P0273
ViraQ Multi-Marker Check 75

CE
0483

IVD

REF P0273

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

P0273 ViraQ Multi-Marker Check 75 is intended to be used as external run control for detection of hepatitis B virus (HBV)-DNA, hepatitis C virus (HCV)-RNA and human immunodeficiency virus type 1 (HIV-1) RNA by the cobas MPX multiplex amplification assay on the cobas 5800/6800/8800 platforms (Table1). P0273 ViraQ Multi-Marker Check 75 is a multi-marker mixture of inactivated HBV-DNA, HCV-RNA, HIV-1 RNA standards in plasma at a concentration of 75 copies/mL giving a low reactive result on the three markers in the Roche cobas MPX® multiplex assay. The run control is intended for repeated testing in consecutive test runs of the cobas MPX assay over time by trained laboratory workers. The run control helps laboratories to ensure sufficient analytical sensitivity and consistent performance of this nucleic acid amplification technology (NAT) assay. P0273 ViraQ Multi-Marker Check 75 should not be used to replace the internal controls or calibrators in the test kits. The test result on the run control should not be used to reject the run or delay the release of test results on donor samples.

Table 1. Assay and platform covered by P0273 Multi-Marker Check 75 control

Assay (manufacturer)	Platform	Test environment
cobas MPX (Roche)	cobas 5800/6800/8800	Blood screening

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

In vitro diagnostic medical device

Country of origin

Expiry date

Number of controls

Caution

CE mark with Notified Body number

Unique device identification

Biological substance Category B

Store below –30 °C

Positive control

Contains human blood derivatives

Read instructions for use

Principle of method

P0273 ViraQ Multi-Marker Check 75 control has been formulated to mimic natural plasma specimens with a low concentration of HBV-DNA, HCV-RNA and HIV-1 RNA. After thawing the run control tubes are ready for use and can be placed at random positions in sample racks on the cobas NAT platforms. The run control contains 75 copies/mL of HBV-DNA, HCV-RNA and HIV-1 RNA, which is equivalent to 14, 27 and 129 International Units (IU)/mL of the viruses respectively. The multi-marker run control has been designed to ensure sufficient analytical sensitivity and consistent performance of multi-dyed real time

polymerase chain reaction (PCR) tests in blood screening laboratories. The viral concentrations in the run control have been set at 4 to 5 times the 95% lower limit of detection (LOD) of the cobas MPX assay for HBV and HCV but is positioned at somewhat greater distance for HIV in the later assay versions (Table 2, 3 and 4)[1]. The positioning of P0273 ViraQ Multi-Marker Check 75 control ensures reactivity rates above 99.9% in the cobas MPX assay and enables laboratories to be alerted in case of a significant reduction of analytical sensitivity of the NAT test system. The run control is a mixture of dilutions of inactivated viral standards. The S0043 HBV-DNA genotype A and S0041 HIV-1 subtype B standards have been pasteurized at 65 °C at low protein concentration before spiking in plasma[2-5]. The S0109 HCV-RNA genotype 3a standard has been prepared by inactivation of window period plasma with betapropiolactone[2,6-8]. 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 inactivated standards have been calibrated in copies/mL and IU/mL against the Viral Quality Control (VQC)-Sanquin and World Health Organization (WHO) International Standards established in the 1990s (Figure 1-3). The low concentration of HBV genotype A, HCV genotype 3 and HIV-1 subtype B are meant to be representative for other genotypes that are prevalent in different geographical regions of the world (and that are detected with similar analytical sensitivity by the commercial NAT assays)[9-18]. The run control generates Ct values in the cobas MPX assay and other real time PCR assays. A non-reactive result on the run control or a significant shift to higher Ct values is indicative of reduced analytical sensitivity of the NAT system and should trigger an investigation of the technical performance of the assay. Statistical analysis of Ct values generated over a certain period allows for comparison of analytical performance of NAT reagent lots and laboratory instruments. For this purpose, users of the cobas MPX assay in combination with the P0273 ViraQ Multi-Marker Check 75 Control can generate a statistical report for post-market performance follow-up[19] using the DataQ Analytics system accessible via the website www.bioqcontrol.com.

