Performance evaluation of ViraQ HBV/HCV/HIV-1 Controls

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1. OBJECTIVE

The objective is to evaluate the performance of the ViraQ run controls for HBV-DNA, HCV-RNA and HIV-1 RNA. The single marker run controls were primarily designed for testing in the Ultrio Plus and Elite assay versions (Grifols) and the ViraQ multi-marker run controls were developed for the cobas MPX assay versions (Roche). In this design verification report the suitability of the ViraQ run controls for these blood screening NAT assays is demonstrated. Moreover it is shown that the single marker run controls can be used for quantitative NAT methods. The design history of the ViraQ Check and Trend Controls is documented in VR4059. This latter report describes the positioning of the run controls in relation to the analytical sensitivity of the target NAT blood screening assays. During the evaluation phase of the ViraQ run controls it was decided to discontinue production of the ViraQ Check and Trend Controls of 250 and 50 cp/mL for HBV-DNA and replace them with controls containing 125 and 25 cp/mL, comparable to the levels in the run controls for HCV-RNA and HIV-1 RNA. Since 2010 several batches of these run controls have been prepared. The present VR4061 report compares the test results on these batches to prove consistency of production and verification of design. As described in the design report VR4059 we needed to develop Trend Controls of 25 cp/mL near the 95% lower limit of detection (LOD) of the Ultrio assay versions in order to monitor variation in analytical sensitivity of the transcription mediated amplification (TMA) reagent batches. The present design verification report VR4061 compares the reactivity rate and distribution of sample to cutoff (S/CO) ratios found on the ViraQ Trend Controls in different Ultrio (Plus and Elite) reagent batches over time. The observed reactivity rate on the run controls is compared with the predicted reactivity rate calculated by probit analysis using the analytical sensitivity data on BioQ standard dilution series (VR4059). We used the available test results on the ViraQ Check and Trend Controls to develop and verify a new statistical method for demonstrating upward or downward trends in the analytical sensitivity of the Ultrio Plus and Elite assays as could be deduced from the changing distribution of S/CO results over time. This was not necessary for the cobas MPX real time PCR assay versions in which Ct values can be monitored using descriptive statistics. At the time of preparation of this report there were test results available of only one batch of multi-marker run control evaluated in the TaqScreen 2.0 and cobas MPX assay versions.

2. INTRODUCTION

Users of nucleic acid amplification technology (NAT) methods test external run controls to have an independent and consistent standard in hand for monitoring proper technical performance of their automated NAT systems and guaranteeing sufficient analytical sensitivity of the daily test runs. By using a well calibrated and rightly positioned run control concentration laboratories are able to control for the correct execution of the NAT test and ensure that the analytical sensitivity of the NAT assay claimed by the manufacturer is maintained over time. In this report we have verified the suitability of two run control concentrations for the Ultrio Plus and Elite Assay versions , i.e. 125 cp/mL and 25 cp/mL in the ViraQ Check and Trend Controls respectively, whereas for the TaqScreen 2.0 and the later introduced cobas MPX assay the ViraQ Multi-Marker Check Controls of 125 and 75 cp/mL were evaluated. In the present report we also evaluated a newly developed statistical method for analyzing the Ultrio Plus or Ultrio Elite test results on our run controls. Both the concentration in the run controls and the statistical evaluation method are essential tools for ensuring sufficient analytical sensitivity of NAT test runs. Both these components of the external quality control system could be verified using the reported data of three national blood screening laboratories: i.e. (1) the South African National Blood Service (SANBS) having tested the ViraQ Check 125 controls in the Ultrio versions, (2) the Irish Blood Transfusion Service (IBTS) having evaluated the ViraQ Trend 25 controls in the Ultrio assay versions and (3) the Sanquin Blood Supply Foundation that tested the ViraQ Multi-Marker Check 125 and 75 controls in two cobas MPX assay versions.

Currently there are two general NAT methods applied for blood screening: i.e. real-time PCR and TMA. Real time PCR generates a Ct value, which can be related to the viral load in the test sample. By using a run control concentration just outside the Poisson distribution detection endpoint range one can follow the Ct values (or the reported log(viral load) in quantitative NAT

methods). Then the Ct values are normally distributed and the Ct value can be directly used for trend analysis according to Westgard rules.

The TMA is a kinetic end-point test, and as a consequence the reported S/CO values reach a saturation point (with a maximum range of S/CO values that does not increase above the 95% LOD). The ViraQ Check Control 125 products contain 4-5 times the 95% LOD in the Ultrio Plus and Elite versions. At this level we expect a 99.5 to 99.9 % reactivity rate (the design requirement in VR4059) while maximum S/CO levels are reached in the saturated response range of the assay in the vast majority of cases. The TMA tests on the ViraQ Trend Controls of 25 cp/mL show more variation in S/CO results which is required to recognize an upward or downward trend in analytical sensitivity of the NAT test runs. For trend analysis it is necessary to define which statistical tests can be used. The S/CO values on the ViraQ Check and Trend Controls are not normally distributed and therefore an alternative distribution model (Gumbel distribution) was required to be able to define statistical valid parameters for trend analysis.

The large amount of available test results on the ViraQ run controls in the Ultrio assay versions enabled us to establish and evaluate the parameters for trend analysis on S/CO. Using the ViraQ Trend Controls of 25 cp/mL we were able to identify differences in analytical sensitivity between TMA reagent batches and compare the performance of NAT instruments. Since the results were collected over a prolonged period of time we were able to also demonstrate batch consistency of the ViraQ run controls. This report verifies the expected reactivity rate and response levels on the run controls in the targeted test systems as could be predicted from the analytical sensitivity studies on our standard dilution panels (VR4059). The results in this design verification report VR4061 show that the ViraQ Trend Controls are suitable for monitoring analytical sensitivity of TMA test runs and that the ViraQ Check Controls are instrumental in ensuring sufficient analytical sensitivity of both Ultrio and cobas MPX assay versions as well as monitoring precision of diagnostic viral load assays.

3. METHODS

3.1 'Design Freeze' of ViraQ run controls

Since the inception of the Check Controls of 125 cp/mL (and 75 cp/mL) and the Trend Controls of 25 cp/mL the composition of the product and the preparation process remained the same for all consecutive batches. In the BioQ quality management system the design phase of the ViraQ run control product family stops ('design freeze') at the time the product is defined and the first batch was prepared for performance evaluation. The consistency of the product composition and the production variables is ensured by and traceable in the batch preparation records that are administered on so called 'batch record forms' (BRFs) which are unique for each product (table 1).

Table 1. ViraQ Check and Trend Control products designed for performance evaluation and corresponding batch record forms

Product	BRF*	Date V1.0	Current version	Date current version#
P0063 ViraQ HCV Check 125	BRF4058	24 sep 2010	7.0	09 jan 2017
P0064 ViraQ HIV Check 125	BRF4059	24 sep 2010	7.0	24 jan 2017
P0065 ViraQ HBV Check 125	BRF4060	04 feb 2010	4.0	19 jan 2017
P0067 ViraQ HCV Trend 25	BRF4062	24 sep 2010	4.1	31 jan 2014
P0068 ViraQ HIV Trend 25	BRF4063	24 sep 2010	3.0	20 jan 2014
P0069 ViraQ HBV trend 25	BRF4064	12 feb 2010	2.0	13 feb 2015
P0154 ViraQ HBV Trend 50^	BRF4154	24 sep 2010	5.0	20 jan 2014
P0254 ViraQ Multi-Marker Check 125	BRF4254	16 okt 2014	1.0	16 okt 2014
P0273 ViraQ Multi-Marker Check 75	BRF4264	24 nov 2016	1.0	24 nov 2016

*BRF= batch record from # In the newer BRF versions details changed, volumes increased but the composition remained the same ^ product is phased out

3.2 Collected test results on ViraQ run controls

During the evaluation phase for CE marking the ViraQ run controls shown in table 2 were registered for performance evaluation only (PEO). Four laboratories of these PEO labelled products reported their test results to us. Table 1 presents an overview of the available test results from these centres and the NAT methods in which the ViraQ run control products were evaluated.

Table 2. Overview of collected test results on ViraQ run controls

Article	NAT method	Type test result	Number test results	Evaluation center$
P0063 ViraQ HCV Check 125	Procleix Ultrio Plus	S/CO	6107	SANBS
	Hologic Aptima	IU/mL	29	OLVG
P0064 ViraQ HIV-1 Check 125	Procleix Ultrio Plus	S/CO	2092	SANBS
	Hologic Aptima	Cp/mL	24	OLVG
P0065 ViraQ HBV Check 125	Procleix Ultrio Plus	S/CO	338	SANBS
	Hologic Aptima	IU/mL	24	OLVG
P0155 ViraQ HBV Check 250	Procleix Ultrio Plus	S/CO	6028	SANBS
P0067 ViraQ HCV Trend 25	Procleix Ultrio	S/CO	192	IBTS
	Procleix Ultrio Plus	S/CO	1330	IBTS
	Procleix Ultrio Elite	S/CO	1178	IBTS
P0068 ViraQ HIV-1 Trend 25	Procleix Ultrio	S/CO	200	IBTS
	Procleix Ultrio Plus	S/CO	1330	IBTS
	Procleix Ultrio Elite	S/CO	1159	IBTS
P0069 ViraQ HBV Trend 25	Procleix Ultrio Elite	S/CO	190	IBTS
P0154 ViraQ HBV Trend 50	Procleix Ultrio Plus	S/CO	1261	IBTS
	Procleix Ultrio Elite	S/CO	1149	IBTS
P0254 ViraQ Multi-Marker 125	Roche MPX 2.0	Ct	40	Sanquin
P0273 ViraQ Multi-Marker 75	Roche cobas MPX	Ct	13	Sanquin

$ SANBS is South African National Blood Transfusion Service (Johannesburg, South Africa), OLVG is Onze Lieve Vrouwe Hospital (Amsterdam, The Netherlands), IBTS is Irish Blood Transfusion Service, (Dublin, Ireland), Sanquin is National Screening Service of Sanquin Blood bank (Amsterdam, the Netherlands).

3.3 Design verification of ViraQ run control levels

A series of ViraQ Check and Trend Controls (BioQControl, Heiloo, the Netherlands) has been developed from inactivated HBV, HCV and HIV standards calibrated in IU/ml and cp/mL against the secondary Sanquin standards in multiple bDNA 3.0 assays (VR4060). The 50% and 95% LODs (CI) on the inactivated BQC standard dilutions were determined by probit analysis on the proportion of reactive results obtained by testing standard dilution series (VR4059). ViraQ Check Controls were formulated at >99.5% reactivity for HBV, HCV and HIV while ViraQ Trend Controls were formulated around 95% reactivity. The analytical sensitivity data of the Grifols Ultrio and Roche cobas MPX versions are reported in VR4059. In this latter report it is shown that Ultrio, Ultrio Plus and Ultrio Elite have similar analytical sensitivity for detecting HIV-1-RNA and HCV-RNA. The Ultrio Plus and Elite assays reach comparable LODs for detection of HBV-DNA, but the original Ultrio assay had a significant lower analytical sensitivity for this marker. Since the Ultrio

assay poorly detects the heat-inactivated BioQ HBV standard and is out phased, a run control has not been developed for this assay. Hence, the ViraQ Check Controls of 125 cp/mL and Trend Controls of 25 cp/mL were mainly designed for the Ultrio Plus and Elite versions and most of the data for evaluation in this report were obtained by these assays (we do not report HBV data for the Ultrio assay). In the present design verification report VR4061 we compared the predicted reactivity rate in Ultrio (Plus and Elite) assays on the ViraQ Check and Trend Control levels from the probit analysis on standard dilution panels (VR4059) with the actually found detection rates on the ViraQ run control products itself. The LODs of the NAT methods on standard dilution panels and the expected reactivity rates on the run control concentrations are shown in VR4059. Originally we designed a multi-marker control of 125 cp/mL for the TaqScreen MPX 2.0 assay, and recently a new multi-marker control of 75 cp/mL was developed for the cobas MPX assay on the cobas 6800/8800 platforms. Although few data are available on these latter run controls we were able to compare the predicted Ct values (based on standard dilution data in VR4059) with the actual values. The suitability of ViraQ Check 125 controls for the quantitative viral load tests is based on the claims for the lower limit of quantification (LOQ) made by the assay manufacturer. In VR4059 the concentration of 125 cp/mL (and equivalent values in IU/mL) in the Check Controls is compared with the LOQ values in IU/mL for three quantitative NAT methods, i.e. the Roche cobas TaqMan, the Abbott real Time assay and the Hologic Aptima tests. It was predicted that that these run control concentrations (when expressed in IU/mL values based on the calibration experiments described in VR4060) are sufficiently above the LOQs of these viral load assays. In the present VR4061 report it is verified that the concentration of the ViraQ Check run control is still within the log linear dynamic range of at least one of these methods (The Hologic Aptima tests). For this latter method we compared the quantitative results on the run controls with the claimed LOQ values. This allowed us to compare the distance between claimed LOQ, the assigned run control concentration and the quantitative values found on the run controls.

3.4 Consistency of manufacturing

The consistency of manufacturing can be checked from the weight records on the BRF forms. The batch is accepted when the weights of stock solution/pre-diluted standard and the negative plasma batch fall within a range between 99.5-100.5% of the target weights on the BRF forms. If all parameters recorded in the BRF forms are satisfactory, batch release control testing takes place at the Sanquin reference laboratory. Since 2 year each Check control batch is tested in Roche cobas MPX tests against a reference batch stored at -80°C. In the batch release protocol both the test batch and the reference batch are tested four times within one run, so generating 4 Ct values of the test batch and 4 Ct values of the reference batch. The difference found in Ct values between the test batch and reference batch is evaluated using a paired t-test assuming equal variance. In this report we have used the Ct values for calculation of a potency value with 95% confidence limits (95%CI). If the 95% CI overlaps the value 1.0 there is no significant difference in the measured concentration of the test batch and the reference batch.

Previously both ViraQ Check and ViraQ Trend Control batches were released by testing dilutions of the ViraQ products at the 95% and 5% LOD in the Ultrio Plus or Elite assays. Until recently this procedure continued for the ViraQ Trend Controls. However since the cobas MPX assay now has 95% LODs being sufficiently below 25 cp/mL for HBV and HIV and near 25 cp/mL for HCV we decided to perform the batch release control tests according to the same procedure as the ViraQ Check Controls by quadruplicate MPX testing by Sanquin.

3.5 Statistics

Run control results have two interpretation levels: a single result within a test run and a combination of test results over time in a number of consecutive test runs. For the latter interpretation a trend analysis is required. For both applications statistical tests must be established and verified with regard to their suitability. The evaluated statistical methods in this validation report are described In the chapters below.

3.5.1 *Comparison of (observed and predicted) reactivity rates*

For the concentrations in the ViraQ run controls we calculated the reactivity rate by probit analysis (and the 95% confidence interval) using the reactivity rates on standard dilution series (data in VR4059). The predicted reactivity rate deduced from the analytical sensitivity studies was compared to the measured reactivity rate on the run controls. The 95% confidence interval for the run control levels was also calculated by probit analysis (assuming Poisson distribution of replicate test results).

We also compared the reactivity rates in different NAT reagent batches and on different run control batches using the overall reactivity as the reference value.

The relative proportions reactive is $(P_1 * N_1) / (P_2 * N_2)$, where is P is proportion positive and N number tested. The reactivity of run controls is expressed in proportions. For each comparison the variability of the difference between proportions is calculated as follows:

To construct a confidence interval for the difference between two sample proportions, we need to know about the sampling distribution of the difference. Specifically, we need to know how to compute the standard deviation of this sampling distribution.

The standard deviation of the sampling distribution is the "average" deviation between all possible sample differences ($p_1 - p_2$) and the true population difference, ($P_1 - P_2$). The standard error of the difference between sample proportions $SE_{p1 - p2}$ is:

$$= \text{sqrt}\{ [p_1 * (1 - p_1) / n_1] * [(N_1 - n_1) / (N_1 - 1)] + [p_2 * (1 - p_2) / n_2] * [(N_2 - n_2) / (N_2 - 1)] \}$$

where P_1 is the population proportion for sample 1, P_2 is the population proportion for sample 2, n_1 is the sample size from population 1, n_2 is the sample size from population 2, N_1 is the number of observations in population 1, and N_2 is the number of observations in population 2. The standard error is used to estimate confidence intervals for difference in proportion positive. In our approach the reference group represents all measurements obtained over a longer time period (the most near outcome for the total large population), while the samples represent a subset. The comparison is used to verify whether significant differences in proportions of reactive results were present.

3.5.2 *Monitoring TMA performance using extreme value distribution on S/CO results*

To monitor performance over time, the distribution of S/CO results reported by the Ultrio assay versions should be established first. It was clear that a normal distribution of S/CO values was not obtained and this cannot be expected because of the nature of the TMA assay. It was found that a type of extreme value distribution was more suitable to describe the data. In probability theory and statistics, the generalized extreme value (GEV) distribution is a family of continuous probability distributions developed within the extreme value theory. In this theory the Gumbel, Fréchet and Weibull families also known as type I, II and III extreme value distributions are combined. We considered the Gumbel and General extreme value distribution (or Fisher-Tippett distribution). Both distributions use the average and median of the set measurements to establish the distribution parameters. It was finally decided to use the Gumbel distribution for evaluating changes in S/CO results over time.

In probability theory and statistics, the Gumbel distribution (Generalized Extreme Value distribution Type-I) is used to model the distribution of the maximum (or the minimum) of a number of samples of various distributions. It is useful in predicting the chance that an extreme event (a negative result will occur. The potential applicability of the Gumbel distribution to represent the distribution of maxima relates to extreme value theory, which indicates that it is likely to be useful if the distribution of the underlying sample data is of the normal or exponential type.

Parameters	μ —location, $\beta > 0$ —scale
Probability density function	$PDF = \frac{1}{\beta} e^{-(z+e^{-z})}$ where $z = \left(\frac{x-\mu}{\beta}\right)$
Cumalative density function	$CDF = e^{-(e^{-z})}$
Measured mean equals	$\mu + \beta\gamma$, where γ is the Euler-Mascheroni constant ~0.5772
Measured median equals	$\mu + \beta \ln(\ln(2))$

Having the mean and median calculated from the data sets both parameters can be calculated.

So Δ (average S/CO – median S/CO) = $\beta\ (\gamma - \ln(\ln(2))$, allowing calculation of β which comes to Δ (average S/CO – median S/CO) = 0.914.β.

Then μ = average S/CO – ($\Delta\gamma$/0.914) and β. = Δ (average S/CO – median S/CO)/0.914

So the Δ is used as parameter to monitor the performance of Ultrio tests as it represents the scale (or skewness) and location of the distribution curve to the minimal value (a non-reactive result).

3.5.3 *Monitoring real time PCR performance using Ct values or viral load (IU/mL or cp/mL).*

Real time PCR or TMA tests report more variation when the input copy number becomes a stochastic variable followed by intermittent detection. This happens with lower viral loads, or a few virions input, near the Poisson distribution detection endpoint range of the assay. In quantitative NAT methods the lower limit of quantification (LOQ) represents the end of the quantitative range of the assay and is determined arbitrarily by comparing the coefficient of variation (%CV) over a wider dose range. The reported Ct value or log viral load on the run control concentration should be sufficiently above the LOQ and just above the range in which an increased variation is expected because of the effect of Poisson distribution. Whether differences in variation of quantitative results reach statistical significance can be evaluated using the chi-square distribution (table 3).

The cumulative Chi-square distribution is used to estimate the probability that the SD of the test population (s) is different from the SD of the reference population (σ):

- n is number of measurements over the evaluated period
- Within the set evaluated: calculate SD on the log(concentration) or Ct value(n>10): s
- Within the reference set: calculate SD on the log(concentration) or Ct value: σ.
- Calculate $X^2 = (n-1)\frac{s^2}{\sigma^2}$

Table 3 gives the Chi-square (X^2) values for different numbers of observations above which the difference between two data sets reaches significance (p<0.05)

Table 3. Chi-square (X^2) values for p=0.05

n-1 (df)	X^2	n-1 (df)	X^2	n-1 (df)	X^2
11	19.69	21	32.67	40	55.76
12	21.04	22	33.92	50	67.51
13	22.36	23	35.17	60	79.08
14	23.69	24	36.42	70	90.53
15	25.00	25	37.65	80	101.88
16	26.30	26	38.89	90	113.15
17	27.59	27	40.11	100	124.34
18	28.87	28	41.34		
19	30.14	29	42.56		
20	31.41	30	43.77		

4. RESULTS

4.1 Comparison of predicted and observed NAT reactivity rates on ViraQ run controls

In the design report VR4059 the reactivity rate on the ViraQ run controls was predicted from the probit curves obtained with replicate NAT test results on the inactivated BioQ standard dilution panels. The same inactivated standards were used for preparation of the ViraQ run controls that were tested in multiple test runs by different NAT methods over time. Table 4 compares the predicted reactivity rates by probit analysis and the observed reactivity rates on the ViraQ run control products. The 95% confidence intervals (CI) of the predicted reactivity rates by probit analysis overlapped the observed reactivity rates indicating that the predicted and observed reactivity rates were comparable.

Table 4 Comparison of predicted and observed reactivity rates on ViraQ run control products

Run control product	NAT method	Observed reactivity rate	Predicted reactivity rate (95% CI)
P0063 ViraQ HCV Check 125	Ultrio Plus	6100/6107 (99.9%)	99.8 (99.0-99.9)%
P0064 ViraQ HIV-1 Check 125	Ultrio Plus	2092/2092 (100%)	99.9 (99.6-99.9)%
P0065 ViraQ HBV Check 125	Ultrio Plus	338/338 (100%)	99.9 (99.1-99.99)%
P0155 ViraQ HBV Check 250	Ultrio Plus	6028/6028 (100%)	100 (99.9-100)%
P0067 ViraQ HCV Trend 25	Ultrio	184/192 (95.8%)	93.7 (88.0-96.8)%^
	Ultrio Plus	1219/1330 (91.7%)	
	Ultrio Elite	1092/1178 (92.7%)	
	All Ultrio versions	2495/2700 (92.4%)	93.7 (88.0-96.8)%^
P0068 ViraQ HIV-1 Trend 25	Ultrio	180/200 (90.0%)	96.7 (92.4-98.6)%^
	Ultrio Plus	1293/1330 (97.2%)	
	Ultrio Elite	1109/1159 (95.7%)	
	All Ultrio versions	2582/2689 (96.0%)	96.7 (92.4-98.6)%^
P0069 ViraQ HBV Trend 25	Ultrio Elite	176/190 (92.6%)	90.8 (81.5-95.6)%#
P0154 ViraQ HBV Trend 50	Ultrio Plus	1253/1261 (99.4%)	
	Ultrio Elite	1127/1149 (98.1%)	
	Ultrio Plus & Elite	2556/2600 (98.3%)	98.2 (93.8-99.4)%
P0254 ViraQ Multi-Marker 125	Roche MPX 2.0	40/40 (100%)	99.7(95.6-99.9)%^{HCV}, 100 (99.9-100)%^{HIV}, 99.8(96.2-99.9)%^{HBV}
P0273 ViraQ Multi-Marker 75	Roche cobas MPX	13/13 (100%)	99.9(94.8-99.9)%^{HCV}, 100(99.1-100)%^{HIV}, 99.7(95.0-99.9)%^{HBV}

^ in Ultrio only #in Ultrio Plus and Elite combined

4.2 Comparison of reactivity rates of TMA reagent batches on ViraQ Trend Control batches

The IBTS tested different ViraQ Trend Control batches in consecutive Ultrio, Ultrio Plus and Ultrio Elite reagent batches. The proportion of reactive results per run control batch and per TMA reagent batch are presented in tables 5a, 5b and 5c. The proportions of reactive results in daily test runs on the consecutive reagent batches were compared to the overall reactivity rate found on all reagent batches of all Ultrio versions taken together.

