Stability of native, lyophilized and inactivated standards

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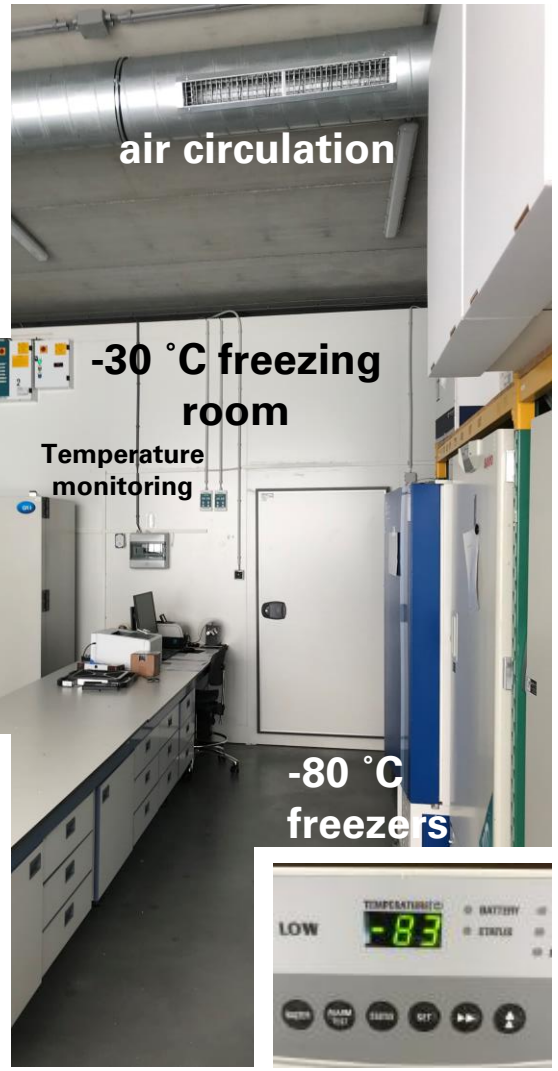
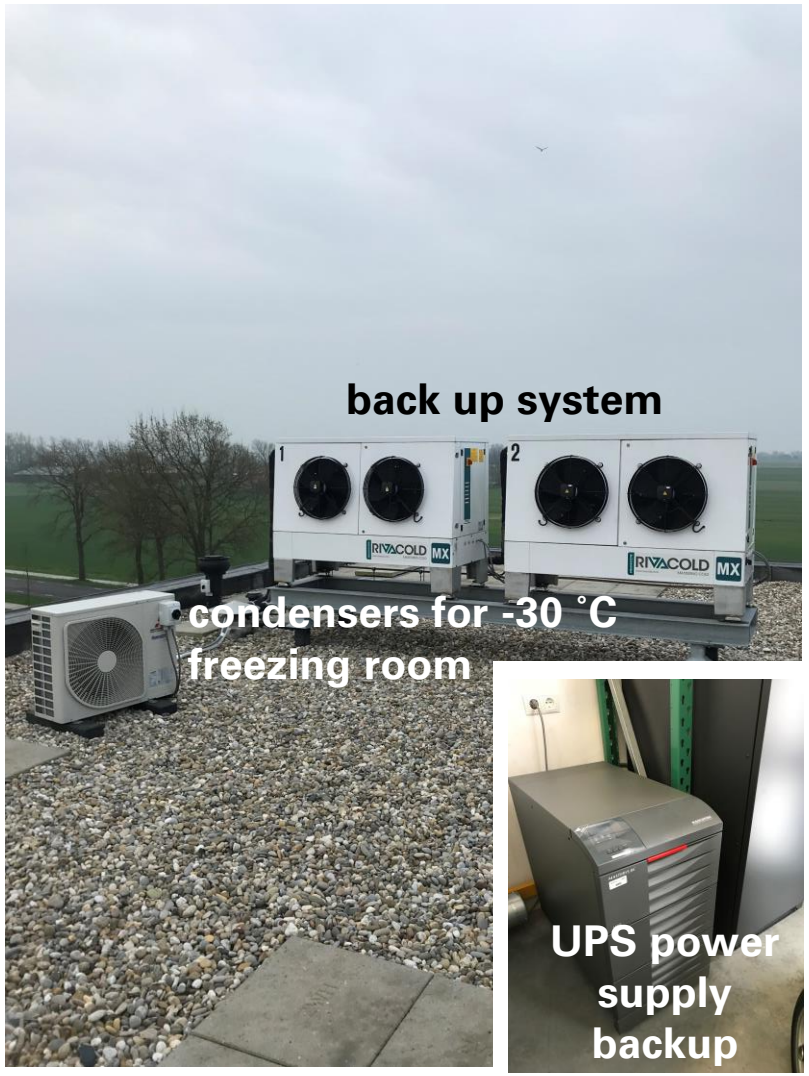
New manufacturing facility