Table 2. NAT detection limits on native and heat-inactivated HBV genotype A standard dilution panels calculated by probit analysis

HBV-DNA standard (1 IU = 5.33 copies)	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0043 VQC-Sanquin HBV-DNA genotype A inactivated	P0251	TaqScreen 2.0	12	2.8 (1.5-4.3)	23.8 (12.4-99.3)
	P0031	cobas MPX	12	2.4 (1.4-4.2)	18.6 (9.1-75.9)
S0011 VQC-Sanquin HBV-DNA genotype A	P0007	TaqScreen 1.0	12	2.9 (1.6-4.9)	26.9 (13.9-71.0)
	P0007	cobas MPX	24	1.9 (1.3-2.7)	13.0 (7.7-29.6)
	P0280	cobas MPX	24	1.0 (0.6-1.5)	9.0 (5.1-24.6)
S0010 Eurohep HBV-DNA genotype A	P0001	TaqScreen 1.0	12	2.3 (1.3-3.8)	14.1 (7.2-56.6)
	P0272	cobas MPX	118	1.9 (1.2-2.5)	9.2 (6.1-19.4)
WHO HBV-DNA 97/750	P0023	cobas MPX	12	1.8 (0.93-2.8)	8.0 (4.4-37.4)

Table 3. NAT detection limits on native and inactivated HCV standard dilution panels calculated by probit analysis

HCV-RNA standard (1 IU = 2.73 copies)	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0109 BioQ HCV-RNA genot. 3a inact.	P0020	TaqScreen 2.0	12	3.1 (2.0-4.7)	21.1 (11.6-68.3)
	P0020	cobas MPX	10	2.5 (1.7-3.6)	17.2 (12.3-24.6)
S0009 VQC-Sanquin HCV-RNA genotype 1	P0019	TaqScreen 1.0	12	4.9 (3.2-7.5)	19.7 (12.0-43.2)
	P0019	cobas MPX	60	2.9 (2.3-3.6)	20.4 (14.3-33.3)
	P0272	cobas MPX	166	3.3 (2.9-3.6)	16.3 (13.7-20.0)

Table 4. NAT detection limits on native and inactivated HIV-1 subtype B standard dilution panels calculated by probit analysis

HIV-1 RNA standard (1 IU=0.58 copies)	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0041 VQC-Sanquin HIV-1 RNA subtype B inactivated	P0251	TaqScreen 2.0	12	2.0 (1.3-2.8)	7.6 (4.9-21.4)
	P0026	cobas MPX	12	1.0 (0.6-1.6)	5.8 (3.0-23.2)
S0012 VQC-Sanquin HIV-1 RNA subtype B	P0025	TaqScreen 1.0	12	4.2 (2.6-6.7)	22.8 (13.0-52.9)
	P0290	cobas MPX	24	1.0 (0.6-1.5)	9.0 (5.1-24.6)
	P0272	cobas MPX	166	1.5 (1.3-1.6)	7.2 (6.1-8.9)
WHO HIV-1 RNA 97/650	P0022	cobas MPX	12	2.7 (1.7-3.9)	5.8 (3.9-24.9)

Traceability to viral nucleic acid copies and International Units

Figure 1, 2 and 3 show the traceability chain for HBV-DNA, HCV-RNA and HIV-1 RNA between P0273 ViraQ Multi-Marker Check 75 control, the inactivated and native VQC-Sanquin standards and the lyophilized 1st and 2nd WHO International Standards. The inactivated S0043 HBV-DNA, S0109 HCV-RNA and S0041 HIV-1 RNA standards (used for preparation of the P0273 ViraQ Multi-Marker Check 75 control) have been calibrated in copies/mL by replicate testing in the Siemens Versant bDNA 3.0 assay[20] against the historically established VQC-Sanquin S0011 HBV-DNA, S0009 HCV-RNA and S0012 HIV-1 standards[21,22]. However, when the inactivated S0109 HCV-RNA genotype 3 standard was later recalibrated against the native S0009 HCV genotype 1 standard using five different quantitative NAT methods it was decided to adjust the originally measured HCV concentration to a 1.67-fold lower value[22].

The primary native VQC-Sanquin standards have been calibrated at:

- 5.33 (5.11-5.55) and 5.20 (4.61-5.80) copies per IU against the 1st and 2nd WHO HBV-DNA (97/746 and 97/750) standards respectively in two bDNA 3.0 experiments[22,23].
- 2.73 (1.4-4.8) copies per IU against the first and second WHO HCV-RNA (96/790 and 96/798) standards according to data from different studies[22,24,25].
- 0.39 (0.34-0.44) copies and 0.58 (0.51-0.66) per IU against the first WHO HIV-1 97/656 and second HIV-1 RNA 97/650 standards respectively in multiple replicate bDNA 3.0 assays[22,26-28].

It must be emphasized that these conversion factors from copies to IU values are NAT method dependent[28] and have not been determined or confirmed for the 3rd WHO HBV

10/264, 3rd WHO HCV 06/100, 4th WHO HCV 06/102, 5th WHO HCV 14/150, 3rd WHO HIV-1 10/152 and 4th WHO HIV-1 16/194 replacement standards.