The TMA reactivity rates on P0067 ViraQ HCV Trend 25 Control are shown in table 5a. The rates per TMA/trend control reagent batch combination varied significantly from 78.4% to 100%. Within one batch of ViraQ Trend Control the TMA batch reactivity rates varied from 78.4 to 95.8% (run control batch 001), 88.1 to 88.9% (run control batch 002), 91.1 to 100% (run control batch 004), 88.6 to 93.2% (run control batch 005) and 93.0 to 95.7% (run control batch 006). The combined TMA batch reactivity rates per ViraQ Trend Control batch varied from 88.3% (run control batch 002) to 94.3% (run control batch 004). The overall Ultrio Plus reactivity rate of 1219/1330 (91.7%) was comparable to the overall Ultrio Elite reactivity rate of 1092/1178 (92.7%).

Table 5a. Proportion of reactive results in daily test runs on P0067 ViraQ HCV Trend 25 Control batches in consecutive Ultrio (U), Ultrio Plus (UP) and Ultrio Elite (UE) reagent batches.

TMA batch	ViraQ batch	p/n	reactivity	delta (95%CI)
U xxxxxx	B4062-001	184/192	95.8%	3.4 (2.8-4.1)%
UP587882	B4062-001	58/74	78.4%	-14.0 (-19.0,-9.1)%
	B4062-001 All	242/266	91.0%	-1.4 (-2.6,-0.3)%
UP587882	B4062-002	88/99	88.9%	-3.5 (-5.7,-1.3)%
UP587882 All		146/173	84.4%	-8.0 (-10.4,-5.7)%
UP592848	B4062-002	252/286	88.1%	-4.3 (-5.7,-2.9)%
	B4062-002 All	340/385	88.3%	-4.1 (-5.3,-2.9)%
UP592848	B4062-004	80/80	100.0%	7.6 (7.3,7.9)%
UP592848 All		332/366	90.7%	-1.7 (-2.7,-0.7)
UP601406	B4062-004	168/179	93.9%	1.4 (0.5,2.4)%
UP601923	B4062-004	195/203	96.1%	3.7 (3.0,4.3)%
UP612037	B4062-004	210/228	92.1%	-0.3 (-1.4,0.8)%
UP621787	B4062-004	82/90	91.1%	-1.3 (-3.2,0.6)%
UP626009	B4062-004	86/91	94.5%	2.1 (0.9,3.3)%
	B4062-004 All	821/871	94.3%	1.9 (1.4,2.3)%
UP All		1219/1330	91.7%	-0.8 (1.3,-0.2)%
UE101801	B4062-005	31/35	88.6%	-3.8 (-7.7-0.0)%
UE616859	B4062-005	158/177	89.3%	-3.1 (-4.8,-1.5)%
UE634122	B4062-005	330/354	93.2%	0.8 (0.0,1.6)%
	B4062-005 All	519/566	91.7%	-0.7 (-1/5,0.0)%
UE101801	B4062-006	439/472	93.0%	0.6 (-0.1,1.3)%
UE106862	B4062-006	134/140	95.7%	3.3 (2.5,4.1)%
	B4062-006 All	573/612	93.6%	1.2 (0.6,1.8)%
UE All		1092/1178	92.7%	0.3 (-0.2,0.8)%
U, UP, UE All		2495/2700	92.4%	reference

The TMA reactivity rates on P0068 ViraQ HIV Trend 25 Control are shown in table 5b. The rates per TMA/trend control reagent batch combination varied significantly from 90.0 to 100%. Within one batch of ViraQ Trend Control the TMA batch reactivity rates varied from 90.0% to 94.5% (run control batch 001), 99.0% (run control batch 002), 96.1 to 100% (run control batch 004), 86.3% to 95.2% (run control batch 005) and 98.5% to 99.3% (run control batch 006). The combined TMA batch reactivity rates per ViraQ Trend Control batch varied from 91.6% (run control batch 001) to 98.6% (run control batch 006). The overall Ultrio Plus reactivity rate of 1293/1330 (97.2%) was comparable to the overall Ultrio Elite reactivity rate of 1108/1159 (95.7%).

Table 5b. Proportion of reactive results in daily test runs on P0068 ViraQ HIV Trend 25 Control batches in consecutive Ultrio (U), Ultrio Plus (UP) and Ultrio Elite (UE) reagent batches.

TMA batch	ViraQ batch	p/n	reactivity	delta (95%CI)
U xxxxx	B4063-001	180/200	90.0%	-5.5 (-6.9,-4.1)%
UP587882	B4063-001	70/73	95.9%	0.4 (-0.6, 1.3)%
	B4063-001 All	250/273	91.6%	-4.0 (-2.9, -5.0)%
UP587882	B4063-002	99/100	99.0%	3.5 (3.2,3.7)%
UP587882 All		169/173	97.7%	2.2 (1.8, 2.5) %
UP592848	B4063-004	67/67	100.0%	4,5 (4.3,4.6)%
UP601406	B4063-004	172/179	96.1%	0.6 (0.0,1.2)%
UP601923	B4063-004	202/204	99.0%	3.5 (3.3,3.7)%
UP612037	B4063-004	219/228	96.1%	0.5 (0.0,1,1)%
UP621787	B4063-004	89/89	100.0%	4.5 (4.3,4.6)%
UP626009	B4063-004	90/91	98.9%	3.4 (3.1,3.7)%
	B4063-004 All	839/858	97.8%	2.3 (2.0,2.5)%
UP All		1293/1330	97.2%	1.7 (1.5,1.9)%
UE616859	B4063-005	120/139	86.3%	-9.2 (-6.9, -11.5)%
UE634122	B4063-005	338/355	95.2%	-0.3 (-0.8,0.2)%
UE101801	B4063-005	67/73	91.8%	-3.8 (-5.7,-1.8)%
	B4063-005 All	525/567	92.6%	-2.9 (-2.6, -2.6)%
UE101801	B4063-006	445/452	98.5%	2.9 (2.7,3.1)%
UE106862	B4063-006	139/140	99.3%	3.8 (3.5,4.0)%
	B4063-006 All	584/592	98.6%	3.1 (2.9,3.3)%
UE All		1109/1159	95.7%	0.2(-0.1, 0.5)%
U, UP, UE All		2582/2689	96.0%	reference

The TMA reactivity rates on P0154 ViraQ HBV Trend Control 50 are shown in table 5c. The rates per TMA/trend control reagent batch combination varied from 97.2% to 100%. The TMA reactivity rates per ViraQ Trend Control batch varied from 97.9% (run control batch 005) to 99.4% (run control batch 004). The overall Ultrio Plus reactivity rate of 1253/1261 (99.4%) was comparable to the overall Ultrio Elite reactivity rate of 1127/1149 (98.1%).

Table 5c. Proportion of reactive results in daily test runs 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	99.0%	0.3 (0.1,0.5)%
UP592848	B4154-003	297/299	99.3%	0.6 (0.5,0.7)%
	B4154-003 All	399/402	99.3%	0.5 (0.4,0.6)%
UP592848	B4154-004	66/67	98.5%	-0.2 (-0.6,0.1)%
UP592848 All		363/366	99.2%	0.4 (0.3,0.5)%
UP601406	B4154-004	179/179	100.0%	1.2 (1.2,1.3)%
UP601923	B4154-004	203/204	99.5%	0.8 (0.7,0.8)%
UP612037	B4154-004	227/228	99.6%	0.8 (0.7,0.9)%
UP621787	B4154-004	90/90	100.0%	1.2 (1.2,1.3)%
UP626009	B4154-004	89/91	97.8%	-1.0 (-1.4,-0.5)%
	B4154-004 All	854/859	99.4%	0.7 (0.6,0.7)%
UP All		1253/1261	99.4%	0.6 (0.5,0.7)%
UE616859	B4154-005	132/132	100.0%	1.2 (1.2,1.3)%
UE634122	B4154-005	345/355	97.2%	-1.6 (-1.9,-1.3)%
UE101801	B4154-005	85/87	97.7%	-1.1(-1.5,-0.6)%
	B4154-005 All	562/574	97.9%	-0.8(-1.0,-0.7)%
UE101801	B4154-006	427/434	98.4%	-0.4(-0.5,-0.2)%
UE101801 All		512/521	98.3%	-0.5(-0.6,-0.3)%
UE106862	B4154-006	138/141	97.9%	-0.9(-1.2,-0.5)%
	B4154-006 All	565/575	98.3%	-0.5(-0.6,-0.3)%
UE All		1127/1149	98.1%	-0.7(-0.8,-0.5)%
UP, UE All		2380/2410	98.8%	reference

It was decided to later evaluate the P0069 ViraQ HBV Trend 25 control product in parallel to P0154 ViraQ HBV Trend 50 in the same test runs (table 5d). By 2-fold reduction of the HBV concentration in the trend control the reactivity rate decreased from 98.3% to 92.6%. The 25 cp/mL concentration is considered more suitable than 50 cp/mL for a trend control.

Table 5d. Comparison of reactivity rate on HBV Trend Control batches of 50 and 25 cp/mL

Ultrio Elite batch	P0154 ViraQ HBV 50			P0069 ViraQ HBV 25			
	B4154-	p/n	%	B4064-	p/n	%	delta (95%CI)
UE101801	004	427/434	98.5%	001	54/58	93.1%	5.4 (3.8,7.6)%
UE106862	004	138/141	97.9%	001	122/132	92.4%	5.5 (4.6, 7.5)%
UE all	004	565/575	98.3%	001	176/190	92.6%	5.7% (5.0,7.3)%

4.3 S/CO values on ViraQ Check and Trend Controls in Ultrio (Plus and Elite) test runs

4.3.1 *P0063 ViraQ HCV Check 125 Control*

At SANBS the batch-ID's of Ultrio Plus and ViraQ Check Control were not recorded. We therefore compared the S/CO distribution of 2012, 2013 and 2014 (table 6 and figure 1). During the three years observation period nine run control batches were tested. The HCV S/CO response values in the Ultrio Plus assay reaches a saturation point above a value of approximately 7.0. Below this level the S/CO response is still in the dynamic range because the TMA reaction is not yet complete. We arbitrarily chose a S/CO cut-off value of 7.0 between saturated and dynamic responses but this threshold value varies per TMA reagent batch. In another experiment in which inactivated S0109 HCV standard dilutions were tested in Ultrio the threshold value between saturated and dynamic S/CO values was 6.0 (VR4059, figure 6). The proportions of S/CO values<7.0 was 3.2% in 2012, 5.9% in 2013 and 7.9% in 2014. The higher proportion of S/CO values<7.0 in 2014 did not coincide with higher proportions of S/CO values <5.0, <4.0, <3.0, <2.0 and <1.0. Although the distribution curve of S/CO results in 2014 has shifted to somewhat lower S/CO values (figure 1), there was no indication of a change in analytical sensitivity. In 2014 only one Ultrio Plus test run had a non-reactive result (0.04%) on P0063 ViraQ HCV Check 125 control, whereas in 2012 and 2013 there were three (0.30%) and four (0.18%) runs with a non-reactive run control test.

Table 6 Comparison of distribution of S/CO results on P0063 ViraQ HCV Check 125 control in Ultrio Plus test runs in 2012, 2013 and 2014

	number tests			proportion			cumulative proportion		
S/CO	2012	2013	2014	2012	2013	2014	2012	2013	2014
0-1	3	4	1	0.30%	0.18%	0.04%	0.30%	0.18%	0.04%
1-2	4	2	18	0.41%	0.09%	0.64%	0.71%	0.27%	0.68%
2-3	3	10	9	0.30%	0.44%	0.32%	1.01%	0.71%	1.00%
3-4	4	7	8	0.41%	0.31%	0.29%	1.42%	1.02%	1.29%
4-5	5	27	28	0.51%	1.19%	1.00%	1.93%	2.21%	2.29%
5-6	3	28	41	0.30%	1.23%	1.47%	2.23%	3.44%	3.76%
6-7	10	55	117	1.02%	2.42%	4.19%	3.25%	5.86%	7.95%
7-8	49	191	488	4.98%	8.42%	17.47%	8.23%	14.28%	25.42%
8-9	340	799	1277	34.55%	35.21%	45.71%	42.78%	49.49%	71.13%
9-10	493	1006	738	50.10%	44.34%	26.41%	92.88%	93.83%	97.54%
10-11	59	131	48	6.00%	5.77%	1.72%	98.88%	99.60%	99.26%
11-12	1	2	2	0.10%	0.09%	0.07%	98.98%	99.69%	99.33%
12-13	0	2	3	0.00%	0.09%	0.11%	98.98%	99.78%	99.44%
13-14	3	1	3	0.30%	0.04%	0.11%	99.28%	99.82%	99.55%
14-15	3	1	4	0.30%	0.04%	0.14%	99.58%	99.86%	99.69%
15-16	3	1	4	0.30%	0.04%	0.14%	99.88%	99.90%	99.83%
>16	1	3	6	0.10%	0.09%	0.18%	100.00%	100.00%	100.00%

Figure 2 shows that at least three of the total of seven non-reactive results had enhanced S/CO levels, but also the 4 lowest non-reactive results are higher than the negative population. The predefined requirement of a reactivity rate >99.5 % to the Check Control was confirmed in each year, indicating that the chosen concentration of 125 cp/mL was suitable to ensure sufficient analytical sensitivity of TMA test runs.

The frequency of non-reactive results (or even weak S/CO responses < 4.0 or <5.0) is too low for recognition of trends. When we examined the cumulative distribution of S/CO values measured over three years (figure 3a) a cut-off value for saturated responses at 7.0 seems the best level. However when comparing the cumulative distribution of S/CO values during three months spread over the years (in order to mimic the use of different Ultrio Plus reagent batches) one would choose threshold values for saturated responses varying between 6.0 and 8.0 depending on the reagent batch in use. Bearing this limitation in mind we decided that a threshold S/CO

value for saturated responses at 7.0 is the best functional level for recognition of trends for higher frequencies of dynamic response values (indicative of lower analytical sensitivity of test runs). Overall we found 5721/6107 (93.7%) saturated HCV response values (S/CO≥7.0), 379/6107 (6.2%) responses in the dynamic range (1.0≥S/CO<7.0) and 7/6107 (0.11%) non-reactive Ultrio Plus results. The 95% confidence intervals were calculated at 91.2-96.0%, 5.6-6.8%, and 0.0-0.3% for saturated, dynamic and non-reactive S/CO values respectively.

Figure 1 Distribution of S/CO values in Ultrio Plus test runs on P0063 ViraQ HCV Check 125 control (blue line 2012, red line 2013 and green line 2014).

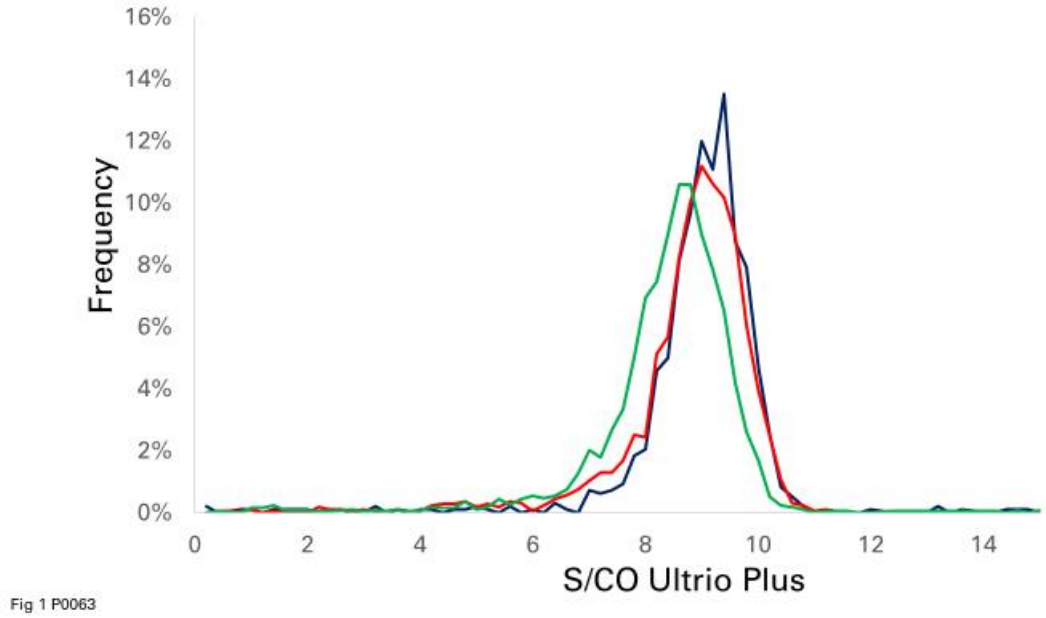


Figure 2 The 30 lowest sorted S/CO values on P0063 ViraQ HCV Check 125 control in 6107 Ultrio Plus test runs of which 7 had S/CO<1.0

Figure 3a Cumulative distribution of S/CO values in 6107 Ultrio Plus test runs on P0063 ViraQ HCV Check 125 control

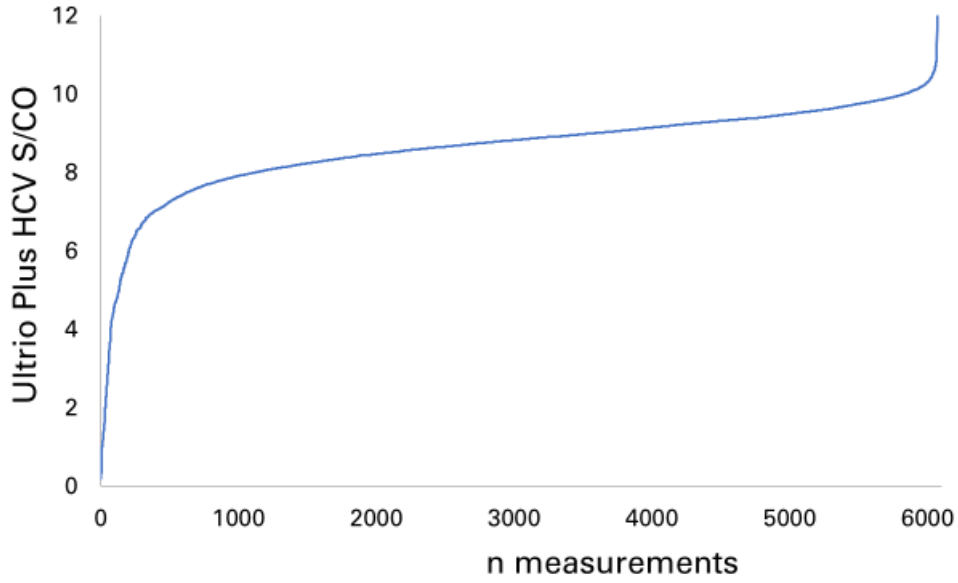


Fig 3a P0063

Figure 3b Cumulative distribution of S/CO values in Ultrio Plus test runs on P0063 ViraQ HCV Check 125 control measured in three different months to mimic properties of three different reagent batches. In this example we have chosen the most different months.

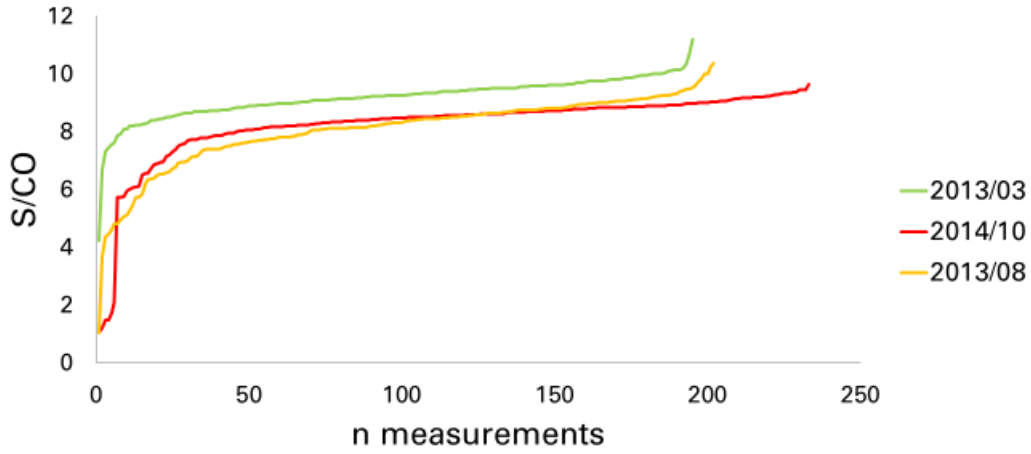


Fig 3b P0063

The dynamic S/CO responses on ViraQ HCV Check 125 occur less frequently than the saturated S/CO values and make that a Gumbel distribution was suitable to describe the data (see statistics in methods). From this type of extreme value distribution it follows that the difference between the median and the average of S/CO values is an indicator of the skewness of the distribution curve. To illustrate this we calculated the mean and median at each time point of testing of the run control for 50 earlier and 50 later S/CO measurements and did the same for the proportion reactive and proportion of saturated reactive responses (figure 4a). From the

sliding values it can be seen that the highest values of Δ (median S/CO – average S/CO) coincided with the lowest proportions of saturated responses (figure 4b). Based on available results we conclude that delta should be below 0.4 when the system is properly functioning. The presence of rare non-reactive results also coincides with high values of Δ (median S/CO – average S/CO), thereby confirming its ability to be a trend indicator for analytical performance of the TMA assay.

Figure 4a Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0063 ViraQ HCV Check 125 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date.

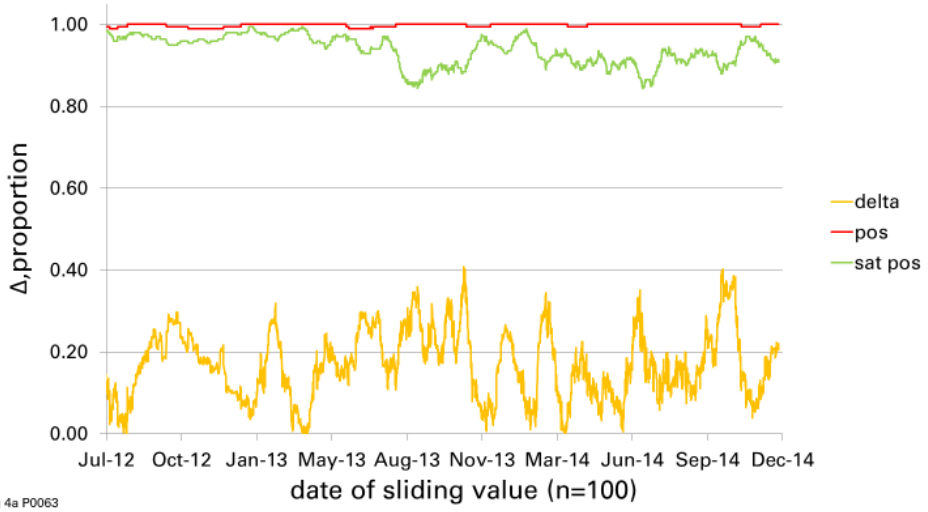


Fig 4a P0063

Figure 4b Relation between sliding Δ (median S/CO – average S/CO) and sliding proportion saturated reactive response values (and observed minimum and maximum, yellow lines) on P0063 ViraQ HCV Check 125 (data figure 4a).