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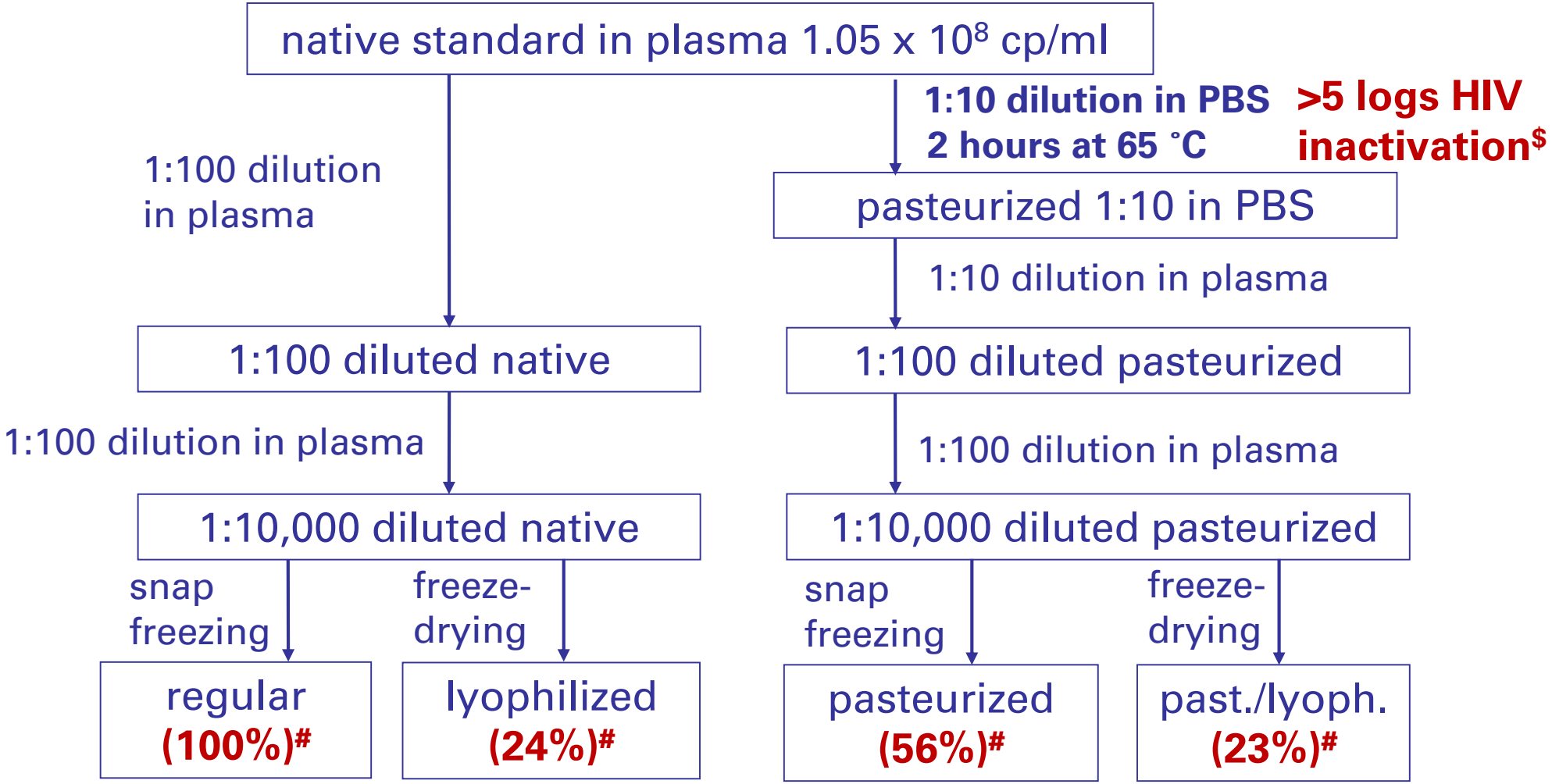
Outline

- Preparation of inactivated standards and run controls
- Viral particle integrity studies
- Stability studies
  - Methods
  - Results
    - long term stability studies in frozen state
    - in use stability studies in liquid phase
- Conclusions

Experimental preparation of pasteurized and/or lyophilized HIV-1 reference samples and RNA recovery#



VQC-Sanquin study performed in 2001 . Each sample was stored at 4 and 21°C and tested in triplicate bDNA and NASBA assays at 0, 8 and 24 hours. Recovery was calculated from 18 bDNA 3.0 results per sample

$ Lelie et al. J Med Virol. 1987;23:297-301.