The copies assigned to the VQC-Sanquin HBV standard were found to be equivalent to those in the Eurohep standard[29] which was used for preparation of the WHO standards. The accurate calibration of the inactivated VQC-Sanquin and BioQControl standards against the WHO, Eurohep and VQC-Sanquin standards in IU/mL and in copies/mL has been confirmed in analytical sensitivity studies of the Grifols Procleix and Roche cobas MPX assays[1,13,22,30]. The BioQControl manufacturing and quality control procedures guarantee consistent virus concentrations in consecutive ViraQ Check Control batches[19,31]. The inactivated BioQControl standards are available in sufficient supply to ensure batch-to-batch consistency of ViraQ run controls for a prolonged period.

Figure 1. Traceability chain between P0273 Multi-Marker Check 75 control, inactivated and native VQC-Sanquin, Eurohep and WHO International Standards for HBV-DNA

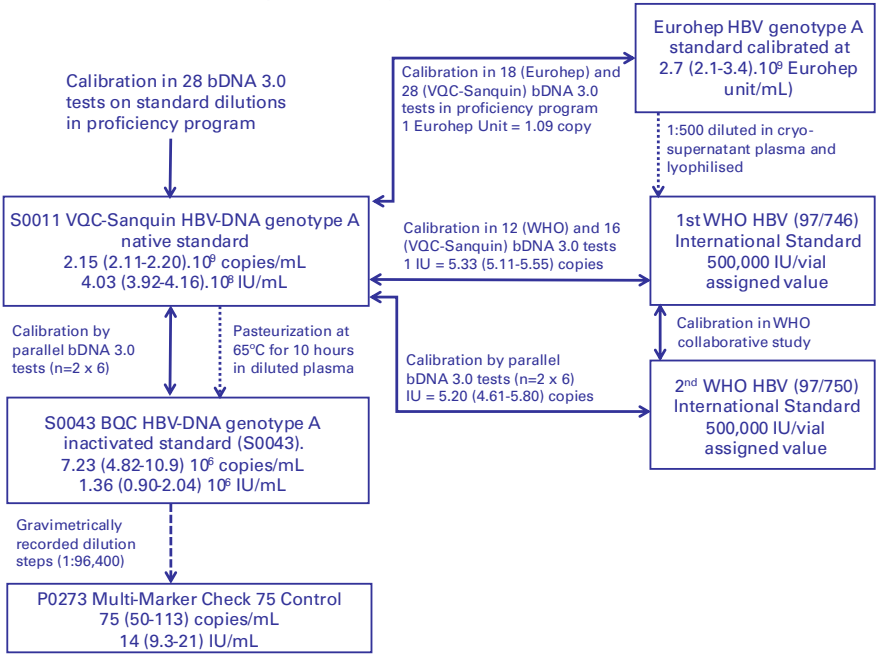


Figure 2. Traceability chain between P0273 Multi-Marker Check 75 control, inactivated BioQControl, VQC-Sanquin and WHO International Standards for HCV-RNA

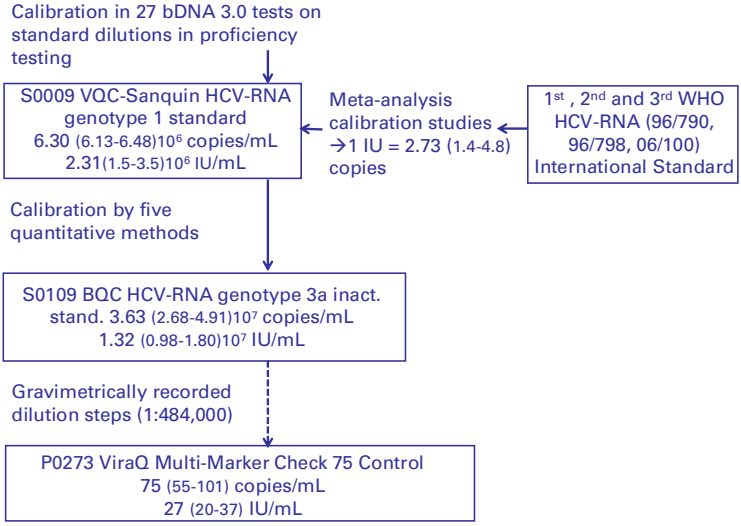
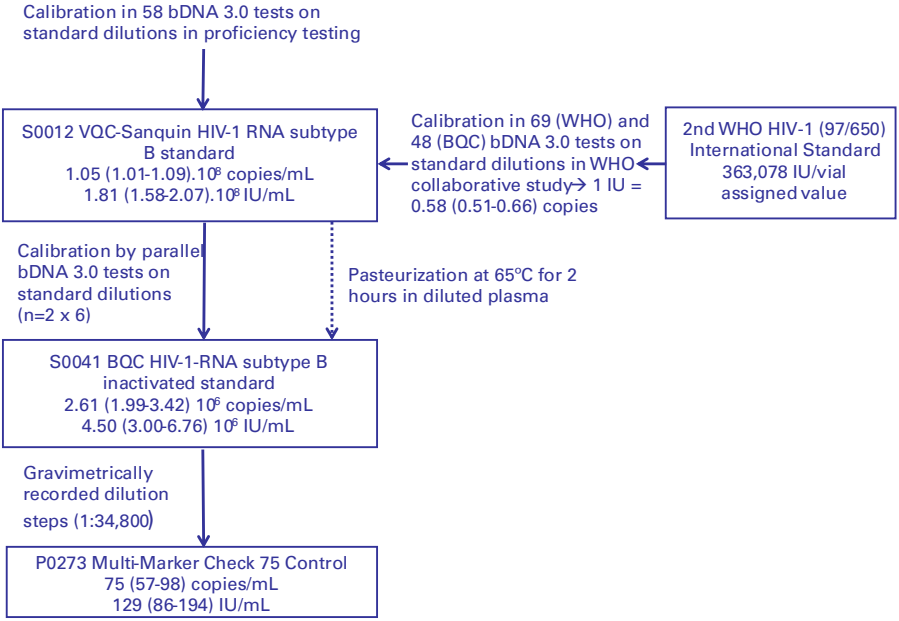