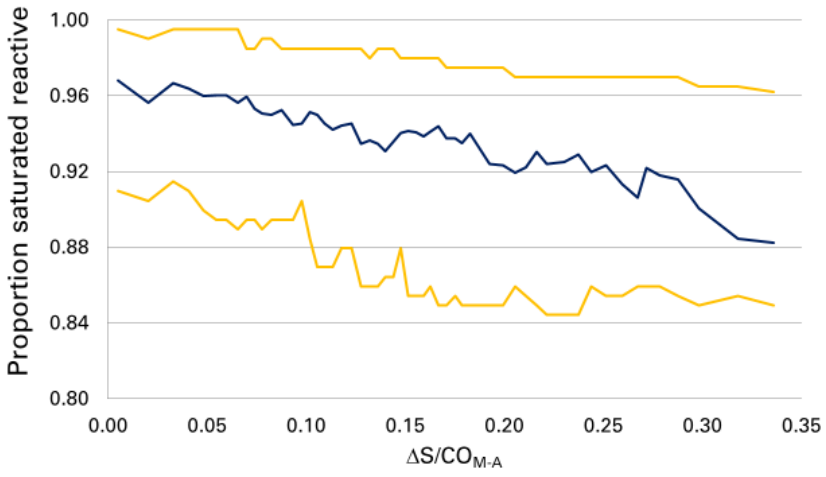


Fig 4b P0063

The parameter $\Delta(S/CO_{M-A})$ can also be applied to compare other experimental conditions such as the TMA reagent batch, the ViraQ run control batch or the testing robot (Tigris or Panther). An example using $\Delta(S/CO_{M-A})$ as performance indicator for experimental group evaluation is

shown in table 7 comparing performance of nine Tigris instruments. [Note that in this case, all data per experimental condition are used without 'sliding']. The result shows that $\Delta(S/CO_{M-A})$'s differ by instrument. For instance, the $\Delta(S/CO_{M-A})$ of instrument #3 is 0.02, smaller than 0.24 of instrument #7. Hence , if the instrument performance indicator $\Delta(S/CO_{M-A})$ has an outlier value it could be used as an alert signal for checking technical performance of the that particular instrument.

Table 7. Example of using $\Delta(S/CO_{M-A})$ for performance evaluation of 9 Tigris instruments used during 3 years.

Instrument #	Average S/CO	Median S/CO	$\Delta(S/CO_{M-A})$	n
1	8.42	8.58	0.16	635
2	8.60	8.74	0.14	792
3	8.72	8.74	0.02	644
4	8.72	8.82	0.10	652
5	8.65	8.82	0.17	763
6	8.77	8.96	0.19	452
7	8.74	8.98	0.24	643
8	8.75	8.98	0.23	631
9	8.98	9.18	0.20	448

4.3.2 *P0067 ViraQ HCV Trend 25*

From August 2011 until June 2015 the P0067 ViraQ Trend 25 control was tested by IBTS in 2700 test runs in the Ultrio, Ultrio Plus and Elite assay versions. The distribution of the S/CO values on the trend control is shown in table 8 and figure 5a,b. As with the Check Control data we assumed a S/CO threshold value for saturated TMA response values at 7.0. There were 196 (7.8%) non-reactive results, 583 (23.2%) dynamic and 1730 (69.0%) saturated responses.

Table 8 Distribution of S/CO results on P0067 ViraQ HCV Trend 25 control in Ultrio, Ultrio Plus and Elite test runs

S/CO	n	%
0-1	196	7.8%
1-2	71	2.8%
2-3	68	2.7%
3-4	86	3.4%
4-5	91	3.6%
5-6	104	4.1%
6-7	163	6.5%
7-8	336	13.4%
8-9	828	33.0%
9-10	509	20.3%
10-11	53	2.1%
11-12	2	0.1%
12-13	1	0.0%
13-14	1	0.0%

Figure 5a Distribution of S/CO values on P0067 ViraQ HCV Trend 25 control

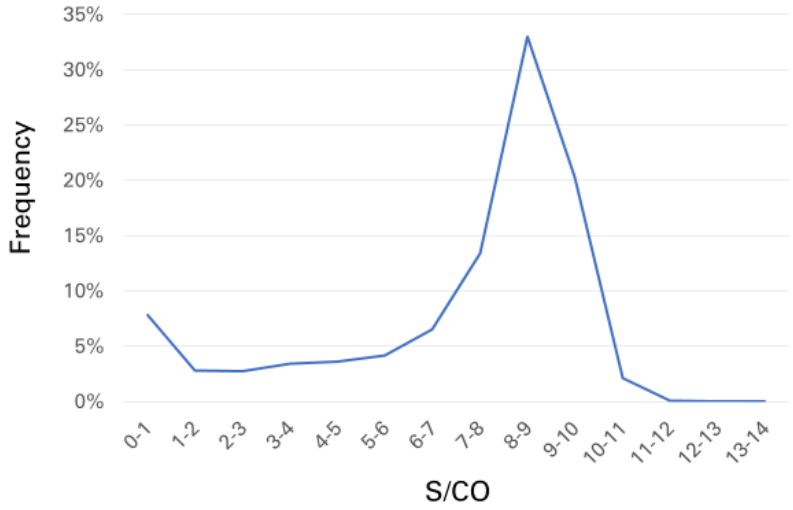


Fig 5a P0067

Figure 5b Cumulative distribution of S/CO values on P0067 ViraQ HCV Trend 25 control

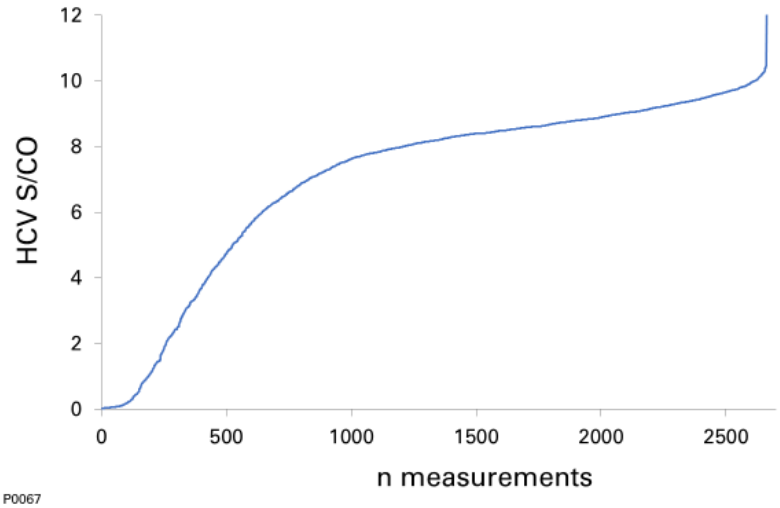


Fig 5b P0067

Figure 6a Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0067 ViraQ HCV Trend 25 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date

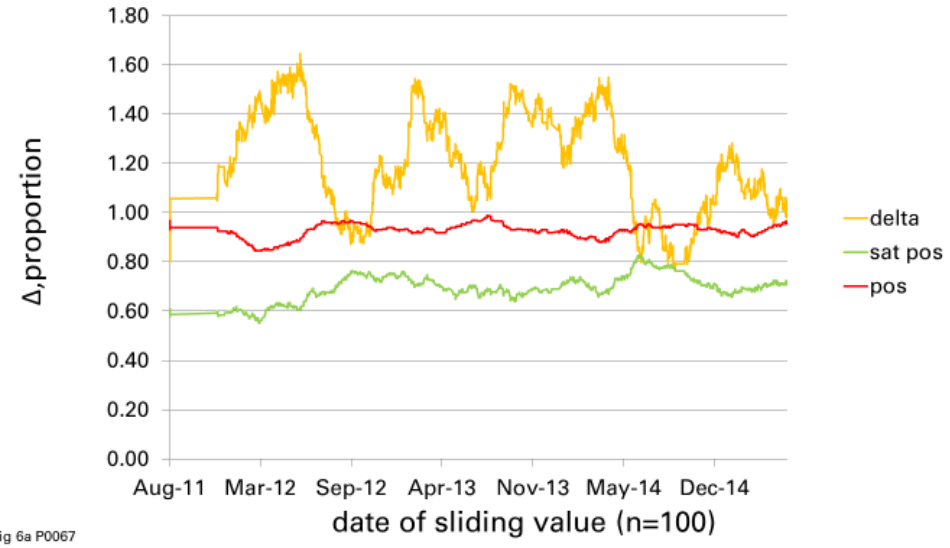


Fig 6a P0067

Figure 6b Relation between sliding Δ (median S/CO – average S/CO) and sliding proportion saturated reactive response values (and observed minimum, maximum yellow lines) on P0067 ViraQ HCV Check 25 (data figure 6a)

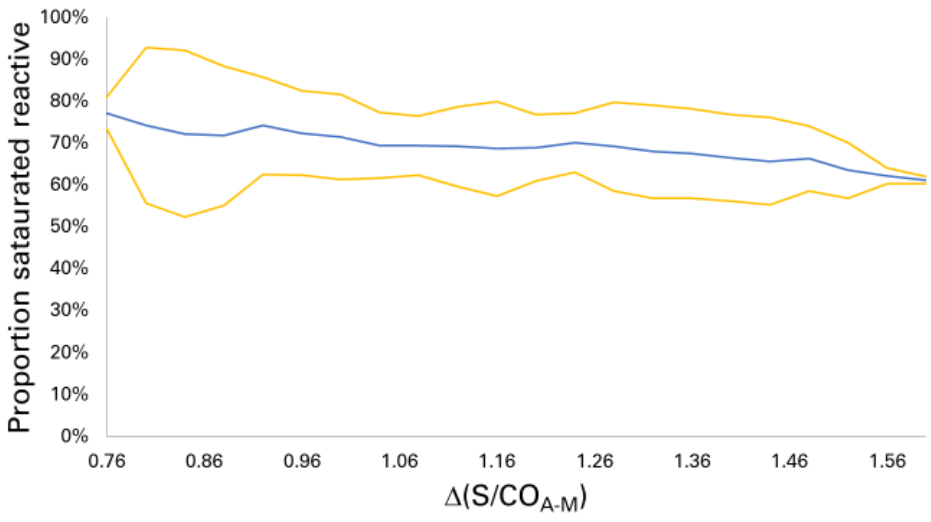


Fig 6b P0067

The Gumbel distribution not only applies to the S/CO values on the HCV Check 125 Control but also to those on the HCV Trend 25 Control in figure 5a and b. Again the sliding value of the performance indicator Δ (median S/CO – average S/CO) was calculated at each time point from 50 earlier and 50 later response values on the Trend Control. Figure 6a shows the course of the sliding value of Δ (median S/CO – average S/CO) in relation to that of the proportion of saturated response values (S/CO≥7). From these data one can confirm the correlation between the value of Δ (median S/CO – average S/CO) and the proportion saturated reactive results

(figure 6b). The highest sliding values of Δ (median S/CO – average S/CO) coincide with the lowest sliding proportions of reactive and saturated reactive results.

The currently evaluated results suggest that a delta above 1.6 may be related to a lower analytical sensitivity of the TMA system.

The parameter $\Delta(S/CO_{M\text{-}A})$ can also be used for comparison of instruments or TMA reagent batches, in which case it is not necessary to estimate sliding values over time from historical results. Figure 6c evaluates delta as a performance indicator of Ultrio (Plus and Elite) reagent batches on different batches of P0067 ViraQ HCV Trend 25 control. The reactivity rates become lower with higher values of the parameter $\Delta(S/CO_{M\text{-}A})$, although for the proportion saturated responses this correlation is less obvious (probably because the threshold S/CO value between saturated and dynamic response values varies and is not always 7.0).

Figure 6c. Correlation between $\Delta(S/CO_{M\text{-}A})$ and proportion reactive (S/CO≥1.0) and saturated (S/CO≥7.0) response levels observed with different Ultrio, Ultrio Plus and Ultrio Elite reagent batches on P0067 ViraQ HCV Trend 25 control. Each point represents a TMA/run control batch combination.

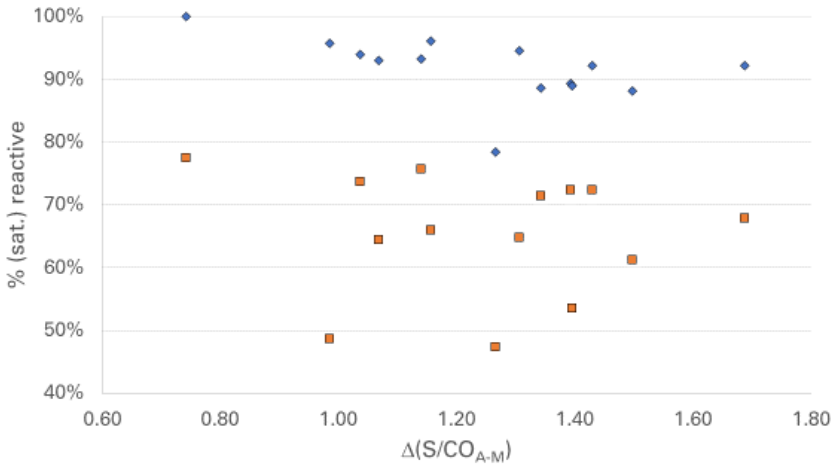


Fig 6c P0067

4.3.3 *P0064 ViraQ HIV-1 Check 125*

Previously SANBS used a customized P0196 HIV-1 subtype C product of a similar concentration as P0064 HIV-1 Check 125. In a previous analysis performed in 2013 it was shown that the P0196 HIV-1 subtype C Check Control was 100% reactive in 1260 test runs. In 2014 it was decided to discontinue the production of P0196 and replace it by the inactivated P0064 HIV-1 subtype B product. At the time of the analysis for this report data were available from 679 Ultrio Plus test runs on P0064 HIV-1 Check 125 controls. Table 10 and figure 7a,b give the distribution of S/CO values on 125 cp/mL samples in the P0064 HIV-1 Check Control.

Table 10 Distribution of test results in Ultrio Plus on P0064 ViraQ HIV-1 Check 125

S/CO	n	%
0-1	0	0.0%
1-2	0	0.0%
2-3	0	0.0%
3-4	0	0.0%
4-5	0	0.0%
5-6	0	0.0%
6-7	0	0.0%
7-8	2	0.3%
8-9	8	1.2%
9-10	95	14.0%
10-11	247	36.4%
11-12	249	36.7%
12-13	66	9.7%
13-14	10	1.5%
14-15	2	0.3%
15-16	0	0.0%

Figure 7a Distribution of S/CO values in Ultrio Plus test runs on P0064 HIV-1 Check 125 Control

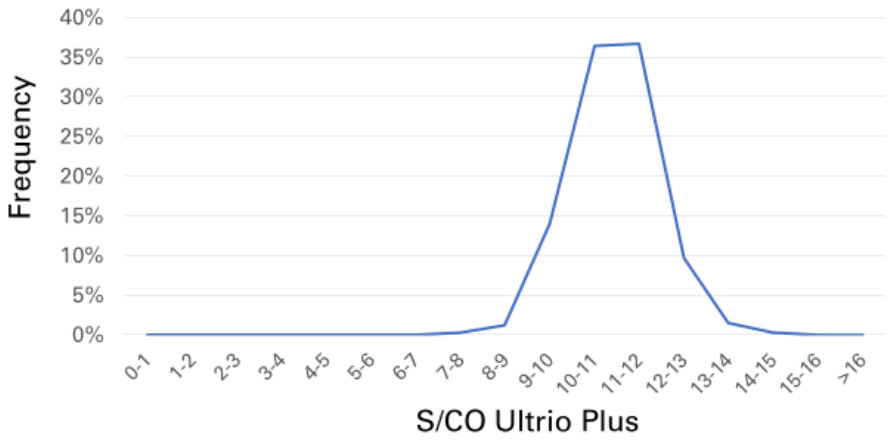


Fig 7a P0064

Figure 7b Cumulative distribution of S/CO values in Ultrio Plus test runs on P0064 HIV-1 Check 125 Control

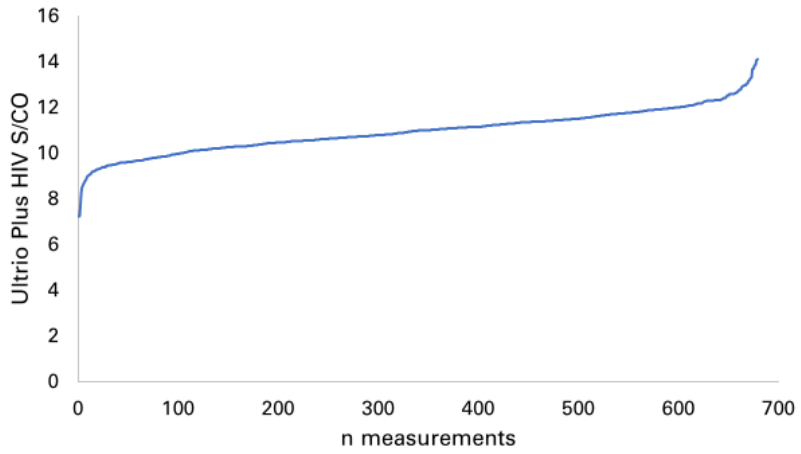


Fig 7b P0064

From this distribution of S/CO values on P0064 HIV-1 Check 125 and P0068 Trend 25 (data below) it was decided to set a threshold S/CO ratio of 8.0 between saturated and dynamic response values in the Ultrio Plus and Elite assay versions. In contrast to Ultrio Plus results on the P0063 HCV Check 125 Control on which in 2014 a considerable proportion of 8% was found in the dynamic range (or non-reactive), only 2 (0.29%) had HIV S/CO values between 6 and 7. Hence nearly all response values on P0064 HIV-1 Check 125 were in the saturated range of the assay, where S/CO values are normally distributed. If a Gumbel distribution is assumed one can again follow the sliding value of Δ (median S/CO – average S/CO) by evaluating 50 earlier and 50 later test results.

Figure 8 Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0064 ViraQ HIV-1 Check 125 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date

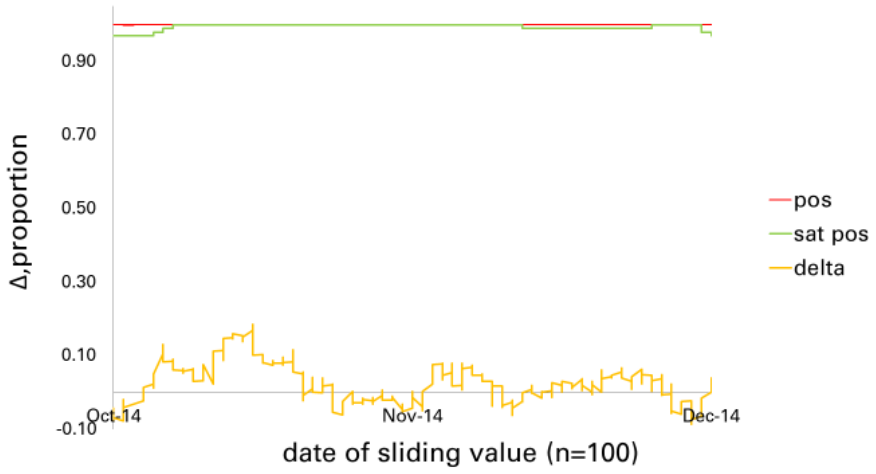


Fig 8 P0064

The highest value of delta on P0064 ViraQ HIV-1 Check 125 Control was found at 0.18, whereas for P0063 ViraQ HCV Check 125 Control this value was 0.40. Since no dynamic responses were found we can conclude that values of delta below 0.18 guarantee sufficient performance. More data need to be collected to establish above which value there is an indication for a reduced analytical sensitivity of test runs.
One can also compare the $\Delta(S/CO_{M-A})$ as a non-sliding parameter for comparing of Tigris instruments (table 11) Note that the values for delta are much smaller than for the HCV Check Control (table 7), except for instrument #9. An outlier value of delta for a certain Tigris instrument could be used to trigger an investigation.

Table 11, Example of using $\Delta(S/CO_{M-A})$ for performance evaluation of 9 Tigris instruments during one quarter of 2014

instrument	average	median	$\Delta(S/CO_{M-A})$	n
1	10.63	10.54	-0.09	72
2	10.75	10.83	0.08	78
3	11.42	11.61	0.19	78
4	10.35	10.29	-0.06	76
5	10.80	10.75	-0.05	76
6	11.20	11.10	-0.10	70
7	10.88	11.01	0.13	75
8	11.09	11.23	0.14	70
9	11.35	11.59	0.24	75

4.3.4 *P0068 ViraQ HIV Trend 25*

From August 2011 until June 2015 the P0068 ViraQ Trend 25 control was tested by IBTS in 2489 test runs in the Ultrio Plus and Elite assay versions. The distribution of the S/CO values on the trend control is shown in table 12 and figure 9a,b. As with the Check Control data we assumed a S/CO threshold value for saturated TMA response values at 8.0. There were 78 (3.5%) non-reactive results, 334 (13.4%) dynamic and 2068 (83.1%) saturated responses.

Table 12 Distribution of S/CO results on P0068 ViraQ HIV-1 Trend 25 control in Ultrio Plus and Elite test runs

S/CO	n	%
0-1	87	3.5%
1-2	61	2.5%
2-3	20	0.8%
3-4	19	0.8%
4-5	12	0.5%
5-6	12	0.5%
6-7	34	1.4%
7-8	176	7.1%
8-9	1087	43.7%
9-10	793	31.9%
10-11	150	6.0%
11-12	21	0.8%
12-13	10	0.4%
13-14	3	0.1%
14-15	4	0.2%

Figure 9a Distribution of S/CO values on P0068 ViraQ HIV-1 Trend 25 control

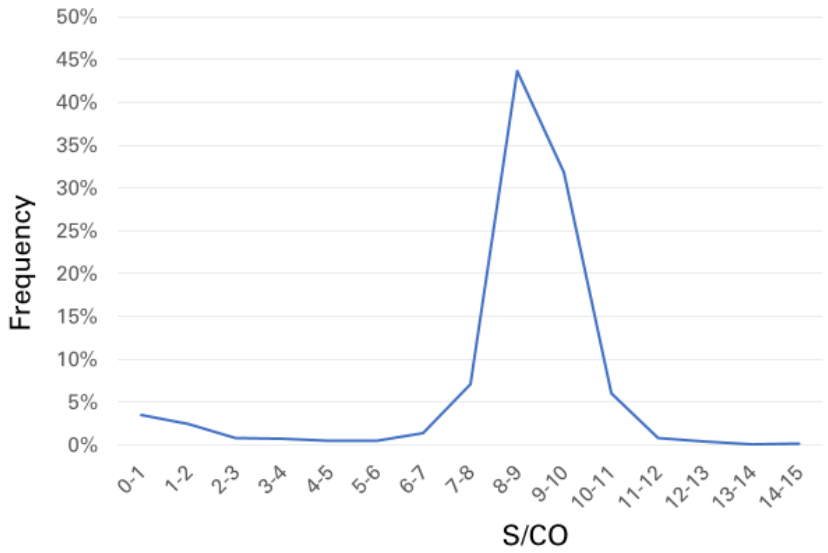


Fig 9a P0068

Figure 9b Cumulative distribution of S/CO values on P0068 ViraQ HIV-1 Trend 25 control

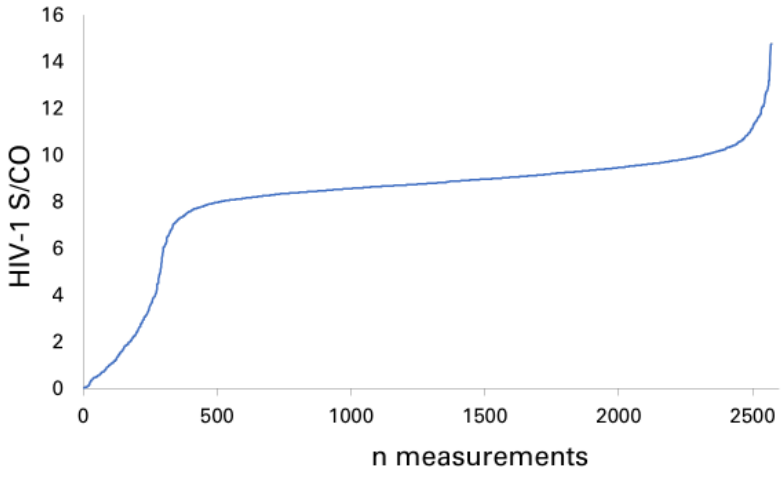


Fig 9a P0068

We also used the Gumbel distribution of the S/CO values on the HIV-1 Trend 25 Control (figure 9a and 9b). Again the sliding value of the performance indicator Δ (median S/CO – average S/CO) was calculated at each time point from 50 earlier and 50 later response values on the Trend Control. Figure 10a shows the S/CO scatter plot together with the course of the sliding value of Δ (median S/CO – average S/CO) in relation to that of the proportion of saturated response values (S/CO≥8). The correlation between the value of Δ (median S/CO – average S/CO) and the proportion saturated reactive results is presented in figure 10b. The highest sliding values of Δ (median S/CO – average S/CO) coincide with the lowest sliding proportions of reactive and saturated reactive results.

