

Bio

QC Control

P0344
ViraQ HCV Quant 1000



REF P0344



The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance



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Intended Use

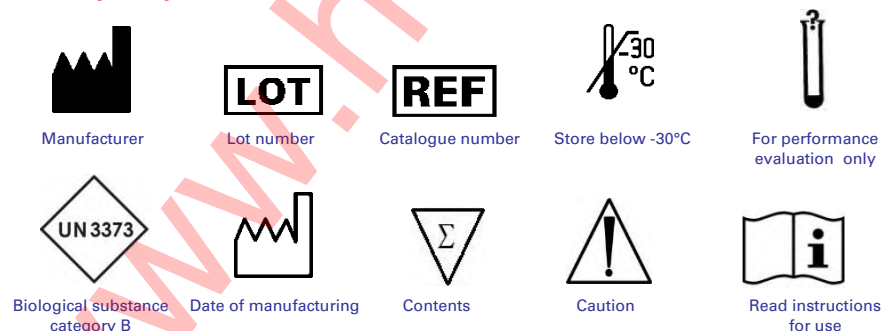
P0344 ViraQ HCV Quant 1000 is intended to be used as external run control for quantitative human immunodeficiency virus type 1 (HCV) RNA amplification assays in combination with the test kits on the platforms defined in table 1. The run control is also suitable for other viral load assays with similar performance characteristics as the methods listed in table 1. The run control helps laboratories to ensure accurate quantification and consistent performance of quantitative HCV nucleic acid amplification technology (NAT) methods with a lower limit of quantification (LOQ) sufficiently below the run control concentration of 1000 copies/mL (~ 368 International Units (IU)/mL). The run control can also be used to compare copy numbers reported by different HCV viral load assays. The run control is intended for performance evaluation only.

Table 1. Test kits and platforms covered by P0344 ViraQ HCV Check 1000 run control

Manufacturer Platform	Test kits	Test environment
Abbott m2000	RealTime HCV assay	Viral load monitoring
Roche cobas Ampliprep/TaqMan	CAP/CTM assay	
Hologic Panther	Aptima HCV Quant	
Cepheid GeneXpert systems	Xpert HCV Viral Load	

The run control should not be used to replace the internal controls or calibrators in the test kits.

Key to Symbols Used



Summary and explanation

In the late 1990s the liquid frozen S009 VQC-Sanquin HCV genotype 1 standard was among the first reference materials for evaluation of NAT methods¹ and used as candidate material in WHO collaborative studies to establish the 1st (and 2nd) International HCV standards^{2,3}. The bDNA 3.0

assay was used as reference method⁴ for calibration in copies/mL. Thorough stability studies have demonstrated that (dilutions of) the S0009 VQC-Sanquin HCV genotype 1 standard is completely stable for more than two decades when stored below -65°C⁵. In the period between 1998 and 2004 the quantitative methods reported similar copy numbers on the VQC-Sanquin standard as in 2018 (table 2 and 3)⁶. Hence the liquid frozen primary S0009 HCV genotype 1 standard calibrated in copies/mL can function as a second anchor for HCV RNA quantification in addition to the WHO standards calibrated in IU/mL which are not stable. The S0009 VQC-Sanquin HCV RNA genotype 1 standard was used for preparation of the P0344 ViraQ Quant control containing 1000 copies/mL. The dilutions were made in human citrate plasma to which EDTA was added in order to mimic the matrix of real patient samples. Since the S0009 HCV genotype 1 standard has been extensively calibrated in both copies and IUs from the 1st International standard P0344 ViraQ HCV Quant can be used as an independent control for testing the accuracy and precision of quantitative HCV NAT methods. The run control has been set at 1000 copies/mL since this level should be accurately detected in a clinical setting.