Figure 3. Traceability chain between P0273 Multi-Marker Check 75 control, inactivated and native VQC-Sanquin and WHO International Standards for HIV-1 RNA



Stability of standards and run control

The long-term stability of the liquid frozen S0043 HBV-DNA, S0109 HCV-RNA and S0041 HIV-1 RNA standards stored at ≤−65 °C has been firmly established[32]; hence the stock solutions from which the run control is prepared have shown to be stable at −80 °C in the BioQControl storage facilities. Real time stability experiments using quantitative NAT assays showed less than 10% degradation of HCV-RNA and HIV-1 RNA (and no degradation of HBV-DNA) in ViraQ Check Controls (and in standard dilutions of higher concentrations) when stored at −30 °C[32]. Hence, it can be guaranteed that the run control is still functional and should generate a reactivity rate greater than 99.9% when stored at −30 °C and used before the expiration date (two years after preparation of the run control batch)[19,31].

Kit Contents (materials provided)

The run control contains human plasma without preservatives and is provided in two formats as detailed in Table 5.

Table 5. Description of P0273 ViraQ Multi-Marker Check 75# kit formats and contents.

Cat. Code	GTIN/UDI-DI code^	Quantity	Tube size	Sample volume	Secondary Packaging
P0273/01	8718719830109	60 tubes	10 mL	1.5 mL (+overfill)	Rack in box
P0273/02	8718719833810	10 tubes	10 mL	1.5 mL (+overfill)	Plastic zip bag

^ Global Trade Item Number = Unique Device Identification - Device Identifier (UDI-DI) code,
# Global Model Number (GMN) = Basic UDI-DI code for P0273 is 871871983P0273EY

To facilitate automation the run control is presented in a polypropylene tube with screw cap comparable in size to vacutainer tubes used for donor sample collection. In addition, the label includes a barcode identifying the product, sequential batch number and multi-marker: MM. The barcode of each run control tube is unique and can be read by the Roche cobas 5800/6800/8800 instrument.

Materials required but not provided

The test kits and liquid handling devices by the NAT manufacturer as specified in Table 1, a water bath of 37 °C for quickly thawing of run control and a vortex instrument for thorough mixing of samples are not provided.

Storage instructions

The run controls should be stored at or below −30 °C for a maximum of two years after preparation of the run control batch and not be used after the expiry date[32]. Once thawed the run control samples should be used within 8 hours. During this period, when the run control is not in use, store sample at 2-8 °C[32]. 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

Although P0273 ViraQ Multi-Marker Check 75 control contains inactivated viral particles[2-8] the plasma may still be potentially bio-hazardous. The matrix is prepared from human blood plasma that tested negative for blood borne viruses (HBV-DNA, HCV-RNA, HIV-RNA, HBsAg, anti-HBc, anti-HBs, 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. The run control should only be used by trained

laboratory workers who are aware of the potential risk of infectious agents in human plasma samples and take the necessary precautions. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[33,34]. In case of a serious incident related to the use of the run control, report it immediately to BioQControl's technical support group (tel: +31 (0)72 2020 730; e-mail: info@bioqcontrol.com) and to the competent authority of the country/EU Member State where the incident occurred.

- 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 controls are 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.

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 ice clot has disappeared remove the run control tube from the water bath.
- Before testing allow the run control tube to adapt to room temperature.
- Mix the run control tube thoroughly prior to use with a vortex instrument.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time from thawing until testing of the control samples.
- Test run control sample within 8 hours after thawing.
- After thawing when not in use, store run control tube at 2-8 °C.

