Stability of ViraQ controls for HBV/HCV/HIV-NAT

Classification	Confidential
Version 1.0 effective from	4 June 2015
Version 2.0 effective from	9 January 2018
Version 3.0 effective from	25 March 2020
Version 4.0 effective from	4 January 2022
Version 5.0 effective from	27 January 2023

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1. OBJECTIVE

The primary objective of this validation report is to document the stability of the ViraQ product family following the ISO 23640 norm. The secondary objective is to compare the stability of the inactivated BioQControl viral standard dilutions with those of the native VQC-Sanquin and lyophilised WHO standards. For this purpose stability data obtained with the current ViraQ product library and the previous PeliSpy products were compared in order to solidify the claim in the instructions for use. Here it is claimed that the run controls are stable when they are stored frozen (≤-30°C) for 2 years and after thawing for at least 8 hours at room temperature or refrigerated storage at 4 (2-8) °C. For this claim 20 % degradation of the analyte was considered acceptable. The rationale for this acceptance criterion is that degradation of a concentration of 125 copies/mL to 100 copies/mL would still guarantee >99.5% reactivity on the ViraQ Check Controls.

Standards evaluated:

As described in VPL4058 the stability of the inactivated standards, i.e. the S0109 HCV-RNA genotype 3a, the S0043 HBV-DNA genotype A and S0041 HIV-RNA subtype B standards has been compared with that of the native S0009 HCV-RNA genotype 1, S0011 HBV-DNA genotype A and S0012 HIV-1-RNA subtype B standards. The heat-inactivated BioQ standards S0041 and S0043 for HIV and HBV originate from the native Sanquin-VQC S0012 and S0011 standards respectively (CE4006). The stability of the chemically inactivated BQC S0109 HCV-RNA genotype 3a standard has been compared with the native S0009 HCV genotype 1 and WHO 06/100 standard (see VPL4058 stability plan). The three VQC-Sanquin standards serve as the primary BQC nucleic acid standards calibrated in copies/mL but are considered secondary standards when calibrated in IU/mL against the WHO standards (VR4060).

2. INTRODUCTION

This report describes the stability at different temperatures of liquid and frozen ViraQ HBV, HCV and HIV-1 run controls, which are dilutions of the inactivated S0043 HBV-DNA, S0041 HIV-1-RNA and S0109 HCV-RNA standards. VPL4058 describes the design of the stability experiments and explains why in addition to experiments performed with the run controls containing 125-250 copies/mL also experiments were performed with standard dilutions of higher concentrations.

This validation report is structured as follows:

a. Real time stability of current ViraQ run controls and historical PeliSpy products (long term frozen stability of BioQ and VQC-Sanquin standards at -30 and -70 °C)
b. In use stability of current ViraQ run controls and historical PeliSpy products (stability in the liquid phase established by accelerated degradation studies at 4, 20 and 37 °C).
c. Recovery of virus after inactivation or lyophilisation and impact on particle integrity and stability

The real time stability of ViraQ run controls is monitored for at least three batches as prescribed in the EN23640:2012 norm[1]. Accelerated stability test data in the frozen state are not available. This would require different temperatures higher than -30°C. However at elevated temperature (such as -10 °C) it is not certain that the solid state structure remains the same.

In the design of the stability experiments we compared the inactivated standards with the native standards. If there is no difference in the stability at -30°C and -70°C or at different temperatures in the liquid phase for both the native and inactivated standards this would indicate that the inactivation method itself does not affect the stability of the nucleic acid standards. The stability plan VPL4058 gives

more details on the design of the experiments and methods used for quantification of the degradation of nucleic acid by replicate quantitative NAT testing.

3. METHODS

All applied methods for the stability tests and calculation of the degradation kinetics of the analytes at different storage temperatures are described in VPL4058 and briefly summarized below.

3.1. Calculation method

Stability is defined as absence of a significant loss of the analyte during the observation period. This is established by plotting time vs ln(analyte) and calculating slope and intercept. The slope and intercept are calculated using SPSS 17.0. When the slope is not significantly different from 0%, loss of reactivity is denied and stability is claimed. For this analysis the 95 % confidence interval on the slope is used to test this hypothesis

The degradation of the analyte is calculated with the reaction kinetics formula:

$$\text{decrease reactivity} = \frac{\mathrm{d}[\text{analyte}]}{\mathrm{dt}} = -\mathrm{K}\,[\text{analyte}]$$

This quotation is following 1st order kinetics for degradation of the analyte. This assumption is justified as the implied enzymes taking part in the degradation reactions are not consumed during the reaction and therefore will not change in concentration.

After resolving the integral one obtains:

$$\ln[\text{analyte}] = \ln[\text{analyte}, \mathrm{t} = 0] - \mathrm{K}\,\mathrm{t}$$

The H_0 hypothesis is K equals zero, if so degradation is absent. The H_1 hypothesis is K does not equal zero and degradation is present. K is the slope between ln(conc) and time and ln(analyte, t=0) is the intercept.

3.2. Design of stability experiments

Stability studies often use quantitative test results at different time points, thereby introducing inter-assay variation. The effect of inter-assay variation can be excluded by having a bench mark temperature at which the preparations are considered stable. The 'stable' preparation stored at -70°C can be used 'to correct' the results of the preparations stored at higher temperature. Note that, inter-assay variation disables establishment of limited degradation when the variation in testing exceeds the degradation.

Stability at -70°C

We compared bDNA test results obtained on preparations stored at -70°C for more than 10 years. Linear regression on ln(conc) vs time yields a slope. When the slope equals zero, stability is confirmed.

In a second approach the longitudinal results found in analytical sensitivity studies of Duplex, Ultrio, Ultrio Plus and Ultrio Elite assays (Grifols) are compared. The dilution panels are stored at -70°C.

For preparation of the panels the native primary VQC-Sanquin standards were used. We compared the 50 and 95 % hit rates (with 95% confidence interval) found in probit analysis. When the 50% confidence limits overlap there is no significant difference in analytical sensitivity or potency of the standard dilution panels.

3.3. HBV-DNA studies

3.3.1. Real time stability studies on currently produced frozen ViraQ HBV-DNA Check controls

Several batches of P0155 ViraQ Check HBV-DNA 250 copies/mL and P0065 ViraQ Check 125 copies/mL were stored for several years at -30°C. By comparing Ct values found in quadruplicate/duplicate Roche Taqscreen 2.0/cobas MPX assays the HBV-DNA content could be followed over time. The concentration in P0154 and P0069 ViraQ Trend HBV-DNA products of 25 and 50 copies/mL is too low for reliable measurements of the Ct value. However since relative degradation is independent from the HBV-DNA concentration the results on the P0155 ViraQ Check 250 copies/mL controls can be extrapolated to the P0065, P0154 and P0069 controls of 125, 50 and 25 copies/mL.

Using linear regression between time and Ct value (log concentration) the slope and 95% CI was calculated in SPSS 21.0. The slope is used to demonstrate 2 year stability and support the product claim.

3.3.2. Historical studies on native HBV-DNA standard dilutions and PeliSpy run controls

The stability of HBV-DNA at -70°C is assessed on longitudinal bDNA 3.0 measurements on dilutions of S0011 HBV-DNA genotype A standard.

Stability of PeliSpy run controls stored at -30°C was assessed over three years by using a 1:10.000 dilution of the native S0011 HBV-DNA standard stored at -70°C tested in parallel in Siemens bDNA3.0 and Roche Monitor assay.

3.3.3. In use stability of ViraQ HBV run controls

The predicted stability after thawing at 4°C, 20°C and 37°C was established during 48 hours with the P0238 mixed standard panel, containing the inactivated and native S0043 and S0011 HBV-DNA standards in a concentration of 2000 copies/mL. At this concentration the precision of the Ct values measured after different storage temperatures is higher than the concentration of 25 to 125 copies/mL present in the ViraQ run controls. As the reaction constant is independent from the concentrations this will likely not affect the outcome.

3.3.4. Particle integrity of inactivated and lyophilised HBV-DNA standards

Heat-inactivation or lyophilisation could destroy or damage HBV particles and if so these processes could have an impact on the stability of HBV-DNA. CE4006 describes the preparation of the heat inactivated S0043 HBV-DNA standard from the native S0011 VQC-Sanquin standard. The density of the standard before and after heat-inactivation was compared with two lyophilised HBV standards (the WHO and ISS standard) in sucrose gradient studies performed by prof W. Gerlich at the University of Giessen. Prof. Gerlich also examined the HBV-DNA recovery after DNAse treatment by quantitative real time PCR assay using HBV plasmids as a control.

3.4. HCV-RNA studies

3.4.1. Real time stability studies on currently produced frozen ViraQ HCV-RNA controls

Some years ago several batches of P0063 ViraQ Check HCV-RNA (stored for several years at -30°C) were tested in quadruplicate Roche Taqscreen 2.0 assays by Dr. M. Koppelman (Sanquin, the Netherlands). By comparing Ct values the HCV-RNA content could be followed over time. The concentration in P0067 ViraQ Trend HCV-RNA is too low for reliable measurements of Ct value. However, since the relative degradation is independent from concentration the results on P0063 HCV Check 125 can be extrapolated to P0067 HCV Trend 25 control.

Recently the real time stability experiment was repeated after a longer storage period of eight ViraQ HCV Check 125 batches from 2-8-2012 until 20-8-2017. In November 2017 the ViraQ Check Control samples stored at -30˚C were tested in quadruplicate or duplicate cobas MPX tests.

The native S0009 HCV genotype 1 standard, and inactivated S0109 HCV genotype 3a standards were diluted to 2000 copies/mL in the P0238 mixed standard panel (table 1). In addition the reconstituted WHO HCV 06/100 standard has been diluted to 1000 IU/mL. The P0238 panel was stored at -80˚C and -30˚C. After 4.3 years of storage sample 3 and 4 of the panel stored at -80˚C were tested in parallel with sample 3 and 4 of the panel stored at -30˚C in 12 replicate tests in the same cobas MPX assay run.

Using linear regression between time and Ct value (log concentration) the slope was calculated in SPSS 21.0. The slope was used to estimate the degradation of HCV-RNA after two years storage at -30˚C and support the product claim (<20% degradation).

Table 1. Composition of P0238 mixed standard panel designed to evaluate stability of ViraQ run control family

Sample-ID	marker	standard	Dilution	concentration	Purpose
1	HBV-DNA	S0011	107500	2000 copies/ml	Reference
	HCV-RNA	S0009	315	2000 copies/ml	Reference
2	HBV-DNA	S0043	3615	2000 copies/ml	Used in controls
	HCV-RNA	S0148	3020	2000 copies/ml	Reference
3	HIV-RNA	S0012	52500	2000 copies/ml	Reference
	HCV-RNA	S0109	3020	2000 copies/ml	Used in controls
4	HIV-RNA	S0041	1310	2000 copies/ml	Used in controls
	HCV-RNA	WHO 06/100	38.7	1000 IU/ml (2730 copies/ml)	Reference

3.4.2. Historical studies on native HCV-RNA standard dilutions and PeliSpy controls

The stability of HCV-RNA at -70°C was assessed by longitudinal bDNA measurements on dilutions of S0009 HCV-RNA genotype 1 standard. Stability at -30°C was assessed over 6 years using a standard dilution of S0009 HCV-RNA genotype 1 (PeliSpy run control) tested in parallel with the same dilution stored at -70°C in the Siemens bDNA 3.0 assay.

3.4.3. In use stability of ViraQ HCV run controls

The stability of ViraQ run controls has been established in an accelerated degradation study of four HCV standards stored for 0, 8, 24 and 48 hours at +4°C, +20°C and + 37°C. The native S0009 HCV genotype 1 standard, the native S0148 HCV genotype 3a and inactivated S0109 HCV genotype 3a standards were diluted to 2000 copies/mL in the P0238 mixed standard panel (table 1). In addition the reconstituted WHO HCV 06/100 standard has been diluted to 1000 IU/mL and was tested in parallel in the P0238 mixed standard panel in the Roche TaqScreen 2.0 assay

3.4.4. Historical in use stability studies with PeliSpy run controls

A PeliSpy run control product was kept at +4°C and at room temperature for 12, 24, 48, 96 and 120 hours and tested in in triplicate in the Organon Teknika NucliSens NASBA assay.

3.4.5. Recovery of HCV-RNA after inactivation and lyophilization of HCV standards

The recovery of HCV-RNA after pasteurization and treatment with betapropiolactone has been examined in the development phase of the PeliSpy and ViraQ run control products. In addition the recovery after lyophilisation has been examined by Sally Baylis et al[2]. The data are reviewed in this report.

3.5. HIV-RNA studies

3.5.1. Real time stability studies on currently produced frozen ViraQ HIV-RNA controls

A number of batches of P0064 ViraQ Check HIV-RNA 125 copies/mL were stored for some years at -30°C. By comparing Ct values found in quadruplicate Roche Taqscreen 2.0 assays the HCV-RNA content could be followed over time. The concentration in P0068 ViraQ Trend HIV-RNA 25 copies/mL is too low for reliable measurements of Ct value. However, since the relative degradation is independent from the concentration the results on the P0064 HIV Check 125 control can be extrapolated to the P0068 HIV Trend 25 control.

In November 2017 a second real time stability experiment has been performed. Seven ViraQ HIV Check 125 batches stored from 1-4-2012 until 24-8-2017 at -30˚C were tested in duplicate or quadruplicate cobas MPX tests.

The native S0012 HIV-1 subtype B standard, and inactivated S0041 HIV-1 subtype B standard were diluted to 2000 copies/mL in the P0238 mixed standard panel (table 1). The P0238 panel was stored at -70˚C an -30˚C. After 4.3 years of storage the sample 3 and 4 of the panel stored at -70˚C were tested in parallel with sample 3 and 4 of the panel stored at -30˚C in 12 replicate tests in the same cobas MPX assay run.

Using linear regression between time and Ct value (log concentration) the slope was calculated in SPSS 21.0. The slope is used to estimate the degradation of HIV-RNA after two years of storage at -30˚C and support the product claim (<20% degradation).

3.5.2. Historical studies on native HIV-1 RNA standard dilutions and PeliSpy run controls

The stability of HIV-RNA at -70°C is assessed on longitudinal bDNA measurements on dilutions of S0012 HIV-RNA subtype B standard.

Stability of PeliSpy run controls stored at -30°C was assessed over 8 years by using a 1:10.000 dilution of the native S0012 HIV-1 RNA standard stored at -70°C tested in parallel in Siemens bDNA3.0 and Organon Teknika NASBA assay.

3.5.3. In use stability of ViraQ HIV-1 RNA run controls

The stability of HIV-1 run controls has been established in an accelerated degradation study of two HIV-1 standards stored for 8,24 and 48 hours after thawing at +4°C, +20°C and + 37°C. The native S0012 and heat-inactivated S0041 HIV-1 standards were diluted to 2000 copies/mL in the P0238 mixed standard panel (table 1) and tested in quadruplicate TaqScreen 2.0 assays after the different storage time and temperatures. To improve the precision of the Ct values we have chosen a concentration of 2000 copies/mL instead of the lower concentrations present in the ViraQ run controls. As the reaction constant is independent from the concentrations this will likely not affect the degradation.

3.5.4. Historical in use stability study on PeliSpy HIV-1 run controls

A PeliSpy run control of 10,500 copies/mL prepared from the native standard S0012 was kept at +4ºC and at room temperature for 12, 24, 48, 96 and 120 hours and tested in in triplicate in the Organon Teknika NucliSens NASBA assay.

3.5.5 Recovery of HIV-1 RNA after pasteurization and lyophilisation and impact on in use stability

Equivalent dilutions of the native S0012 and heat-inactivated S0043 HIV-1 standard were lyophilised by Dr Weycamp (Queen Beatrix Hospital, Winterswijk, the Netherlands). The HIV-RNA recovery in the native and pasteurized standards before and after lyophilisation and reconstitution was measured in triplicate bDNA 3.0 (Bayer/Siemens) and triplicate NASBA (Organon Teknika/Biomerieux) assays. In addition the recovery was measured in triplicate bDNA and NASBA assays after storing the four preparations at 12, 24 and 48 hours at +4ºC and at room temperature.