Principle of method

P0344 ViraQ HCV Check 1000 control has been formulated to mimic natural plasma specimens with a specified HCV-RNA viral load of 1000 copies/mL^{4,6}). The run control has been designed to ensure sufficient accuracy and precision of viral load results by quantitative HCV NAT methods. After thawing the run control tubes are ready for use and can be placed between patient samples at random positions in the work list. The HCV-RNA concentration in the run control has been set at 1000 copies/mL, equivalent to a clinical decision level in therapy monitoring⁷. The run control enables laboratories to be alerted in case of a significant change in quantitative results over time. The run control is prepared by gravimetrically recorded dilution steps of the S0009 HCV-RNA genotype 1 standard, which originate from a plasma pool with high viral loads intercepted at the blood bank. The plasma matrix in which the run control is diluted is manufactured from plasma units that tested negative for all relevant markers of blood borne viruses. The S0009 HCV standard has been historically calibrated in both copies/mL and IU/mL against the 1st (and 2nd) WHO International Standards (figure 1). The quantitative values obtained by different assays on P0344 HCV Quant 1000 control are representative for a native HCV genotype 1 virus but not for HCV preparations of other genotypes. For comparison of quantitative HCV results of different assays on non-genotype 1 standards the P0142 HCV 1000 copies/mL genotype reference panel can better be used. A quantifiable result within the expected range between 500 and 2000 copies/mL on the run control indicates that the NAT method has been

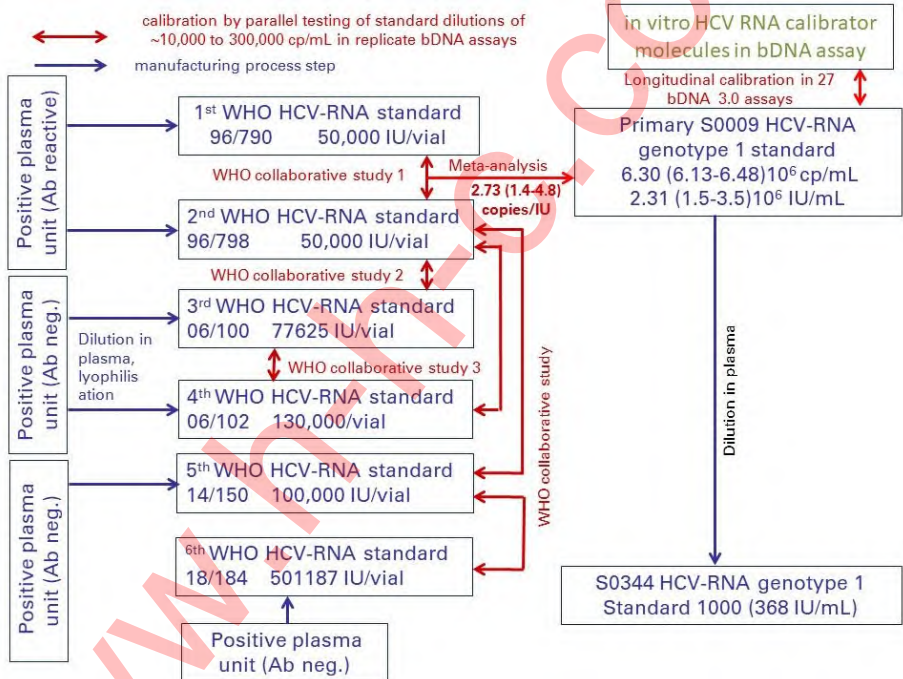
performed with sufficient accuracy and precision. A result outside the expected range is indicative of reduced accuracy and precision and should trigger investigation of the technical performance or recalibration of the assay. The run control generates Ct values or viral loads (expressed in copies/mL) in real time PCR and TMA assays. Statistical analysis of the assay response values generated over a certain period of time allows for comparison of NAT reagent batches and laboratory instruments. The BQC manufacturing and quality control procedures guarantee consistent virus concentrations in consecutive ViraQ HCV Quant 1000 batches⁹. The BQC HCV genotype 1 standard is available in sufficient supply to ensure batch to batch consistency of ViraQ run controls for a prolonged period of time.

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Traceability to HCV RNA copies and International Units

Figure 1 shows the traceability chain between the P0344 HCV Quant 1000 control, the primary S0009 VQC-Sanquin genotype B standard and the 1st HCV 96/790 standard and 2nd WHO HCV 96/796 International Standards.

Figure 1. Traceability chain between P0344 HCV Quant 1000 control, the S0009 VQC-Sanquin HCV genotype 1 standard and the WHO International Standards



Calibration of S0009 VQC-Sanquin HCV genotype 1 standard in copies/mL

The viral concentration in the S0009 VQC-Sanquin HCV RNA genotype 1 standard was established by laboratories testing dilutions of these standards in the VQC proficiency program organized between 1996 and 2004. Table 2 compares the geometric mean values in copies/mL as reported by different quantitative NAT methods when adjusted to 1000 copies/mL values^{5,6}. It was decided to use the Siemens bDNA 3.0 assay

as the reference method⁴ for quantification and assign the value of 6.30 (6.13-6.48).10⁶ copies/mL to the undiluted S0009 VQC-Sanquin standard⁵.