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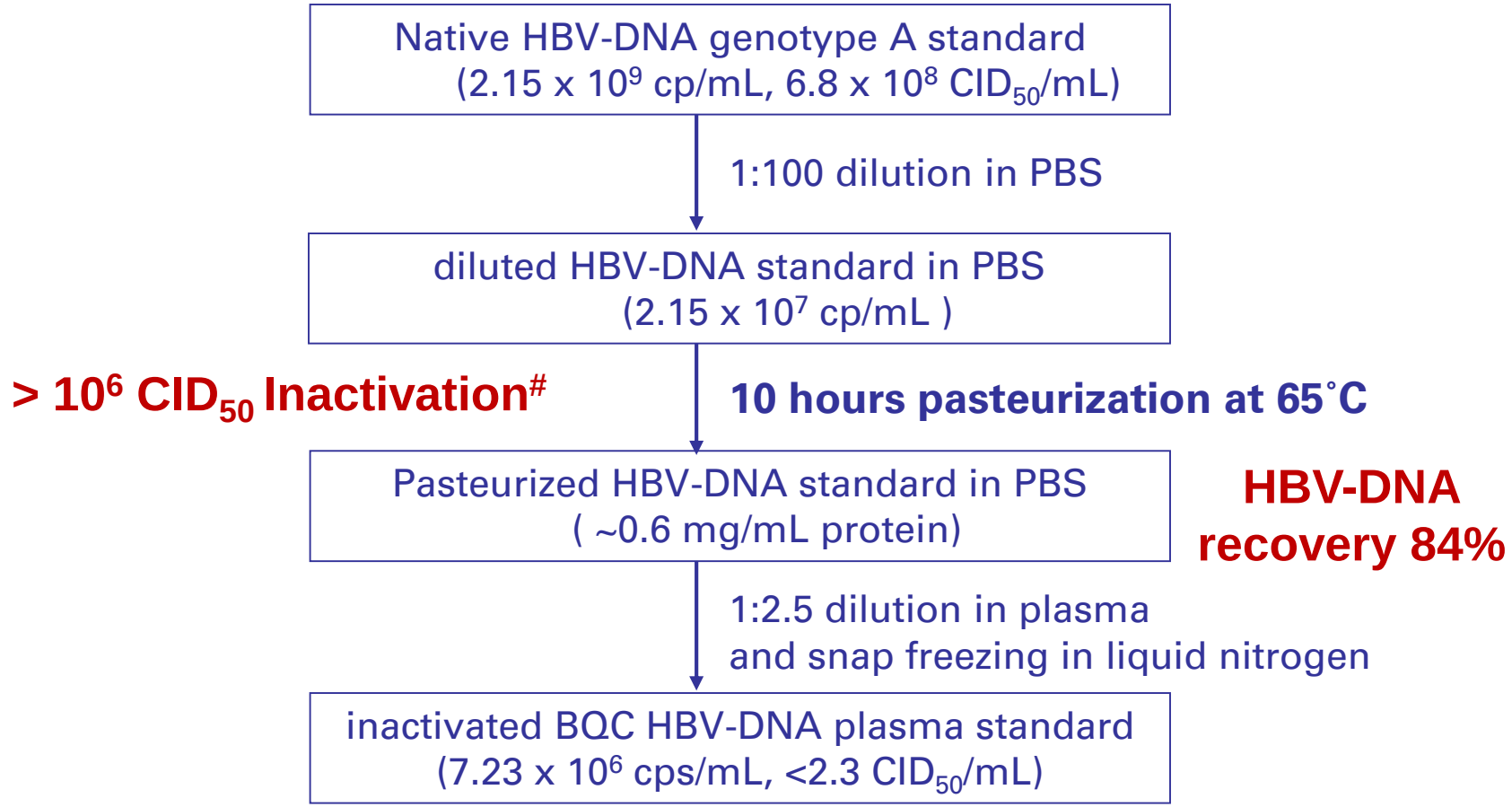
Preparation of heat inactivated S0043 HBV-DNA standard



Lelie PN et al J.Med.Virol. 1987:23:289-95

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Preparation of chemically inactivated HCV standard and ViraQ HCV Trend 25 Control

S0146 HCV-RNA genotype 3a window period plasma unit 4.39.10^7 cp/mL

0.14% betapropiolactone 5h 23˚C, 18h 4˚C,

HCV-RNA recovery 94%

S0109 HCV-RNA genotype 3a inactivated standard; 4.11.10^7 cp/mL#

adjusted to 6.06. 10^7 cp/mL in 2nd bDNA calibration experiment

dilution steps and snap freezing in liquid nitrogen and storage at -80˚C

S1239 HCV-RNA genotype 3a inactivated standard (1:100 of S0109)

S1241 HCV-RNA genotype 3a inactivated standard (1:10 of S1239)

S1088 HCV-RNA genotype 3a inactivated standard (1:10 of S1241)