4. RESULTS

The results are presented by marker for the real time (frozen) and in use (liquid) stability experiments respectively.

4.1. HBV-DNA

4.1.1. Stability S0011 HBV-DNA genotype A standard at -70°C

The S0011 HBV-DNA standard stored at -70°C is used as a reference to preparations stored at higher temperatures. To demonstrate stability at -70ºC replicate bDNA test results covering the period

1996 to 2008 were assessed (table 2) by plotting ln [copies/ml] vs years. The slope obtained by linear regression and the confidence limits were used to judge stability. When the slope does not differ significantly from zero stability is claimed.

Table 2. Stability of S0011 HBV-DNA genotype A primary reference standard tested in dilutions falling well in the dynamic range of the bDNA assay.

Test	Year	average	ln average	Ln stdev	n	Lower and upper limit 95 % Conf. Interval	
bDNA 1.0	1996	3.73E+09	2.20E+01	0.015	9		
	1997	2.38E+09	2.16E+01	0.001	2		
	1998	2.94E+09	2.18E+01	0.163	4		
	1999	2.69E+09	2.17E+01	0.426	2		
	Slope ln(conc) vs year		-0.070	0.079	17	-0.412	0.272
bDNA 3.0	2001	2.15E+09	2.15E+01	0.092	16		
	2006	2.07E+09	2.15E+01	0.028	3		
	2006	1.40E+09	2.11E+01	0.072	3		
	2008	2.71E+09	2.17E+01	0.099	6		
	Slope ln(conc) vs year		0.009	0.059	28	-0.245	0.264
Both	Slope ln(conc) vs year		0.000	0.017	45	-0.043	0.042

S0011 HBV-DNA genotype A standard is stable when stored at -70°C; in both bDNA versions no significant decrease in reactivity was found. The confidence interval (96 to 105%) did not differ significantly from 100%.

4.1.2. Real time stability data for long term frozen storage at -30°C

In four batches of one ViraQ run control product variant (P0155) containing 250 copies/mL of HBV-DNA the real time stability was monitored in December 2014 by testing in quadruplicate TaqScreen 2.0 assays (table 3a).

In November 2017 a second real time stability experiment was performed with four batches of ViraQ HBV Check Control (which included also two batches of P0065 ViraQ Check containing 125 copies/mL of HBV-DNA). In this experiment the HBV-DNA concentration was measured in duplicate cobas MPX tests (table 3b).

The relative concentrations in table 3a and 3b were calculated by taking 2^(-delta (Ct value sample – average Ct value all measurements)). All samples were tested at Sanquin under supervision of Dr Marco Koppelman.

Table 3a Stability data of P0155 ViraQ HBV Check 250 control tested in quadruplicate TaqScreen 2.0 assays in 2014

Article nr.	Batch-ID	Date prepared	Ct value	Years	Relative concentration#	Temperature
P0155	B4155-001	10/01/2010	31.4	4.18	105%	-40°C
			31.2		121%	
			31.4		105%	

			31.7		86%	
	B4155-004	7/31/2012	31.8	2.38	80%	-30°C
			31.5		98%	
			31.5		98%	
			31.2		121%	
	B4155-005	4/02/2013	32.3	1.71	56%	
			31.2		121%	
			32.1		65%	
			31.3		113%	
	B4155-010	7/10/2014	31.3	0.44	113%	
			31.9		74%	
			31.1		130%	
			31.6		92%	
			33.6		89%	

as compared to average Ct value of experiment as 100%

Table 3b. Stability data of P0155/P0065 ViraQ HBV Check 250/125 control tested in duplicatre cobas MPX assays in 2017

Article nr.	Batch-ID	Date prepared	Ct value	Years	Relative concentration#	Temperature
P0155	B4155-004	7/31/2012	32.31	5.30	111%	-30°C
			32.05		133%	
	B4155-005	04/02/2013	32.39	3.82	105%	
			32.39		105%	
P0065	B4060-004	13/02/2015	33.34^	2.76	109%	
			33.63^		89%	
	B4060-012	21/09/2017	33.94^	0.15	72%	
			33.62^		89%	

as compared to average Ct value of experiment as 100%. ^Ct values in analyses were corrected for 2-fold lower HBV concentration in P0065

Linear regression on time versus Ct value increase showed that the slope did not significantly differ from zero indicating that stability is demonstrated over the period of the measurements (table 4). Note that for the first time point the storage was at -40°C instead of -30°C which would cause a degradation being a factor 2.61 lower according to the Arrhenius equation.

Table 4. Calculated slope of degradation of HBV-DNA during storage of P0155, P0065 ViraQ run control products at -30 °C

Data in:	Degradation after 2 years by linear regression	95 % confidence interval
table 3a	94.8 %	60.7 - 148.6 %
table 3a#	104.0 %	85.5 – 126.8%
Table 3b	123.6 %	83.2 – 156.0%

including -40°C measurements

4.1.3. Historical stability study of native HBV-DNA standard at -30°C

The consistency of HBV-DNA concentrations was established in three PeliSpy products (1:10.000 dilutions of S0011 HBV-DNA genotype A standard) as measured in triplicate Roche Monitor and Siemens bDNA 3.0 assays in the same test run (Figure 1 and table 5). All samples were stored at -30°C.

Figure 1 Stability of three PeliSpy batches(1:10,000 dilution of S0011 HBV-DNA genotype A standard) measured in Roche HBV Monitor (concentrations at t=0 were measured indifferent test runs)

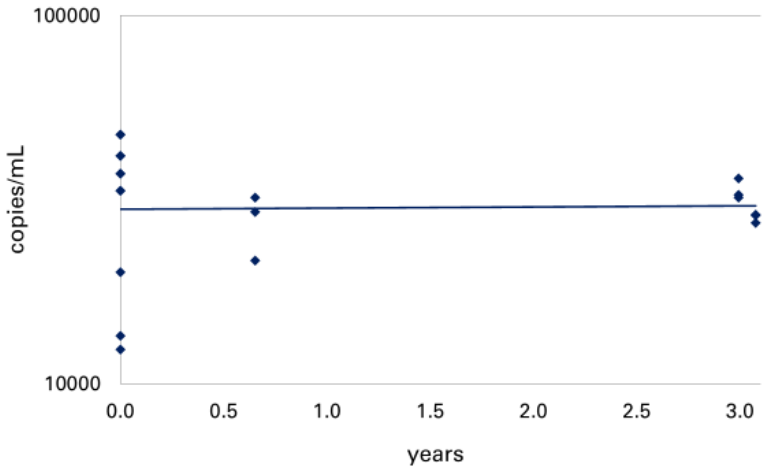


Fig 1

Table 5. Stability of S0011 HBV-DNA genotype A standard dilutions stored at -30°C

Test	Year	geo mean copies/mL	ln average	Ln stdev	n	Upper and lower limit 95 % Conf. Interval	
Roche HBV Monitor	2001	35974	10.59	0.14	8		
	2003	40389	10.21	0.21	3		
	2004	29555	10.31	0.14	9		
	Slope ln(conc) vs year		0.007	0.062	20	-0.123	0.137
Siemens bDNA 3.0 assay	2001	20720#§	9.94	0.12	3		
	2003	21086#	9.96	0.13	3		
	2004	20626§	9.93	0.04	3		
	Slope ln(conc) vs year		-0.001	0.010	9	-0.127	0.124

#p=0.48 §p=0.40, On average 100.7 (88-115)% is recovered after one year storage at -30°C

4.1.4. In use stability of ViraQ HBV run controls

The predicted stability after thawing at +4°C, +20°C and + 37°C was established with P0238 mixed standard panel, containing the inactivated and native S0043 and S0011 HBV-DNA standards in a concentration of 2000 copies/mL. Table 6a and b show the Ct values on the respective standard dilutions in this accelerated degradation experiment

Table 6a: Accelerated HBV-DNA degradation study on sample 1 of P0238 mixed standard panel (S0011 native HBV-DNA standard) tested in Roche TaqScreen 2.0 assay

Test date	Temperature	Time (hours)	Ct values measured				Average
	S0011 HBV-DNA native						
4/25/2013	-70°C	start	27.6				27.6
5/1/2013	-70°C	8	28.3	27.9	27.8	27.9	28.3
5/1/2013	2-8°C	8	28.4	28.1	28.0	27.6	28.4
5/1/2013	+20°C	8	27.9	27.9	27.5	27.8	27.9
5/1/2013	+37°C	8	28.1	27.7	27.8	27.9	28.1
5/2/2013	-70°C	24	27.9	28.0	28.3	28.0	27.9
5/2/2013	2-8°C	24	28.2	27.9	28.2	27.7	28.2
5/2/2013	+20°C	24	28.0	27.7	28.1	27.7	28.0
5/2/2013	+37°C	24	27.8	28.1	27.9	28.0	27.8
5/3/2013	-70°C	48	28.2	28.1	28.1	28.3	28.2
5/3/2013	2-8°C	48	27.9	28.1	27.8	28.2	27.9
5/3/2013	+20°C	48	28.2	27.8	27.9	28.2	28.2
5/3/2013	+37°C	48	28.2	28.4	28.1	28.4	28.2

Table 6b: Accelerated HBV-DNA degradation study on sample 2 of P0238 mixed standard panel (S0043 inactivated HBV-DNA standard) tested in Roche TaqScreen 2.0 assay

Test date	Temperature	Time (hours)	Ct values measured				Average
	S0043 HBV-DNA inactivated						
4/25/2013	-70°C	start	28.6				28.6
5/1/2013	-70°C	8	28.2	28.3	28.2	28.1	28.2
5/1/2013	2-8°C	8	28.2	28.5	28.0	28.0	28.2
5/1/2013	+20°C	8	28.0	27.9	28.0	28.0	28.0
5/1/2013	+37°C	8	27.8	27.9	28.0	28.0	27.8
5/2/2013	-70°C	24	28.2	28.4	28.4	27.9	28.2
5/2/2013	2-8°C	24	28.3	28.0	28.1	27.9	28.3
5/2/2013	+20°C	24	27.8	27.9	27.8	28.1	27.8
5/2/2013	+37°C	24	27.7	28.2	27.7	27.5	27.7
5/3/2013	-70°C	48	28.4	28.3	28.5	28.7	28.4
5/3/2013	2-8°C	48	28.2	28.5	28.2	28.1	28.2
5/3/2013	+20°C	48	28.2	28.5	28.0	28.3	28.2
5/3/2013	+37°C	48	28.4	28.2	27.7	28.1	28.4

The slope for Ct value vs time was calculated for the three temperatures to establish if degradation occurs, and if so whether the slope (delta Ct value per hour) differs significantly from zero (Table 7).

Table 7: Calculated in use stability of native and inactivated HBV-DNA standards

			Recovery (95 % C.I.) as compared to t=0	
HBV standard	Temp	Ct/hour (95 % C.I.)	After 4 hours	After 8 hours
S0011 Native	4°C	-0.004 (-0.009-0.001)	101%(100%-103%)	102% (99%-105%)
	20°C	-0.002 (-0.006-0.003)	101% (99%-102%)	101% (99%-104%)
	37°C	0.003 (-0.002-0.007)	99% (98%-100%)	99% (96%-101%)
S0043 Inactivated	4°C	-0.005 (-0.011-0.001)	101% (100%-103%)	103% (99%-106%)
	20°C	-0.004 (-0.011-0.004)	101% (99%-103%)	102% (98%-106%)
	37°C	-0.007 (-0.014-0.001)	102% (100%-104%)	104% (100%-108%)
Table 7 continued			Recovery (95 % C.I.) as compared to t=0	
HBV standard	Temp	Ct/hour (95 % C.I.)	After 24 hours	After 72 hours
S0011 Native	4°C	-0.004 (-0.009-0.001)	107% (98%-116%)	122% (95%-157%)
	20°C	-0.002 (-0.006-0.003)	103% (95%-110%)	110% (86%-135%)
	37°C	0.003 (-0.002-0.007)	95% (89%-103%)	86% (71%-110%)
S0043 Inactivated	4°C	-0.005 (-0.011-0.001)	109% (98%-120%)	128% (95%-173%)
	20°C	-0.004 (-0.011-0.004)	107% (94%-120%)	122% (82%-173%)
	37°C	-0.007 (-0.014-0.001)	112% (98%-126%)	142% (95%-201%)

Table 7 and Figure 2 show that both native and inactivated HBV-DNA standards are stable in liquid format from 4 to 37°C. Extrapolation to 72 hours indicates no loss of reactivity.

Figure 2. In use stability of inactivated HBV-DNA standard (4°C blue lines; room temperature orange lines and 37 °C red lines; average recovery thick lines; 95% confidence limits thin lines; individual measurements diamonds).

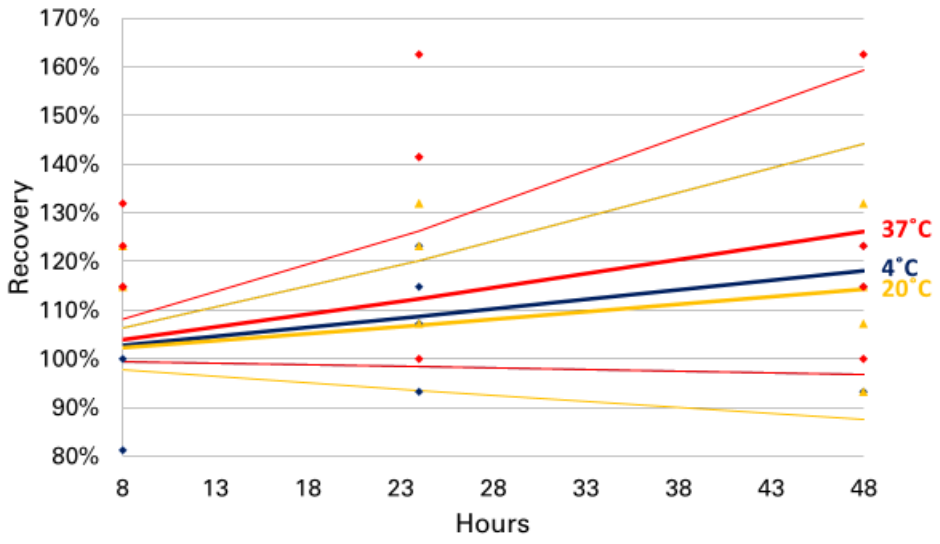


Fig 2

4.1.5. Impact of pasteurization and lyophilization on HBV particle integrity

The recovery after pasteurization of the VQC-Sanquin HBV-DNA standard was established at 84% (CE4006). Chudy et al[2] reported similar HBsAg recoveries after lyophilisation of the WHO HBsAg genotype panel. Hence the stability for these processes seems higher for HBV than for HIV and HCV particles. Harvey Holmes (NIBSC, personal communication) showed that addition of RNAse inhibitor during reconstitution restored the HIV-RNA concentration of the WHO standard which indicated that free HIV-RNA is degraded by RNAse during the reconstitution process. Since HBV-DNA is more stable one could theoretically measure free HBV-DNA together with particle bound HBV-DNA in lyophilised and pasteurized HBV-DNA standards. Therefore Prof W.H. Gerlich (University of Giessen, Germany) kindly performed a sucrose density gradient study of a number of HBV-DNA standards (Figure 3). The native VQC-Sanquin and pasteurized BQC standard banded at the same density as the lyophilised WHO and ISS standards. Treatment of the HBV standard with DNAse did not reduce the HBV-DNA concentration in real time PCR assay (X-region) kindly performed by Prof Gerlich and Dr Bremer (figure 4).