Table 2: Quantification of S0009 VQC-Sanquin HCV RNA genotype 1 standard in proficiency studies performed between 1996 and 2004. The quantification in the Siemens bDNA 3.0 assay was chosen as the reference method for calibration in copies/mL

Assay	n	geomean cp/mL	(95% CI) cp/mL
Roche Amplicor 1.0	90	641	590-697
Roche COBAS Amplicor 2.0	73	695	645-738
Roche MWP Amplicor 2.0	35	630	557-711
Bayer bDNA 2.0	43	1560	1494-1635
Siemens bDNA 3.0	27	1000	973-1026

Calibration of S0009 VQC-Sanquin HCV genotype 1 standard in IU/mL

A Dilution of S0009 was included as sample CC in 2 WHO studies. In the 1st study² a S0009 dilution was evaluated against the 1st and 2nd WHO International standards; samples AA and BB. In the 2nd study several preparations were calibrated on the 1st WHO standard³. The 2nd WHO International standard was prepared equal but in a different lyophilisation batch. The consecutive 3rd to 6th WHO International Standards were not stable, and therefore we refer not to these standards (the WHO has changed the source material from antibody positive to antibody negative; plasma originating from the antibody window phase).

Stability of HCV standards and run control

The long term stability of the liquid frozen S0009 HCV genotype 1 standard dilutions stored at ≤65°C has been firmly established⁵; hence the stock solutions from which the run control is prepared have shown to be stable for more than three decades in the BQC storage facilities. Stability experiments using quantitative NAT assays showed no degradation of HCV RNA for S0009 HCV standard dilutions when stored at -30°C⁵ for several years. Hence, it can be guaranteed that the run control is still functional and should generate quantitative results within the expected range of 500-2000 copies/mL when stored at -30°C and used before the expiration date (two years after preparation of the run control batch).

Kit Contents (materials provided)

The run control contains tissue culture derived HCV virus human plasma without preservatives and is provided in one format as detailed in table 7.

Table 7. Description of kit formats and contents

Cat. Code	Description of contents	Primary packing	Secondary packing
P0344	10 x 1.2 mL run control	2 mL vial	Plastic zip bag

Materials required but not supplied

The test kits and liquid handling devices provided by the NAT manufacturer such as those as specified in Table 1.

Storage instructions

The run controls should be stored at or below -30°C for a maximum of two years⁷. When stored below -65°C the run controls can be stored for a maximum of five years. Once thawed the run control samples should be used within 8 hours. During this period, when not in use, store sample at 2-8°C⁵. Do not refreeze the controls after thawing to prevent formation of cryoprecipitates. Any control sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

The P0344 ViraQ HCV Quant 1000 contains native HCV virus particles in a plasma matrix and should be treated as bio-hazardous. The plasma matrix is prepared from human blood plasma that tested negative for blood borne viruses (HBV-DNA, HCV-RNA, HIV-RNA, HBsAg, anti-HBc, anti-HIV, anti-HCV and anti-Treponema *pallidum*). No test method can offer complete assurance that products derived from human blood cannot transmit (unknown) infectious agents. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials^{9,10}.

- Do not pipette by mouth.
- Use personal protective equipment, including lab coats, gloves and safety glasses.
- Do not eat, drink or smoke in areas where the run control is handled.
- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the run controls for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the run control samples to avoid formation of cryoprecipitates that could alter reactivity or cause pipetting errors in the automated sampling systems.
- Store run controls in an upright position.
- A laboratory protocol for (possible) transmission of HCV must be in place

Reagent preparation

- Thaw the run control quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after thawing remove the run control tube from the water bath.
- Vortex the run control.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the control samples.
- Use within 8 hours after thawing
- After thawing when not in use: store at 2-8°C

Test procedure and calculations

The run control should be tested in a manner identical to that of clinical specimens and the result be calculated according to the instructions for use of the NAT procedure.

Quantitative detection of HCV RNA by viral load assays

For monitoring the accuracy and precision in viral load assays one can use a Levey-Jennings QC chart for trend analysis.

Levey-Jennings QC chart.