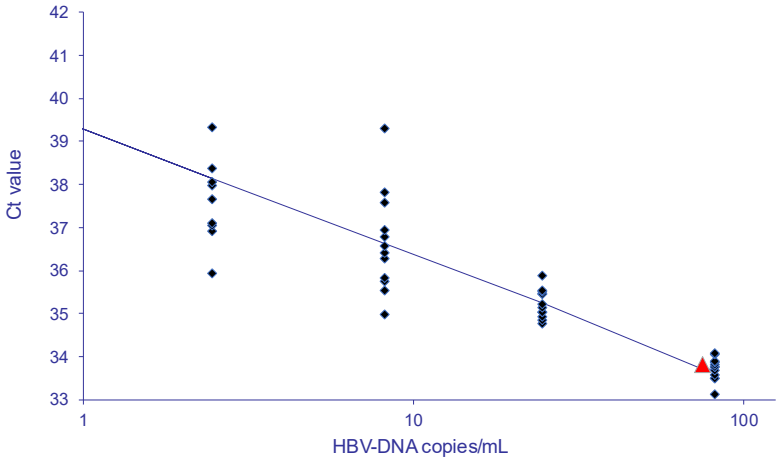
Performance Characteristics

The cobas MPX assay gives qualitative results (reactive, nonreactive or invalid) and if reactive a Cycle threshold (Ct) value is generated for HIV, HBV and HCV as well as for an internal cobas MPX control. P0273 ViraQ Multi-Marker Check 75 Control is expected to be reactive for all three markers in more than 99.9% of cobas MPX test runs.

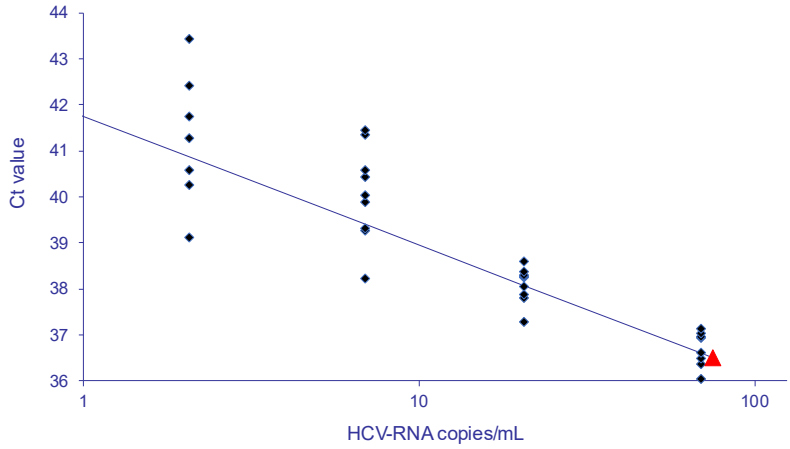
The positioning of P0273 ViraQ Multi-Marker Check 75 control is near the Poisson detection endpoint range of the cobas MPX assay but at enough distance to guarantee sufficient reproducibility of Ct values. This has been demonstrated by testing dilution panels of the inactivated viral standards used for preparation of the run control (Figure 4). At the concentration of 75 copies/mL a normal distribution of Ct values can be assumed for statistical analysis (Figure 5). Hence for monitoring the performance of the cobas MPX assay one can use untransformed Ct values in a Levey-Jennings QC chart for trend analysis.

Figure 4. Distributions of Ct values on inactivated BioQControl standard dilutions in cobas MPX assay. The red triangle is the run control level at 75 copies/mL.

S0043 HBV-DNA genotype A standard (dilution panel P0031)



S0109 HCV-RNA genotype 3 standard (dilution panel P0020)



S0041 HIV-1 subtype B standard (dilution panel P0026)