Figure 10a Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0068 ViraQ HIV-1 Trend 25 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date

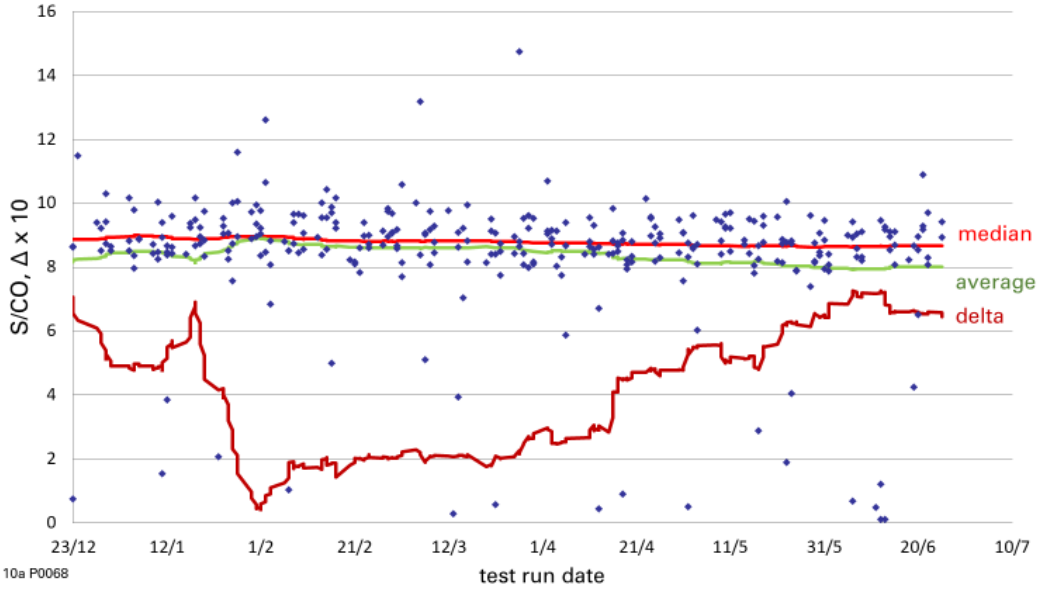


Fig 10a P0068

Figure 10b Relation between sliding Δ (median S/CO – average S/CO) and sliding proportion saturated reactive response values (and 95% CI, yellow lines) on P0068 ViraQ HIV-1 Check 25 (data figure 10a)

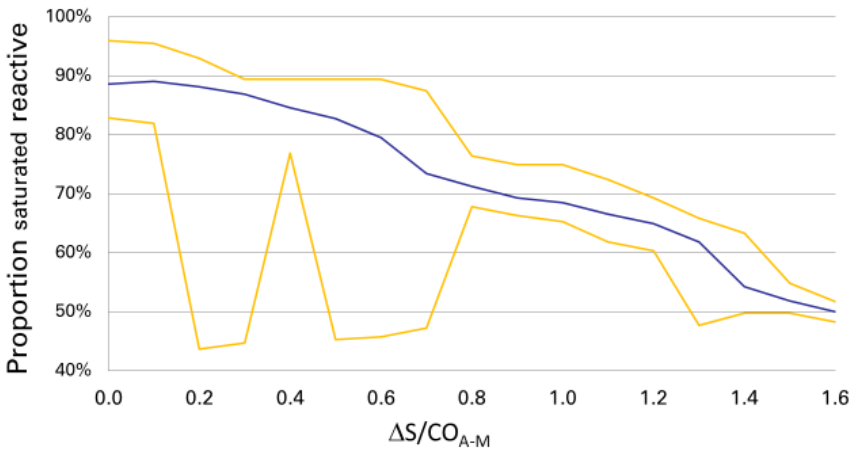


Fig 10b P0068

The currently evaluated results suggest that values of delta below 0.8 are associated with sufficient analytical sensitivity of the TMA system.

The parameter $\Delta(S/CO_{M-A})$ can also be used for comparison of instruments or TMA reagent batches, in which case it is not necessary to estimate sliding values over time from historical results. Figure 10c evaluates delta as a performance indicator of Ultrio (Plus and Elite) reagent batches on different batches of P0068 ViraQ HIV Trend 25 control. The reactivity rates become lower with higher values of the parameter $\Delta(S/CO_{M-A})$, although for the proportion saturated

responses this correlation is affected by the choice of the threshold S/CO value between saturated and dynamic response values. This threshold value varies per TMA reagent batch and is not always 8.0.

Figure 10c. Correlation between $\Delta(S/CO_{M-A})$ and proportion reactive (S/CO≥1.0) and saturated (S/CO≥8.0) response levels observed with different Ultrio, Ultrio Plus and Ultrio Elite reagent batches on P0068 ViraQ HIV Trend 25 control. Each point represents a TMA/run control batch combination.

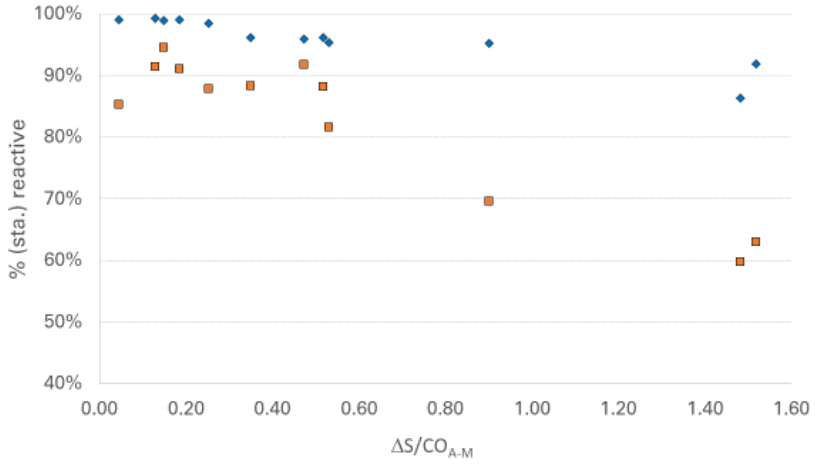


Fig 10c P0068

4.3.5 *P0065 ViraQ HBV Check 125*

Before P0065 ViraQ HBV Check 125 controls were introduced SANBS has first used the P0155 ViraQ HBV Check 250 control. This latter run control of 250 cp/mL was tested in 6026 Ultrio Plus test runs by SANBS in 2012, 2013 and 2014 and was found to be 100% of the time reactive with saturated S/CO values ≥12.0. Only S/CO ratios of 339 test runs on the P0065 run control of 125 cp/mL were analysed at the time of writing this report (table 14). In this data set 1.5% of the Ultrio Plus response values were in the dynamic range (S/CO<12.0).
Since only 1.5% of the S/CO values on P0065 HBV Check 125 control were in the dynamic range we decided not to assume a Gumbel distribution and examine Δ (median S/CO – average S/CO) as a trending parameter for the Check Control but only for the Trend Control (see below)

Table 14. Distribution of S/CO values in Ultrio Plus on HBV Check 125 run control

S/CO	n	%
0-1	0	0.0%
1-2	0	0.0%
2-3	0	0.0%
3-4	0	0.0%
4-5	0	0.0%
5-6	0	0.0%
6-7	0	0.0%
7-8	0	0.0%
8-9	0	0.0%
9-10	0	0.0%
10-11	0	0.0%
11-12	5	1.5%
12-13	18	5.3%
13-14	58	17.1%
14-15	148	43.7%
15-16	103	30.4%
>16	7	2.1%
	339	100%

Figure 11a. Distribution of S/CO values in Ultrio Plus on HBV Check 125 run control

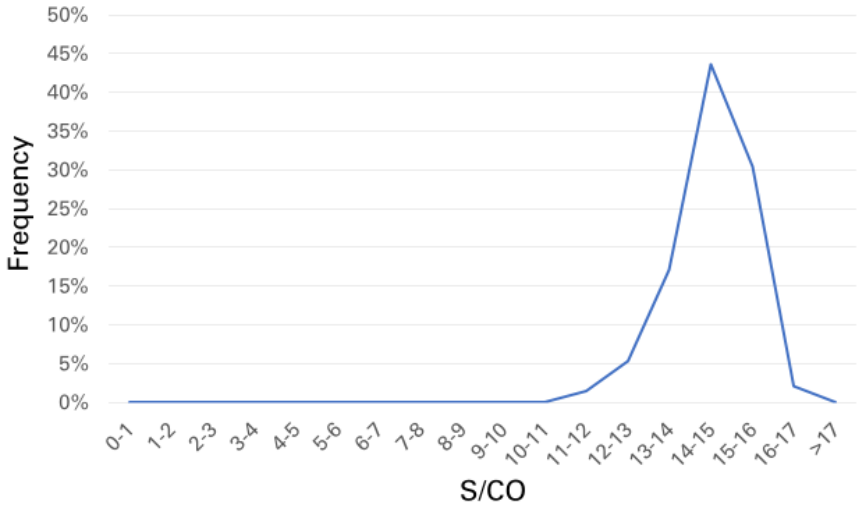


Fig 11a P0065

Figure 11b. Cumulative distribution of S/CO values in Ultrio Plus on HBV Check 125 run control

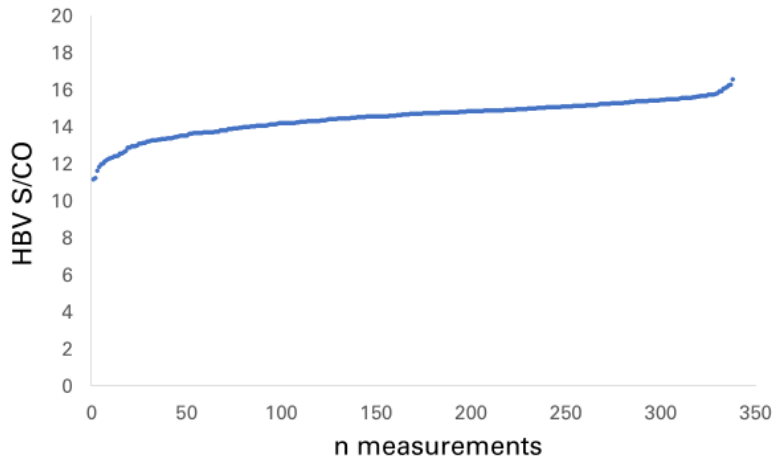


Fig 11b P0065

4.3.6 *P0069 ViraQ HBV Trend 25 and P0154 HBV Trend 50*

Before P0069 ViraQ HBV Trend 25 control was introduced IBTS tested P0154 ViraQ Trend 50 containing 50 cp/mL in 2843 Ultrio Plus and Elite test runs. Table 15 and figure 12a and 12b give the distribution of S/CO values on this run control product. At the time of writing this report the distribution of only 190 S/CO values were analysed on the P0069 ViraQ HBV Trend 25 run control that contains 25 cp/mL of HBV-DNA (table 16, figure 13a, 13b, 13c). With the two-fold reduction of the HBV-DNA concentration in the trend control the proportion of non-reactive results increased from 1.7% to 7.4% and the proportion S/CO values in the dynamic range (<12.0) increased from 4.2% to 5.8%. Note that the proportion of S/CO values in the dynamic range was much higher for HCV trend control (23.2%) than for the HBV trend control (7.4%), whereas the proportion of non-reactive results was comparable (7.8% vs 7.4%). Obviously the dynamics of the TMA reaction for HBV is different from that of HCV.
Since nearly all S/CO response values generated by Ultrio Plus and Elite on the HBV Check Controls were in the saturated range of the assays we only assumed a Gumbel distribution for the S/CO values on the HBV Trend Controls. Again the sliding value of the performance indicator Δ (median S/CO – average S/CO) was calculated at each time point from 50 earlier and 50 later response values on the Trend Control. Figure 12c and 14a shows the course of the sliding value of Δ (median S/CO – average S/CO) in relation to that of the proportion of saturated response values (S/CO≥12) for P0154 HBV Trend 50 and P0069 HBV Trend 25 control respectively. From these data one can confirm the correlation between the value of Δ (median S/CO – average S/CO) and the proportion saturated reactive results (figure 12d and 14b). The highest sliding values of Δ (median S/CO – average S/CO) coincide with the lowest sliding proportions of reactive and saturated reactive results. The data show that values for Δ (median S/CO – average S/CO) below 0.45 are appropriate for P0154 HBV Check 50, but for P0069 HBV Trend 25 values below 1.50 are expected. The parameter $\Delta(S/CO_{M-A})$ can also be used for comparison of instruments or TMA reagent batches, in which case it is not necessary to estimate sliding values over time from historical results. Figure 12e evaluates delta as a performance indicator of Ultrio Plus and Elite reagent batches on different batches of P0154 ViraQ HBV Trend 50 control. The reactivity rates above cutoff and saturation level (S/CO≥12.0) become lower with higher values of the parameter $\Delta(S/CO_{M-A})$.

Table 15 Distribution of S/CO values in Ultrio Plus on P0154 HBV Check 50 run control

S/CO	n	%
0-1	44	1.7%
1-2	7	0.3%
2-3	5	0.2%
3-4	3	0.1%
4-5	5	0.2%
5-6	6	0.2%
6-7	9	0.3%
7-8	6	0.2%
8-9	6	0.2%
9-10	14	0.5%
10-11	17	0.7%
11-12	30	1.2%
12-13	257	9.9%
13-14	936	36.0%
14-15	838	32.2%
15-16	367	14.1%
16-17	46	1.8%
17-18	4	0.2%

Table 16 Distribution of S/CO values in Ultrio Plus on P0069 HBV Check 25 run control

S/CO	n	%
0-1	14	7%
1-2	1	1%
2-3		0%
3-4		0%
4-5		0%
5-6		0%
6-7		0%
7-8		0%
8-9		0%
9-10	3	2%
10-11	3	2%
11-12	4	2%
12-13	70	37%
13-14	82	43%
14-15	13	7%
15-16		0%
>16		0%

Figure 12a. Distribution of S/CO values in Ultrio Plus and Elite on O0154 HBV Trend 50 run control

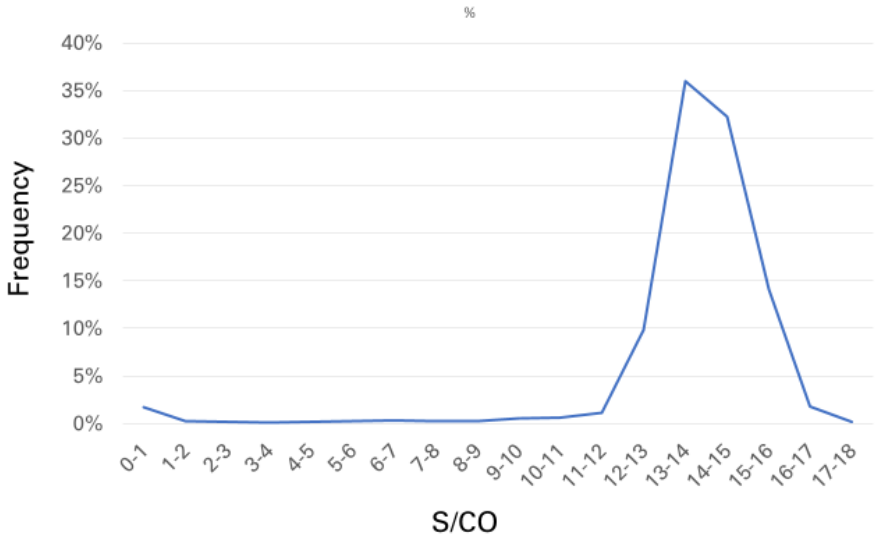


Fig 12a P0154

Figure 12b. Cumulative distribution of S/CO values in Ultrio Plus and Elite on P0154 HBV Trend 50 run control

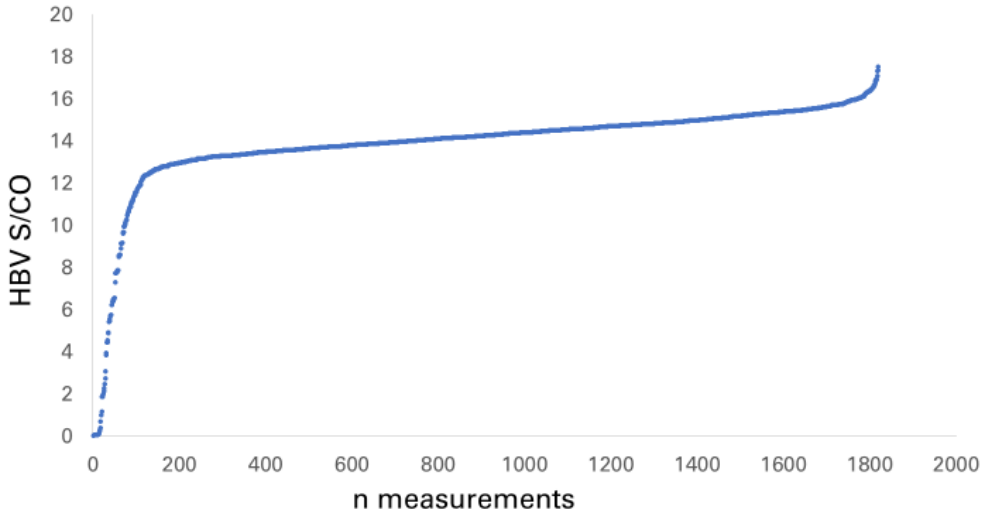


Fig 12b P0154

Figure 12c Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0154 ViraQ HBV Trend 50 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date

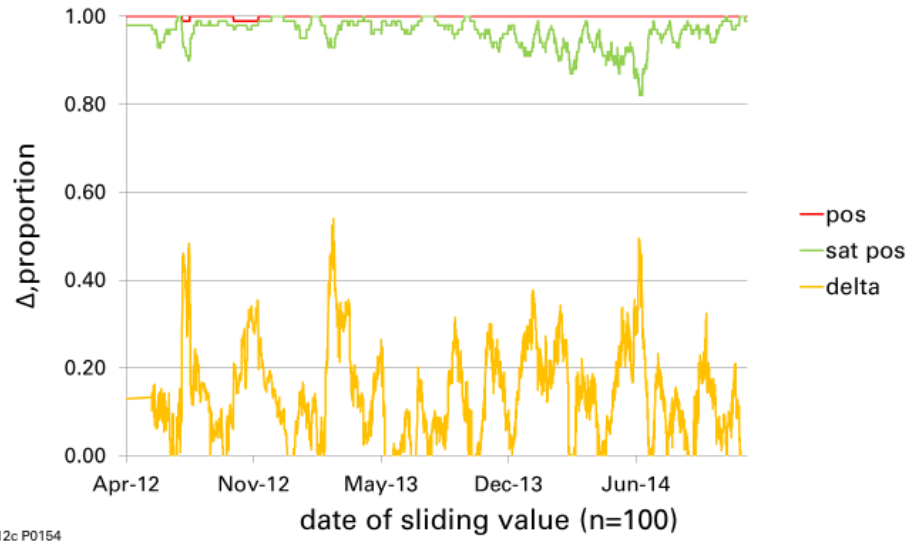


Fig 12c P0154

Figure 12d Relation between sliding Δ (median S/CO – average S/CO) and sliding proportion saturated reactive response values (and 95% CI, yellow lines) on P0154 ViraQ HBV Check 50 (data figure 14a)

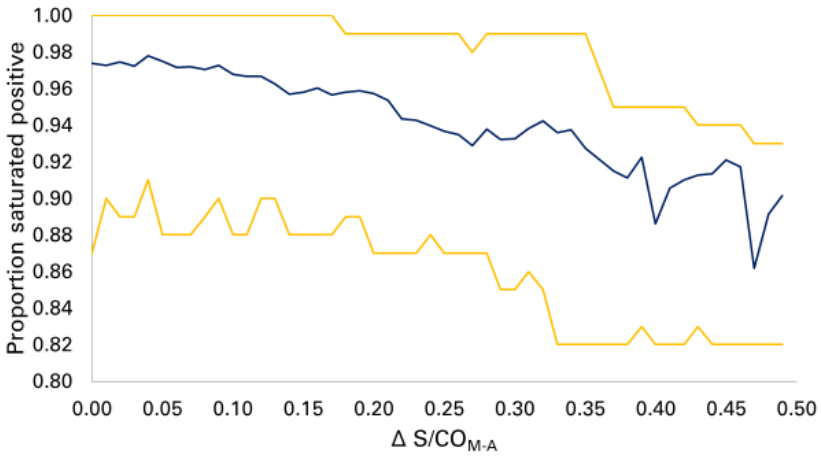


Fig 12d P0154

Figure 12e. Correlation between $\Delta(S/CO_{M-A})$ and proportion reactive (S/CO≥1.0) and saturated (S/CO≥12.0) response levels observed with different Ultrio Plus and Ultrio Elite reagent batches on P0154 ViraQ HBV Trend 50 control. Each point represents a TMA/run control batch combination.