Figure 3. Density of native, pasteurized and lyophilised HBV-DNA standards (study kindly performed by Prof. Wolfram Gerlich, University of Giessen, Germany)

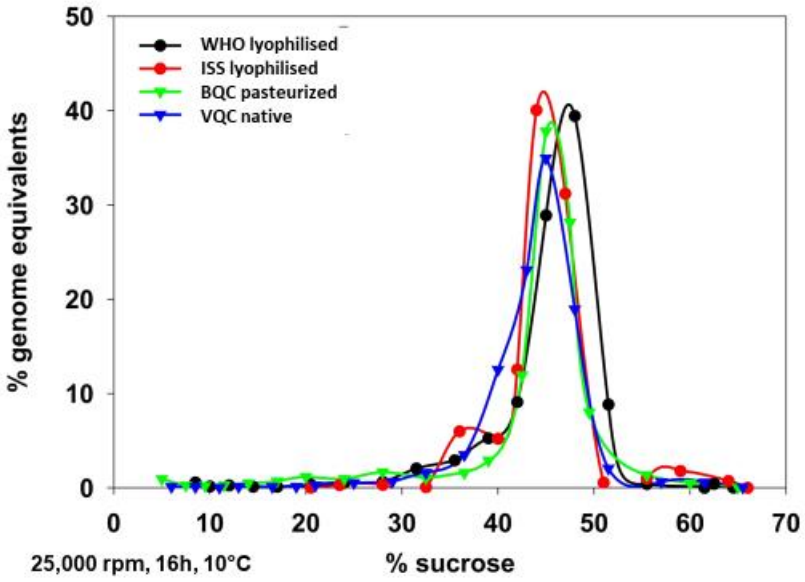


Fig 3

Figure 4. Digestion of free cloned or virus encapsidated HBV-DNA in standards (200 µl serum, 500 Units DNAse (Benzonase), 1 h 37°C, real-time PCR of X-region (study kindly performed by Prof. Wolfram Gerlich, University of Giessen, Germany).

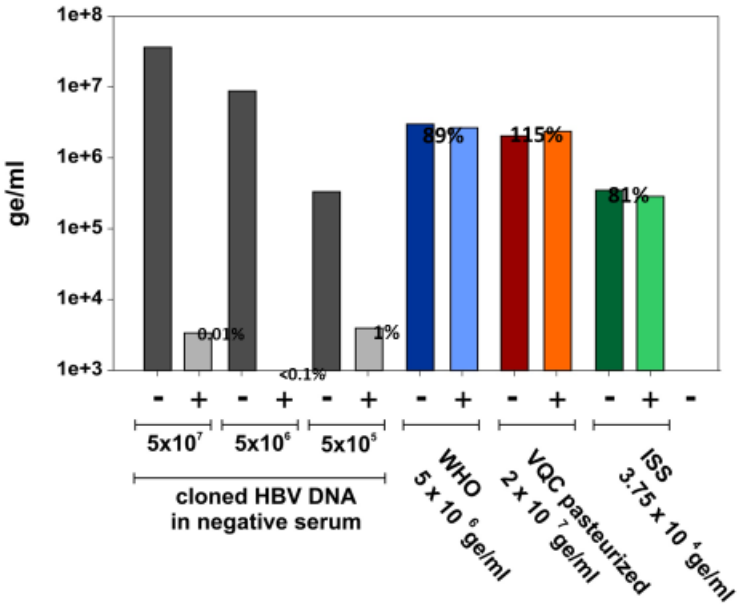


Fig 4

4.1.6. Summary and conclusion of HBV stability studies

The native S0011 HBV-DNA standard was stable during 12 years storage at -70°C, while also no degradation was established during three years storage at -30°C. The real time stability data did not show degradation of four P0155 and two P0065 ViraQ HBV Check HBV run control batches stored frozen at -30°C for up to 5.3 years. On the basis of these data we claim 5 year stability of HBV-DNA in ViraQ HBV run control products (P0065, P0069, P0154, P0155, when stored at -30°C or lower.

The in use stability data on dilutions of native S0011 and inactivated S0043 HBV-DNA standards is sufficient for the claimed 8 hours usage of ViraQ run control products after thawing and storage at +4 °C or room temperature, since during 48 hours of storage at +4 °C, +20 °C and +37 °C no degradation was observed. Finally it was demonstrated by Prof W. Gerlich and collegues that there was no free HBV-DNA in the heat inactivated S0043 HBV-DNA standard and in the lyophilised WHO standard.

Conclusion: native and heat inactivated HBV-DNA standard dilutions are stable for at least 5 years when stored at -30°C or lower and can be stored after thawing at the laboratory for 8 hours at +4 °C or at room temperature until actual testing takes place on the laboratory test platform.

4.2. HCV-RNA

4.2.1. Stability of S0009 HCV-RNA genotype 1 standard at -70˚C

S0009 HCV genotype 1 standard stored at -70°C is used as a primary reference standard for other HCV preparations stored at higher temperature. Table 8 shows the quantitative bDNA test results on S0009 HCV standard dilutions falling well in the dynamic range of the assay, which were collected in the period from 1996 to 2008. The slope ln [conc]/year obtained by linear regression analysis and the 95% confidence limits were used to judge stability. When the slope equals zero and the confidence limits of the potency of the stored preparation overlaps 100% stability is claimed.

Table 8. Stability of S0009 HCV-RNA genotype 1 primary reference standard

Test	Year	average	ln average	Ln stdev	n	95 % conf.interval	
bDNA 1.0	1996	8.76E+06	1.60E+01		4		
bDNA 2.0	1997	1.17E+07	1.63E+01	0.276	3		
	1998	6.31E+06	1.57E+01	0.034	4		
	1999	7.01E+06	1.58E+01		1		
	2000	9.59E+06	1.61E+01	0.317	5		
	2000	1.06E+07	1.62E+01	0.195	30		
	Slope	100.3%	0.003	0.115	43	-0.362	0.368
bDNA 3.0	2001	6.21E+06	1.56E+01	0.183	8		
	2003	6.43E+06	1.57E+01	0.115	6		
	2003	6.77E+06	1.57E+01	0.127	3		
	2004	8.45E+06	1.59E+01	0.195	4		
	2008	5.00E+06	1.54E+01	0.096	6		
	Slope	96.6%	-0.035	0.035	27	-0.147	0.077
all	slope	98.2%	-0.018	0.018	70	-0.057	0.022

In the observation period 98.2 (94-102)% was recovered after one year. Hence there was no significant degradation observed with storage at -70˚C.

Another manner to examine stability of the S0009 HCV genotype 1 standard is to compare analytical sensitivity studies on dilution panels over time. Table 8 gives an overview of the reported 95% and 50% LODs in Gen-Probe/Hologic Ultrio versions on S0009 HCV genotype 1 standard dilution panels.

Table 8. Stability of S0009 HCV-RNA genotype 1 standard dilution panels as demonstrated by consistent LODs in validation studies of the Ultrio versions performed over time.

Study	year	assay	95 % LOD (C.I.)	50 % LOD (C.I.)
Lelie et al[4]	2000	Duplex	25 (19-35)	2.3 (1.8-2.9)
Koppelman et al[5]	2004	Ultrio	25 (14-72)	2.9 (1.9-4.4)
Grabarczyk et al[6]	2010	Ultrio	25 (17-39)	2.9 (2.1-3.9)
Grabarczyk et al[6]	2010	Ultrio Plus	15 (11-23)	1.7 (1.3-2.3)
Grabarczyk et al[6]	2013	Ultrio Elite	13 (8-21)	1.5 (1.0-2.2)

Over a 13 years observation period the 50 % LOD did not change significantly since 95% confidence intervals overlap. This further confirms consistency and stability of the standard dilution panels when stored at -70 °C.

4.2.2. Real time stability of ViraQ HCV Check Controls stored at -30°C

In eight batches of P0063 ViraQ HCV Check Control (125 copies/mL) real time stability has been monitored in two experiments (Table 9a and 9b). Available samples were tested on 16-12-2014 (table 9a in TaqScreen MPX 2.0 (table 9a) and on 16-11-2017 in the cobas MPX assay (table 9b). Real time PCR tests of both experiments were performed at Sanquin (supervisor Dr Marco Koppelman).

Table 9a: Real time stability of four batches of P0063 ViraQ HCV Check 125 control stored at -30°C as demonstrated by quadruplicate TaqScreen 2.0 tests in 2014

Batch-ID	Date prepared	Ct value	Years	Relative concentration#	Temperature
B4058-001	19-9-2008	36.3	3.6	130%	-40°C
		36.6		105%	
		36.6		105%	
		37.3		65%	
B4058-003	1-2-2012	36.3	2.3	130%	-30°C
		36.1		149%	
		37.7		49%	
		36.8		92%	
B4058-005	12-2-2012	36.9	2.0	86%	
		37.2		69%	
		37.4		60%	
		36.9		86%	
B4058-013	12-12-2014	36.6	0.0	105%	
		36.4		121%	
		36.7		98%	
		37.0		80%	

as compared to average Ct value of experiment as 100%

Figure 5a. Degradation of HCV-RNA at -30°C determined linear regression on log (relative concentration) versus time (years) as calculated from Ct values in TaqScreen 2.0 assay (data presented in table 9a).

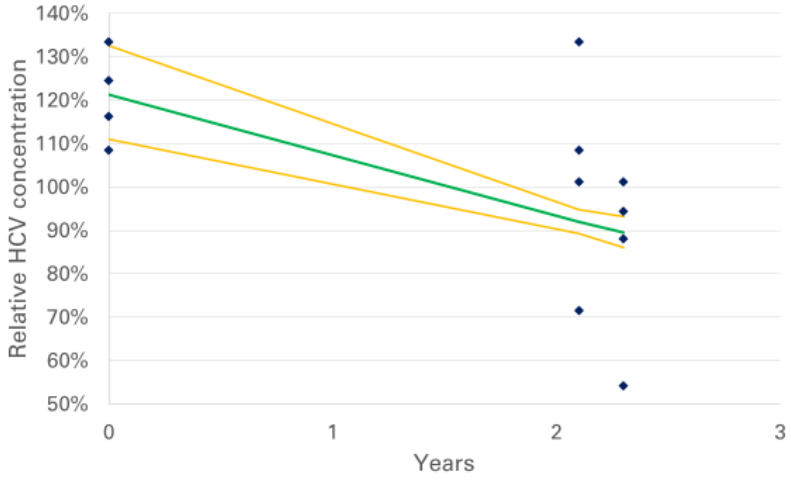


Fig 5a

Table 9b. Real time stability of eight P0063 ViraQ HCV Check 125 batches stored at -30°C as demonstrated by quadruplicate or duplicate cobas MPX assays in 2017)

Batch-ID	Date prepared	Ct value	Years	Relative concentration#	Temperature
B4058-005	12-2-2012	37.69		69%	-30°C
		37.27		92%	
		37.13		101%	
		37.44	5.30	82%	
B4058-006	14-3-2013	37.72		67%	
		37.16	4.68	99%	
B4058-009	23-1-2014	38.83		Outlier	
		36.94	3.82	116%	
B4058-016	30-3-2015	36.71		136%	
		36.69	2.64	137%	
B4058-018	20-8-2015	36.89		120%	
		36.68	2.25	138%	
B4058-019	24-6-2016	36.51		156%	
		37.60	1.40	73%	
B4058-021	13-12-2016	36.70		137%	
		37.81	0.93	63%	
B4058-022	22-5-2017	37.04		108%	
		37.56	0.49	75%	

as compared to average Ct value of experiment as 100%

Figure 5b. Degradation of HCV-RNA at -30°C determined by linear regression on log (relative concentration) versus time (years) as calculated from Ct values in cobas MPX assay (data presented in table 9b)

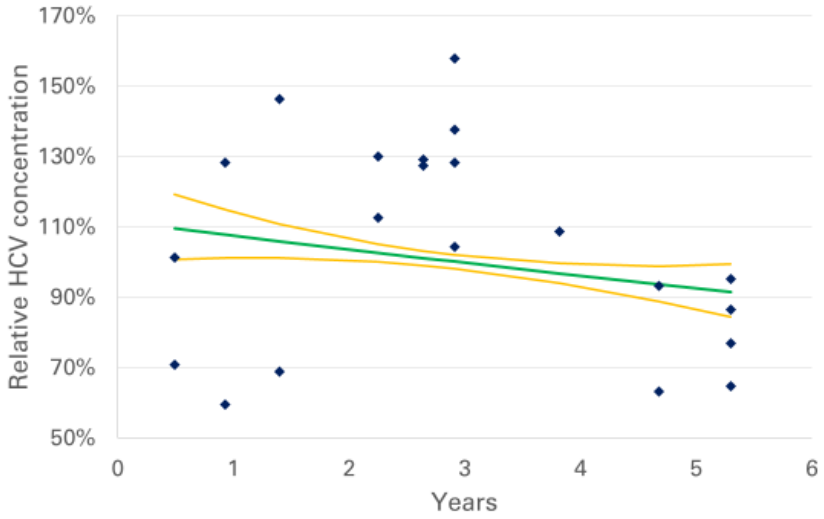


Fig 5b

Linear regression on relative concentration (or on Ct value) versus storage time at -30˚C showed that the slope in the first experiment (data in table 9a) performed in 2014 (almost) differed significantly from zero. In the second experiment (data table 9b) the decay (95% CI) after two years was estimated at 7.2% (-10.2%, 21.8%) and did not differ significantly differ from zero.

Table 10: Calculated slope of degradation of HCV-RNA during storage of P0063 ViraQ HCV Check 125 Control based on Ct values

Data in:	Degradation after 2 years by linear regression	95 % confidence interval
table 9a	76.8%	57.8-102.3 %
table 9a#	91.2%	88.9-93.5%
table 9b	92.8%	78.2-110.2%

including -40°C measurements

4.2.3. Long term stability of inactivated S0109 HCV standard stored at -30°C and -70°C

The inactivated S0109 HCV genotype 3 standard was diluted to 2000 copies/mL in sample 3 of the P0238 mixed standard panel (table 1). The reconstituted WHO HCV 06/100 standard was diluted to 1000 IU/mL in sample 4 of this panel. After 4.3 years of storage of the P0238 panel at -70˚C and -30˚C samples 3 and 4 of the panel were tested in 12 replicates in the cobas MPX assay. The individual Ct values found on the standards stored at -30 and -70 ˚C are shown in table 11.

Table 11. Measured Ct values in 12 cobas MPX replicate tests on S0109 and WHO 06/100 HCV-RNA standard dilutions in P0238 mixed standard panel stored at -30˚C and -70˚C for 4.3 years

S0109		WHO 06/100	
-30°C	-70°C	-30°C	-70°C
33.00	32.35	32.66	32.64
32.98	32.53	33.55	32.51
33.00	32.29	33.21	32.44
33.07	32.20	32.74	32.60
33.14	32.27	33.12	32.45
33.09	32.28	32.95	32.39
32.88	32.49	33.03	32.62
33.01	32.59	32.87	32.17
33.01	32.43	32.71	32.21
33.17	32.29	32.63	32.35
32.80	32.55	32.84	32.32
32.97	32.34	32.62	32.35

Table 12 shows the difference in Ct values on the standard dilutions stored at -30˚C and -70˚C with the 95% CI estimated by paired t-test. It was estimated that the loss of HCV-RNA in the inactivated S0109 and WHO 06/100 standards after 2 years was 18(15-22)% and 15(9-19)% respectively.

Table 12 Estimation of degradation of HCV-RNA after two years storage of HCV standards at -30˚C (from data presented in table 11)

HCV standard	delta Ct (95%CI) after 4.3 years	delta Ct (95% CI) after 2 years	recovery after 2 years
S0109 inactivated	0.63 (0.49-0.76)	0.29 (0.23-0.35)	82 (78-85%)
WHO 06/100	0.49 (0.31-0.67)	0.23 (0.14-0.31)	85 (81-91%)

4.2.4. Long term stability of S0009 HCV standard stored at -30 ˚C and -70 ˚C

A 1:231 dilution of S0009 HCV genotype 1 standard was prepared on 10 occasions, in 3 cases stored at -70° and 7 cases at -30°C. After up to 8 years of storage all preparations were tested in 6-fold in 2 runs Roche Amplicor HCV Monitor 2.0. Figure 6 depicts all measurements and the regression lines.