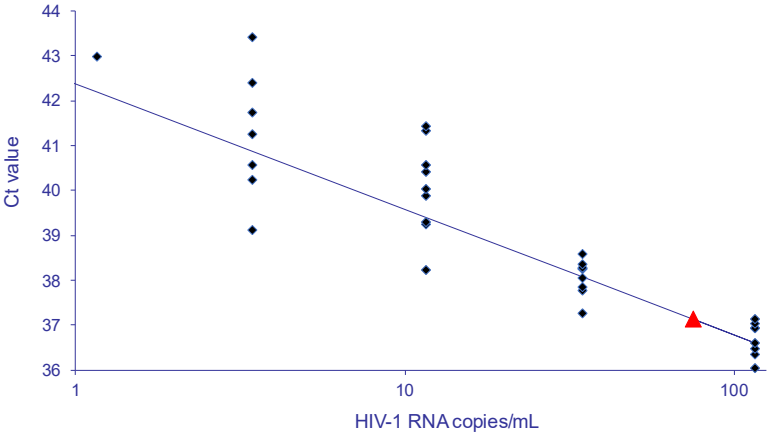
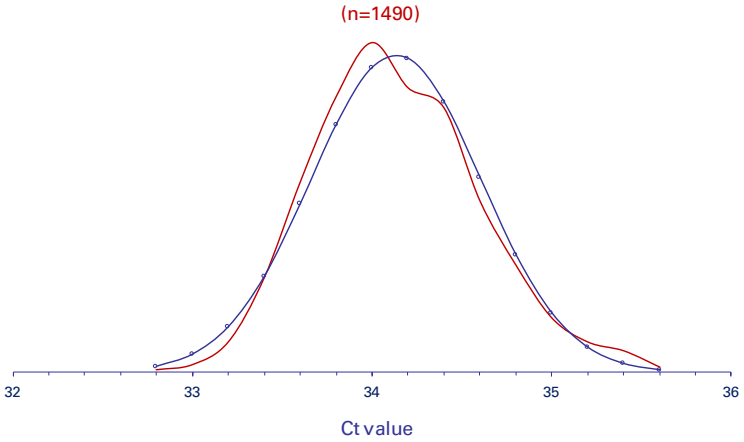


Figure 5. Example of normal distribution of Ct values on inactivated HBV-DNA concentration of 75 copies/mL in P0273 ViraQ Multi-Marker Control. The red line gives the actual distribution of Ct values in 1490 cobas MPX test runs, and the blue line represents a normal distribution.



Expected assay response values on run control

The expected results for the P0273 ViraQ Multi-Marker Check 75 Control are as follows:

1. HIV-1 RNA range Ct values: 33.3 – 35.7
2. HBV-DNA range Ct values: 33.1 – 35.6
3. HCV-RNA range Ct values: 35.8 – 38.6

Each cobas MPX reagent lot appears to have its own dose response line and distribution of Ct values on P0273 ViraQ Multi-Marker Controls. This depends on the analytical sensitivity of the cobas MPX reagent lots that are in use. Thus, it cannot be guaranteed that the assay response values will always fall within these ranges. P0273 ViraQ Multi-marker Check 75 run control serves as an independent standard for monitoring the reproducibility of the analytical sensitivity of cobas MPX test runs and reagent lots over time.

Interpretation of Results

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. Subsequent test runs can be analysed by appropriate statistical approaches on the Ct values obtained on the external control samples. A software system (DataQ Analytics) is available via the website www.bioqcontrol.com for entering Ct values and generating a statistical report with the following calculations for preparing a Levey-Jennings Chart: Use Student-t-values belonging to the 95% and 99% predictive intervals (Table 6). Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: Average – 99% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 95% Lower limit: Average – 95% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 99% Upper limit: Average + 99% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 95% Upper limit: Average + 95% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$

Table 6. Student t value to calculate predictive interval for 30 test runs.

Run (n)	t-value at 95% int.	t-value at 99% int.
30	2.045	2.756
infinite	1.960	2.576

Test the run control at least 30 times during the reference period. Since Ct values are used no log transformation is required and predictive intervals can be calculated from the arithmetic mean and standard deviation (SD). The Levey-Jennings chart (Figure 6) is designed to identify individual aberrant values outside the 95% and 99% predictive intervals. By collecting additional data, the chart characteristics may be updated.

Use Nelson rules[35] to identify deviations in the Levey-Jennings trend analysis.

The cobas MPX test results on P0273 ViraQ Multi-Marker Check 75 control are expected to be reactive in 99.9-100% of cases according to probit analysis on the inactivated S0043 HBV, S0109 HCV and S0041 HIV-1 standard dilutions in the P0031, P0020 and P0026 reference panels. Repeatedly non-reactive results are indicative of a significantly reduced analytical sensitivity of the NAT system. A single event of a non-reactive result is however possible without deterioration of the test system and can be explained by Poisson distribution.

Levey-Jennings Chart

The Ct values obtained in consecutive cobas MPX test runs for HIV-RNA, HBV-DNA, and HCV-RNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ Analytics system available on www.bioqcontrol.com as shown in the example of Figure 6.

Table 7 summarizes the Levey-Jennings chart parameters in Figure 6, which were deduced from the Ct values reported on three P0273 ViraQ Multi-Marker batches (B4264-008, -009 and -010) by different laboratories over a period of ten months.