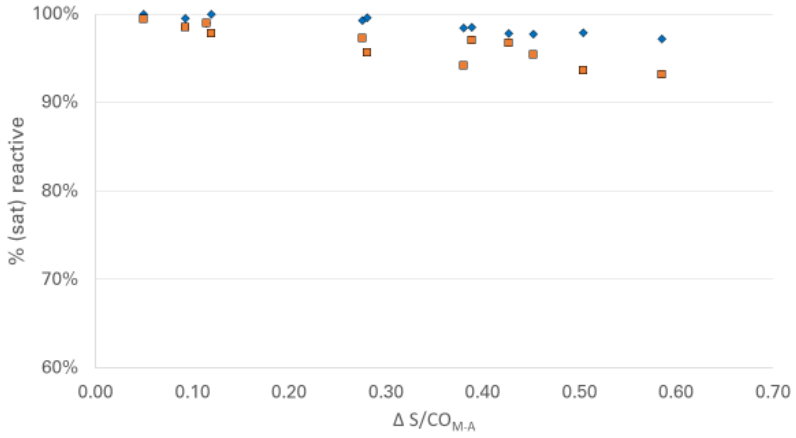


Fig 12e P0154

Figure 13a. Distribution of S/CO values in Ultrio Plus and Elite on P0069 HBV Trend 25 run control

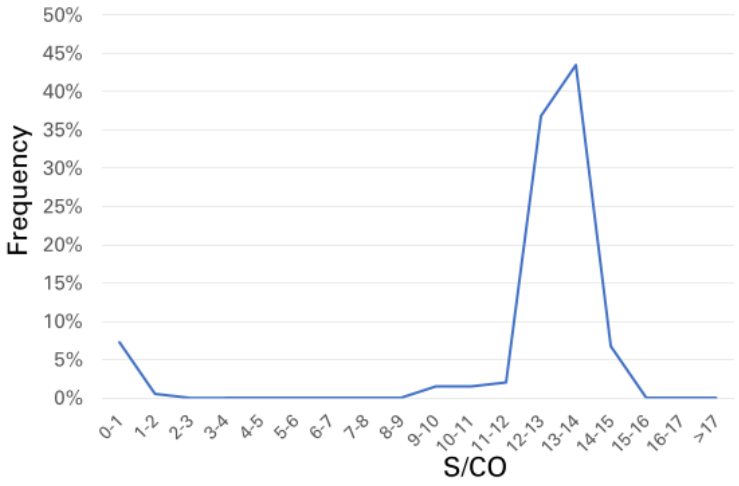


Fig 13a P0069

Figure 13b. Cumulative distribution of S/CO values in Ultrio Plus and Elite on P0069 HBV Trend 25 run control

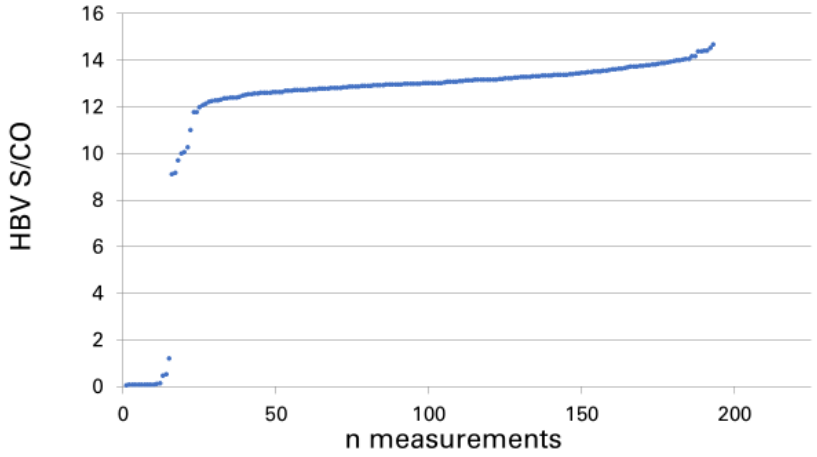


Fig 13b P0069

Figure 13c. Distribution of S/CO values in Ultrio Plus and Elite on P0069 HBV Trend 25 and P0154 HBV Trend 50 run control

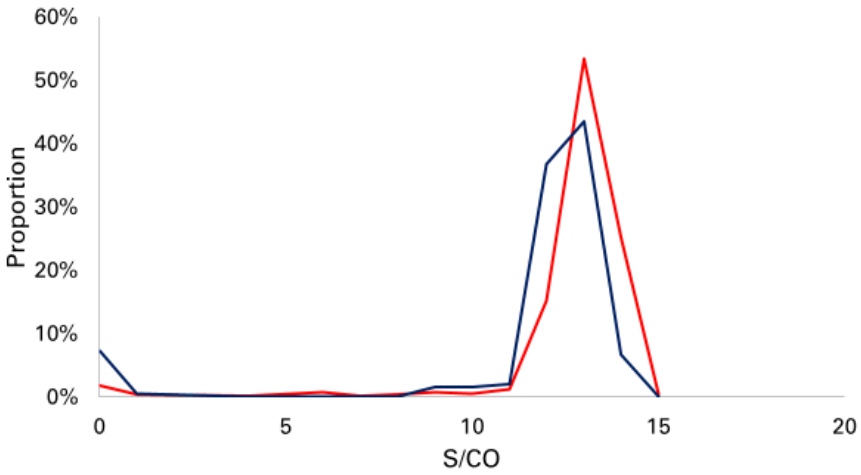


Fig 13c P0069, P0154

Figure 14a Sliding course of Δ (median S/CO – average S/CO) over time in relation to proportions reactive and saturated reactive on P0069 ViraQ HBV Trend 25 Control. Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date

Fig 14a P0069

Figure 14b Relation between sliding Δ (median S/CO – average S/CO) and sliding proportion saturated reactive response values (and 95% CI, yellow lines) on P0069 ViraQ HBV Check 25 (data figure 14a)

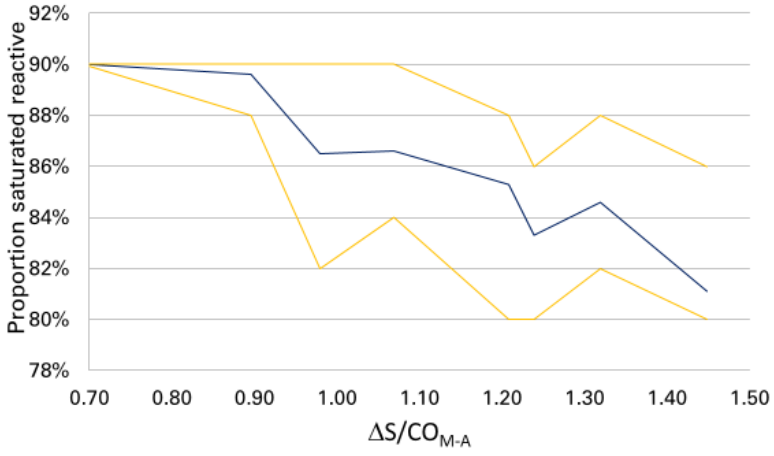


Fig 14b P0069

4.4 Summary of distribution of S/CO values in Ultrio Plus and Elite on ViraQ Check 125 and Trend 25 controls

Figure 15 summarizes the above described distributions of the six ViraQ HBV, HCV and HIV-1 Check 125 and Trend 25 control products that are currently in use by customers. The differences in distributions are probably related to (1) the true viral concentrations in the run controls in cp/mL, (2) the 50% and 95% LOD in cp/mL of the Ultrio versions and (3) the dynamics of TMA reaction for the different structures of the viral genomes. The proportions of dynamic response values on ViraQ Check and Trend Controls are probably higher for HCV (6.2% and 23.2%) than

for HIV (1.5% and 13.4%) because of the true viral concentrations. As discussed in VR4060 the HCV-RNA concentration in the S0109 HCV genotype 3 standard was 1.5 fold lower in a second calibration experiment in the bDNA 3.0 assay and two-fold lower on the basis of Ct values in the Roche cobas MPX assays. For HBV the reactivity rate on the 25 cp/mL trend control is lower than for HIV because the analytical sensitivity for HBV is approximately 2-fold less (VR4059). Despite the lower analytical sensitivity of TMA for HBV the proportion of saturated responses on ViraQ Trend Controls is higher for HBV than for HIV (87.0% vs 80%). Only 5.7% of HBV response values on ViraQ Trend Controls were found in the dynamic range as compared to 16.0 and 24.0% for HIV and HCV respectively. Obviously the efficiency of the TMA reaction for HIV-RNA and HCV-RNA is higher than for HBV-DNA (for which less low S/CO values and more nonreactive results are found).

Figure 15. Cumulative distributions of S/CO values in Ultrio Plus and Elite on ViraQ Check 125 and ViraQ Trend 25 Controls

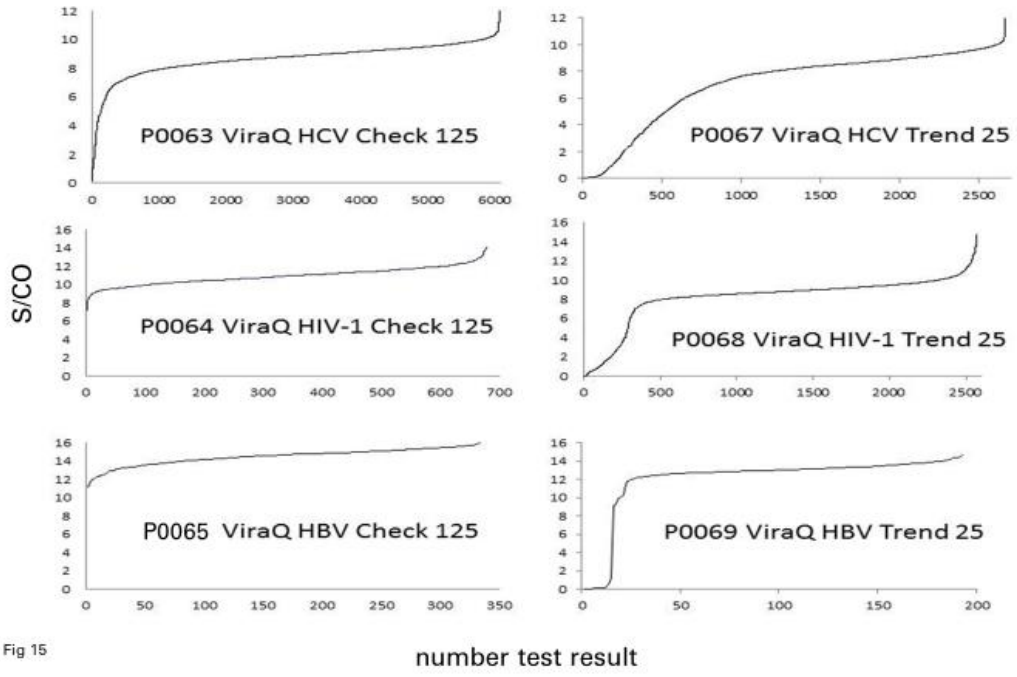


Fig 15

Table 17a Distribution categories of S/CO values in Ultrio Plus and Elite assays for HBV-DNA, HCV-RNA and HIV-1 RNA

	S/CO categories in Ultrio versions		
Marker	Non-reactive	Dynamic	Saturated
HBV-DNA	<1.0	1.0-12	≥12
HCV-RNA		1.0-7	≥7
HIV-1 RNA		1.0-8	≥8

Table 17b Distribution of S/CO values in Ultrio Plus and Elite on ViraQ Check 125 and Trend 25 controls

Run control	n	Non-reactive	Dynamic	Saturated
P0063, HCV Check	6107	0.1 (0-0.3) %	6.2 (5.6 - 6.8) %	93.7 (91.2-96.0) %
P0064, HIV-1 Check	679	0 %	1.5 (0.0 - 4.0) %	98.5 (97.6-100) %
P0065, HBV Check	338	0 %	1.5 (1.3 - 1.7) %	98.5 (97.3-100) %
P0067, HCV Trend	2508	7.8 (6.7-8.9)%	23.2 (21.5-25.3)%	69.0 (66.2-72.7)%
P0068, HIV-1 Trend	2489	3.5 (2.8-4.2)%	13.4 (12.0-14.9)%	83.1 (79.5-86.7)%
P0069, HBV Trend	190	7.4 (3.5-11.2)%	5.8 (2.4-9.2)%	92.6 (73.6-100)%

Table 17 gives the proportions of the three S/CO distribution ranges and the 95% CI's that were found in the evaluated data sets. It must be emphasized that users of the run control can find proportions of non-reactive, dynamic, or saturated S/CO values that are outside the 95% CI in table 17b. This was illustrated when the annual distributions on ViraQ HCV Check 125 were compared and the proportions of dynamic ranges varied between 3.2% to 7.9%. These variations are caused by differences in the levels of saturated S/CO values that vary per TMA reagent batch. So the arbitrarily chosen threshold S/CO value of 7.0 between saturated and dynamic S/CO values for HCV may vary between 6.0 and 8.0 depending on the Ultrio reagent batch. We therefore developed a simple statistical parameter, that is related to the skewness of the S/CO distribution curves, i.e. the delta between the average and median S/CO values, The higher the value of Δ (average S/CO – median S/CO) the more responses are found in the dynamic and nonreactive response range of the TMA assay. By following Δ (average S/CO – median S/CO) over time customers can follow trends in analytical sensitivity of assay runs, instruments or TMA reagent batches.

4.5 Evaluation of Ct values on ViraQ Multimarker controls of 125 and 75 cp/mL on cobas MPX assay versions

In VR4059 we decided from the analytical sensitivity data on the Roche cobas TaqScreen MPX V2.0 that a run control of 125 cp/mL is required for this assay (at least to guarantee >99.5% reactivity for HCV). Since the analytical sensitivity of the later introduced cobas MPX assay further improved we later also designed a 75 cp/mL multi-marker run control. In this report we compared the predicted Ct values deduced from the dose response relation in the standard dilution series (VR4059) with those observed on the two multi-marker run controls in consecutive real time PCR test runs performed by Sanquin (Amsterdam, the Netherlands).

4.5.1 *Roche cobas TaqScreen MPX V2.0*

Figure 16 a, b and c show the Ct values found on P0254 ViraQ Multi-Marker 125 in consecutive test runs of the cobas TaqScreen MPX V2.0 assay.

Figure 16a-c. Ct values on P0254 ViraQ Multi-Marker 125 in consecutive test runs of the cobas TaqScreen MPX V2.0 assay, the orange lines represent the 95 % confidence interval calculated from the run control results

Figure 16a HBV-DNA

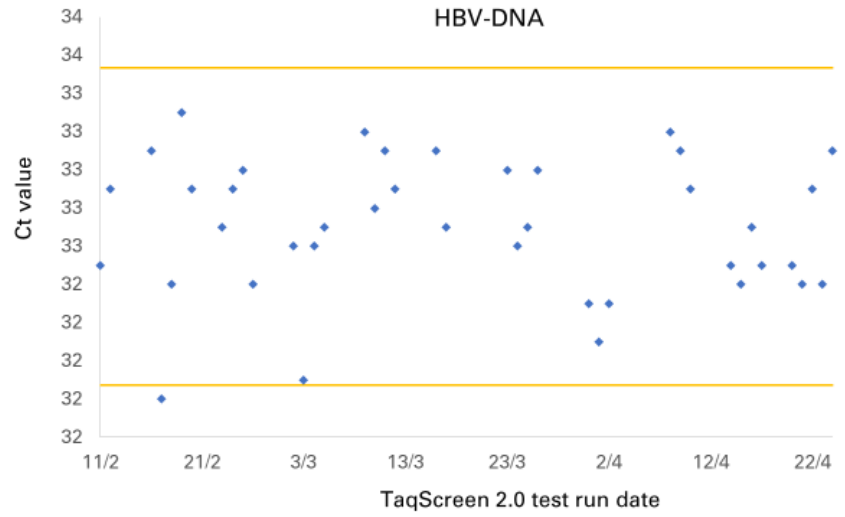


Fig 16a P0254

Figure 16b HCV-RNA

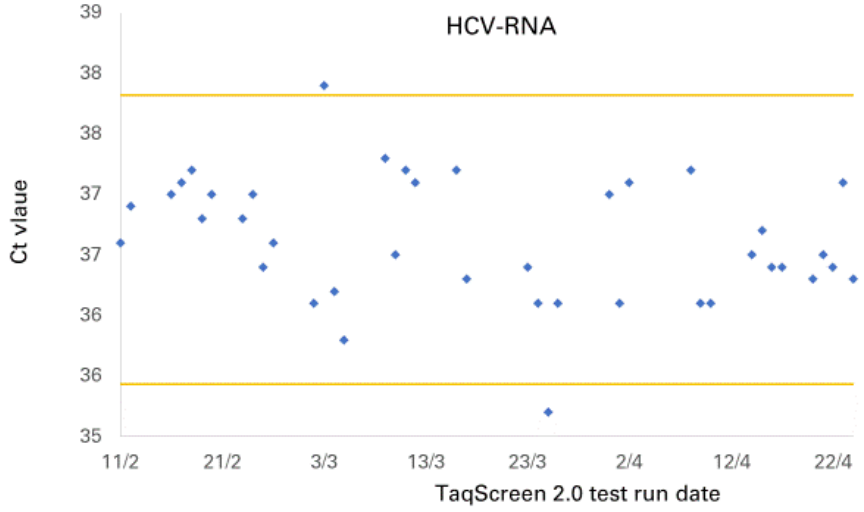


Fig 16b P0254

Figure 16c HIV-1 RNA

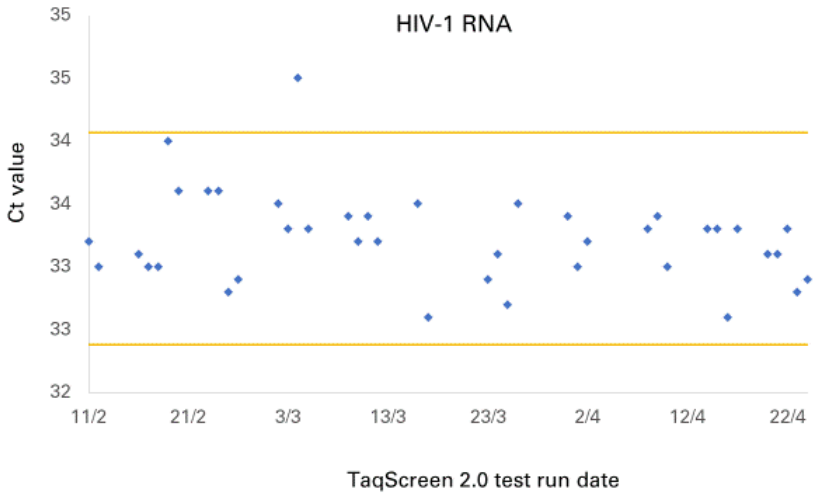


Fig 16c P0254

Dr. M Koppelman (Sanquin, Amsterdam) tested a two-fold P0254 Multimarker standard dilution panel that was composed of the same inactivated standards as the later evaluated P0254 ViraQ Check 125 Control in the cobas Taqscreen 2.0 assay. The predicted and observed Ct values from the dilution panel and run control respectively were comparable for HBV-DNA, but those on the run control were somewhat lower for HCV and HIV (table 18a). It is not known whether this difference is caused by cobas TaqScreen MPX reagent batch variation, difference in HCV and HIV concentrations in the reference samples or loss of RNA in the run control samples. More TaqScreen 2.0 data on P0254 Multimarker run control need to be evaluated in order to establish whether a significant difference in Ct values (and possibly analytical sensitivity) exists between PCR reagent batches. The run control data (n=40) in figure 16 were derived from 2 TaqScreen reagent batches. As can be seen the two reagent batches generated comparable Ct values on P0254 ViraQ Check 125 control.

Table 18a. Comparison of predicted and observed average Ct value (SD) from data on P0254 standard dilutions and P0254 ViraQ Multimarker Check 125 Control in cobas TaqScreen MPX v2.0 assay

Marker	Predicted from P0254 dilution panel (500, 250,125 cp/mL)		Observed from P0254 run control (125 cp/mL)		Difference between mean Ct values (95% CI, unpaired t-test)
	n	mean (SD)	n	mean (SD)	
HIV-1-RNA	19	32.6 (0.24)	40	33.2 (0.36)	0.62 (0.46-0.78)
HBV-DNA	19	32.8 (0.37)	40	32.7 (0.36)	0.14 (-0.07-0.35)
HCV-RNA	18	35.8 (0.46)	40	36.6 (0.51)	0.81 (0.55-1.10)

We also analyzed the total of 59 results (from at least three TaqScreen 2.0 reagent batches) together to calculate the expected 95% confidence interval of Ct values on 125 cp/mL samples in P0254 Multimarker run control (table 18b).

Table 18b. Expected 95% and 99% confidence intervals (CI) of Ct-values on P0254 ViraQ Check 125 Control (preliminary results from 3 reagent batches)

Marker	95% CI	99 % CI
HIV-1-RNA	32.4-34.1	32.1-34.3
HBV-DNA	31.9-33.5	31.6-33.8
HCV-RNA	35.4-37.8	35.1-38.1

4.5.2 *Roche cobas MPX test on the 6800/8800 System.*

Figure 17 a, b and c show the Ct values found on P0273 ViraQ Multi-Marker 75 in consecutive test runs of the cobas MPX assay.

Figure 17a-c. Ct values on P0273 ViraQ Multi-Marker 75 in consecutive test runs of the cobas MPX assay, the orange lines represent the 95 % confidence interval calculated from the run control results

Figure 17a HBV-DNA

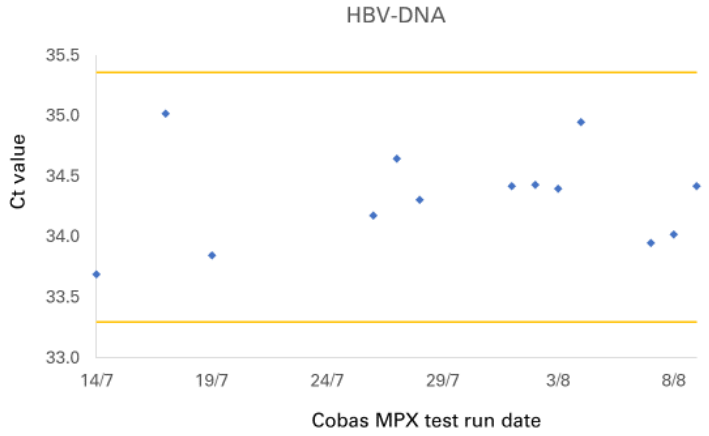


Fig 17a P0273

Figure 17b HCV-RNA

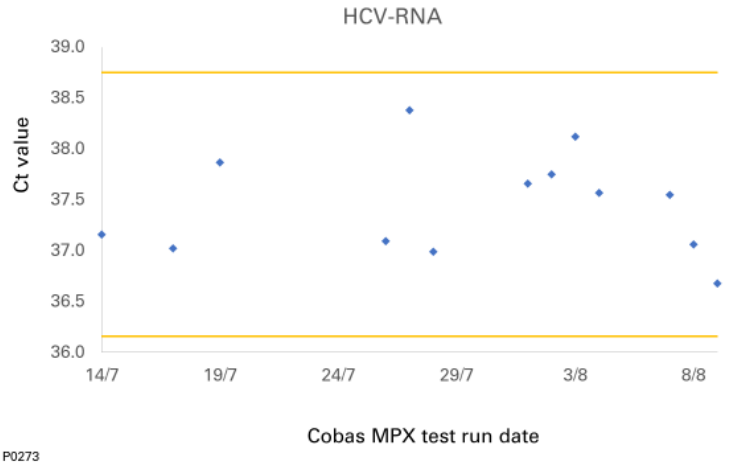


Fig 17b P0273

Figure 17c HIV-RNA

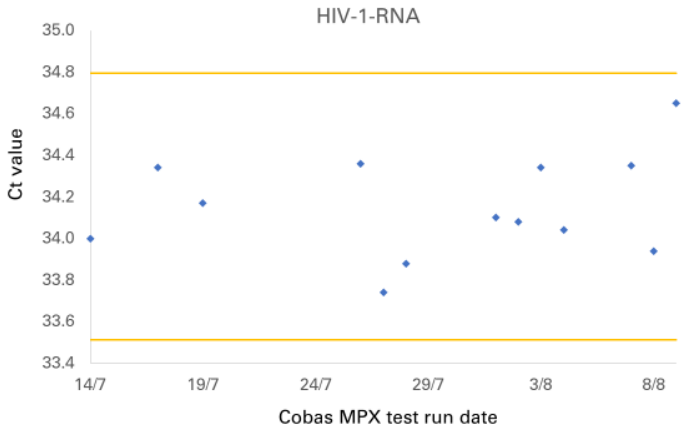


Fig 17c P0273

Recently Dr. M Koppelman (Sanquin, Amsterdam) also tested the P0026 HIV-1, P0031 HBV and P0020 HCV inactivated standard dilution series and P0273 ViraQ Check 75 Control in the cobas MPX assay on the cobas 6800 instrument. The predicted and observed Ct values from the dilution panel and run control respectively were comparable for all markers (table 19a). The small difference found for HIV-1 RNA may be related to PCR reagent variation or batch variation of the reference samples. More data on P0273 Multimarker run control need to be evaluated in order to establish whether significant differences exist in Ct values (and possibly analytical sensitivity) between PCR reagent batches.

Table 19a. Comparison of predicted and observed average Ct value (SD) from data on P0026, P0031 and P0020 standard dilution panels and P0273 ViraQ Multimarker Check 75 Control in cobas MPX assay

Marker	Predicted from dilution panels#		Observed from P0273 run control (75 cp/mL)		Difference mean (95% CI, unpaired t-test)
	n	mean (SD)	n	mean (SD)	
HIV-1-RNA	24	33.7 (0.32)	13	34.2 (0.25)	0.4 (0.2-0.6)
HBV-DNA	24	34.4 (0.49)	13	34.3 (0.40)	0.1 (-0.1-0.4)
HCV-RNA	19	37.1 (0.43)	13	37.5 (0.50)	0.4 (0.0-0.7)

#24.6 and 81.9 cp/mL samples from P0026 HIV-1 subtype B inact. reference panel
#35.4 and 118.2 cp/mL samples from P0031 HBV genotype A inact. reference panel
#34.5 and 116 cp/mL samples from P0020 HCV genotype 3a inact. reference panel

We also analyzed the total of 32-37 results (from two cobas MPX reagent batches) together to calculate the expected 95% confidence interval of Ct values on 75 cp/mL samples in P0273 Multimarker run control (table 19b). Since we do not know the variation in cobas MPX reagent batches we recommend to establish the confidence limits from the run control and PCR reagent batch combination in use.