Linear regression between years and ln(copies/mL) generated a slope that was not significantly different from zero (0.007; 95 % C.I. -0.026 to 0.041 for 30°C and 0.014; 95 % C.I. -0.014 to 0.043 for -70°C). When expressed as potency 100.7% (97.4%-104.2) was found for 30°C and 101.4% (98.6%-104.4) for -70°C. The stability of S0009 HCV genotype 1 for both temperatures was not different. At both temperatures the S0009 standard dilutions were stable.

Figure 6. Stability of S0009 HCV-RNA genotype 1 standard at -30°C (red diamonds) and -70°C (blue diamonds), the lines represent averages and 95 % confidence intervals.

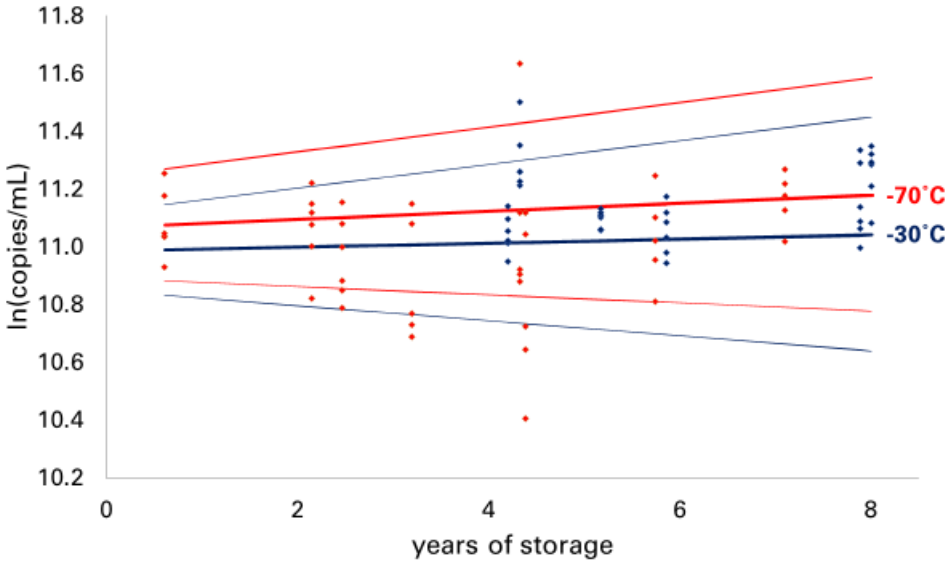


Fig 6

In a similar experiment the bDNA 3.0 assay was used for testing the same dilution of the S0009 HCV genotype 1 standard stored at -30 and -70°C for 4 years..

Table 13 Stability of S0009 HCV genotype 1 standard dilution stored for 4 years at -30 and -70 °C as established in replicate bDNA 3.0 assays

bDNA 3.0	-70°C	-30°C
Number tests	12	12
copies/mL (95% C.I.)	124,451(102,332-151,350)	126,814(114,107-140,936)

This latter stability experiment (table 12) confirms the stability study on S0009 HCV standard dilutions in figure 6. Obviously the native S0009 HCV genotype 1 standard was more stable than the inactivated S0109 HCV genotype 3 standard. The higher stability of the native S0009 HCV genotype 1 standard can also be influenced by the presence of anti-HCV antibodies in this standard. The inactivated P0109 HCV genotype 3 standard was derived from an anti-HCV negative window period donation.

4.2.5. In use stability of ViraQ HCV run controls

The stability of ViraQ run controls has been established in an accelerated degradation study of four HCV standards stored for 8, 24 and 48 hours at +4°C, +20°C and + 37°C (table 14). The native S0009 HCV genotype 1 standard and the native S0148 and inactivated S0109 HCV genotype 3a standards were diluted to 2000 copies/mL in the P0238 mixed standard panel. In addition the reconstituted WHO HCV 06/100 standard has been diluted to 1000 IU/mL and was tested in parallel in the P0238 mixed standard

panel in the Roche TaqScreen 2.0 assay. The objective of this study was to determine the utility time after thawing and to ensure whether the ViraQ run controls are sufficiently stable when placed in the NAT testing robots.

Table 14. TaqScreen 2.0 test results for HCV-RNA on sample 1, 2, 3 and 4 in 2000 copies/mL P0238 mixed standard panel containing dilutions of S0009, S0148, S0109 and WHO 06/100 HCV standards respectively.

Test date	Temperature	Time (hours)	Ct values measured				Average
	S0009 HCV-RNA genotype 1						
4/25/2013	-70°C	Start	30.9				32.1
5/1/2013	-70°C	8	31.0	30.7	30.6	31.2	30.9
5/1/2013	2-8°C	8	30.9	30.8	30.9	30.9	30.9
5/1/2013	+20°C	8	31.1	31.2	31.0	30.7	31.0
5/1/2013	+37°C	8	31.7	31.4	31.3	31.5	31.5
5/2/2013	-70°C	24	31.2	31.0	30.9	30.5	30.9
5/2/2013	2-8°C	24	31.0	30.8	31.0	30.7	30.9
5/2/2013	+20°C	24	31.0	31.1	30.9	30.7	30.9
5/2/2013	+37°C	24	31.7	32.3	31.9	32.3	32.1
5/3/2013	-70°C	48	30.8	30.9	30.6	31.4	30.9
5/3/2013	2-8°C	48	31.1	31.2	31.4	31.2	31.2
5/3/2013	+20°C	48	31.2	31.2	31.1	31.0	31.1
5/3/2013	+37°C	48	32.2	32.4	32.4	32.5	32.4
	S0148 HCV-RNA genotype 3a		Ct values measured				Average
4/25/2013	-70°C	Start	31.7				
5/1/2013	-70°C	8	31.0	31.6	31.0	31.3	31.2
5/1/2013	2-8°C	8	31.1	32.3	31.5	31.7	31.7
5/1/2013	+20°C	8	31.4	31.4	31.9	31.4	31.5
5/1/2013	+37°C	8	32.6	32.3	32.3	32.1	32.3
5/2/2013	-70°C	24	31.5	31.2	31.3	30.8	31.2
5/2/2013	2-8°C	24	31.3	31.9	31.5	31.5	31.6
5/2/2013	+20°C	24	31.4	31.5	31.4	31.9	31.6
5/2/2013	+37°C	24	31.5	32.2	32.0	32.0	31.9
5/3/2013	-70°C	48	31.0	31.1	30.9	31.1	31.0
5/3/2013	2-8°C	48	31.1	31.9	31.3	31.7	31.5
5/3/2013	+20°C	48	31.3	31.5	31.5	31.5	31.5
5/3/2013	+37°C	48	31.8	32.1	32.6	32.0	32.1
	S0109 HCV-RNA genotype. 3a inactivated						
4/25/2013	-70°C	start	32.2				
5/1/2013	-70°C	8	31.8	31.5	32.7	32.0	32.0
5/1/2013	2-8°C	8	32.3	32.0	32.4	32.1	32.2
5/1/2013	+20°C	8	32.4	31.9	32.6	32.6	32.4
5/1/2013	+37°C	8	33.3	33.4	33.2	33.7	33.4
5/2/2013	-70°C	24	31.9	31.9	31.9	31.6	31.8
5/2/2013	2-8°C	24	32.5	32.0	32.2	32.3	32.3
5/2/2013	+20°C	24	32.5	32.7	32.4	32.9	32.6
5/2/2013	+37°C	24	33.1	33.2	33.9	33.1	33.3
5/3/2013	-70°C	48	31.5	31.8	31.9	31.8	31.8
5/3/2013	2-8°C	48	32.5	32.5	32.3	32.1	32.4
5/3/2013	+20°C	48	32.2	32.0	32.1	32.2	32.1
5/3/2013	+37°C	48	33.5	33.0	33.4	33.0	33.2

Table 14 continued

Test date	Temperature	Time (hours)	Ct values measured				Average
	WHO HCV 06/100		Ct values measured				Average
4/25/2013	-70°C	Start	33.5				
5/1/2013	-70°C	8	33.3	33.5	33.8	33.5	33.5
5/1/2013	2-8°C	8	33.7	33.8	33.8	33.5	33.7
5/1/2013	+20°C	8	33.6	33.0	33.0	33.2	33.2
5/1/2013	+37°C	8	33.5	33.7	33.6	33.2	33.5
5/2/2013	-70°C	24	33.7	33.3	33.5	33.4	33.5
5/2/2013	2-8°C	24	33.6	33.9	33.8	33.8	33.8
5/2/2013	+20°C	24	33.5	33.9	34.4	32.9	33.7
5/2/2013	+37°C	24	33.5	33.8	33.5	33.9	33.7
5/3/2013	-70°C	48	33.2	32.6	33.7	32.7	33.1
5/3/2013	2-8°C	48	34.0	33.4	33.5	34.1	33.8
5/3/2013	+20°C	48	34.4	33.9	33.4	34.0	33.9
5/3/2013	+37°C	48	33.7	33.8	33.6	33.9	33.8

The slope for Ct value vs time was calculated for the three temperatures to establish if degradation is present, and if so the slope (delta Ct value per hour) differs significantly from zero (figure 7 and 8, table 15).

Table 15. Calculated degradation of HCV-RNA in 4 standards in the liquid phase at different storage temperatures

Marker	Temp	Ct/hour (95 % C.I.)	Recovery (95 % C.I.)	
			After 4 hours	After 8 hours
Genotype 1	4°C	0.006 (-0.001-0.014)	98% (96%-100%)	97% (93%-101%)
	20°C	0.003 (-0.006-0.012)	99% (97%-102%)	98% (94%-103%)
	37°C	0.028 (0.018-0.039)§	92% (90%-95%)	85% (81%-91%)
Genotype 3a native	4°C	0.007 (-0.002-0.016)	98% (96%-100%)	96% (91%-101%)
	20°C	0.007 (-0.003-0.017)	98% (95%-101%)	96% (91%-102%)
	37°C	0.015 (0.000-0.031)§	96% (92%-100%)	92% (84%-100%)
Genotype 3a inactivated	4°C	0.012 (0.004-0.020)§	97% (95%-99%)	94% (90%-98%)
	20°C	0.007 (-0.004-0.018)	98% (95%-101%)	96% (91%-102%)
	37°C	0.023 (0.004-0.041)§	94% (89%-99%)	88% (80%-98%)
3rd WHO int. stand.	4°C	0.014 (0.004-0.024)§	96% (94%-99%)	93% (87%-98%)
	20°C	0.022 (0.005-0.038)§	94% (90%-99%)	89% (81%-97%)
	37°C	0.015 (0.005-0.026)§	96% (93%-99%)	92% (87%-97%)

§ $p<0.05$

After 8 hours storage of the S0109 inactivated HCV genotype 3a standard at 4 °C a significant degradation of 6% (2-10%) was estimated, but the degradation was not significantly different from 0% when the standard was stored at room temperature. There was no significant degradation observed at these temperatures for the native S0009 HCV genotype 1 and S0148 genotype 3a standards. By contrast a significant degradation of 7 (2-13)% and 11 (3-19%) was observed in the reconstituted WHO 06/100 standard after 8 hours storage at 4 and 20 °C respectively.

Figure 7. In use stability of S0109 inactivated HCV standard established in accelerated degradation study (4°C blue lines; room temperature orange lines; 37°C red lines; average recovery thick lines ;95% confidence limits thin lines; individual measurements diamonds)

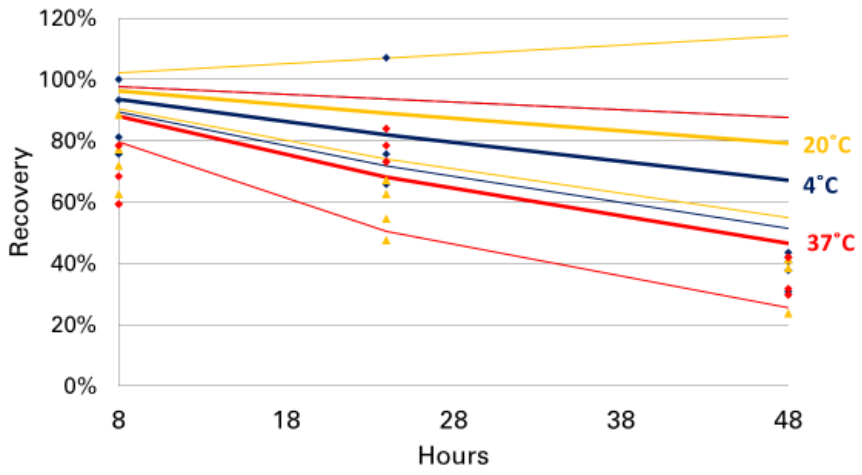


Fig 7

Figure 8 In use stability of WHO HCV-RNA 06/100 standard established in accelerated degradation study. (4°C blue lines; room temperature orange lines; 37°C red lines; average recovery thick lines ;95% confidence limits thin lines; individual measurements diamonds)

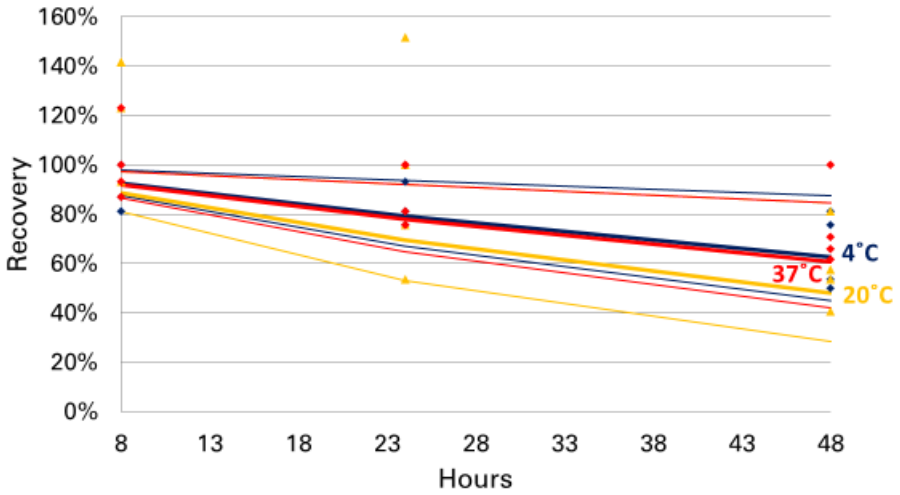


Fig 8

4.2.6. Historical in use stability study on S0009 HCV genotype 1 standard dilutions

A standard dilution of the native S0009 HCV-RNA genotype 1 plasma pool diluted to 27,000 copies/mL was tested in bDNA 3.0 assay during storage for, 12, 24, 48, 72, 96 and 120 hours at 4 and 20°C. (table 16, table 17, figure 9).