Preparation time <4 hours

reference batch stored at ≤ -70°C

P0067 ViraQ HCV Trend 25 Control
1: 242.3 dilution of S1088

regular batches

R4003-xxx Plasma units

P0008 neg human plasma pool

Filling, snap freezing and storage at ≤ -30˚C

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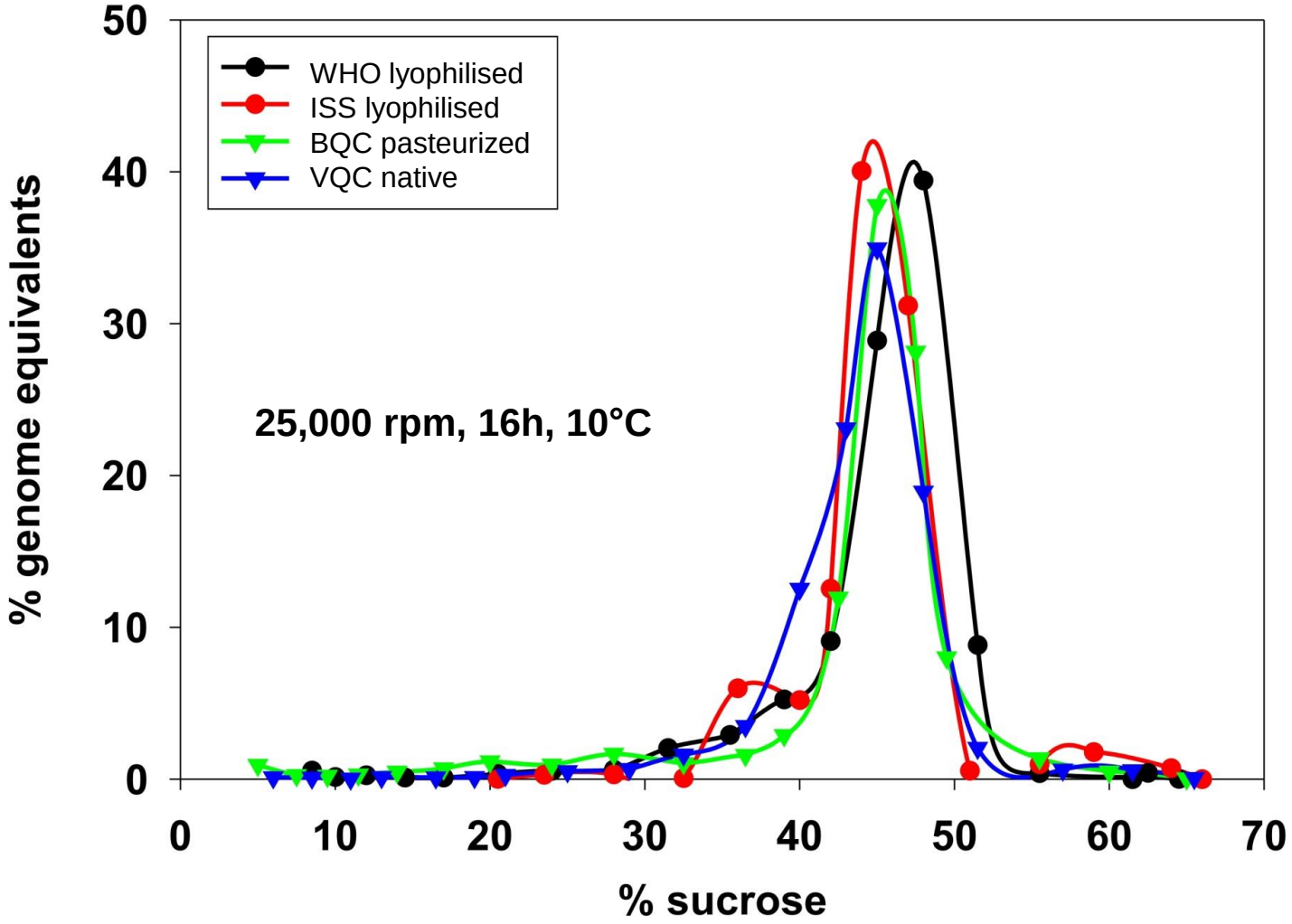
Density of HBV standards in sucrose gradient

Experiment kindly performed by Prof W. Gerlich, University of Giessen, Germany



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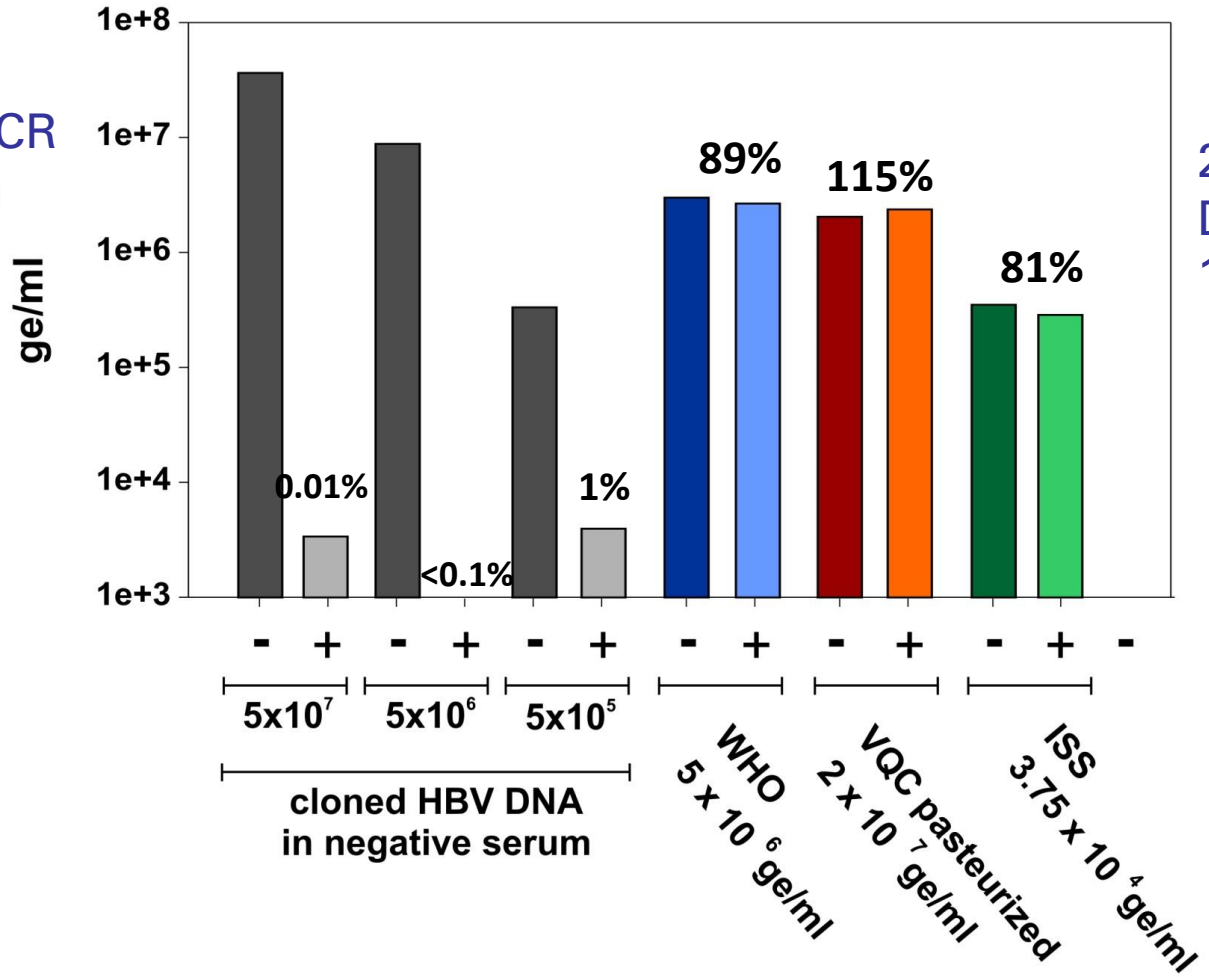
Treatment of free and virus encapsidated HBV-DNA in viral standards with DNAse

