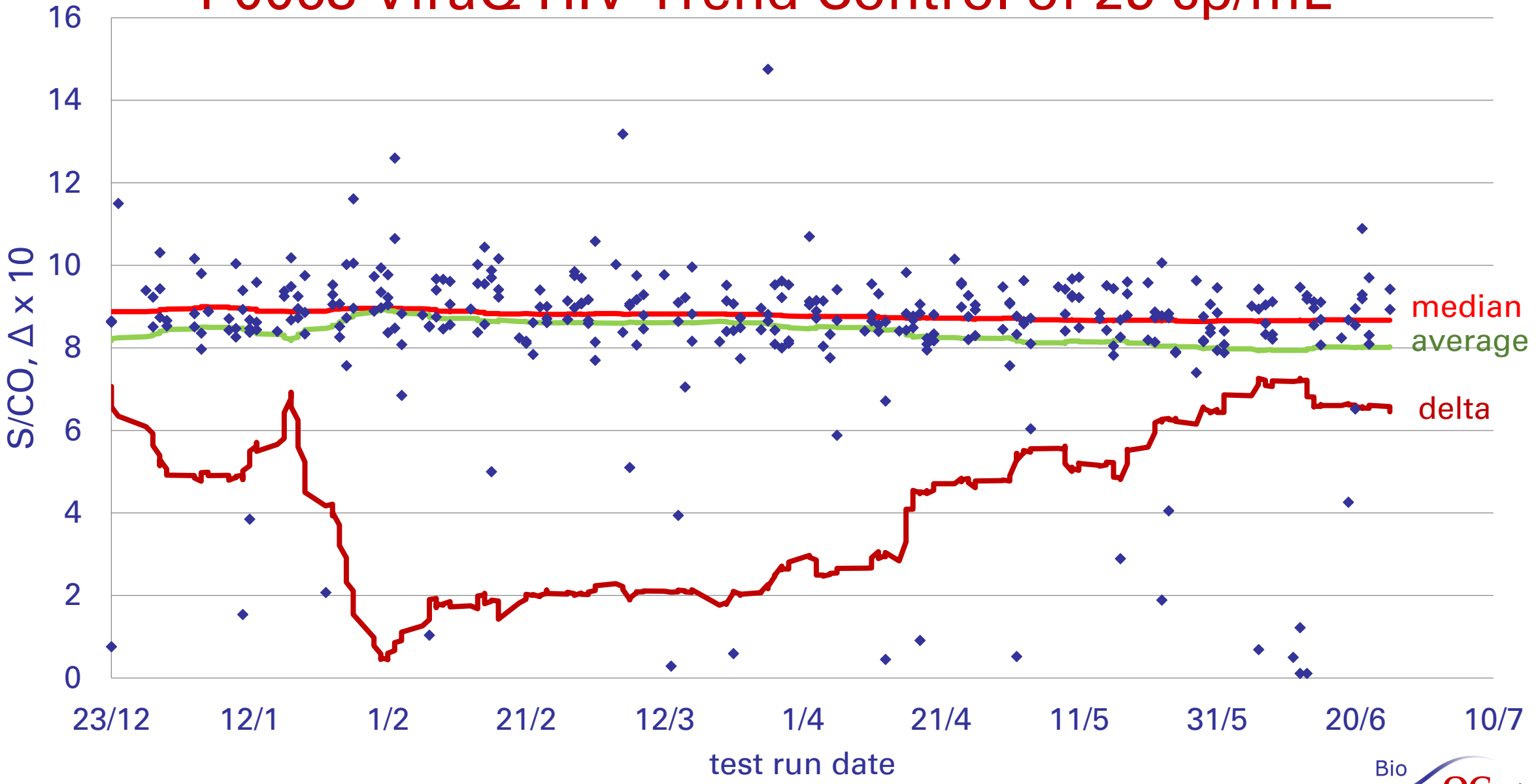


Reactivity rates of Ultrio (Plus and Elite) reagent batches on P0068 ViraQ HIV Trend 25 control batches

TMA batch	P0068 ViraQ batch	reactive/ test runs	reactivity rate	Delta % vs all (95% CI)
Ultrio 1	B4063-001	180/200	90.0%	-6.0 (-7.4/-4.6)%
Ultrio Plus 1		70/73	95.9%	0.1 (-1.1/0.8)%
	B4063-002	99/100	99.0%	3.0 (2.7/3.2)%
Ultrio Plus 2		285/299	95.3%	-0.7 (-1.3/-0.2)%
	B4063-003	67/67	100.0%	4.0 (3.8/4.1)%
Ultrio Plus 3		173/180	96.1%	0.1(-0.5/0.7)%
Ultrio Plus 4		202/204	99.0%	3.0 (2.8/3.2)%
Ultrio Plus 5		219/228	96.1%	0.1(-0.5/0.6)%
Ultrio Plus 6		89/89	100.0%	4.0 (3.8/4.1)%
Ultrio Plus 7		90/91	98.9%	2.9 (2.6/3.2)%
Ultrio Elite 1	B4062-004	120/139	86.3%	-9.7(-12.0/-7.4)%
Ultrio Elite 2		338/355	95.2%	-0.8(-1.3/-0.3)%
Ultrio Elite 3		67/73	91.8%	-4.2 (-6.1/-2.3)%
Ultrio Elite 3	B4062-005	445/452	98.5%	2.5 (2.2/2.6)%
Ultrio Elite 4		139/140	99.3%	3.3 (3.1-3.5)%
All		2583/2690	96.0%	reference

Bio QControl

S/CO ratios in Ultrio Elite on P0068 ViraQ HIV Trend Control of 25 cp/mL



Bio QControl

Conclusions

- ViraQ Check 125 and 75 Controls were set at 3-6 times the 95% LOD of NAT methods (and ~10 times for HIV in cobas MPX versions)
- A nonreactive result on ViraQ Check Controls is a warning signal for a significantly reduced analytical sensitivity of the NAT system.
- ViraQ Multi-Marker Check Controls are positioned in the lower part of the log-linear dose response line of the cobas MPX assay versions. Monitoring Ct values can be done with Levey-Jennings charts
- ViraQ Check and Trend Controls are positioned near the Poisson detection endpoint range where S/CO values are no longer normally distributed. Since a Gumbel (extreme value) distribution can be used to describe the data it follows that Δ (median – average) S/CO is a parameter for the skewness of the distribution curve or the proportion of weak (and nonreactive) results. Hence Δ (median – average) S/CO is a parameter for monitoring the analytical sensitivity of TMA reagent lots and instruments.
- ViraQ HBV Trend 50 Controls gave higher than expected reactivity rates of 99.4% in Ultrio Plus and 98.1% in Ultrio Elite
- ViraQ Trend 25 Controls were set at 91-97% reactivity rate according to probit analysis on the inactivated standard dilutions and 92-96% hit rates were observed by IBTS
- ViraQ Trend Controls were instrumental to reveal small but significant differences in analytical sensitivity of Ultrio Plus and Elite reagent batches