Table 16. In use stability of a S0009 HCV-RNA genotype 1 standard dilution of 27,000 copies/mL at 4 and 20°C

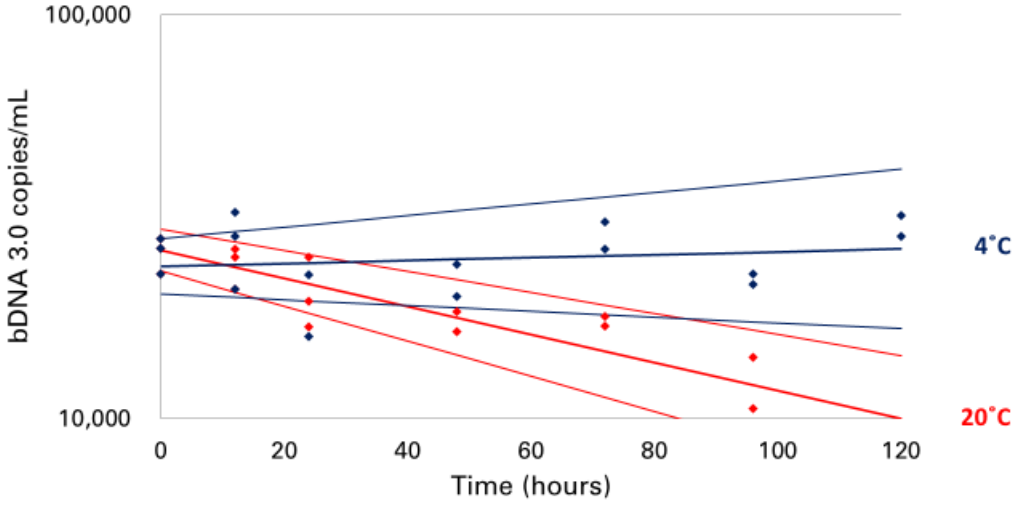
	Values found in bDNA 3.0 on 27.000 copies/mL samples	
time (hours)	4°C	room temp.
0	27,900	27,900
0	26,395	26,395
0	22,814	22,814
12	20,866	20,866
12	32,394	25,043
12	28,243	26,311
24	22,731	25,099
24	15,970	19,485
24		16,854
48	20,024	16,412
48	24,064	18,361
72	30,655	17,883
72	26,226	16,908
96	22,842	14,178
96	21,521	10,579
120	28,271	7,286
120	31,871	9,858

Table 17. Linear regression on ln(conc) vs time on bDNA 3.0 data in table 16

Temp.	Ln(conc)/hour (95 % C.I.)	Recovery (95 % C.I.)	
		After 8 hours	After 120 hours
4°C	0.001 (-0.002-0.003)	101% (99%-103%)	110% (82%-149%)
20°C	-0.008 (-0.010- -0.006)	94% (92%-95%)	38% (30%-49%)

The S0009 HCV genotype 1 standard was stable during 5 days storage at 4 °C, but a significant degradation of 6 (5-8%) of HCV-RNA was observed after 8 hours storage at room temperature (figure 9).

Figure 9. Degradation of HCV-RNA in S0009 HCV genotype 1 standard during storage at 4 and 20 °C determined by triplicate bDNA 3.0 measurements (blue line 4 °C, red line room temperature; think line average trend; thin lines 95% confidence limits, diamonds actual measurements).



4.2.7. Potency of WHO HCV standards according to analytical sensitivity studies in Procleix assays

To examine the potency of WHO HCV replacement standards, a large amount of analytical sensitivity data obtained in validation studies performed by users of Procleix Ultrio assay versions were subjected to parallel line probit analysis. Table 18 shows the 50% and 95% LODs and the potency of the WHO HCV replacement standards as compared to the first established WHO 96/790 HCV standard as the reference preparation.

Table 18. Potency of WHO replacement standards according to analytical sensitivity studies in Procleix Ultrio versions

HCV-RNA standard	N	50% LOD (CI) copies/mL#	95% LOD (CI) copies/mL#	Potency (CI)
1st WHO HCV IS (96/790)	48	2.3 (1.6-3.3)	16.7 (11.6-24.4)	reference
2nd WHO HCV IS (96/798)	593	2.4 (2.1-2.6)	17.1 (15.1-20.0)	1.04 (0.71-1.50)
3rd WHO HCV IS (06/100)	750	3.1 (2.7-3.4)	22.3 (19.0-32.6)	0.78 (0.67-0.89)

1 IU = 2.73 copies (VR4060)

The potency of the third WHO 06/100 standard was significantly lower than that of the first and second international standards, suggesting a lower in use stability or loss of potency during lyophilized storage.

Five WHO 06/100 standard dilution series (three batches of BQC, one batch of ISS and one batch of Hong Kong Red Cross(HKRC)) were tested by in total 16 laboratories. After preparation the dilution

series were stored at -70°C. Table 19 compares the potencies of different WHO standard dilution batches in the Ultrio versions.

Table 19. Potencies found in Ultrio tests between five different WHO 06/100 IS dilution series

Standard dilution series		potency (95 % C.I.)
BQC 1	BioQControl 2	0.80 (0.58-1.08)
	BioQControl 3	0.69 (0.50-0.94)
	ISS 4	0.87 (0.57-1.32)
	HKRC 5	0.60 (0.40-0.91)
BQC 2	BioQControl 3	0.87 (0.72-1.03)
	ISS 4	1.09 (0.79-1.52)
	HKRC 5	0.76 (0.55-1.04)
BQC 3	ISS 4	1.26 (0.90-1.76)
	HKRC 5	0.87 (0.63-1.20)
ISS 4	BioQControl 3	0.79 (0.56-1.10)
	HKRC 5	0.69 (0.45-1.05)
HKRC 5	BioQControl 3	1.14 (0.82-1.57)
	ISS 4	1.44 (0.94-2.21)

The first batch of the WHO standard dilutions prepared by BioQControl had the highest potency and the third the lowest. The third BioQControl batch was prepared from a second reconstitution of lyophilized ampoules transported at room temperature by NIBSC. Both the third BioQControl and the HKRC batch of standard dilutions had a significantly lower potency than the first BioQControl batch tested several years earlier.

4.2.8. Impact of inactivation and lyophilization on recovery and stability of HCV standards

Unfortunately the HCV-RNA recovery after 2 hours pasteurization of a 1:10 dilution of the S0009 HCV-RNA standard in PBS was approximately 5% (data not shown). It therefore was decided not to choose heat treatment for inactivation of BQC HCV standards. The S0009 VQC-Sanquin HCV-RNA standard was antibody positive (like the 1st and 2nd WHO HCV 96/790 and 96/798 standards) which may have improved the stability. The S0009 HCV-RNA standard is no longer available in large amount (since after acquisition of VQC by Acrometrix it has been used up as raw material). We therefore decided to establish a new BioQ HCV standard starting from a high titer window period plasma that has been inactivated by beta-propiolactone (CE4006). The HCV-RNA recovery after this chemical inactivation step was 94% (CE4006). The stability of this chemically inactivated BioQ HCV-RNA genotype 3a standard was compared with the same plasma before inactivation and for reference also the S0009 VQC-Sanquin HCV was included in the mixed standard panel (see above). Since the 3rd WHO HCV IS 06/100 standard is known to be not as stable as the previous 1st and 2nd WHO HCV 96/790 and 96/798 standards we also incorporated this material for reference in the accelerated stability studies.

No lyophilisation experiments have been performed with the S0009 VQC-Sanquin HCV genotype 1 standard. However in the WHO collaborative study of Baylis et al[3] the HCV-RNA concentration of the lyophilized IS 06/100 and 06/102 standard was compared to the plasma bulk before lyophilization and to the IS 96/798 as the reference preparation (Table 20). Interestingly the HCV-RNA recovery was higher in the second freeze-dry run of the 06/102 standard than the first freeze-dry run of the 06/100 standard (45% versus 67% when taking the quantitative results of three methods together and 31% versus 51% based

on all qualitative and quantitative assays performed in the collaborative study). The core antigen recovery of the 06/100 standard after lyophilization was also 40% which indicates that the capsids are also destroyed by the freeze drying process. Obviously the lyophilisation process of WHO standards is not consistent.

Table 20. HCV-RNA recovery after lyophilisation of Polish HCV genotype 1 window period plasma to establish the WHO IS 06/100 and 06/102 standards

quantitative assays	n labs	IS 96/798	IS 06/100	IS 06/102	unlyophilised
		IU/mL	IU/mL	IU/mL	IU/mL
Abbott RT	7	1,00E+05	1,82E+05	2,88E+05	4,57E+05
Siemens bDNA	4	1,00E+05	1,58E+05	2,19E+05	2,95E+05
Roche CTM	6	1,00E+05	1,51E+05	2,34E+05	3,63E+05
Mean three methods	17	1,00E+05	1,63E+05	2,45E+05	3,66E+05
recovery lyophilisation			45%#	67%	100%
Overall mean six methods report	25	1,00E+05	1,55E+05	2,57E+05	5,01E+05
recovery lyophilisation			31%	51%	100%

#40% in Abbott HCV core antigen test

4.2.9. Summary and conclusion on stability of HCV standards

Two real time stability experiments with a number of P0063 ViraQ Check HCV 125 run control batches that were stored for more than five years at -30°C predicted a degradation of 7 and 22% after two years, respectively. The estimated degradation in these two experiments appeared not to be significantly different from 0% but in another long term storage experiment a significant degradation was established. When 2000 copies/mL samples of the inactivated S0109 HCV standard were kept at -30 and -70°C for 4.3 years and compared by multiple replicate real time PCR tests in the cobas MPX assay a significant degradation of 18 (15-22)% was estimated after two years storage at -30°C. Since the degradation is less than the acceptance criterion of 20% we claim two year stability for P0063 Check 125, P0067 Trend 25, P0254 Multi-marker 125 and P0273 Multi-marker 75 run control products when stored at -30°C or lower. A loss of 20% of HCV-RNA in ViraQ HCV Trend Control (from 25 to 20 copies/mL) reduces the predicted reactivity rate in Ultrio versions from 93.7 (88.0-96.9)% to 91.2 (84.6-95.2)%. A 20% decrease in the HCV-RNA concentration (from 125 to 100 copies/mL) in ViraQ HCV Check controls would reduce the predicted reactivity rate from 99.8 (99.7-99.9)% to 99.6 (98.4-99.9)% in Ultrio versions.

The results of experiments for the in use stability (after thawing) show that some degradation of HCV-RNA in the S0109 HCV standard occurs under regular laboratory circumstances. During storage at 4 ºC or room temperature for 8 hours the loss of HCV-RNA is limited to 4-6 %, below the acceptance criterion of 10 % (see VPL4058). The product insert of ViraQ run controls prescribes to test the run controls within eight hours after thawing and shorten the time between thawing and testing on the NAT robot as much as possible.

We found evidence of a significantly lower stability of the antibody negative S0148, S0109 and WHO 06/100 HCV-RNA standards than the antibody positive S0009 HCV standard when stored frozen at -30°C. In contrast to the window period plasma derived standards the antibody positive S0009 HCV

standard (that has been extensively calibrated against the first and second antibody positive WHO 96/790 and 96/798 standards) turned out to be also stable at -30˚C for up to eight years. Because of the higher stability the latter S0009 standard has been used as an alternative to the WHO HCV 06/100 and 06/102 standards in analytical sensitivity studies.

Comparison of the antibody positive S0009 HCV-RNA genotype 1 standard dilutions with the antibody negative S0148 and S0109 HCV-RNA genotype 3a and WHO HCV 06/100 standard dilutions at 4, 20 and 37°C did not show important differences in degradation kinetics. The inactivated antibody negative S0109 HCV-RNA genotype 3a standard tended to be less stable at 4°C than the antibody positive S0009 HCV standard, but the difference in degradation kinetics at room temperature was not significant.

Since it was reported that the 3rd and 4th WHO standards were less stable we included the 3rd WHO 06/100 standard in our stability studies with the P0238 2000 copies/mL mixed standard panel. Immediately after thawing of the standard dilution of 1000 IU/mL stored at -70˚C the concentration was 714 IU/mL when compared with the S0009 HCV genotype 1 standard as the reference (calibration data not shown). This result is in line with the lower potency found on WHO 06/100 standard dilution panels in Ultrio (Plus and Elite) validation studies. Probably significant degradation of HCV-RNA has occurred in the WHO 06/100 standard during lyophilized storage, reconstitution and subsequent freeze thaw cycles of the reconstituted standard dilutions for preparation of test panels.

Interestingly the recovery in two subsequent freeze dry runs of the same plasma bulk was 45% and 67% in the 06/100 and 06/102 standards respectively. Obviously the loss of HCV-RNA after lyophilisation and reconstitution was not reproducible in the two international HCV standard preparations [Baylis er al[3]]. Not only the 06/100 standard but also the 06/102 standard is known to have lost potency as noticed in validation experiments (Christoph Niederhauser, Berne, Switzerland, personal communication).

4.3. HIV-1 RNA

4.3.1. Stability of S0012 HIV-1 RNA subtype B standard at -70˚C

S0012 HIV-1 genotype B standard stored at -70°C is used as a primary reference standard for other preparations stored at higher temperature. Table 21 shows the quantitative bDNA test results on HIV standard dilutions (falling well in the dynamic range of the assay), which were collected in the period from 1999 to 2008. Using these data we assessed stability by plotting ln[copies/ml] vs. years. The slope ln [conc]/year obtained by linear regression analysis and the 95% confidence limits were used to judge stability. When the slope does not differ significantly from zero stability is claimed. During the 11 year observation period 100.7 (91.3-11.2)% of HIV-1 RNA was recovered according to regression analysis. Hence no degradation of HIV-1 RNA in the S0012 HIV-1 subtype B standard occurs during storage at -70˚C or lower.

Table 21. Stability of S0012 HIV-1-RNA group M subtype B primary reference standard

Test	year	average	ln average	Ln stdev	n	Lower and upper 95% confidence limits	
bDNA 1.0	1997	4.71E+07	17.6668	0.3952	13		
bDNA 2.0	1999	1.14E+08	18.5516	0.3199	18		
	1999	1.06E+08	18.4775	0.2060	31		
	2000	1.13E+08	18.5445	0.3291	8		
	Slope	138%	0.320	0.085	57	-0.044	0.684
bDNA 3.0	2001	8.75E+07	18.2869	0.0710	3		
	2002	1.63E+08	18.9069	0.1162	6		
	2002	9.66E+07	18.3861	0.2580	33		
	2002	1.66E+08	18.9272		2		
	2003	8.99E+07	18.3147	0.1047	6		
	2004	4.18E+07	17.5475		2		
	2008	1.56E+08	18.8666	0.1137	6		
	Slope	101.9%	0.019	0.094	58	-0.223	0.261

4.3.2. Stability of S0012 HIV-1 RNA standard at -70˚C according to analytical sensitivity studies

Another manner to demonstrate long term stability of the S0012 HIV-1 standard at -70˚C is to compare 95% and 50% LODs on dilution panels of this standard in analytical sensitivity studies performed during the last two decades. Table 22 gives an overview of the reported 95% and 50% LODs in analytical sensitivity studies of the Grifols Ultrio versions. Over a 14 years observation period the 50 % LOD did not change significantly since 95% confidence limits overlap. This confirms both assay consistency and stability of the standard dilution panels when stored at -70 °C.

Table 22. Stability of S0012 HIV-1-RNA subtype B standard dilution panels

Study	year	assay	95 % LOD (C.I.)	50 % LOD (C.I.)
Lelie et al[4]	2000	Ultrio	13 (8-22)	1.5 (1.1-2.1)
Koppelman et al[5]	2004	Ultrio	21 (12-52)	2.4 (1.8-3.2)
Vermeulen et al[7]	2009	Ultrio Plus	8.3 (5-15)	1.3 (0.9-1.8)
Grabarczyk et al[6]	2010	Ultrio	11 (8-16)	1.5 (1.2-1.8)
Grabarczyk et al[6]	2010	Ultrio Plus	13 (9-19)	1.7 (1.3-2.2)
Grabarczyk et al[6]	2013	Ultrio Elite	15 (10-24)	2.0 (1.4-2.9)

4.3.3. Real time stability of ViraQ HIV run controls stored frozen at -30˚C

In two real time stability experiments the HIV-1 RNA concentration in nine batches of P0064 ViraQ HIV-1 Check Control (125 copies/mL) stored at -30˚C has been monitored in real time PCR tests. In the first study of December 2014 the stability of four batches stored for 3.6 years was evaluated by comparison of Ct values of quadruplicate Roche TaqScreen 2.0 tests (table 23a). The relative concentrations are calculated by taking 2^(-delta (Ct value test sample minus average Ct value)). In a later experiment in November 2017 six batches of P0064 ViraQ HIV-1 Check Control 125 were tested in the cobas MPX assay after storage at -30˚C for up to 5.3 years (table 23b). All samples were tested at Sanquin (supervisor Dr Marco Koppelman).