Experiment kindly performed by Prof. W. Gerlich, University of Giessen, Germany.

real-time PCR of X-region



200 µl serum, 500 Units DNAse (Benzonase), 1 h 37°C

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Design of stability studies (methods)

Evaluation stability at -70˚C

- Evaluated by measurements on standard (dilutions) from 1996 until now

Stability evaluation for shelf life at - 30˚C (high volume products)

- Real time stability of ViraQ Check Controls was monitored in at least three batches (EN23640:2012 norm) stored up to 5 years at -30˚C.
- Equivalent (and identical) frozen standard dilutions (2000 - 30,000 cp/mL) were stored at -30˚C and -70˚C for more than 4 (up to 8) years and tested in multiple replicate quantitative NAT tests (bDNA 3.0, NASBA, cobas MPX) within the same run.

In use stability

- Accelerated degradation studies were performed by storing standard dilutions (2000 - 30,000 cp/mL) at 4, 20 and 37˚C and testing in multiple replicate qPCR tests after 0, 8, 24, 48 (and 72 and 120) hours

Estimation of viral degradation at certain temperature

With first order degradation kinetics:

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

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

With data at multiple time points K (and 95 % CI) was calculated by linear regression analysis using ln(conc) vs t (K is the slope).

When data on one time point were available with a (stable) reference material for comparison the difference in Ct value was calculated using a paired t-test and translated to recovery (95% C.I.). Then -K is ln(recovery)/t.

S0009 HCV-RNA genotype 1 standard is stable when stored at ≤ -70°C

Assay	Year	n	geomean cp/mL
bDNA 1.0 bDNA 2.0	1996 1997 1998 1999 2000 2000	4 3 4 1 5 30	8.76E+06 1.17E+07 6.31E+06 7.01E+06 9.59E+06 1.06E+07
	1996-2000	*43*	*Recovery 100 (96-104)%*
bDNA 3.0	2001 2003 2003 2004 2008	8 6 3 4 6	6.21E+06 6.43E+06 6.77E+06 8.45E+06 5.00E+06
	2001-2008	*27*	*Recovery 97 (83-108%)*
all	*1996-2008*	*70*	*Recovery 98 (94-102)%*

Consistent analytical sensitivity on standard dilution panels# prepared from S0009 HCV-RNA genotype 1 over 17 years

Study	year	n	assay	95 % LOD (C.I.)	50 % LOD (C.I.)
Lelie et al[1]	2000	65	Duplex	25 (19-35)	2.3 (1.8-2.9)
Koppelman et al[2]	2004	48	Ultrio	25 (14-72)	2.9 (1.9-4.4)
Grabarczyk et al[3,4]	2010	36	Ultrio	25 (17-39)	2.9 (2.1-3.9)
Grabarczyk et al[3,4]	2010	48	Ultrio Plus	15 (11-23)	1.7 (1.3-2.3)
Grabarczyk et al[4]	2013	24	Ultrio Elite	13 (8-21)	1.5 (1.0-2.2)
Coleman et al[5]	2016	24	Ultrio Elite	14 (9-24)	1.9 (1.3-2.7)

Standard, standard dilutions and reference panels were all stored at -70°C

1. Lelie et al. Transfusion. 2002,42:527-36. 2. Koppelman et al. Transfusion 2005;45:1258-66 . 3. Grabarczyk et al. Transfusion 2013;53:2512-2524 4, Grabarczyk et al. Transfusion 2015;55:2246-55 . 5. Coleman et al, manuscript in preparation