Table 7. Average Ct values with 95% and 99% predictive intervals as found on three batches of P0273 ViraQ Multi-Marker Check 75 Control in 1177 cobas MPX test runs in the period from 4-7-2023 to 18-4-2024 (data in Figure 6)

Marker	n	Average Ct	95% pred. int. Ct	99% pred. int. Ct
HIV-RNA	1177	34.32	33.47 – 35.17	33.20 – 35.44
HBV-DNA	1164	34.11	33.33 – 34.88	33.08 – 35.13
HCV-RNA	1176	36.85	35.88 – 37.82	35.58 – 38.13

Figure 6. Levey-Jennings QC charts of cobas MPX assay Ct values for HIV-1 RNA, HBV-DNA and HCV-RNA on three batches of P0273 ViraQ Multi-Marker Check 75 Control.

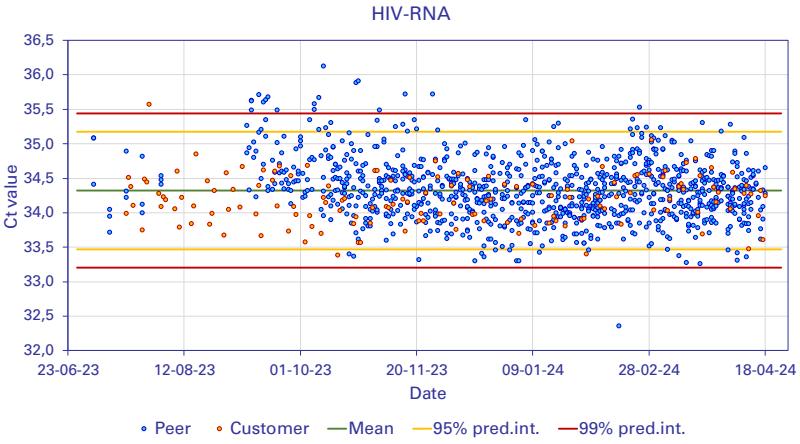
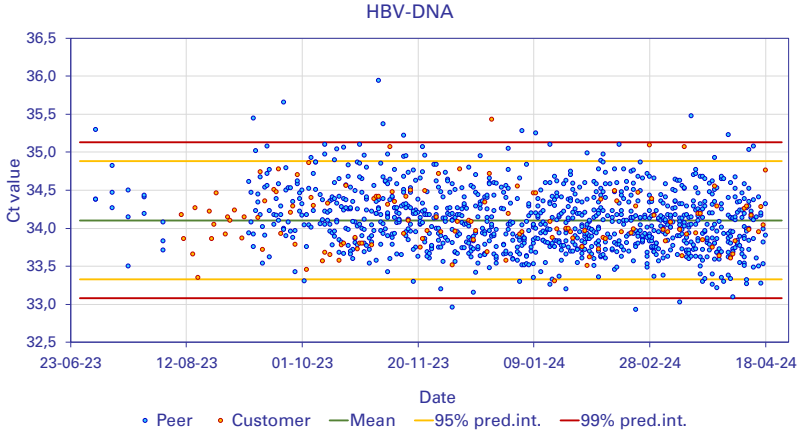
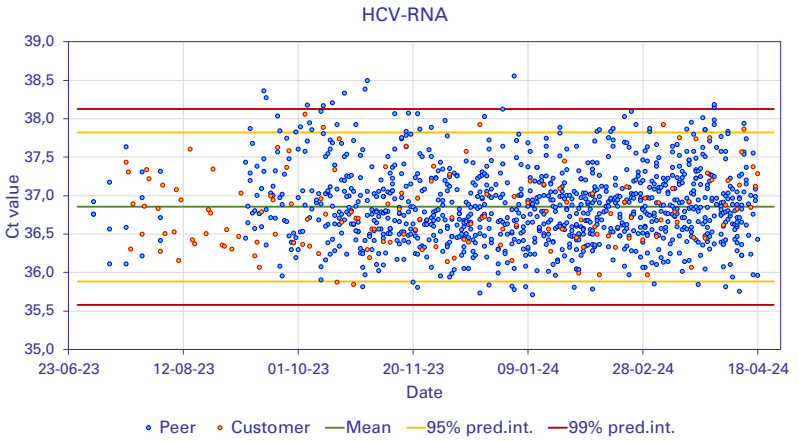


Figure 6 continued





Post-market performance follow-up of run control batches

One can use P0273 ViraQ Multi-Marker Check 75 in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the Ct values on different run control batches, cobas MPX reagent lots or cobas 5800/6800/8800 instruments. For example, Table 8, 9 and 10 compare the variation in mean Ct values on P0273 ViraQ Multi-Marker batches and cobas MPX reagent lots for HIV-1 RNA, HBV-DNA and HCV-RNA respectively. There is some variation in Ct values on consecutive cobas MPX reagent lots and run control batches as is visualized in Figure 7 showing the HBV-DNA results over a period of seven years (same data as in Table 9).