Table 23a. Real time stability of P0064 HIV-1 Check 125 control batches stored at -30 ºC measured by quadruplicate tests in coabs TaqScreen 2.0 assay.

Batch-ID	Date prepared	Ct value	Years	Relative concentration#	Temperature
B4059-001	10/14/2010	33.0	3.6	111%	-40°C
		33.1		104%	
		33.8		64%	
		33.6		73%	
B4059-003	2/1/2012	33.4	2.3	84%	-30°C
		34.3		45%	
		33.6		73%	
		33.5		78%	
B4059-004	4/1/2012	33.3	2.1	90%	
		33.9		59%	
		33.0		111%	
		33.4		84%	
B4059-007	5/15/2014	33.0	0.0	111%	
		33.1		104%	
		33.3		90%	
		33.2		97%	

as compared to average Ct value in experiment

Table 23b. Real time stability of P0064 HIV-1 Check 125 control batches stored at -30 °C measured by duplicate or quadruplicate tests in cobas MPX assay

Batch-ID	Date prepared	Ct value	Years	Relative concentration#
B4059-004	4/1/2012	34.11	5.6	95%
		34.53		71%
		34.07		97%
		34.37		79%
B4059-006	27/1/2014	34.01	3.8	101%
		34.44		75%
		34.08	3.5	97%
		33.93		107%
B4059-011	13/3/2015	35.15	2.7	46%
		34.16		91%
B4059-014	7/9/2015	33.78	2.2	119%
		33.52		142%
B4059-017	24/1/2017	33.92	0.80	147%
		33.54		157%
B4059-018	22/9/17	33.47	0.15	108%
		33.38		140%

by comparison against average Ct value in experiment

Figure 10. Degradation of inactivated S0041 HIV-1 standard during storage of P0064 ViraQ HIV-1 Check 125 Control batches at -30°C measured in cobas MPX assay. (diamonds are relative concentrations to average Ct value in real time PCR experiment: the green regression line represents the estimated HIV-1 RNA degradation and the yellow lines represent the 95% confidence limits).

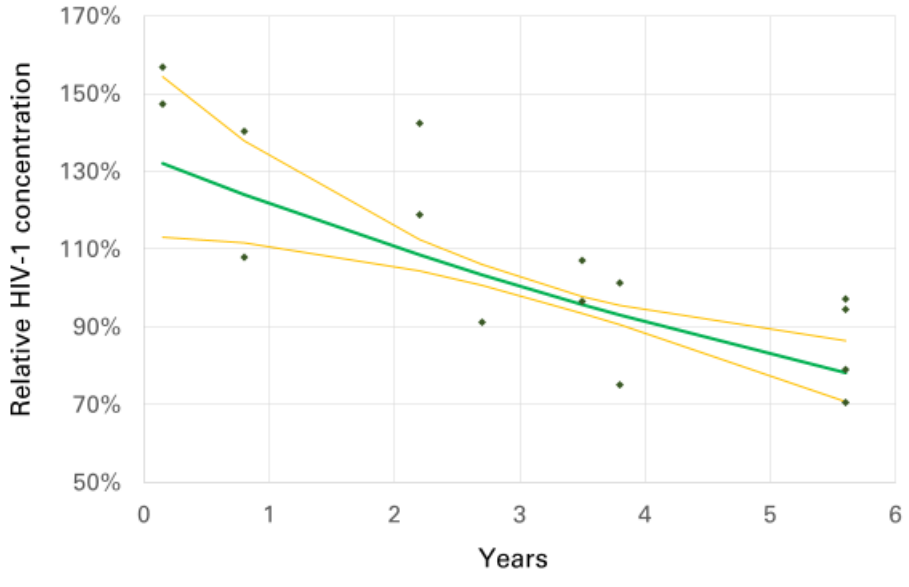


Fig 10

Figure 10 shows the relative concentration calculated from the Ct values (diamonds) in the cobas MPX assay on six batches of P0064 ViraQ HIV-1 Check 125 control stored for different time periods at -30ºC and the trend line represents the degradation of HIV-1 RNA according to regression analysis. After two years of storage at -30˚C it was estimated that 16.6 (2.5-28.7)% of HIV-1 RNA in the ViraQ HIV run controls was lost (table 24).

Table 24. Calculated degradation of HIV-1 RNA during storage of P0064 ViraQ HIV-1 run control batches at -30 ˚C during two years.

Data in:	Degradation after 2 years by linear regression	95 % confidence interval
table 23a	76.7 %	57.4 - 120.2 %
table 23a#	89.1 %	72.1 - 91.0 %
table 23b	83.4%	71.3 - 97.5 %

including -40°C measurements

4.3.4. Long term stability of native and inactivated HIV standards stored at -30˚C and -70˚C

The native S0012 and inactivated S0041 HIV-1 subtype B standards were diluted to 2000 copies/mL samples in the P0238 mixed standard panel that was specially prepared for stability studies. After 4.3 years of storage of the P0238 panel at -30 ºC and -70 ºC sample 3 and 4 of this panel (containing the native S0012 and S0041 standard respectively (table 1)) were tested in 12 replicate cobas MPX tests by Sanquin. The Ct values of this long term stability experiment are summarized in table 25.

Table 25. Measured Ct values in 12 cobas MPX replicate tests on native S0012 and inactivated S0041 HIV-1 RNA standard dilutions in P0238 mixed standard panel stored at -30˚C and -70˚C for 4.3 years

S0012		S0041	
-30°C	-70°C	-30°C	-70°C
29.30	28.96	30.35	29.74
29.51	28.27	30.67	29.71
29.28	28.66	30.58	29.97
29.38	28.66	30.32	29.77
29.21	28.80	30.50	29.77
29.24	28.63	30.25	29.44
29.15	28.98	30.40	29.71
29.21	28.81	30.21	29.53
29.13	28.97	30.18	29.41
29.33	28.76	30.14	29.80
29.18	28.73	30.15	29.73
29.31	29.00	30.17	29.70

Table 26 shows the difference in Ct values on the standard dilutions stored at -30˚C and -70˚C with the 95% CI estimated by paired t-test. It was estimated that the loss of HIV-1 RNA in the native S0012 and inactivated S0041 HIV-1 standards after 2 years was 15(10-20)% and 19(16-21)% respectively.

Table 26 Estimation of degradation of HIV-1 RNA after two years storage of standards at -30˚C (from data presented in table 25)

HIV-1 standard	delta Ct (95%CI) after 4.3 years	delta Ct (95% CI) after 2 years	recovery after 2 years
S0012 native	0.50 (0.32-0.69)	0.23 (0.15-0.32)	85 (80-90%)
S0041 inactivated	0.64 (0.53-0.75)	0.30 (0.24-0.35)	81 (79-84%)

4.3.5. Historical real time stability studies with native S0012 HIV-1 subtype B standard

A 1:10,000 dilution of the S0012 HIV-1 subtype B standard was kept frozen at -70 ˚C. PeliSpy Monitor batches of the same 1:10,000 standard dilution were kept frozen at -30 ˚C. Different batches of the HIV-1 standard dilution and PeliSpy Monitor run control had been stored for different time periods and all samples were tested in parallel in three EasyQ HIV-1 test runs. Figure 11 shows the individual quantitative results on a log scale with the degradation slopes calculated by regression analysis. There was no significant decay in the concentration of the cultured HIV-1 standard when stored for seven years at -70˚C, but a significant degradation of 14 (10-18)% per year was observed during 8 years of storage at -30˚C. It must be emphasized that the actual temperature in the -30˚C freeze unit has often been -25˚C (or lower for a short period of time when products needed to be handled). At least once products were relocated to another freezer when the cooling system was broken down. It therefore cannot be excluded that the 1 mL volume PeliSpy samples sometimes reached temperatures above -20 ˚C. We therefore decided to repeat the long term storage experiment with the 2000 copies/mL mixed standard panel for both the native S0012 and the heat inactivated S0041 HIV-1 RNA standards.

Figure 11. Long term stability data on tissue culture derived VQC-Sanquin HIV-RNA subtype B standard at -30 ˚C and -70 ˚C, the red diamonds represent -30˚C and the blue diamonds -70˚C. The regression lines represent average and 95 % confidence interval of HIV-1 degradation.

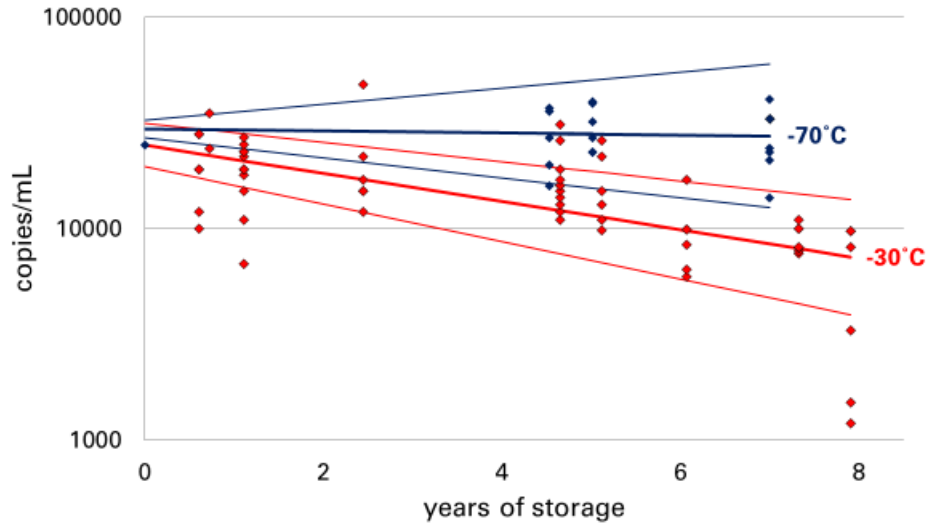


Fig 11

After one year at -30˚C 86 % (82-90%) and at -70°C 99 % (90-109%) was recovered. Hence, also in the historical study the native HIV-1-RNA subtype B standard also was stable at -70 ˚C, but not at -30˚C.

4.3.6. In use stability of ViraQ HIV-1 run controls

The stability of HIV-1 run controls has been established in an accelerated degradation study of two HIV-1 standards stored for 8,24 and 48 hours after thawing at +4°C, +20°C and + 37°C. The native S0012 and heat-inactivated S0043 HIV-1 standards were diluted to 2000 copies/mL in the P0238 mixed standard panel (table 1) and tested in quadruplicate TaqScreen 2.0 assays after different storage times and temperatures. The objective of this study is to ensure that the ViraQ run controls are sufficiently stable when handled in the laboratory and placed in the NAT testing robots.

To improve the precision of the Ct values we have chosen a concentration of 2000 copies/mL instead of the lower concentrations present in the ViraQ run controls. As the reaction constant is independent from the concentrations this will likely not affect the degradation. Table 27 summarizes the individual Ct values that were found on the native S0012 and the inactivated S0041 standard dilutions in the TaqScreen 2.0 test after the different storage times and temperatures.

Table 27: TaqScreen 2.0 test results for HIV-1 RNA on sample 3 and 4 in 2000 copies/mL P0238 mixed standard panel containing dilutions of the native S0012 and inactivated S0043 HIV-1 subtype B standards

Test date	temperature	time (hours)	Ct values measured				Average
	S0012 HIV-RNA native						
4/25/2013	-70°C	start	29.0				
5/1/2013	-70°C	8	29.3	29.1	29.1	28.7	29.1
5/1/2013	2-8°C	8	29.1	29.1	29.0	29.0	29.1
5/1/2013	+20°C	8	29.0	29.0	28.6	28.8	28.9
5/1/2013	+37°C	8	29.1	28.8	29.2	29.0	29.0
5/2/2013	-70°C	24	29.3	29.2	28.9	29.2	29.2
5/2/2013	2-8°C	24	29.1	29.0	29.2	28.9	29.1
5/2/2013	+20°C	24	29.0	28.9	29.0	29.1	29.0
5/2/2013	+37°C	24	29.1	29.4	29.2	29.2	29.2
5/3/2013	-70°C	48	28.6	28.8	28.7	28.9	28.8
5/3/2013	2-8°C	48	29.3	29.3	29.2	29.2	29.3
5/3/2013	+20°C	48	29.5	29.2	28.9	29.0	29.2
5/3/2013	+37°C	48	30.4	29.6	29.3	29.9	29.8
	S0043 HIV-RNA inactivated.		Ct values measured				Average
4/25/2013	-70°C	start	29.2	29.0	29.1	28.9	29.1
5/1/2013	-70°C	8	28.8	29.0	29.0	28.8	28.9
5/1/2013	2-8°C	8	29.0	29.0	28.8	28.9	28.9
5/1/2013	+20°C	8	29.0	28.6	28.6	28.5	28.7
5/1/2013	+37°C	8	29.2	29.1	28.9	28.9	29.0
5/2/2013	-70°C	24	29.0	28.8	29.2	28.6	28.9
5/2/2013	2-8°C	24	28.9	29.0	29.1	28.9	29.0
5/2/2013	+20°C	24	28.6	29.3	29.0	28.7	28.9
5/2/2013	+37°C	24	28.8	29.0	29.0	28.9	28.9
5/3/2013	-70°C	48	28.8	28.9	28.6	28.8	28.8
5/3/2013	2-8°C	48	29.3	29.2	29.3	29.4	29.3
5/3/2013	+20°C	48	29.3	29.0	29.2	29.3	29.2
5/3/2013	+37°C	48	29.3	29.6	29.6	29.3	29.5

The slope for Ct value vs time was calculated for the three temperatures to establish if degradation is present, and if so the slope (delta Ct value per hour) differs significantly from zero (figure 12a and 12 b, table 28).

Table 28: Calculated degradation of HIV-1 RNA in native and inactivated HIV-1 subtype B standards in the liquid phase at different storage temperatures.

			Recovery (95 % C.I.)	
Marker	Temp	Ct/hour (95 % C.I.)	After 4 hours	After 8 hours
Native	4°C	0.010 (0.003-0.017)§	97% (95%-99%)	95% (91%-98%)
	20°C	0.010 (0.002-0.018)§	97% (95%-100%)	95% (91%-99%)
	37°C	0.022 (0.012-0.033)§	94% (91%-97%)	88% (83%-94%)
Inactivated	4°C	0.011 (0.007-0.016)§	97% (96%-98%)	94% (92%-96%)
	20°C	0.011 (0.003-0.019)§	97% (95%-99%)	94% (90%-98%)
	37°C	0.013 (0.007-0.020)§	96% (95%-98%)	93% (90%-96%)

§ p<0.05

Figure 12a. In use stability of S0012 standard established in accelerated degradation study. (4°C blue lines; room temperature orange lines; 37°C red lines; average recovery thick lines ;95% confidence limits thin lines; individual measurements diamonds).