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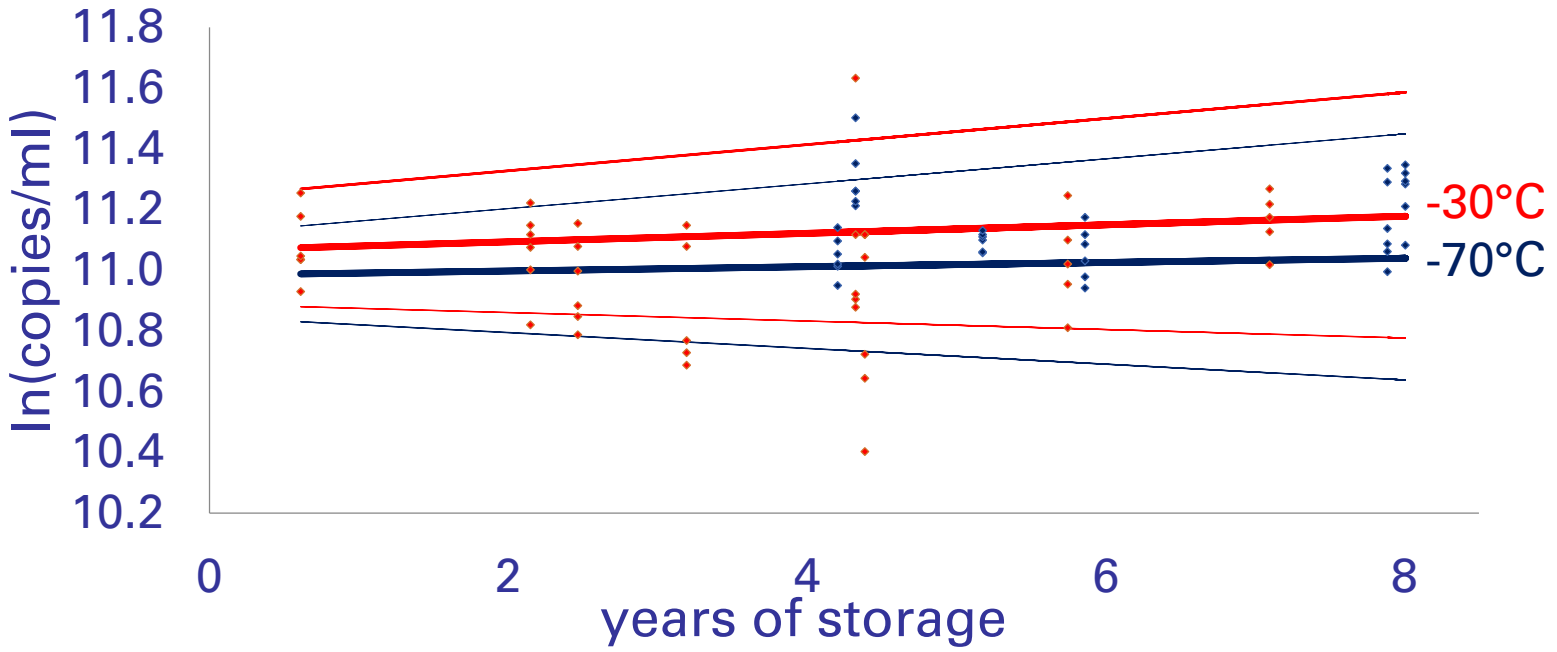
Stability of S0009 HCV-RNA genotype 1 standard

PeliSpy samples and equivalent standard dilutions stored at -70°C and -30°C were tested in triplicate NASBA tests in the same test runs



standard dilutions stored at -70°C and -30°C were tested after 4 years in 12 bDNA 3.0 replicate tests

temp	-70°C	-30°C
cp/mL	124,451	126,814
(95% CI)	(102,332-151,350)	(114,107-140,936)

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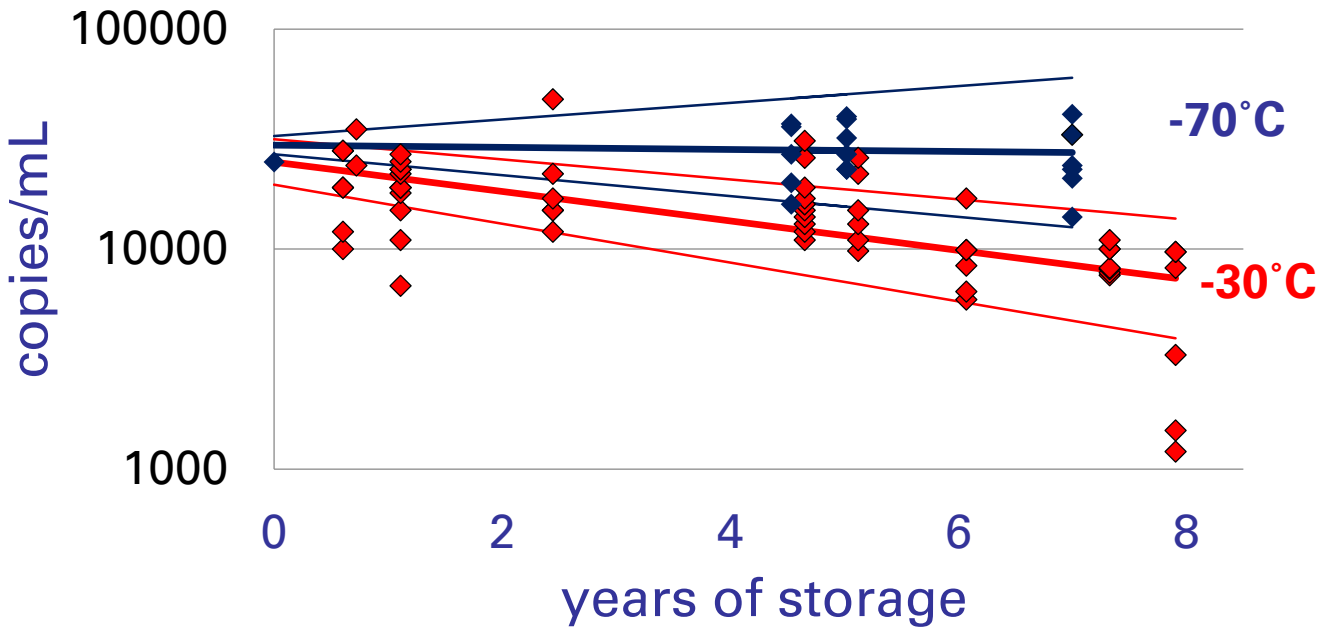
Stability of S0012 HIV-1 RNA subtype B standard