Table 19b. Expected 95% and 99% confidence intervals (CI) of Ct-values on P0254 ViraQ Check 125 Control (preliminary results from 2 reagent batches)

Marker	95% CI	99 % CI
HBV-DNA	33.3 - 35.4	33.0 - 35.7
HCV-RNA	36.1 - 38.4	35.8 - 38.7
HIV-1 RNA	33.0 - 34.7	32.8 - 34.9

We also used the Ct values in cobas MPX to compare the potency of the copies assigned to the HCV genotype 3a standard dilutions in panel P0020 as compared to those in HCV genotype 1 samples in panel P0019. According to this analysis the potency of the copies in the S0109 HCV genotype 3a standard is 0.413 (0.33-0.51) of those in the S0009 HCV genotype 1 standard. If the concentration in the inactivated S0109 HCV RNA genotype 3 standard would be 2.42 fold adjusted (according to calibration by Ct analysis against the S0009 HCV genotype 1 standard) the Ct values would have been 1.27 fold lower. Hence the average Ct value on 75 cp/mL samples would reduce from 37.5 to 36.2), still significantly higher than the Ct values for HIV and HBV.

4.6 Evaluation of ViraQ Check Controls in quantitative TMA assays

In VR4059 we showed that the viral concentration in ViraQ Check Controls is set at enough distance from the LOQs of the real time PCR and TMA assays of Roche, Abbott and Hologic). Hence, it is expected that they can be used for monitoring precision of viral load measurements by these quantitative NAT systems. So far, we only evaluated the Check Controls in consecutive test runs of the Hologic Aptima tests (figure 18a-c). Table 20a compares the geometric mean (and 95%CI) of the IU/mL values reported by the Aptima tests with the viral concentration that was established in the original calibration of the ViraQ Check Controls (VR4060). For HCV and HBV the IU/mL (and 95% CI) are comparable indicating that there is consensus of calibration of the quantitative TMA and ViraQ Check Control in IU/mL. For HIV the Aptima test reports in cp/mL and also here there was no significant difference, although the deviation factor was somewhat higher than for HCV and HBV (table 20a). Hologic reports a conversion factor of 0.35 copy per IU, whereas BioQ Control uses a factor 0.58. Hence when Aptima would have reported in IU/mL instead of in cp/mL values the factor would be 0.75 instead of 1.24. It must be emphasized that the copy to IU conversion factors were found to be different for our S0012 HIV-1 standard when compared to the data of the first (97/656) and second (97/650) International Standards in the WHO collaborative study of Holmes et al (0.42 and 0.58 respectively) (VR4060). When we use the conversion factor to the first international standard the geometric mean value reported by the Aptima test would be 54.2 IU/mL as compared to 52.5 IU/mL in the ViraQ Check Control (factor 1.03).

We found that the coefficient of variation was higher for HCV and HBV than for HIV, but still in line with the claim in the package insert of the Aptima assays (table 20b). Only for HCV the %CV was somewhat higher but this may be caused by the fact that the inter-assay variation measured in this study is usually higher than the intra-assay variation that was reported in the Aptima package insert.

Figure 18a-c. Viral load values reported in consecutive TMA test runs (Hologic Aptima) on ViraQ Check Controls

Figure 18a HBV-DNA

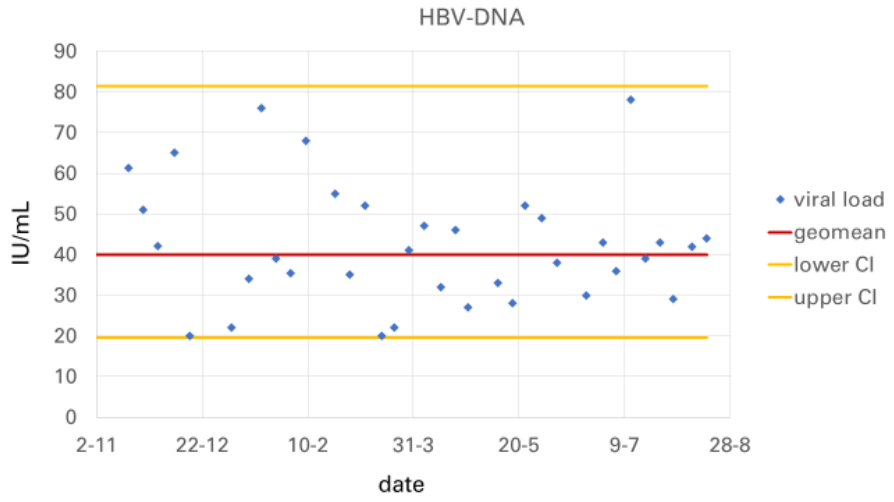


Fig 18a P0155

Figure 18b HCV-RNA

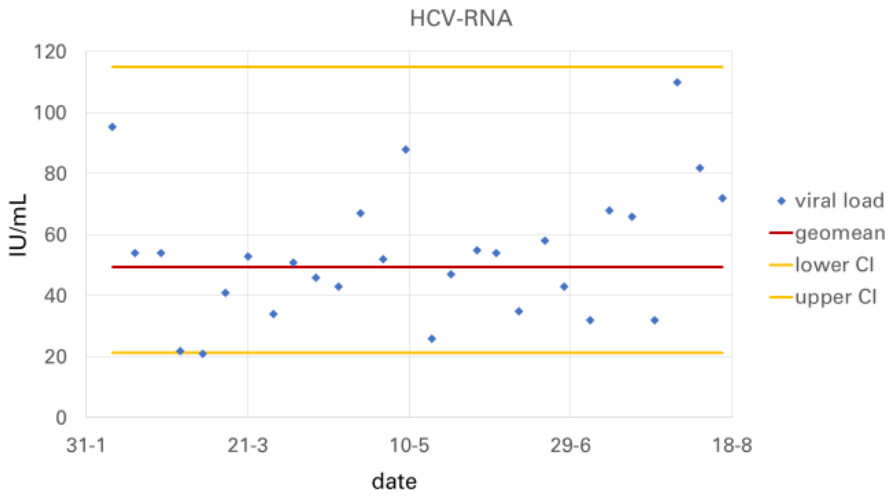


Fig 18b P0063

Figure 18c HIV-1 RNA

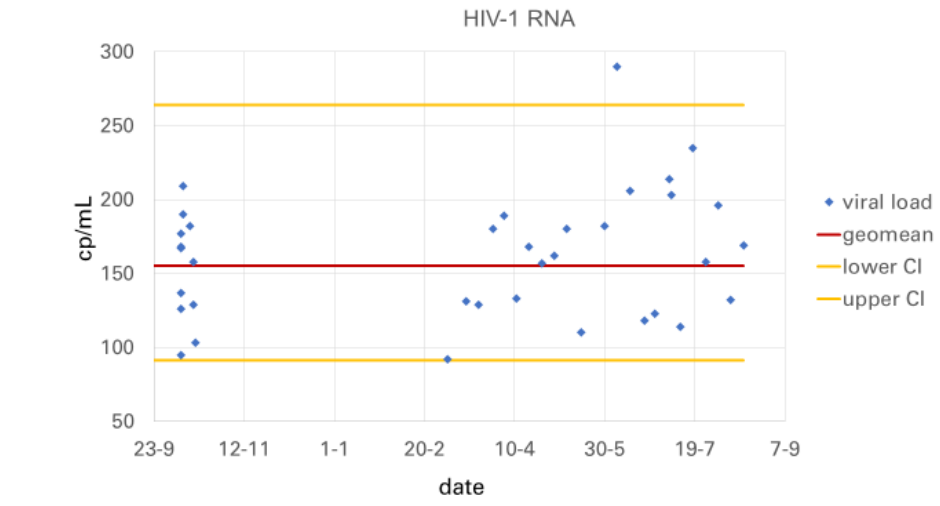


Fig 18c P0064

Table 20a. Comparison of viral concentration in ViraQ Check Controls and geometric mean viral load values found in quantitative TMA (Aptima) test runs.

ViraQ Check Control	Quantitative Aptima tests	LOQ IU/mL[a]	n test runs	Aptima geomean (CI) IU/mL[a]	ViraQ IU/mL[a]	Factor values Aptima to Vira Q
P0063	HCV Quant	10	28	49 (21-115)	46 (28-77)	1.06
P0064	HIV Quant Dx	30[a]	37	155 (91-264)[a]	125 (95-164)[a]	1.24
P0155	HBV Quant	5.6	36	40 (20-81)	47 (30-70)	0.85

[a]For HIV values are not in IU/mL but in cp/mL

Table 20b. Comparison of claimed and observed precision of the Aptima tests by comparison of standard deviation of log values

ViraQ Check Control	Quantitative Aptima tests	n test runs	Aptima log (SD) IU/mL[a]	Aptima SD claimed	%CV on measured log values
P0063	HCV Quant	28	1.69 (0.18)	0.14	10.7%
P0064	HIV Quant Dx	37	2.19 (0.11)[a]	0.14	5.0%
P0155	HBV Quant	36	1.60 (0.15)	0.20	9.4%

[a]For HIV values are not in IU/mL but in cp/mL

4.7 Consistency of manufacturing according to batch release control testing

Since the availability of the cobas MPX TaqScreen 2.0 and the later cobas MPX assay the ViraQ Check Control batches are released by quadruplicate testing against a reference batch stored at -80˚C in the same test run. The batch is released when there is no significant difference between the Ct values of test batch and reference batch in a paired t-test. For this report we used the 4 Ct values found on the test batch and the 4 Ct values found on the reference batch to calculate a potency value and 95% CI. Table 21a summarizes the potency (and 95%CI) values of consecutive batches of the ViraQ Check 125 Controls and table 21b for two ViraQ Multi-marker Check 75 controls

Table 21a. Potencies of consecutive batches of ViraQ Check 125 Controls

P0063 ViraQ HCV Check 125		P0064 HIV Check 125		P0065 HBV Check 125	
batch tested against ref batch B4058-009	potency (95%CI)	batch tested against ref batch B4059-007	potency (95%CI)	batch tested against ref batch B4060-001,4,5,8	potency (95%CI)
B4058-014	92 (67-126)%	B4059-010	111 (83-148)%	B4060-004	125 (85-183)%
B4058-015	102 (74-140) %	B4059-011	77 (62-97)%	B4060-005	126 (101-157)%
B4058-016	113 (38-338)%	B4059-012	144 (117-176)%	B4060-006	87 (71-105)%
B4058-017	125 (43-364)%	B4059-013	111 (95-130)%	B4060-008	132 (113-153)%
B4058-018	115 (73-181)%	B4059-014	109 (94-127)%	B4060-009	98 (81-120)%
B4058-019	109 (65-184)%	B4059-015	99 (84-116)%	B4060-010	75 (63-88)%
B4058-020	92 (45-189)%	B4059-016	101 (83-123%)	B4060-011	99 (86-114)%
B4058-021	116 (53-254)%	B4059-017	102 (89-117)%	B4060-012	131 (115-150)%
B4058-022	124 (97-159)%	B4059-018	100 (85-118)%		

Table 21b. Potencies of two batches of ViraQ Multi-marker Check 75 Controls

virus	batch tested against ref batch B4254-001	potency (CI)	virus	batch tested against ref batch B4264-001	potency (CI)
HCV	B4264-001	121 (100-146)%	HCV	B4264-002	105 (85-131)%
HIV	B4264-001	103 (89-119)%	HIV	B4264-002	94 (82-109)%
HBV	B4264-001	106 (93-120)%	HBV	B4264-002	131 (98-174)%

In some cases the 95% CI does not overlap with the value 100%. However we found that on repeat batch release tests the potency did overlap the value 100%. For example on initial testing the potency of B4059-15 was 129 (108-154)% and on repeat tests it became 99 (84-116)%. Another example was batch B4059-16 in which the potency values changed from 146 (131-163)% to 101 (83-123)% on repeat testing. These changes in MPX Ct values may have been caused by not testing in the same test runs (or even by using different PCR reagent batches). Since the cobas MPX assay is a qualitative test one cannot guarantee that the intra-assay variation guarantees that the individual tests are consistent and the Ct values can be used for calculation of a potency with quadruplicate tests. For P0065 HBV Check 125 different batches had been used as reference batch which makes comparison of potencies more difficult to interpret. For some time P0155 ViraQ HBV Check 250 was used by SANBS with potencies of batch B4155-10, 011, 012 and 013 being 97 (92-113)%, 68 (53-88)%, 93 (75-117)% and 87 (76-100)% respectively.

So far only two batches of P0273 Multi-marker 75 Check Control have been manufactured. The reference batch was tested against the reference batch of P0254 Multi-marker Check 125 Control and the corrected potency result was calculated (table 21b). In this case all potency values overlapped the value 100%.

4.8 Acceptance testing of NAT system components using run controls

ViraQ Check and Trend controls can also be used for acceptance testing of a component of a NAT system such as a NAT reagent batch (or transport integrity check), a (re)-installation qualification of an instrument or training of an operator. The ViraQ Check Controls cannot be used for acceptance testing of Procleix Ultrio versions because the reactivity rate is near 100% and the majority of S/CO ratios are in the saturation range of the assay. For this purpose the ViraQ Trend controls are suitable because of their positioning near the 95% LOD of the assay. So far we only have sufficient data of Ultrio (Plus and Elite) batches to develop an acceptance test procedure using ViraQ Trend Controls.

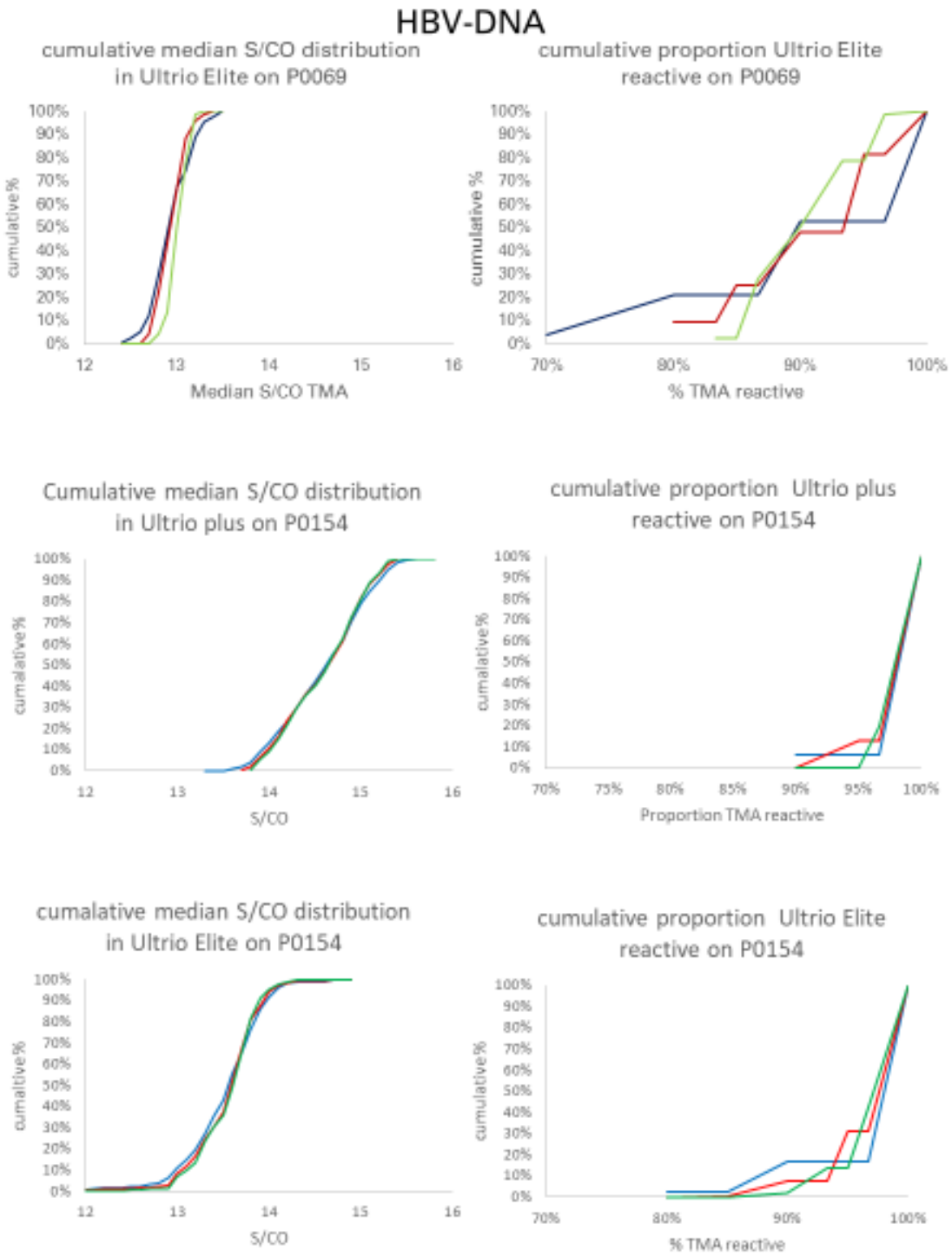
4.8.1 *Acceptance testing of Procleix NAT system components using ViraQ Trend Controls*

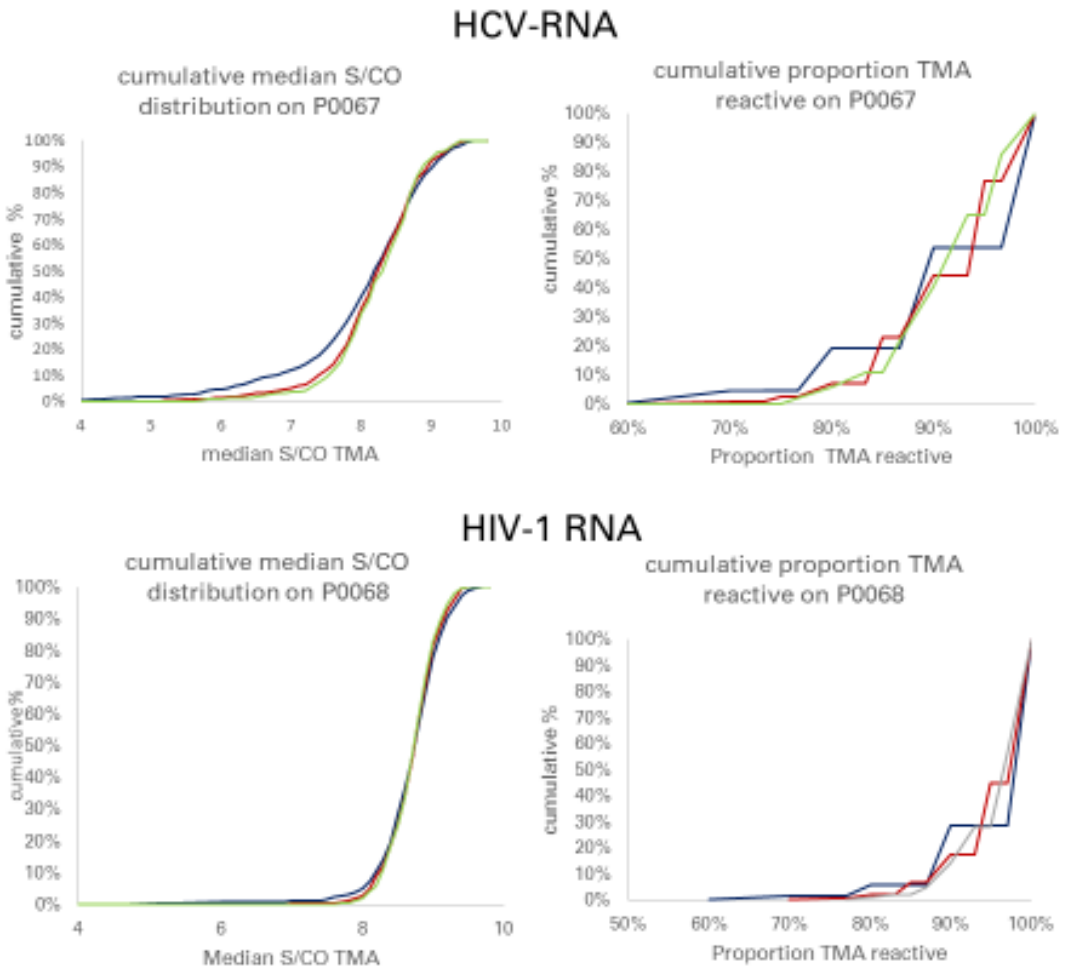
Acceptance criteria were established by a simulation study with sliding sets of 10, 20 or 30 sequential results out of a data base of Ultrio Plus and Elite test runs obtained during a four year observation period by IBTS. For P0067 HCV, P0068 HIV and P0154 trend controls data of respectively 13, 13 and 11 TMA reagent batches could be analyzed but for P0069 HBV trend control only 190 test runs with two Ultrio Elite reagent batches were available (since for a long time P0154 ViraQ Trend 50 was used by IBTS). By assessing the data sets we decided that an acceptance test algorithm could be established by accepting 95% of the data sets of 20 sequential results on the basis of the proportion reactive results (criterium 1) and the median S/CO value (criterium 2). Figure 19 gives the cumulative distribution of data sets according to reactivity rate and median S/CO value for 10, 20 and 30 sequential test runs. Table 22 gives the threshold values of these parameters for 10, 20 or 30 sequential test results above which 95% approval rate can be achieved (this means that 5% of data sets will not be approved when either one of the two criteria are not fulfilled). We recommend to repeat the batch or instrument acceptance test procedure if one of the two criteria are not fulfilled. Hence, with inclusion of the repeat test a rejection rate of 5% x 5% = 0.25% is expected. A root cause analysis of the reduced analytical sensitivity of the NAT system is then justified.

Table 22. Acceptance criteria of Procleix Ultrio Plus and Elite NAT reagent batches or Tigris/Panther instruments established by a simulation study on a four year data base of IBTS

Data base for simulation			acceptance criteria		
Vira Q Trend Control	number test runs	number of TMA reagent batches	simulated replicates per run	reactivity rate	Median S/CO
P0067 HCV 25	2700	13	10	≥70%	≥6.0
			20	≥80%	≥6.5
			30	≥80%	≥7.0
P0068 HIV-1 25	2689	13	10	≥80%	≥8.0
			20	≥85%	≥8.1
			30	≥87%	≥8.2
P0069 HBV 25 on Ultrio Elite	190	2	10	≥80%	≥12.6
			20	≥85%	≥12.7
			30	≥87%	≥12.8
P0154 HBV 50 on Ultrio Plus	1261	7	10	≥90%	≥13.9
			20	≥95%	≥13.9
			30	≥97%	≥14.0
P0154 HBV 50 on Ultrio Elite	1139	4	10	≥90%	≥13.0
			20	≥90%	≥13.0
			30	≥93%	≥13.1

Figure 19. Cumulative distribution of the median S/CO and proportion reactive results in Ultrio Plus and Elite assays in sliding sets of sequential data (blue n=10, red n=20 and green n=30).





From table 22 and figure 19 it can concluded n=20 is the most optimal sample size. The curves of the median and reactivity rate of n=20 are comparable to those of n=30. For HBV P0069 there were only 190 test results. More data need to be collected for the P0069 trend control to solidify the threshold values for acceptation.