Test the run control at least 10 times during the reference period, apply log transformation on values expressed in IU/mL or copies/mL, estimate the geometric mean, standard deviation (SD) and its confidence interval (CI) as described below. [If Ct values are used no log transformation is required and confidence intervals can be calculated from the arithmetic mean and SD]. The Levey-Jennings chart is designed to identify individual aberrant values outside the 95% and 99% confidence intervals. With collecting additional data the chart characteristics may be updated. The quantitative values for [HCV RNA] are 'log normal' distributed.

- Calculate from each measurement the log(concentration) in IU/mL or copies/mL.
- Calculate mean and SD on these log values
- Take anti-log of the mean of log values, i.e. the geometric mean of the measurements in IU/mL or copies/mL.

Use table 8 to obtain Student-t-values belonging to the 95% and 99% CI for different number of observations (n). Calculate the log(95% and 99% CI) as follows:

- Log (99% Lower limit): $\log(\text{Average}) - (99\%) \text{ Student-t-Value} \times \log(\text{SD})$
- Log (95% Lower limit): $\log(\text{Average}) - (95\%) \text{ Student-t-Value} \times \log(\text{SD})$
- Log (95% Upper limit): $\log(\text{Average}) + (95\%) \text{ Student-t-Value} \times \log(\text{SD})$
- Log (99% Upper limit): $\log(\text{Average}) + (99\%) \text{ Student-t-Value} \times \log(\text{SD})$

Table 8. Relation of Student t value and numbers of runs (n) to calculate CI's.

Run (n)	t-value at 95% C.I.	t-value at 99% C.I.
10	2.306	3.355
20	2.101	2.878
30	2.048	2.763
infinite	1.960	2.576

Use the Westgard rules¹¹ to identify deviations in the Levey Jennings trend analysis.

Comparison of variation in quantitative values between result sets

For this analysis result sets could represent e.g. laboratory, reagent batch, instrument, operator, etcetera.

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

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

Use table 9 to determine if the precision of the quantitative NAT method has significantly changed.

Table 9. Chi-square (X^2) values for p=0.05

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

Interpretation:

Chi-square: $X^2_{(Calculated)} < X^2_{(P=0.05)}$: precision is not significantly changed.

Chi-square: $X^2_{(Calculated)} \geq X^2_{(P=0.05)}$: precision has changed significantly.

Interpretation of test results on run control

P0344 ViraQ HCV Quant 1000 should be used in conjunction HCV viral load assays with an LOQ sufficiently below 500 copies/mL. Table 10 gives the expected frequency of different categories of results on the run control in these viral load assays.

Table 10. Interpretation of a single quantitative NAT test result on P0344 ViraQ HCV Check 1000 control and expected frequency of viral load measurements above the lower limit of quantification (LOQ) of the current commercial real time PCR and TMA assays.

Result	HIV-cp/mL	Expected frequency	Interpretation
Reactive quantifiable	≥LOQ	100%	This is an expected result.
Reactive quantifiable	≥500 ≤2000	>95%	This is an expected result.
Reactive quantifiable	<500 >2000	<5%	This is an unexpected result but is possible. An investigation of technical performance or calibration of the NAT system is recommended
Reactive nonquantifiable or nonreactive	<LOQ	0%	This is an unexpected result. An investigation of technical performance of the NAT system is required

The linear range of the quantitative NAT methods tests starts at enough distance below the run control concentration of 1000 copies/mL to expect quantifiable results (above the LOQ) within a range between 500 and 2000 copies/mL in more than 95% of test runs⁵.

One should be careful with comparing the copies/mL and IU/mL levels because different methods and standards have been used for calibration of the run control and (calibrators of) the NAT systems.

Limitations

- P0344 ViraQ HCV Quant 1000 Control must not be substituted for the mandatory controls or calibrators provided with NAT test kits for calculating the cut-off and/or quantitative test results or for setting criteria not to release clinical test results.
- The expected distributions of assay response values on P0344 ViraQ HCV Quant 1000 Control that are presented in this package insert were based on evaluation studies involving a limited number of assays and reagent batches. Therefore it cannot be guaranteed that slightly different results will be found on other assay versions or reagent batches.
- P0344 ViraQ HCV Quant 1000 should not be used for establishing accuracy of quantitative NAT results expressed in IU/mL. For this purpose only a dilution of the current WHO International Standard can be used.

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