2003: *PeliSpy samples and equivalent standard dilutions stored at -70°C and -30°C were tested in multiple NASBA tests in the same runs*



2017: *2000 cp/mL standard dilutions were stored for 4.3 years at -70°C and -30°C and tested in 12 cobas MPX tests in one run*

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%)

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Annual decay of viral RNA plasma standards when stored at -30°C

Standard dilutions of 2000 cp/mL were stored for 4.3 years at -70°C and -30°C and tested in 12 replicate tests in the cobas MPX assay

Viral RNA standard	Characteristics standard	physical state standard	annual decay^ % (95% CI)
S0009 HCV gt 1	anti-HCV+	native	stable
S0109 HCV gt 3	window period	inactivated	9 (7-11)%
WHO HCV 06/100	window period	lyophilised	8 (5-9)%
S0012 HIV-1 gt B	tissue culture	native	7 (5-10)%
S0041 HIV-1 gt B	tissue culture	inactivated	9 (7-11)%

^determined by using paired t-test on difference in Ct values of cobas MPX tests on 12 samples stored at -70˚C and 12 samples stored at -30˚C transformed to recovery per year

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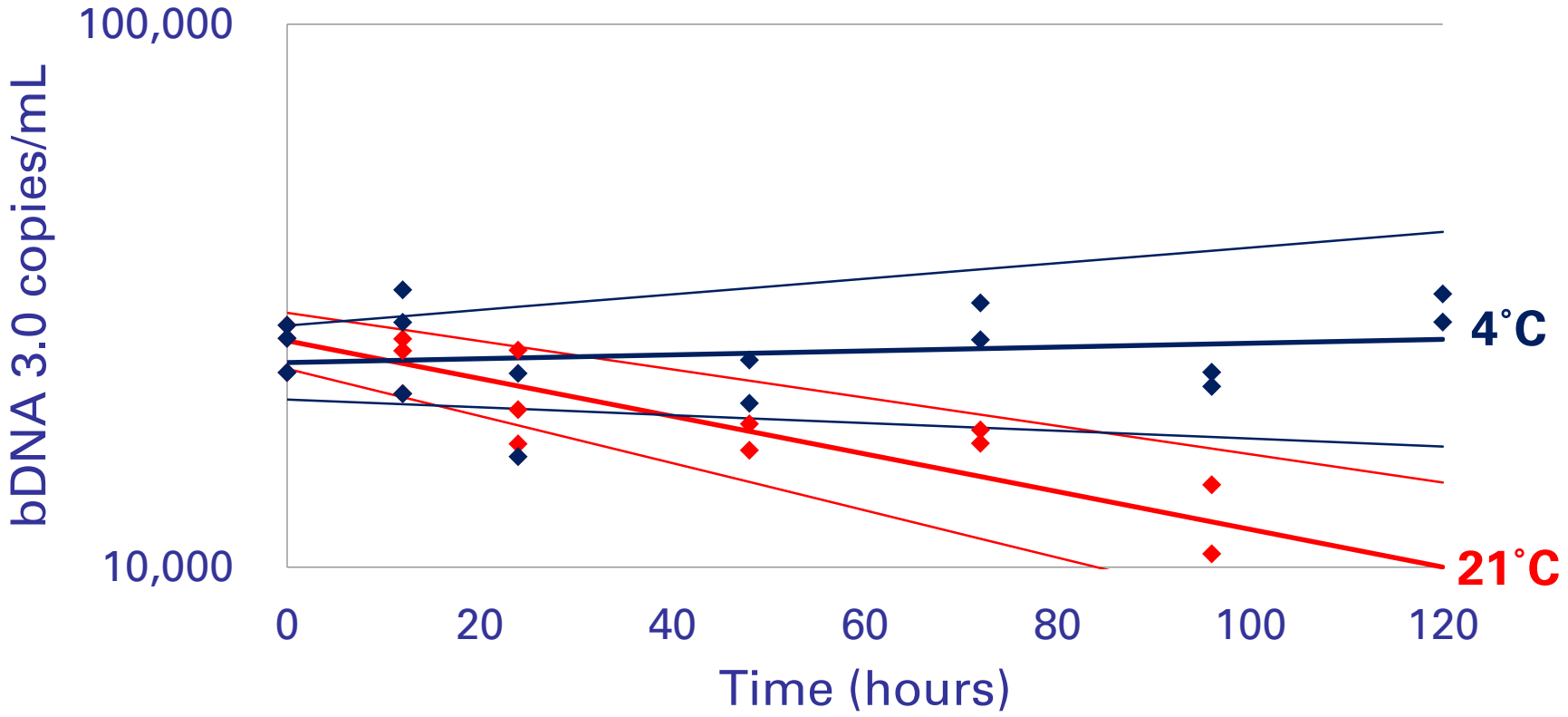
Stability of S0009 HCV-RNA genotype 1 standard at 4°C and 21°C



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In use stability of HCV standards after thawing

Standard dilutions of 2000 cp/mL were thawed and stored for 8, 24 and 48 hours at 4, 21 and 37°C and tested in quadruplicate TaqScreen 2.0 assays in the same run against reference sample at t=0 (tested immediately after thawing)