5. CONCLUSION AND DISCUSSION

5.1 Suitability of ViraQ Check 125 Controls for qualitative and quantitative NAT methods

This performance evaluation report of the ViraQ Check and Trend controls demonstrates that concentrations of 125 cp/mL of the inactivated BioQ HBV, HCV and HIV-1 standards are suitable for external quality control of both the qualitative (Ultrio Plus and Elite) and quantitative (Aptima) TMA assay versions. (At the time of writing this report no data were available yet on Abbott real time and Roche Taqman viral load assays). The concentration of 125 cp/mL in ViraQ Check Controls was also useful in a multi-marker format for the cobas TaqScreen MPX 2.0 assay. Since the latest MPX version for the cobas 6800/8800 platform has improved analytical sensitivity (VR4059) a ViraQ Check Control of 75 cp/mL was developed and shown to be suitable for this assay. The concentrations of 125 and 75 cp/mL (set at 4-5 times the 95% LOD and LOQs (VR4059)) in ViraQ Check Controls guarantee that - if the assays are >99.9% of the time reactive - NAT testing is performed with sufficient analytical sensitivity. Hence - even without monitoring assay response values with an internet based data exchange system for interlaboratory comparison – the testing of ViraQ Check Controls is useful and enables customers to ensure sufficient analytical sensitivity of their NAT system. If the proportion of nonreactive results is higher than 0.1% it may be that a TMA reagent batch of lower analytical sensitivity is in use or that NAT instruments are functioning in a suboptimal manner. For HCV it cannot be excluded that the proportion of nonreactive results is somewhat higher (0.2-0.3%) with some TMA reagent batches. We consider to adjust the concentration of the inactivated HCV genotype 3 standard by a factor 1.5 (based on a second calibration experiment in the bDNA 3.0 reference assay) to harmonize the reactivity on ViraQ HIV and HCV run controls.

5.2 Monitoring (semi-)quantitative NAT response levels on ViraQ Check Controls

The data presented in this evaluation report show that quantitative NAT response values (cp/mL, IU/ mL or Ct values) on the ViraQ Check Controls can be longitudinally monitored in the same manner as S/CO values on the SeraQ run controls for immunoassays. After introduction of a new NAT reagent batch (or ViraQ Check Control batch) one can establish the geometric mean viral load or arithmetic mean Ct value of the initial 20-30 test runs and monitor the response values outside the 95% (or 99%) confidence limits using Westgard rules. However for the Ultrio assay versions monitoring of S/CO values on Check Controls is statistically meaningless because >98% of the S/CO values reach the saturation endpoint of the TMA assay. For P0063 ViraQ HCV Check Control a lower proportion (>93%) of the S/CO response values will be found in the saturation range of the Ultrio assay, which allows monitoring responses in the dynamic range as happens with ViraQ Trend Controls with viral concentrations near the 95% LOD (see chapter below). After planned recalibration of the S0109 HCV genotype 3 standard the proportion of S/CO results on 125 cp/mL samples is also expected to be >98% (comparable to the ViraQ HIV and HBV Check Controls). For the cobas MPX versions the ViraQ Multi-marker Check Controls of 125 and 75 cp/mL have shown to react with similar Ct values as expected by testing standard dilution panels. The position of these multi-marker controls at the edge of the Poisson detection endpoint range of the cobas MPX assay allows for using the average Ct value and the standard deviation as parameters for monitoring analytical sensitivity of assay runs over time (and for comparing analytical sensitivity of PCR reagent batches). The same holds for the use of ViraQ Check Controls in viral load assays that so far have only been evaluated in the quantitative Hologic Aptima TMA tests. The positioning of the ViraQ Check Controls at 4-5 times the 95% LOD or LOQ (with 99.7-99.9% reactivity rate) allows for monitoring the performance of Aptima reagent batches over time by comparing mean and standard deviation (%CV).

5.3 Monitoring TMA reactivity rates on ViraQ Trend Controls

Since >98% of the Ultrio Plus and Elite S/CO values on 125 cp/mL concentrations in the ViraQ Check Controls reach the saturation endpoint of the assay and >99.9% of test results are reactive one cannot use these controls to monitor trends towards higher or lower analytical sensitivity of assay runs. We therefore developed ViraQ Trend Controls containing 25 cp/mL

concentrations of the inactivated BioQ HBV, HCV and HIV-1 standards, near the 95% LOD of the assays. Probit analysis on HIV-1, HCV and HBV standard dilutions (VR4059) predicted reactivity rates of 96.7 (92.4-98.6)%, 93.7 (88.0-96.8)% and 90.8 (81.5-95.6)% respectively and this was confirmed by actual testing of the ViraQ Trend Controls showing reactivity rates of 96.0% for HIV, 92.4% for HCV and 92.6% for HBV. Adjustment of the HCV-RNA concentration in the Trend Control by a factor of 1.5 (according to a second bDNA recalibration experiment against the VQC-Sanquin HCV genotype 1 standard) is expected to generate a reactivity rate near 95%, similar to the rate on the HIV Trend control.

Doubling of the HBV concentration from 25 to 50 cp/mL in ViraQ Trend Controls enhanced the reactivity rate from 92.6% to 98.3%. (Probit analysis on dilutions of the inactivated standard showed a 95% LOD of 35 cp/mL as described in VR4059)). Since the analytical sensitivity of these Ultrio versions for HBV is approximately two-fold less than for HCV and HIV (and since the ViraQ HBV Trend 25 control generating around 93% reactivity is still functional) we do not intend to adjust the trend control concentration for HBV.

The Trend Controls were found to be instrumental to monitor the analytical sensitivity of Ultrio Plus and Elite reagent batches. Within one ViraQ Trend Control batch reactivity rates varied up to 10%, indicating that more than two-2-fold differences in analytical sensitivity between TMA reagent batches can exist. Overall the reactivity rates on Ultrio Plus and Ultrio Elite reagent batches were comparable, also for HIV-1 (97.2% versus 95.6%) indicating that inclusion of HIV-2 oligonucleotides in Ultrio Elite has not much affected the analytical sensitivity for HIV-1. Although theoretically the viral concentration in Trend Control batches should not vary more than 1% on the basis of weight records the reactivity rates varied up to 6%. (for HIV 91.2%, 97.8%, 92.4% and 98.6% and for HCV 91.0%, 88.3%, 94.3%, 91.7% and 93.6%). It is unlikely that variation in the viral concentration in ViraQ Trend Control batches or matrix effects have caused these differences in reactivity rates. Probably the variation in analytical sensitivity of TMA reagent batches has also contributed to these results (due to variations in manufacturing or deterioration of TMA reagents). The only way to prove consistency in reactivity rates on ViraQ Trend Controls is to test different controls (stored at -80 °C) in parallel in multiple replicate assays in the same Ultrio Elite reagent batch for some time.

5.4 Monitoring TMA S/CO response levels on ViraQ Trend Controls

The S/CO ratios in Ultrio (Plus and Elite) on ViraQ Trend Controls are not normally distributed. A considerable proportion of S/CO ratios (23%, 13% and 6% for HCV, HIV and HBV respectively) are found in the dynamic range where the Ultrio (Plus and Elite) tests have not yet reached the saturation endpoint of the TMA reaction. A Gumbel distribution was found suitable to describe the data. From the mathematical equations of this type of extreme value distribution it follows that one can use the difference between the average and median S/CO values as a measure of the skewness of the distribution curve. The higher the value of delta (average minus median), the more weak reactive and nonreactive results (with S/CO rations below the saturation point of TMA) are found on the ViraQ Trend Controls. It was shown that Δ (average minus median S/CO) can be used as a parameter to indirectly monitor the analytical sensitivity of TMA assay runs over time but also to compare the performance of Tigris/Panther instruments or Ultrio Plus/Elite reagent batches. One also can use the proportion of dynamic responses as a parameter but the problem is that the threshold S/CO value between saturated and dynamic responses varies per TMA reagent batch. Therefore Δ (average minus median S/CO) is a more standardized and simpel trending parameter. So for interpreting a single S/CO or a small number of S/CO results one can use the expected proportion of nonreactive dynamic responses in table 17b as guide. For statistically valid comparisons of analytical sensitivity based on S/CO value distributions (generated by for example different instruments or consecutive TMA reagent batches on ViraQ Trend Controls) it is recommended to use Δ (average minus median S/CO). This delta can also be used as a sliding parameter over time as shown in this report. Once more laboratories would use ViraQ Trend Controls and an internet based data exchange system is available one could

establish alert values of the delta parameter above which the analytical sensitivity of the TMA assay is considered too low and an investigation on performance of Tigris/Panther instruments or quality of Ultrio Plus/Elite reagent batches is required.

5.5 Consistency of manufacturing of ViraQ Check and Trend Controls

According to the weight records the expected variation in viral concentration of ViraQ Check and Trend Controls is expected to be less than 1%. A certain impact of a changing matrix in the ViraQ run control batches (TMA inhibiting substances in the manufacturing plasma pools) cannot be excluded. Another potential source of variability is loss of viral RNA during the manufacturing process or the storage time at -30˚C at the manufacturing (and testing) site. Probably the most important source of variation during manufacturing is the time the run control batch is handled at room temperature before the tubes are frozen in liquid nitrogen. This period becomes longer with larger batches and should be less than 4 hours to ensure minimal degradation of RNA (see stability data in VR4058). The only data we had to compare the potency of consecutive ViraQ Check Control batches were the Ct values obtained by batch release control testing in the cobas MPX assays. In the batch release control testing the newly prepared ViraQ batch is compared with the reference batch that was earlier produced and stored at -80˚C. The Ct values of quadruplicate assays of test batch and reference batch (that should be tested in parallel in the same cobas MPX test run) were used to compare the potencies of consecutive batches. With few exceptions the 95% confidence limits overlap the expected 100% potency value and in few cases where this criterium was not fulfilled it did on repeat testing.

5.6 Comparison of ViraQ run controls with CE marked products of other manufacturers

So far we have no access to NAT test data of CE marked run controls of other manufacturers to which we can compare the performance of our ViraQ run controls. One of the competitor products is chosen as an example for comparison in table 23 in which different quality aspects of run controls are evaluated.

Table 23. Comparison of different quality aspects of ViraQ run controls and a CE marked competitor product

Quality aspect	**Bio Quality Control**	**NRL/Exact/Thermo Scientific/Diamed**
Viral stock solutions	inactivated standards stored at -80˚C	raw material batches or standards?
Calibration of standards	in copies and IU by replicate bDNA tests against, native Sanquin, lyophilised WHO and chimp infectivity standards	quantification of starting material in VL assay?
	In IUs in WHO collaborative studies	
	Viral concentrations verified in other replicate VL assays	
	Viral concentrations verified in potency tests in Ultrio and cobas MPX versions	
frozen stability	native standards: >20 years at <-65˚C, 2 years -30˚C, verified by replicate RT PCR, bDNA and NASBA tests	tested on product in qualitative NAT tests? Was concentration too high to see loss of RNA?
	inactivated standards: >15 years at -80˚C and 2 years at -30˚C	

in use stability at 4, 21, 37C	<5% loss of RNA at 8h +4˚C and 21˚C, verified by replicate RT-PCR and bDNA tests up to 48 hours	
particle integrity	75% loss of RNA by lyophilisation and 40% by pasteutization, 15% loss of HBV-DNA, no free DNA in DNAse digestion and banding at Dane particle density in sucrose gradient	no characterization
inactivation	methods of proven efficacy in plasma and vaccine industry at proper protein concentration	How? just 2 hours 60˚C on undiluted plasma?
matrix	EDTA/Citrate plasma to mimic real samples, quick thaw required	base matrix (not screened for anti-HBc, anti-HBs?)
positioning of run controls	Check Controls at 4-5 times 95% LOD verified on native and inactivated standard dilutions in multi-center analytical sensitivity studies in Ultrio and cobas MPX versions	distance to LODs not known and to our knowledge not verified in analytical sensitivity studies. Are concentrations too high for meaningful EQC?
	Trend Controls near 95% LOD	not available
verification in field studies	design verified, predicted responses in analytical sensitivity studies confirmed	No verification of design, just data collection of responses in EDC net
monitoring TMA reagent batch consistency	Possible with Trend Controls and with more than half log lower analytical sensitivity also visible by Check Controls	not possible when concentrations are too high
statistical evaluation of TMA responses on run controls	Gumbel extreme value distribution possible because positioning of controls near LOD gives a fraction of responses in the dynamic range. Use average minus median as trending parameter.	Monitoring outliers in Levey Jennings charts, but not meaningful when S/CO responses are in saturated range of assay.
statistical evaluation of real time PCR responses on run controls	Monitoring Ct value or viral load in Levey Jennings Charts. Precision can be followed from mean and SD because of position near 99.5% LOD (start Poisson detection endpoint range)	Monitoring Ct value or viral load in Levy Jennings Charts but SD does not increase with lower analytical sensitivity when concentration is too high.
internet data exchange system	in fact not required because package insert gives predicted response values and statistical evaluation method. Minimum peer group required for interlaboratory comparison.	EDC net available and user network created in collaboration with VQC-Sanquin using PeliSpy standards. Currently large user group but is there a discipline to set confidence limits for each reagent and run control batch combination and are statistical evaluation tools valid?

6. UPDATE OF PERFORMANCE EVALUATION DATA AFTER REVIEW BY NOTIFIED BODY

This performance evaluation report has been reviewed by our previous Notified Body (LRQA-PEI) in the 4th quarter of 2018 and has led to CE marking of the ViraQ Controls in the 1st quarter of 2019. in In the 2nd quarter of 2019 (just after we received CE marking of the ViraQ Controls evaluated in this this report) we were informed that, due to the Brexit, LRQA stopped its Notified Body service. In the 4th quarter of 2019 we signed an agreement with a new Notified Body (MDC) that for re-certification did the review of the design dossiers again.

Issues in design dossier/performance evaluation review by PEI.

The batch consistency data in chapter 4.7 of this report were based on only 4 replicate cobas MPX tests per batch. After discussion with the reviewers of PEI the batch release procedure was changed by testing 8 replicate cobas MPX data per batch. A specific validation report VR4091 describes the current batch release criteria and summarizes batch release data obtained so far.

Since the report does not include actual performance data on the ViraQ Check Controls of 125 copies/mL (P0063, P0064 and P0065 for HCV, HIV-1 and HBV respectively) in the Abbott RealTime assay and the Roche CAP/CTM or cobas 6800/8800 viral load assays the PEI reviewers only accepted to include the Hologic Aptima assays in the intended use of these products.

Issues in design dossier/performance evluation review by MDC.

One remark was that the performance evaluation data in this report were rather old. Therefore we refer to data presented at our pre-IPFA-PEI workshop in Athens (May 15, 2018) that can be reviewed at our website www.bioqcontrol.com and are added as additional test reports to the technical files.

6.1 Update performance evaluation P0063, P0064 and P0065 ViraQ Check 125 Controls

Presentation Vermeulen, SANBS, South Africa, at pre-IPFA-PEI workshop. May 15, in Athens

For more recent data than described in this report we refer to the presentation of Marion Vermeulen (SANBS, South-Africa), entitled "The use of ViraQ Check Controls for qualitative and quantitative NAT" given at our last workshop in Athens. Apart from data already described in this report additional Ultrio Elite data collected in 2017 and 2018 were presented in power point slide 4 to 10. These data confirm the performance characteristics in this report and the expected values of the parameters given in the package inserts.

In addition performance data on the Abbott RealTime m2000 viral load assay were presented (slide 11,12). These latter performance evaluation data would allow us to include the Abbott RealTime assays in the intended use of the P0064 HIV-1 and P0065 HBV run control products but since P0063 HCV data on the Abbott platform are still lacking this has not been done so far (and thus the expected performance data in Abbott realTime assay have not been included in the package inserts).

6.2 Update performance evaluation of P0067, P0068 and P0069 ViraQ Trend 25 Controls

Presentation Boland, Ireland, at pre-IPFA-PEI workshop. May 15, in Athens

In the presentation of Fiona Boland slides 25-29 present data for P0067 ViraQ HCV Trend 25 control. The data are summarized in table 24.

Table 24. Summary of performance data on P0067 ViraQ HCV trend 25 control

Trend batch	Timeframe	Master Lot	Reactivity rate	S/CO Mean	S/CO Median	S/CO Delta
B4062-009	Sept '17 - Mar '18	Overall	535/568 (94.2%)	6.96	8.10	1.14
	Sept '17 - Jan '18	180939	426/445 (95.7%)	7.19	8.14	0.953
	Jan '18 - Mar '18	186731	114/128 (89.1%)	6.11	7.61	1.496
B4062-008	Feb '17 - Sept '17	Overall	535/564 (94.9%)	7.46	8.40	0.937
	Feb '17 - Mar '17	146182	x/31^	8.56	8.78	0.224
	Feb '17 - May '17	154471	x/235^	7.79	8.69	0.897
	May '17 - Aug '17	159260	x/223^	7.08	8.27	1.181
	Aug '17 - Sept '17	180939	x/75^	7.09	8.01	0.921
B4062-007	*Feb '16 - Feb '17*	*Overall*	*998/1083 (92.2%)*	*7.17*	*8.28*	*1.105*
	Feb '16 - May '16	119400	x/285^	6.24	7.53	1.294
	May '16 - Sept '16	131154	x/315^	7.72	8.64	0.92
	Sept '16 - Mar '17	146182	x/483^	7.37	8.47	1.097
		Total	2068/2215 (93.4%)			

^ reactivity rate not reported

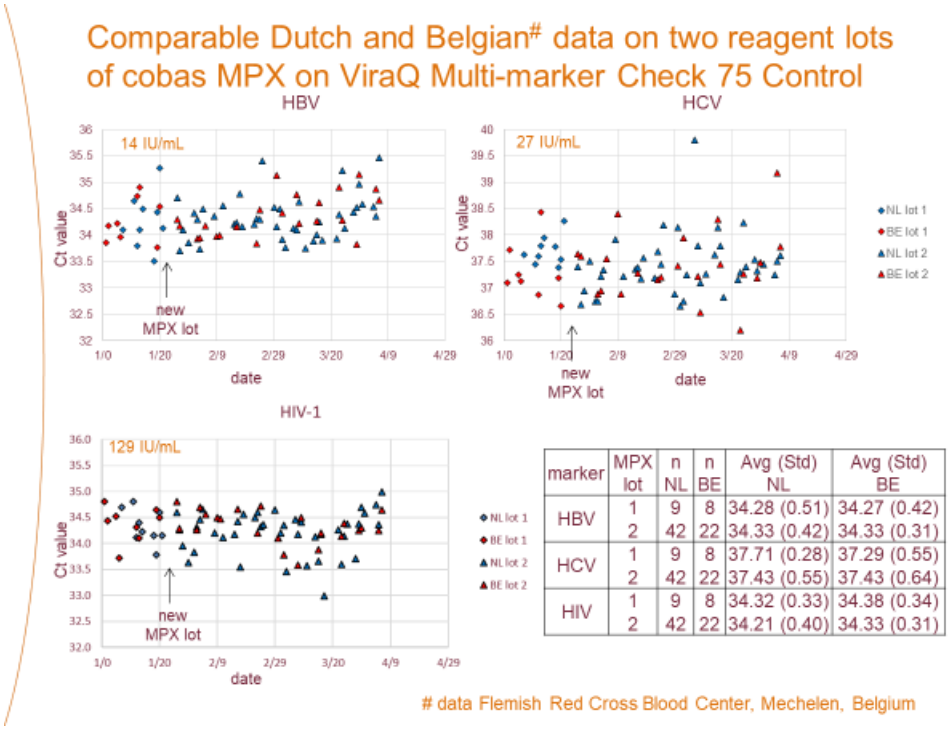
For some combinations of Ultrio Elite Master Lots and Trend Control batches the reactivity rate was not reported but the delta between the average S/CO and median S/CO was presented. The highest delta value was seen on Master Lot 186731 but still lower than the alert value of above 1.60 proposed in our package insert. The reactivity rate corresponding to the delta value of 1.496 was 89.1%, significantly lower than the rate of 95.7% on the previous Master Lot that was subjected to the same trend control batch B4062-009. Further data presented by Fiona Boland suggested that one particular Panther instrument showed a reduced reactivity rate of 80% on this master lot as compared to another instrument generating a rate of 95% (although this result could also be influenced by a too low number of test runs per instrument for this particular reagent master lot).

6.3 Update performance evaluation of P0273 ViraQ Multi-Marker Control

Presentation Koppelman, Sanquin, Netherlands, at pre-IPFA-PEI workshop. May 15, in Athens

Marco Koppelman compared results on different external controls in his presentation. Figure 20 below compared cobas MPX data on the P0273 ViraQ Multi-Marker Check control using data collected in 2018 from the Flemish Red Cross and Sanquin. The average Ct values are comparable to the ones observed before (table 19a) and in line with the expected values (table 19b).

Figure 20. Performance evaluation data in cobas MPX assay on the P0273 ViraQ Multi-Marker Check control obtained in 2018 from the Flemish Red Cross (Mechelen, Belgium) and Sanquin (Amsterdam).



marker	MPX lot	n NL	n BE	Avg (Std) NL	Avg (Std) BE
HBV	1	9	8	34.28 (0.51)	34.27 (0.42)
	2	42	22	34.33 (0.42)	34.33 (0.31)
HCV	1	9	8	37.71 (0.28)	37.29 (0.55)
	2	42	22	37.43 (0.55)	37.43 (0.64)
HIV	1	9	8	34.32 (0.33)	34.38 (0.34)
	2	42	22	34.21 (0.40)	34.33 (0.31)

6.4 Comparison with other CE marked controls

At the workshop in Athens Aneta Kopacz (IHTM, Warsaw, Poland) presented parallel testing data in cobas MPX and Ultrio Elite on ViraQ and "QConnect Controls" that are in routine use in Poland. In all data comparisons it was clear that the competitive products had a much higher concentration of virus in the samples making them less functional to recognize poor NAT performance. In the package inserts of the CE marked QConnect controls there was no information on quantification, calibration, stability and expected performance in the assays.

7. EXTERNAL REFERENCES: PRESENTATIONS AT BIOQCONTROL WORKHOP, MAY 15 2018, ATHENS

- Marion Vermeulen, SANBS, Johannesburg, South Africa. The use of ViraQ Check Controls for qualitative and quantitative NAT. www.bioqcontrol.com
- Fiona Boland, IBTS, Dublin, Ireland. Monitoring the analytical sensitivity of Procleix reagent batches using ViraQ HEV Check controls and HBV, HCV and HIV Trend controls. www.bioqcontrol.com
- Marco Koppelman, Sanquin, Amsterdam, Netherlands. Evaluation of run controls for cobas 6800 MPX and HEV assay. www.bioqcontrol.com
- Aneta Kopacz. IHTM, Warsaw, Poland. Evaluation of external NAT controls from two manufacturers. www.bioqcontrol.com

8. ADDITIONAL PERFORMANCE DATA AFTER RECALIBRATION HCV STANDARD IN 2022

VR4060 describes that the HCV-RNA concentration in the inactivated S0109 HCV genotype 3 standard needed to be adjusted 1.67 fold based on calibration against the S0009 VQC-Sanquin HCV genotype 1 standard by 5 different quantitative methods. As a consequence of this adjustment the distance to the 95% LOD in the cobas MPX assay for HCV-RNA shifted from 2-3 times to 4-5 times the 95% LOD (VR4059) in line with the design specifications for ViraQ Check Controls (CE4558). Moreover the recalibration is expected to bring the concentration of the P0067 HCV Trend 25 control from somewhat below the 95% LOD of the Ultrio Elite assay to somewhat above the 95% LOD.

8.1 Impact of recalibration of HCV standard on performance of P0273 ViraQ Check Control in the cobas MPX assay

Figure 21 compares the distribution of Ct values for HCV-RNA on two batches of the P0273 ViraQ Multi-Marker Check 75 run control product, of which batch 005 was based on the old calibration in copies/mL using the bDNA 3.0 assay and batch 007 was based on the average calibration in copies/mL by multiple methods (VR4060). According to the new calibration the HCV-RNA concentration in the older P0273 batch 005 was in fact 45 copies/mL as compared to 75 copies/mL in PEO batch 007. One can clearly see the impact of the 1.67-fold increase in HCV concentration on the Ct value distribution in Figure 21. Five of the 49 test results on the old product of 45 copies/mL are in the range between 38 and 43. These outlier Ct values are caused by the impact of Poisson distribution on PCR reactivity by the lower viral load in batch B4264-005. The tailing is not seen with the PEO batch 007 containing 75 copies/mL of HCV-RNA according to the new calibration. The tailing to higher Ct values is also not seen for HBV-DNA and HIV-RNA in this new batch B4264-007 (Figure 22).