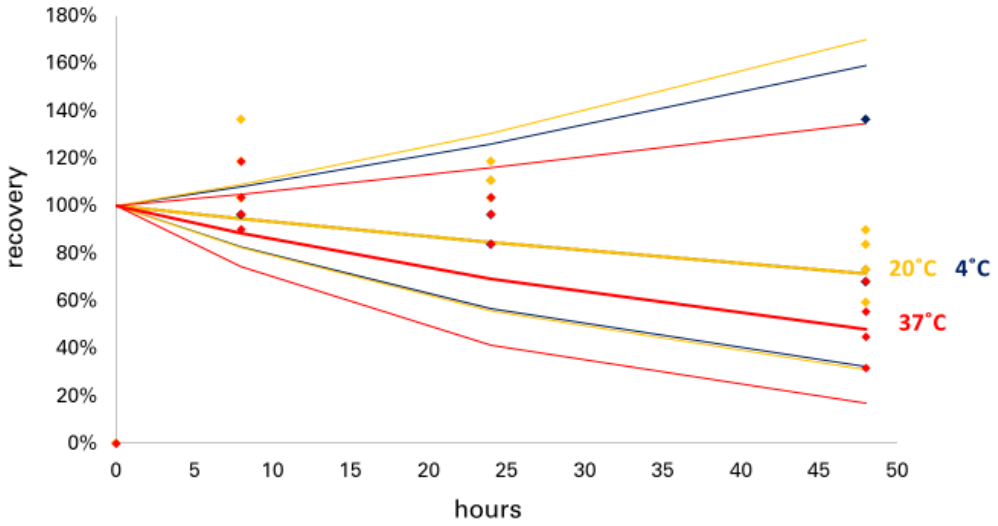


Fig 12a

Figure 12b. In use stability of S0041 standard established in accelerated degradation study. (4°C blue lines; room temperature orange lines; 37°C red lines; average recovery thick lines ;95% confidence limits thin lines; individual measurements diamonds).

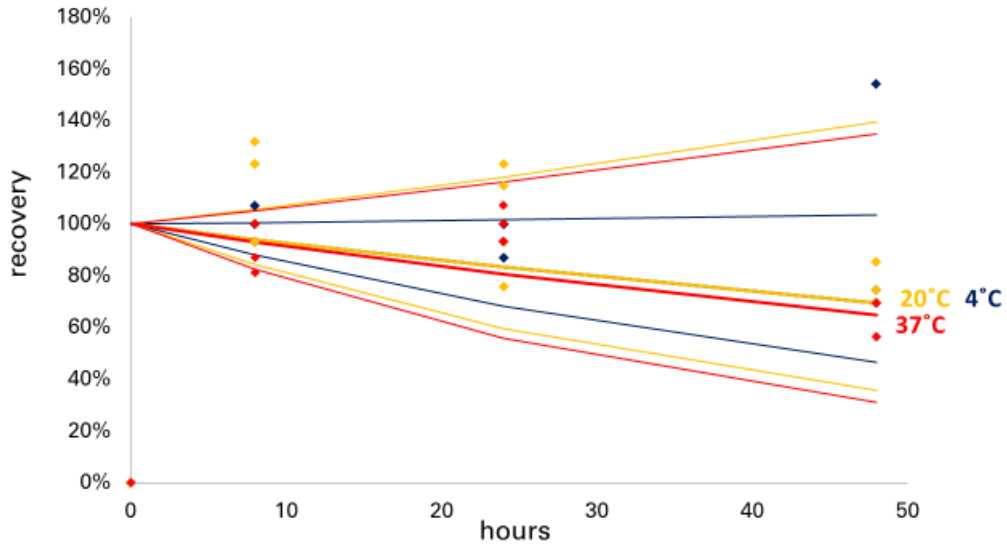


Fig 12b

After 8 hours storage of the inactivated S0041 HIV-1 subtype B standard at 4 °C and room temperature a significant degradation of 6 (2-10)% was estimated. Likewise a significant degradation of 5 (1-9%) was found after storing the native S0012 HIV-1 standard for 8 hours at these temperatures.

4.3.7. Historical in use stability study on S0012 HIV-1 standard dilutions

In 2002 the in use stability of PeliSpy HIV Monitor, a 1:10.000 dilution of S0012 HIV-RNA group M subtype B standard was evaluated at 4°C and room temperature during 5 days. The bioMerieux (Organon Teknika) NASBA assay was used. Each time point (12, 24, 48, 72, 96, 120 hours) was measured in triplicate tests (Figure 12). At both temperatures significant degradation could be established by regression analysis (table 29).

It has been estimated by regression analysis that 3 (2-4%) of HIV-1 RNA has been lost 8 hours after thawing and storage at room temperature (Table 25).

Table 29. Estimated recovery of HIV-1 RNA in PeliSpy Monitor 10,500 copies/mL run control assuming first order kinetics of degradation (data figure 12).

	Recovery (95 % C.I.)	
ours	4°C	Room temperature
	98.0 (96.4-99.6)%	97.0 (95.6-98.4)%
4	94.1 (89.5-98.9)%	91.3 (87.4-95.4)%
8	88.5 (80.1-97.8) %	83.4 (76.4-90.9)%

Figure 13. In use stability of PeliSpy HIV-1 Monitor 10,500 copies/mL run control, (blue diamonds and lines 4°C; red squares and lines room temperature; thick lines estimated concentration; thin lines 95 % confidence limits)

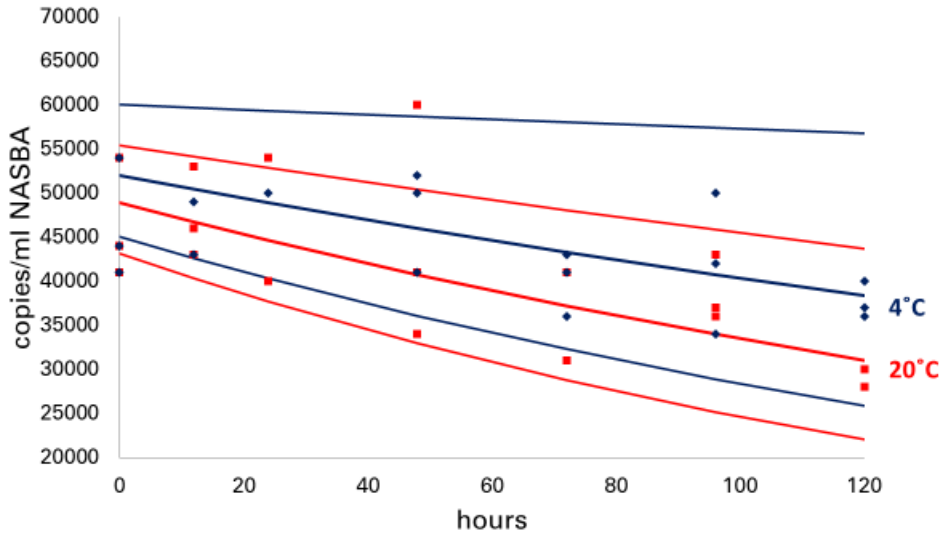


Fig 13

4.3.8. Impact of heat-inactivation and lyophilisation on recovery and stability of S0012 HIV-1 standard

The native S0012 VQC-Sanquin HIV-1 RNA standard has been used for preparation of the S0041 heat-inactivated standard (CE4006). The 1:10,000 dilutions of the S0012 standard that had and had not been subjected to 2 hours pasteurization have been lyophilized by Dr Weycamp (Queen Beatrix Hospital, Winterswijk, the Netherlands). Figure 14 summarizes the preparation scheme of the four equivalent 1:10,000 dilutions of the S0012 standard to obtain HIV-1 in the following physical conditions:

1. native liquid
2. native lyophilized
3. pasteurized liquid
4. pasteurized lyophilized

Samples were kept at 4°C and room temperature. Stored samples were tested in triplicate in both the Organon Teknika NASBA and the Siemens bDNA 3.0 assays at time point zero and after 8 and 24 hours.

For all preparations regardless the inactivation or lyophilisation status there was no significant degradation of HIV-1 after 8 and 24 hours storage in the liquid phase at 4°C and room temperature (table 30 and 31)

Figure 14. Preparation scheme of 1:10,000 dilutions of S0012 HIV-1 standard with and without incorporation of a pasteurization and lyophilisation step

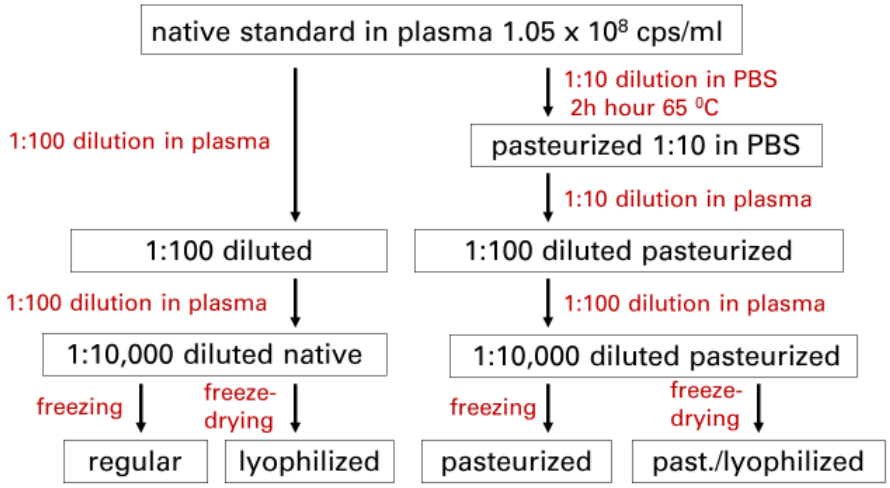


Fig 14

Table 30. Geometric mean HIV-1 RNA concentrations in HIV-1 preparations stored for different temperatures and percent recovery after pasteurization and lyophilisation

preparation	storage temp	storage time	geomean# HIV-1 RNA copies/mL NucliSens	geomean# HIV-1 RNA copies/mL bDNA 3.0	HIV-1 RNA recovery§ NucliSens	HIV-1 RNA recovery§ bDNA 3.0
liquid	4°C	0	41977	17439	100%	100%
liquid	20°C	0	36919	15164	100%	100%
liquid	4°C	8	41977	16209	100%	100%
liquid	20°C	8	36551	15004	100%	100%
liquid	4°C	24	41977	16715	100%	100%
liquid	20°C	24	35828	14703	100%	100%
lyoph.	4°C	0	8999	4250	21%	24%
lyoph.	20°C	0	7702	3299	21%	22%
lyoph.	4°C	8	8102	4307	19%	27%
lyoph.	20°C	8	6627	3515	18%	23%
lyoph.	4°C	24	7089	4656	17%	28%
lyoph.	20°C	24	6217	3096	17%	21%
liquid-inact.	4°C	0	19512	9208	46%	53%
liquid-inact.	20°C	0	18882	8463	51%	56%
liquid-inact.	4°C	8	18327	9344	44%	58%
liquid-inact.	20°C	8	16641	9170	46%	61%
liquid-inact.	4°C	24	18156	9077	43%	54%
liquid-inact.	20°C	24	15616	8697	44%	59%
inact-lyoph.	4°C	0	10222	3380	24%	19%
inact-lyoph.	20°C	0	9933	3010	27%	20%
inact-lyoph.	4°C	8	8798	4359	21%	27%
inact-lyoph.	20°C	8	6697	3591	18%	24%
inact-lyoph.	4°C	24	8140	3801	19%	23%
inact-lyoph.	20°C	24	6926	3805	19%	26%

Table 31. Geometric mean HIV-1 RNA concentration (and 95% CI) of bDNA 3.0 test results. Recovery after treatment and degradation after 8 hour storage

Physical status	4°C geomean	95 % C.I. (low	high)	RT geomean	95 % C.I. (low	high)
native	17003	(15073	19181)	15162	(13408	17145)
lyophilised	4219	(3863	4607)	3421	(3097	3779)
inactivated	9285	(8025	10743)	8725	(7403	10283)
inact.,lyophilised	3707	(3044	4513)	3140	(2773	3555)
	Relative content compared to native material					
lyophilised	25%	(26%	24%)	23%	(23%	22%)
inactivated	55%	(53%	56%)	58%	(55%	60%)
inact.,lyophilised	22%	(20%	24%)	21%	(21%	21%)
	Degradation after 8 hours					
liquid	99%	(93%	106%)	99%	(93%	106%)
lyophilised	103%	(98%	108%)	97%	(92%	103%)
inactivated	99%	(92%	108%)	100%	(92%	110%)
inact.,lyophilised	102%	(92%	114%)	107%	(100%	115%)

It turned out that pasteurization of HIV-1 in 1:10 diluted plasma in PBS is a less harsh treatment than lyophilisation of undiluted plasma. Lyophilisation of native HIV-1 gave the same loss of RNA (~76%) as lyophilisation and pasteurization together. Obviously lyophilisation disrupts the majority of viral particles or capsids. Pasteurization destroys less than half (approximately 44%) of HIV-1 particles. Obviously the residual amount of virus after pasteurization and/or lyophilisation is rather stable when stored at 4°C or at room temperature (table 30 and 31).

We concluded from these experiments that the heat inactivated S0041 HIV-1 standard was suitable for preparation of quality control samples and that accelerated degradation experiments should be performed to establish in use stability (data presented above).

4.3.9. Summary and conclusion on stability of HIV-1 RNA standards

From five year stability data of several batches of ViraQ HIV-run controls stored frozen at -30°C it was estimated that 17 (2-29)% degradation of HIV-RNA occurred in two years. A similar estimate of 19 (16-21)% degradation was found in another long term (4.3 year) stability experiment comparing the HIV-1 concentration of the inactivated S0041 HIV-RNA standard at -30˚C and -70˚C. The loss of HIV-1 RNA in the native S0012 standard was comparable in this experiment and estimated at 15 (10-20)% after two years storage at -30˚C. In the historical long term (8 year) real time stability study of PeliSpy (10,500 copies/mL) run controls 14 (10-18)% degradation per year was calculated, but as described above the temperature has occasionally been higher than -25°C. From these data we claim that after two tears storage at -30˚C there is less than 20% loss of HIV-1 RNA in the P0064 ViraQ Check 125, P0068 ViraQ Trend 25, P0273 Multi-marker Check 125 and P0253 Multi-marker Check 75 Controls. A reduction of 20% of the HIV-1 RNA concentration has a minor effect on the predicted NAT reactivity rates on the Check and Trend Controls (data not shown). Hence, the acceptance criterium of less than 20% RNA degradation

for our claim of two year stability of ViraQ controls when stored at -30˚C or below has been met by the data in this report.

There was a considerable loss of ~44% of HIV-RNA after heat inactivation. The inactivation potentially could have affected the integrity of the HIV particles and their stability but our data do not demonstrate a difference in degradation kinetics between native and inactivated virus.

Our data did not show any significant degradation of HIV-1 RNA in the native S0012 and inactivated S0041 standards when stored at -70°C. For the moment we claim more than 15 years stability of S0012 and S0041 standards and standard dilution panels when stored at -70°C.

The results for in use stability (after thawing) show that some HIV-1 RNA degradation occurs under regular laboratory circumstances. Assuming a maximum duration of 8 hours between thawing of ViraQ run controls and NAT testing the loss is limited to approximately 6 % when operating at 4°C or room temperature. The insert recommends to minimize the time period between thawing and actual testing on the robot.

4. DISCUSSION

Recently the initiators of VQC-Sanquin (Alkmaar, the Netherlands) and BioQControl (Rijswijk, the Netherlands) have relocated to a new facility (Heiloo, the Netherlands) and further improved the infrastructure for ensuring continuous liquid frozen storage of viral plasma standards below -30˚C and -70˚C. In the late 1990s native HBV, HCV and HIV-1 plasma standards have been established in VQC-Sanquin (VR4060), which have been kept frozen at ≤70˚C until today. Later in BioQControl inactivated standards have been prepared (CE4006) and calibrated against the native VQC-Sanquin standards (VR4060). In VQC-Sanquin the PeliSpy run controls and PeliCheck reference panels were prepared from the native liquid frozen plasma standards stored at -70˚C, but in BioQControl the ViraQ run controls are manufactured from inactivated standards (also stored at ≤ -70˚C). For HIV and HBV heat-inactivated standards have been prepared from the native VQC-Sanquin standards but for HCV a chemically inactivated standard was produced from another source plasma (CE4006). In this report (showing data collected over 20 years) the degradation kinetics of HBV-DNA, HCV-RNA and HIV-1 RNA in the native and inactivated standards were compared at different storage temperatures, i.e. at -70˚C, -30˚C, +4˚C, + 20 ˚C and +37˚C.