HCV standard	Temp	ΔCt/hour (95 % CI)	Recovery (95 % C.I.)	
			After 4 hours	After 8 hours
S0009 HCV 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%)
S0146 HCV 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%)
S0109 HCV 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 HCV IS 06/100	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%)

§ $p<0.05$, decay is significant

Decay of viral RNA plasma standards at 4°C

Standard dilutions of 2000 cp/mL were stored for 8, 24 and 48 hours at 4, 20 and 37 ˚C and tested in 4 replicate TaqScreen 2.0 tests in the same run against reference sample that was tested immediately after thawing

Viral RNA standard	Charateristics standard	physical state standard	8 hour decay at 4˚C# % (95% CI)
S0009 HCV gt 1	anti-HCV+	native	stable
S0109 HCV gt 3a	window period	inactivated	6 (2-10)%
WHO HCV 06/100	window period	lyophilised	7 (2-13)%
S0012 HIV-1 gt B	tissue culture	native	6 (2-10)%
S0041 HIV-1 gt B	tissue culture	inactivated	5 (1-9)%

calculated from slope of Ct values by regression analysis

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Conclusions

- VQC-Sanquin HBV-DNA genotype A, HCV-RNA genotype 1 and HIV-1-RNA subtype B standards stored at ≤ 70°C are stable and can serve as 1st order standards calibrated in copies/mL and as secondary standards calibrated in IU/mL, directly traceable to the 1st WHO standards.
- For the inactivated secondary BioQ standards calibrated against primary VQC-Sanquin standards we did not observe stability issues when stored at ≤ 70°C.
- The manufacturing process of controls and reference panels using quick thawing, diluting, aliquoting and snap freezing is consistent.
- For BQC lyophilisation is not a process which can be sufficiently controlled or done at large scale.
- HBV standards and controls were stable for at least 5 years at -30˚C (data not shown).
- Stability of (inactivated) HCV-RNA and HIV-RNA standards at -30°C must be assessed for each individual reference standard
  - ✓ ViraQ HCV, HIV-1 controls can be stored for a maximum of 2 years at -30˚C to guarantee less than 20% RNA degradation and maintaining >99.5% reactivity in NAT target assays.
  - ✓ ViraQ Controls should be tested within same day (max. 8 hours) after thawing to guarantee less than 6% RNA degradation
  - ✓ HCV and HIV standard dilution panels (used to assess analytical sensitivity) must be stored at -70°C.

Appendix (more data)

Consistent analytical sensitivity on S0012 HIV-1-RNA subtype B standard dilution panels

Study	year	n	assay	95 % LOD (C.I.)	50 % LOD (C.I.)
Lelie et al[1]	2000	91	Duplex	13 (8-22)	1.5 (1.1-2.1)
Koppelman et al[2]	2004	48	Ultrio	21 (12-52)	2.4 (1.8-3.2)
Vermeulen et al[3]	2009	24	Ultrio Plus	8.3 (5-15)	1.3 (0.9-1.8)
Grabarczyk et al[4,5]	2010	60	Ultrio	11 (8-16)	1.5 (1.2-1.8)
Grabarczyk et al[4,5]	2010	48	Ultrio Plus	13 (9-19)	1.7 (1.3-2.2)
Grabarczyk et al[5]	2013	24	Ultrio Elite	15 (10-24)	2.0 (1.4-2.9)

1. Lelie et al. Transfusion. 2002,42:527-36. 2. Koppelman et al. Transfusion 2005;45:1258-66 . 3. Vermeulen et al. Transfusion 2013;53:2384-2398. 4. Grabarczyk et al. Transfusion 2013;53:2512-2524 5. Grabarczyk et al. Transfusion 2015;55:2246-55 .

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Time table for preparation of P0063 ViraQ HCV Check 125 control batches

Batch-ID	start	End	Duration
B4058-023	9:50	13:00	3:10
B4058-022	8:45	12:30	3:45
B4058-021	8:15	11:08	2:53
B4058-020	8:45	10:50	2:20
B4058-019	8:30	11:10	2:40
B4058-018	9:18	11:30	2:12
B4058-017	9:52	12:00	2:08
B4058-016	9:38	12:18	2:40
B4058-015	9:41	12:10	2:08
B4058-014	9:49	12:20	2:31
B4058-013	9:52	12:14	2:22

Batch sizes increased over time. Start is at putting the viral standard in a water bath at 37°C and end is at snap freezing the aliquoted vials in liquid nitrogen. As QC parameter a 4 hours limit for the preparation time is accepted (>98 % (95 %-101%) recovery). The dilution error is less than 1 % (from gravimetrical recording).

Worst case end-user reactivity on ViraQ HCV run controls

- Preparation < 4 hours →recovery >97%
- Use within 8 hours at 4-20˚C → recovery >94%.
- Use within 2 years storage at -30˚C →recovery >82%

Impact of 20 % HCV-RNA degradation in ViraQ Controls on NAT reactivity

NAT Assay	n	Hit rate according to probit analysis on standard dilutions			Hit rate according to probit analysis on standard dilutions with 20% HCV-RNA degradation		
		Trend 25 cp/mL	**Check 75 cp/mL**	**Check 125 cp/mL**	**Trend 25 cp/mL**	**Check 75 cp/mL**	**Check 125 cp/mL**
Ultrio	52	93.7%		99.8%	91.2%		99.6%
cobas MPX	10		99.9%			99.8%	
TaqScreen 2.0	12			99.7%			99.5%