Figure 21. Distribution of Ct values for HCV-RNA on two batches of P0273 ViraQ Multi-Marker Check 75 before and after recalibration of the inactivated S0109 HCV genotype 3 standard (VR4060).

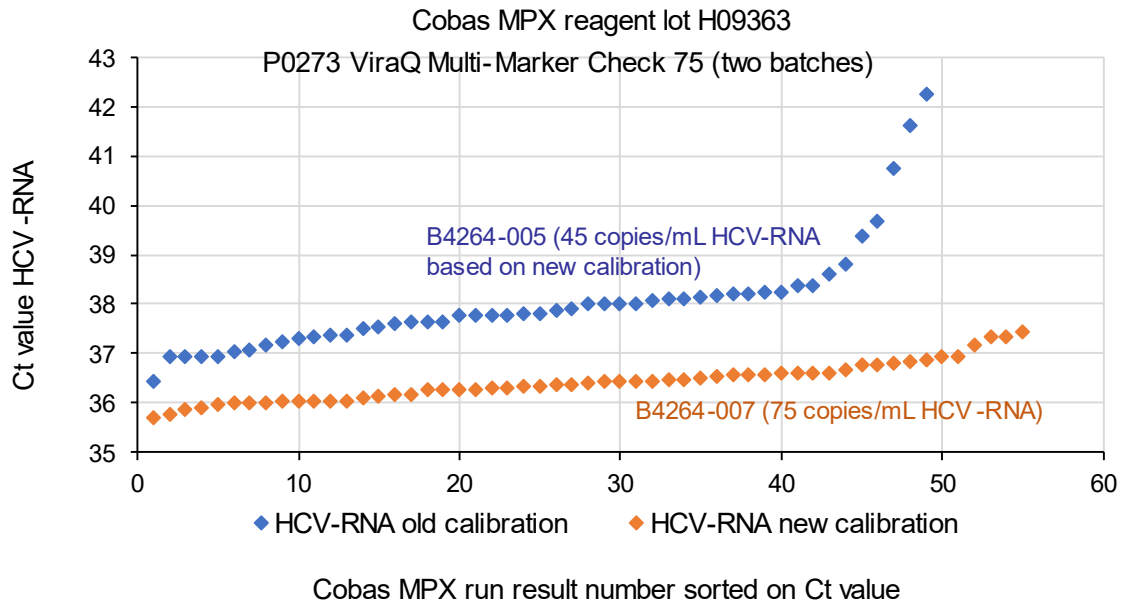
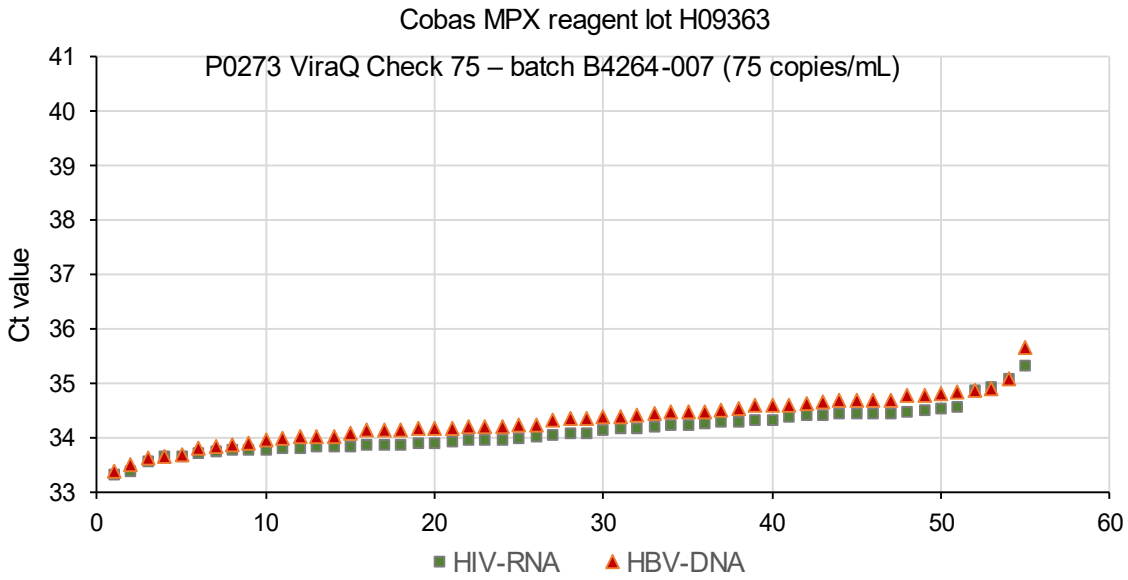


Figure 22. Distribution of Ct values for HBV-DNA and HIV-RNA on new P0273 ViraQ Multi-Marker Check 75 lot B4264-007 with adjusted HCV concentration (see Figure 21).



When the cobas MPX Ct values of different batches of the P0273 ViraQ Multi-Marker Check 75 Control are compared we see consistent confidence intervals for HBV-DNA and HIV-RNA with a %CV of 1.2%. The 1.67-fold adjustment of the HCV concentration in batch 007 significantly reduced the %CV from 2.9% to 1.1%.

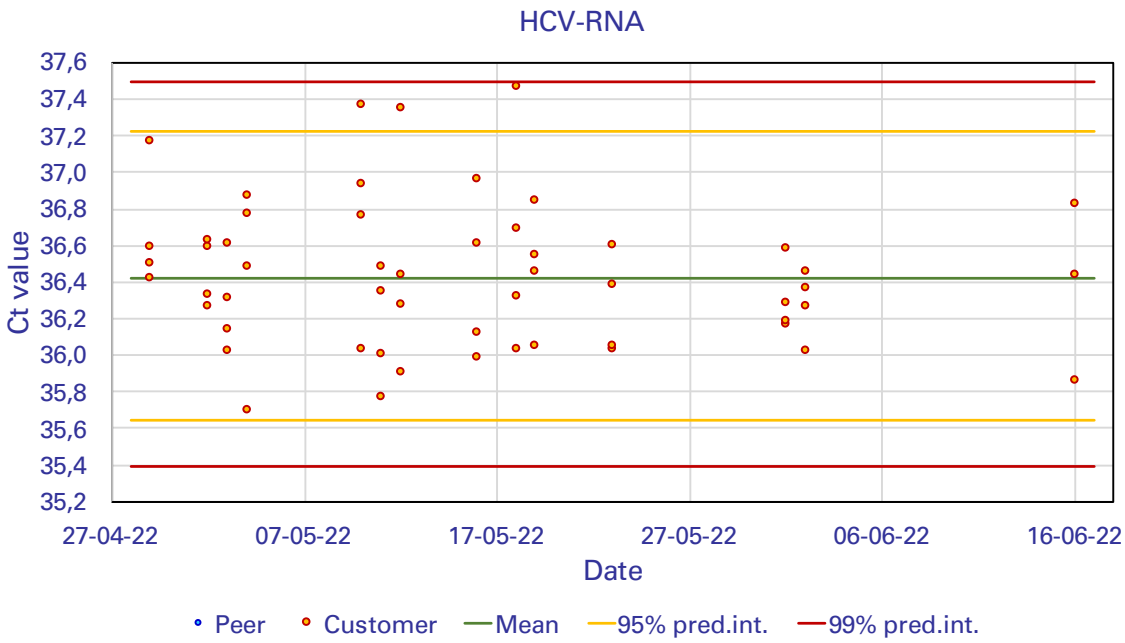
Table 25. Comparison of Ct values measured on different batches of P0273 ViraQ Multi-Marker Check 75 in cobas MPX assay

B4264 batches	Marker	Cps/mL	n	Average Ct	95 % confidence interval	99 % confidence interval
002-004	HBV-DNA	75	883	34.1	33.3-35.0	33.0-35.3
007	HBV-DNA	75	55	34.3	33.5-35.2	33.2-35.5
002-004	HCV-RNA	45^	884	37.7	36.4-38.9	36.0-39.3
005	HCV-RNA	45^	49	38.0	35.8-40.3	35.1-41.0
007	HCV-RNA	75	55	36.4	35.6-37.2	35.4-37.5
002-004	HIV-1-RNA	75	881	34.2	33.5-34.9	33.2-35.1
007	HiV-1 RNA	75	55	34.1	33.3-34.9	33.1-35.2

^new calibration

We therefore replaced the Levey-Jennings chart for HCV-RNA in the new version 8.0 of the KI4268 package insert using the data obtained by Sanquin on batch 007 (Figure 23)

Figure 23. Levey-Jennings QC chart for HCV-RNA obtained after performance evaluation study with P0273 ViraQ Multi-Marker Check 75 batch B4264-007 in cobas MPX assay



Conclusion

The recalibration of the inactivated S0109 HCV genotype 3 standard to 1.67-fold lower copy numbers (VR4060) was necessary to meet the design specifications (CE4558) and will reduce the risk of false alerts caused by nonreactive results on the P0273 ViraQ run control in the cobas MPX assay.

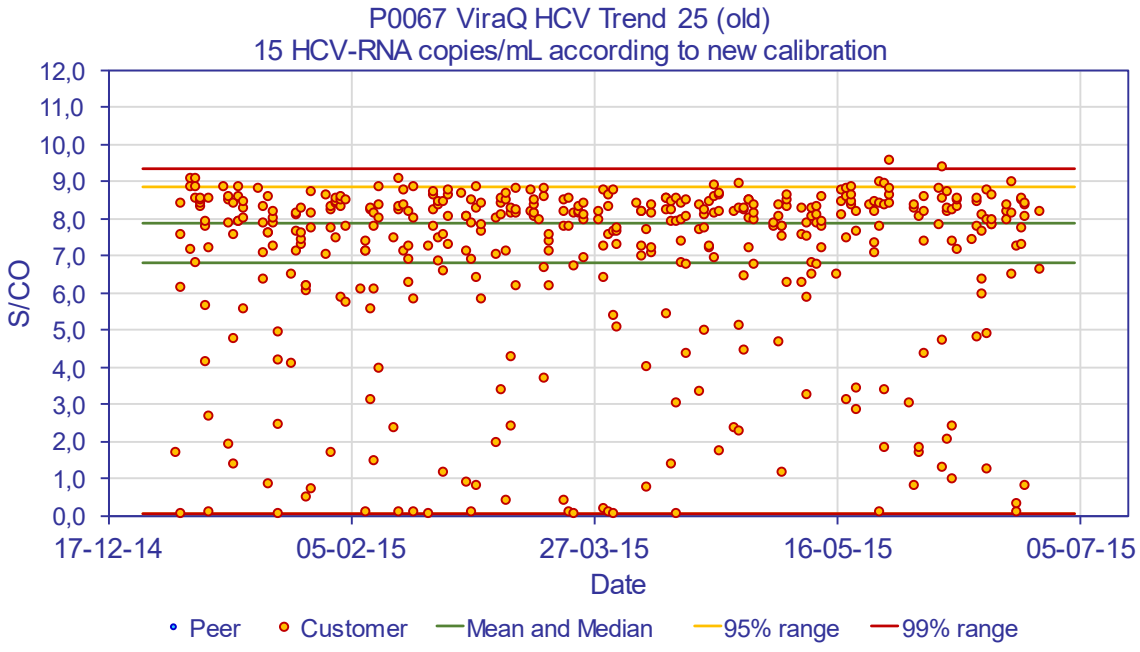
Discussion

One may wonder why this calibration issue was not discovered during the initial design phase of the ViraQ run control products and why the run control was not prepared from the well calibrated native VQC-Sanquin HCV genotype 1 standard. We would have preferred using this standard that was available in a large volume (10 L). Unfortunately the company that had acquired the Sanquin VQC business (Acrometrix, Benicia) in 2005 has used up almost the total volume of the primary HCV genotype 1 standard as raw material for their panel production. Our inactivated S0109 HCV genotype 3 replacement standard was only tested in 6 replicates against the primary S0009 HCV genotype 1 standard in the bDNA3.0 assay. There are small differences in genotype detection efficiency of quantitative NAT methods and the Roche cobas MPX assay quantified the S0109 genotype 3 standard 2.5-fold lower than in the initial calibration experiment with the bDNA 3.0 assay. A second bDNA 3.0 calibration experiment with only 3 replicate tests quantified the So109 standard 1.5-fold lower than the initial bDNA 3.0 experiment. We therefore decided to base the calibration on multiple methods (VR4060) and the geometric mean result was taken as the final calibration result against the primary genotype 1 standard. With the old calibration there is real risk of nonreactive HCV results in the cobas MPX assay on the P0273 run control, especially when the control reaches its expiry date after 2 years storage at -30°C. At that time 10-20% of the original concentration in the control has been lost (45-9=36 copies/mL) as we found in stability studies (VR4058). So far nonreactive results have not been reported on the P0273 control but to prevent this from happening with wider use of the run control we consider the recalibration of the S0109 standard and the design correction of the P0273 run control as an urgent matter.

8.2 Impact of recalibration of HCV standard on performance of P0067 ViraQ Trend Control in the Ultrio Elite assay

In Figure 24 one can see an example of a Levey-Jennings chart for the Ultrio Elite assay on the P0067 HCV Trend 25 Control as generated by the macros in the Excel statistical report developed by our statistician Dr. Jos Weusten. The red and orange lines give the 99% and 95% predictive intervals, calculated from the 0.5/99.5% and 2.5/97.5% percentiles of the available dataset. The green lines give the mean and median S/CO of the dataset, in this example 6.80 and 7.88 respectively. The delta between the two (1.08) is an indicator of the skewness of the distribution curve. The more nonreactive and weak responses in the Ultrio Elite assay are found on the Trend control of 25 copies/mL the greater the distance between the mean and median S/CO values becomes. Therefore the delta S/CO (median minus average) is an indicator of the analytical sensitivity of the Ultrio Elite reagent lot and/or the HCV concentration in the Trend Control. One can see that the lower 95% and 99% confidence limits in the dataset of Figure 24 are reaching values far below the S/CO=1 cutoff level. The reason is that 28/329 (6.5%) of the results were found nonreactive and a large proportion of S/CO values were in the lower dynamic range of the assay whereby the amplification reaction in the Ultrio Elite assay does not yet reach the saturated signal (S/CO between 1.0 and 7.0). According to the new calibration of the inactivated S0109 HCV genotype 3 standard the concentration in batch B4062-006 was 1.67-fold lower than 25 copies/mL or 15 copies/mL

Figure 24 Levey-Jennings chart of Ultrio Elite S/CO values on P0067 ViraQ HCV Trend 25 Control batch B4062-006 before recalibration of the inactivated S0109 HCV genotype 3 standard



To understand the impact of increasing the HCV concentration from 15 to 25 copies/mL as a consequence of the recalibration of the S0109 standard we tested batch B4062-013 (15 copies/mL) and a PEO labeled reference batch B4062-014 (25 copies/mL) in parallel in the same Ultrio Elite test runs. Figure 25 and 26 present the comparison of the Ultrio Elite S/CO values on the old and new trend control of 15 and 25 copies/mL respectively (based on the new calibration). In 56 runs 2 (4%) were nonreactive on 15 copies/mL and none on 25 copies/mL. The proportion of not yet saturated results were 19 (34%) and 4 (7%) on 15 and 25 copies/mL respectively. Again the 95% and 99% lower limits on the Trend Control of 15 copies/mL were below cut off value and the distance between the median and mean (delta S/CO) was 0.81.

However on the recalibrated control containing 25 copies/mL the 95% and 99% lower limits of the distribution were found at S/CO= 5.84 and 5.38 respectively which made the Levey-Jennings QC plot more functional for the users. The delta between median and mean reduced to 0.09.

Figure 25. Levey-Jennings charts of two batches of P0067 ViraQ Trend control before and after recalibration of the HCV standard

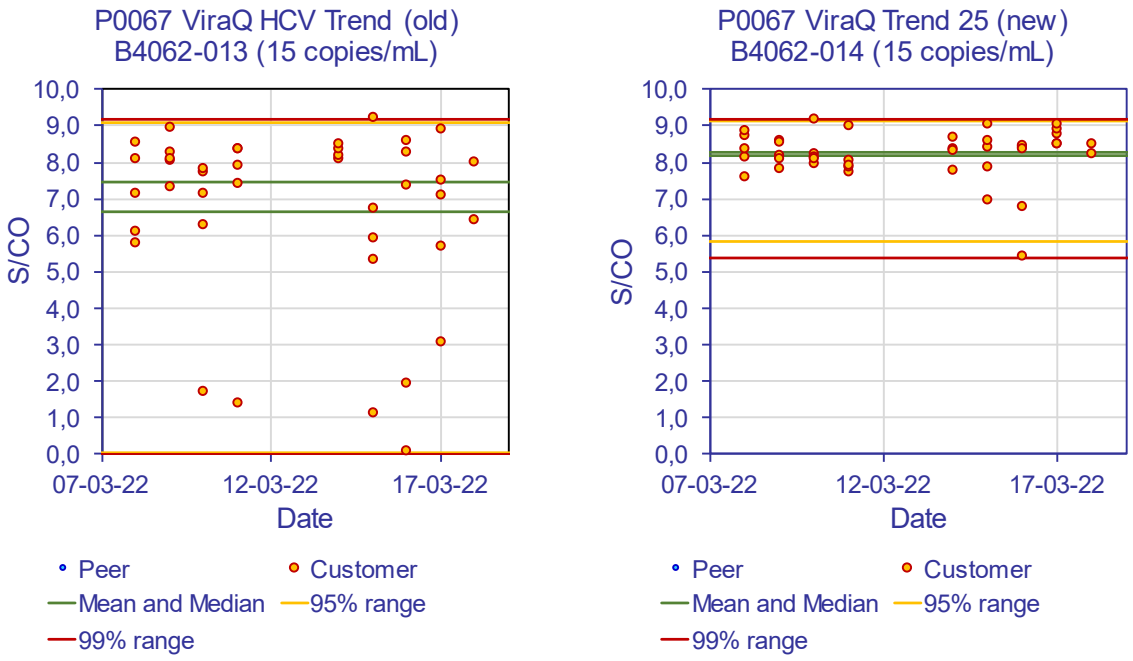
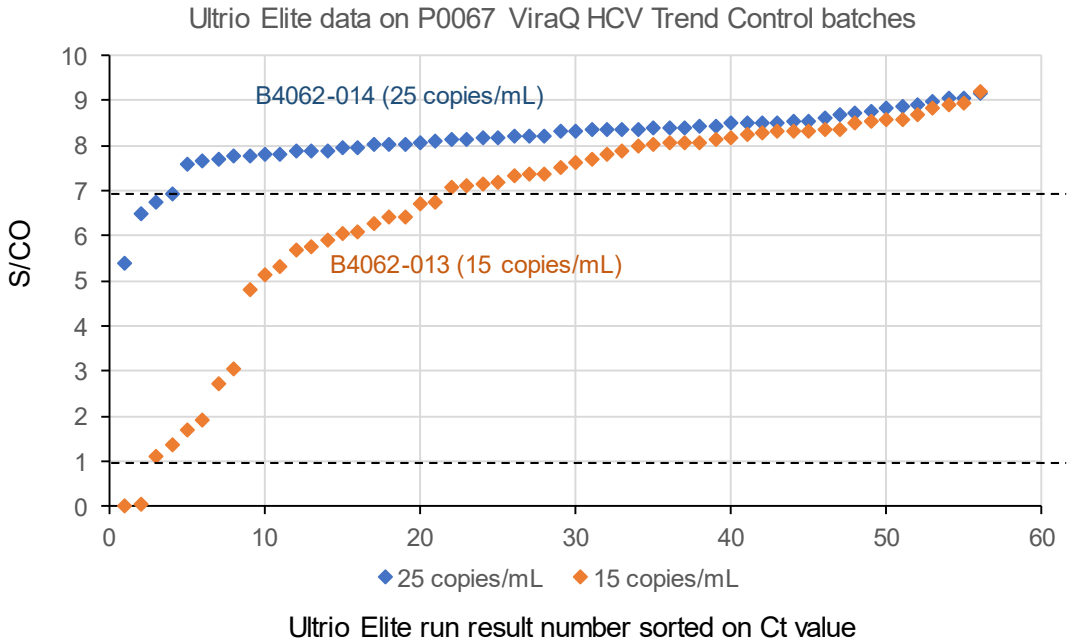


Figure 26. Distribution of Ultrio Elite S/CO values on two batches of P0067 HCV Trend Control before and after recalibration of the S0109 HCV standard



Conclusion

The recalibration of the inactivated S0109 HCV genotype 3 standard significantly reduced the proportion of nonreactive and weak S/CO values on the P0067 HCV Trend Control in the Ultrio Elite assay, which made the statistical report with the Levey-Jennings chart more functional and the interpretation of the results probably easier for the users.

Discussion

The recalibration of the HCV standard is expected to make the S/CO values distribution on the P0067 ViraQ HCV Trend Control more comparable to those found on the ViraQ P0068 HIV Trend Control and the P0154 HBV Trend 50 Control. So far there is only one customer using the Trend Controls (IBTS, Ireland) and this user judges the performance of Ultrio Elite reagent lots by the percentage reactive and the delta value between median and mean S/CO on the trend control. In the future one can also use the level of the 95% and 99% range limits as additional parameters to compare the analytical sensitivity of Ultrio Elite reagent lots. More data need to be collected on future batches of the P0067 HCV Trend Control in combination with Ultrio Elite reagent lots to know how the statistical parameters fluctuate over time and on different instruments and reagent lots. These data should also be used to formulate new Ultrio Elite reagent lot acceptance criteria on the modified P0067 HCV Trend Control.

8.3 Impact of recalibration of HCV standard on performance of P0063 ViraQ Check Control in the Ultrio Elite assay

The current positioning of the P0063 HCV Check 125 Control in the Ultrio Elite assay is in fact ideal with a concentration of 4-5 times the 95% LOD. So far only once a nonreactive result was reported on the run control. Hence there is no reason to use another formulation to reduce the risk of nonreactive results. According to the new calibration this control should in fact be renamed as P0063 HCV Check 75 Control, since it contains 75 copies/mL. In order to be consistent we decided to also adjust the concentration in the P0063 HCV Check Control 1.67-fold, which increases the distance to the 95% LOD from 4-5 times to 7-9 times the 95% LOD, comparable to the P0064 HIV-1 Check 125 Control. This change would still be compatible with the Design Specifications (CE4558) that required a distance between 3 and 10 times the 95% LOD. This 1.67-fold adjustment of the HCV concentration will make the control more robust and reduce the risk of nonreactive results in case of poor performance of the Ultrio Elite assay. However, the Levey-Jennings chart parameters of our new statistical reports still allow being alerted in case of reduced analytical sensitivity of an Ultrio Elite reagent lot or Panther instrument. We therefore first need to collect performance evaluation data on a PEO batch of the P0063 run control with 1.67-fold higher concentration before this change can be effectuated. At the time of writing this new chapter of version 5.0 of this report no data were available yet.

9. RELATED DOCUMENTS

CE4027	Product Information P0064 ViraQ HIV-1 Check 125
CE4135	Product information P0063 ViraQ HCV Check 125
CE4249	Product information P0065 ViraQ HBV Check 125
CE4558	Design Specifications of ViraQ Check Controls
CE4560	Design Specifications of ViraQ Trend Controls.
CE4006	Preparation of inactivated secondary viral standards : Safety assessment of viral quality control samples for viral serology and NAT assays in blood screening laboratories
VR4058	Stability report ViraQ run control product family
VR4059	Positioning of ViraQ Check and Trend Controls compatible with analytical sensitivity of NAT assays
VR4060	Calibration of native and inactivated HBV/HCV/HIV-1 standards
VR4061	Performance evaluation of ViraQ HBV/HCV/HIV-1 controls
VR4091	Definition of batch release criteria for ViraQ Controls