The data in this report show that the native HBV-DNA standard dilutions in plasma were stable for 20 years at -70˚C and that both the native and pasteurized standard dilutions were stable for at least 5 years at -30˚C. After thawing HBV-DNA was stable at 4, 20 and even at 37 ˚C for the entire 48 hours of the accelerated degradation study.

The data in this report show that the antibody negative and chemically inactivated S0109 HCV standard was significantly less stable at -30˚C than the native antibody positive S0009 HCV standard. The inactivated HCV standard (that was prepared from a window period plasma unit) showed a decay of approximately 9% per year when stored at -30 ˚C whereas the native VQC-Sanquin standard had no degradation and comparable stability at -30˚C and -70˚C for 8 years. The latter VQC-Sanquin standard is known to be stable for 20 years at -70˚C as proven by the quantitative data from proficiency studies and validation studies presented in this report. The degradation kinetics of the inactivated HCV standard at -30˚C was comparable to that of the 3rd WHO 06/100 international HCV standard (after reconstitution and spiking in plasma). This WHO standard was also derived from an antibody negative window period

donation and known to be less stable in the lyophilised state than the 1st and 2nd WHO HCV standards (that were both derived from the same antibody positive plasma unit diluted in a cryo-supernatant plasma pool). After thawing the degradation kinetics of the WHO 06/100 and the inactivated BioQ standard were almost comparable showing approximately 7-11% and 4-6% loss of RNA after 8 hours at 4-20˚C respectively. A similar degradation of HCV-RNA of 3-6% was observed in the VQC-Sanquin standard after 8 hours storage at room temperature, but in contrast to the inactivated standard the native antibody positive standard was completely stable for 5 days when stored at 4˚C. The data in this report indicate that the HCV concentration in the lyophilised ampoules of the WHO 06/100 standard has become at least 25-30% lower (after reconstitution and spiking in plasma) during the period it has been used in our studies. Similar observations were made by Niederhauser and collegues using the WHO standards for quality control testing (Berne, Switzerland, personal communication). The liquid frozen and more stable VQC-Sanquin HCV standard has been extensively calibrated against the 1st and 2nd WHO HCV standards (VR4060) and is currently used as an alternative to the WHO standards in analytical sensitivity studies.

The tissue culture derived VQC-Sanquin HIV-1 subtype B standard has also been shown to be stable for 20 years at -70˚C according to quantitative results of proficiency studies and analytical sensitivity studies presented in this report. However the long term frozen stability studies over 5-8 years showed that both the native and heat-inactivated standard were not stable at -30 ˚C. It was calculated that after 2 years 15 (10-20)% and 19 (16-21)% of HIV-RNA was lost in the native and inactivated HIV-1 standards respectively. In the liquid phase both standards showed similar 5-6% degradation of HIV-RNA after 8 hours of storage at 4-20˚C.

The real time stability studies with several batches of ViraQ run controls (containing 125 or 250 copies/mL) in the -30˚C freezing room have confirmed the degradation kinetics of the viral standard dilutions of higher concentrations shown in this report. It can be concluded that less than 20% degradation of HIV-1 and HCV-RNA occurs in two years when the run controls are stored at ≤-30˚C, whereas HBV-DNA in ViraQ run controls was found to be stable for at least 5 years. Moreover it can be guaranteed that less than 10% degradation of HIV-1 RNA and HCV-RNA (and no degradation of HBV-DNA) occurs at 4-20˚C when ViraQ run controls are tested within 8 hours after thawing. It can be guaranteed that ViraQ Check and Trend Controls near the expiration date are still functional and generate >99.5% and >90% reactivity rate in the target NAT systems. We therefore claim two year stability of ViraQ run controls when stored at ≤-30˚C and when tested within 8 hours after thawing on the automated NAT systems. To avoid cryo-precipitation (and potential loss of virus) samples should be quickly thawed in a water bath of 37˚C or under the tap (under hand warm water, under shaking of tube) until the ice clot has been just removed.

The WHO International Laboratories historically perform lyophilisation of biological standards which is known to increase the stability of proteins at -20 ˚C and allow long term storage at 4˚C and shipment of ampoules at room temperature. However limited stability data are available for lyophilised WHO nucleic acid standards. The data presented in this report indicate that the WHO HCV 06/100 standard is not stable in the lyophilised state or after reconstitution. Baylis et al showed an inconsistent recovery of 45% and 67% of two lyophilisation runs of the WHO 06/100 and 06/102 standards respectively with similar recovery of HCV antigen. We ourselves also examined the recovery of HIV-RNA after lyophilisation and found 24% as compared to 56% after pasteurization (and 22% after both pasteurization and lyophilisation). Obviously lyophilization is a more harsh treatment than heat-inactivation. These manipulations can affect the integrity of the viral particles, for example introduce

subpopulations of less stable (damaged) particles or induce aggregates of virus and proteins from which nucleic acid cannot be so easily extracted. We did however not see a difference in stability between the native and inactivated HBV, HCV and HIV-1 standards. The only factor that may have an impact on HCV stability is binding of antibodies to the virus as the data of this report suggest. The low recovery of HCV and HIV-RNA after lyophilization can be restored by addition of RNAse inhibitor during reconstitution (unpublished experiments of Harvey Holms, NIBSC, UK). Hence one could imagine that free HBV-DNA may be present in the standards after lyophilization or pasteurization. However the data of Prof Gerlich et al with sucrose gradient ultracentrifugation and digestion of HBV-DNA did not provide evidence for this hypothesis.

Our stability data of HIV-1 RNA and HCV-RNA may have also implications for other applications such as biobanks or sample archives of blood banks for look back testing. For application of NAT prolonged storage of low viral load window period samples at -30˚C may eventually generate false negative NAT results.

We conclude that there is no advantage of lyophilization of viral nucleic acid standards and that long term stability can be guaranteed by using liquid frozen standard dilutions in plasma when kept at ≤-70˚C. In our BioQControl facilities the old native VQC-Sanquin standards and the inactivated BioQ standards are stored at -80˚C and have given consistent results in NAT methods for over 20 years.

5. CONCLUSION

ViraQ run controls can be handled with sufficient stability during at least two years storage at -30°C and within 8 hours at °4C (or room temperature) after thawing. These circumstances can easily be met in blood bank and diagnostic laboratories.

6. ADDITIONAL STABILITY STUDIES REQUESTED BY NOTIFIED BODY

The data above were reviewed by experts of the Paul Ehrlich Institute (PEI) who performed the review of the design dossiers of ViraQ Controls for our previous Notified Body (LRQA, Birmingham). In the 1st quarter of 2018 it was decided that the batch release testing in the cobas MPX assay by Sanquin (head Dr. M Koppelman) should be based on 8 replicate tests for both the new batch and a reference batch of the same product stored at -80˚C (VR4091). The same batch release test protocol was also to be used for the stability tests on three consecutive batches of the run controls. The PEI experts did not accept the stability data on the 2000 copies/mL P0238 Multimarker/standard panel and single marker ViraQ Control batches of 125 copies/mL in this report as being representative of the ViraQ Multi-Marker Controls of 125 and 75 copies/mL and the ViraQ Trend Controls of 25 copies/mL. We therefore subjected ViraQ Control Multi-Marker batches of 125 and 75 copies/mL and ViraQ Trend Controls of 25 copies/mL that had passed the expiration date after two years of storage at -30˚C to the approved batch release control procedure (QCF4007 version 7.0 or higher). Table 31 summarizes the data obtained until March 2022 on these products tested at the expiry date or thereafter.

The most informative stability experiments are when the same run control batch is stored at -30ºC and -80ºC at the expiry date. We performed such experiments with P0273 Multi-Marker Check 75 batch B4264-001 and P0254 Multi-Marker Check 125 Control batch B4254-002. Both experiments showed no significant difference in potency for the three markers after two years storage at -30ºC and -80ºC respectively. The P0254 Multi-Marker Check 125 Control batch B5254-002 was also tested after three and four years storage at 30ºC and -80ºC. The inactivated HIV-1 standard in the P0254 product seemed the least stable with a significant 25 (16-33)% and 21 (11-31)% loss of potency after three and four years

respectively. At two year the HIV-1 recovery was 86 (72-103)% similar as 81 (79-84)% that was found in the long term stability studies described above (with 12 replicate tests at year 4.3 on the 2000 copies/mL P0238 multi-marker panel). Interestingly the stability of HCV-RNA in P0254 batch B4254-002 seemed somewhat better than of HIV-RNA since up to four years storage at -30ºC and -8030ºC the potency did not differ significantly.

Table 31. Real time stability tests performed at the expiry date of ViraQ Control batches after two years storage at -30˚C according to batch release procedure QCF4007 (version 7.0) or one year thereafter.

QCF4007	Cat no	n^	Marker	cp/mL	Batch-ID		stability	potency (95% CI)
					ref batch	test batch		
86	P0273	8	HBV	75	B4264-001	B4264-001	2 year	99 (81-120)%
86	P0273	8	HCV	75	B4264-001	B4264-001	2 year	93 (69-125)%
86	P0273	8	HIV-1	75	B4264-001	B4264-001	2 year	88 (74-105)%
102	P0273	8	HBV	75	B4264-001	B4264-002	2 year	70 (57-86)%
102	P0273	8	HCV	75	B4264-001	B4264-002	2 year	77 (60-98)%
102	P0273	8	HIV-1	75	B4264-001	B4264-002	2 year	70 (59-83)%
130	P0273	8	HBV	75	B4264-004	B4264-003	2 year	115 (95-137)%
130	P0273	8	HCV	75	B4264-004	B4264-003	2 year	93 (61-141)%
130	P0273	8	HIV-1	75	B4264-004	B4264-003	2 year	85 (72-100)%
108	P0254	8	HBV	125	B4254-002	B4254-002	2 year	102 (85-122)%
108	P0254	8	HCV	125	B4254-002	B4254-002	2 year	99 (84-118)%
108	P0254	8	HIV-1	125	B4254-002	B4254-002	2 year	86 (72-103)%
131	P0254	8	HBV	125	B4254-002	B4254-002	3 year	98 (82-116)%
131	P0254	8	HCV	125	B4254-002	B4254-002	3 year	91 (65-127)%
131	P0254	8	HIV-1	125	B4254-002	B4254-002	3 year	75 (67-84)%
182	P0254	8	HBV	125	B4254-002	B4254-002	4 year	121 (102-143)%
182	P0254	8	HCV	125	B4254-002	B4254-002	4 year	85 (66-110)%
182	P0254	8	HIV-1	125	B4254-002	B4254-002	4 year	79 (69-89)%
080	P0069	4	HBV	25	B4254-002	B4064-003	2 year	82 (57-119)%
97	P0069	8	HBV	25	B4064-004	B4064-004	2 year	122 (86-173)%
115	P0069	8	HBV	25	B4064-004	B4064-005	2.5 year	81 (54-122)%
99	P0067	8	HCV	25	B4062-011	B4062-010	2 year	128 (69-235)%
141	P0067	8	HCV	25	B4062-011	B4062-011	2 year	45 (19-108)%
179	P0067	4	HCV	25	B4062-011	B4062-011	3 year	132 (44-390)%
180	P0067	8	HCV	25	B4062-011	B4062-012	2 year	94 (39-229)%
100	P0068	16	HIV-1	25	B4063-011	B4063-010	2 year	51 (43-60)%
142	P0068	8	HIV-1	25	B4063-011	B4063-011	2 year	66 (53-82)%
181	P0068	8	HIV-1	25	B4063-013	B4063-013	2 year	83 (64-109)%

^ number of replicate cobas MPX tests on batch.

In another storage experiment of the second batch of the P0273 Multi-Marker Check 75 control (B4264-002) the potency of the three markers was 70-77%, significantly lower than the reference batch B4264-001. By contrast the potencies of the third batch of this product (B4264-003) was not significantly different from the reference batch (B4264-004).

The batch release data on the Trend control batches of 25 copies/mL are more difficult to interpret since the concentrations are too close to the 95% LOD of the cobas MPX tests because of which confidence limits are very wide (VR4091, VR4095). One batch release control test was performed on the same B4064-004 batch of P0069 ViraQ HBV Trend 25 control stored at -30 and -80˚C. As in a previous

batch release test using the old protocol the potency was not significantly different from 100% as we had expected from the long-term stability tests presented in this report. Also the potency in the third stability test on the P0069 HBV Trend Control (B4064-005) did not significantly differ from the reference batch.

Three stability tests have been performed on the P0067 HCV and P0068 HIV-1 Trend controls respectively. The potency of the HCV trend control was not significantly different from the reference batch stored at -80˚C but the HIV trend control had a 1.5 to 2-fold lower potency in two experiments, but had not significantly decreased in the third experiment.

In conclusion the data on the ViraQ Controls of 125 copies/mL and 75 copies/mL confirm that at the expiration date (2 years after preparation) at least 80% of the analyte is still present. The data of one stability experiment after three years storage of the P0254 ViraQ Multi-Marker Control of 125 copies/mL solidifies this claim and makes it possible to also claim longer than two years stability.

In use stability

On initial review of the technical file of the P0063 ViraQ HCV Check 125 Control the new Notified Body (mdc, Stuttgart) mentioned that there were no in use stability data available. However, the accelerated stability data on the 2000 copies/mL P0238 Multi-marker/standard panel in chapters 4.1.4, 4.2.4 and 4.3.6 for HBV, HCV and HIV respectively show that 8 hours after storage at 4˚C at least 94% of HIV-RNA and HCV-RNA has recovered. At the time these experiments were designed it was decided to prepare a panel with higher 2000 copies/mL concentration of the inactivated standards to be able to more reliably measure the decay at 4 ˚C, room temperature and 37˚C. In reaction to the comment of mdc a stability plan has been prepared (VPL4007) that plans to use the discontinued P0254 ViraQ Multi-Marker control product to confirm the claimed stability for all ViraQ Controls tested within 8 hours storage at 2-8 ˚C as well as for repeated freezing and thawing (to establish the potential impact of freezing and thawing of stock solutions during production). So far, this study has not been performed because it has a low priority. In use stability tests on all ViraQ product variants is considered not very informative. Customers place the run control sample immediately after thawing on the test robots.

7. RELATED DOCUMENTS

VPL4058 Validation plan Stability ViraQ run control product family

CE4006 Preparation of inactivated secondary viral standards: Safety assessment of viral quality control samples for viral serology and NAT assays in blood screening laboratories

VR4059 Positioning of ViraQ Check and Trend Controls compatible with analytical sensitivity of NAT assays

VR4060 Calibration of native and inactivated viral standards and traceability to nucleic acid copies and International Units

VR4061 Performance evaluation of ViraQ run controls for detection of HBV, HCV and HIV-1 in different NAT assays

VR4091 Definition of batch release criteria of ViraQ HBV/HCV/HIV-1 Controls

VR4085 Reassessment of batch release criteria of ViraQ HBV/HCV/HIV-1 Controls

8. APPENDIXES

VR4058_A01	Real time HIV stability VQC data.xlsx
VR4058_A02	Consistency HCV and HIV NAT Half Products
VR4058_A03	HIV-RNA native degradation at -30
VR4058_A04	V000255 Consistency HBV-DNA 30.000 versus heat-inactivated 24.000
VR4058_A05	ViraQ real time stability
VR4058_A06	ViraQ in use stability
VR4058_A07	VQC Stability Inactivated and Lyophilised viral NAT Standards
VR4058_A08	Consistency HBV-DNA 30.000 versus heat-inactivated 24.000_S2048_s2289
VR4058_A09	Comparision P0230_HBV gelijkheid natief en geinactiveerd
VR4058_A10	VQC Stability Results PeliSpy NAT in Liquid Phase
VR4058_A11	Real time stability experiments 2017

9. LITERATURE

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