In 2022 the HCV-RNA concentration in the P0273 ViraQ Multi-Marker run control was increased 1.67-fold after recalibration of the HCV standard using multiple quantitative

methods. The impact of increasing the HCV-RNA concentration from 45 to 75 copies/mL on the distribution of Ct values is shown in Figure 8. The distribution curve of Ct values on the first five P0273 run control batches of 45 copies/mL was skewed to the right since several outlier Ct values above 40.0 were found due to Poisson distribution. After adjustment of the HCV-RNA concentration to 75 copies/mL a normal distribution was found as was observed for HIV-RNA and HBV-DNA (Figure 9). Over seven years nonreactive results have not been reported on ten P0273 ViraQ Multi-Marker Check 75 run control batches.

Table 8. Comparison of mean HIV-RNA Ct values (and 95% CI) on P0273 ViraQ Multi-Marker Check 75 Control batches and between consecutive cobas MPX reagent lots

P0273 ViraQ run control batch	cobas MPX reagent lot	n	Average HIV Ct (95% CI) on MPX reagent lot	n	Average HIV Ct (95% CI) on run control batch
B4264-001	J11224	13	34.15 (34.00 - 34.30)	**13**	**34.15 (34.00 - 34.30)**
B4264-002	284023	23	34.17 (34.04 - 34.30)	**185**	**34.22 (34.17 - 34.27)**
	296869	45	34.17 (34.05 - 34.28)		
	312109	68	34.26 (34.19 - 34.33)		
	E08514	19	34.22 (34.06 - 34.38)		
	(blank)	30	34.25 (34.11 - 34.40)		
B4264-003	E08514	50	33.98 (33.90 - 34.07)	**671**	**34.17 (34.14 - 34.20)**
	E16887	93	34.11 (34.03 - 34.18)		
	E24868	50	34.10 (34.01 - 34.18)		
	E29921	81	34.05 (33.97 - 34.12)		
	E31293	20	34.04 (33.87 - 34.22)		
	E31294	75	34.24 (34.14 - 34.33)		
	E31351	56	33.95 (33.87 - 34.04)		
	F23147	90	34.17 (34.10 - 34.25)		
	G15705	91	34.46 (34.39 - 34.53)		
	G30218	55	34.36 (34.24 - 34.49)		
	Y28593	10	34.16 (33.83 - 34.49)		
B4264-004	*F23147*	*7*	*34.05 (33.63 - 34.48)*	**209**	**34.30 (34.26 - 34.35)**
	G15705	86	34.35 (34.28 - 34.41)		
	G30218	71	34.24 (34.17 - 34.31)		
	H09363	45	34.36 (34.25 - 34.47)		
B4264-005	G15705	39	34.39 (34.26 - 34.52)	**226**	**34.23 (34.18 - 34.28)**
	H09363	48	34.37 (34.27 - 34.46)		
	H14558	131	34.14 (34.08 - 34.21)		
	H28856	*5*	*34.15 (33.80 - 34.50)*		
	H33238	*3*	*34.07 (33.67 - 34.47)*		
B4264-006	H09363	26	34.11 (33.99 - 34.23)	**114**	**34.19 (34.13 - 34.25)**
	H14558	13	34.10 (33.86 - 34.34)		
	H29158	57	34.24 (34.16 - 34.33)		
	H33238	19	34.21 (34.06 - 34.37)		
B4264-007	H09363	55	34.12 (34.01 - 34.23)	**55**	**34.12 (34.01 - 34.23)**
B4264-008	H29158	41	34.34 (34.23 - 34.44)	**191**	**34.27 (34.21 - 34.32)**
	H33238	14	34.41 (34.16 - 34.66)		
	J11224	12	34.56 (34.29 - 34.84)		
	J23629	122	34.20 (34.13 - 34.27)		

(continued)

Table 8 *(continued)*

P0273 ViraQ run control batch	cobas MPX reagent lot	n	Average HIV Ct (95% CI) on MPX reagent lot	n	Average HIV Ct (95% CI) on run control batch
B4264-009	J18176	65	34.82 (34.71 - 34.93)	**724**	**34.42 (34.39 - 34.46)**
	J23629	247	34.49 (34.43 - 34.55)		
	K00715	325	34.35 (34.31 - 34.40)		
	(blank)	87	34.21 (34.14 - 34.28)		
B4264-010	*J23629*	*4*	*34.12 (33.59 - 34.65)*	**377**	**34.17 (34.13 - 34.20)**
	J30027	14	34.19 (34.00 - 34.39)		
	K00715	79	34.07 (34.00 - 34.14)		
	K05368	17	34.23 (34.01 - 34.45)		
	K07140	193	34.15 (34.10 - 34.20)		
	K20606	61	34.32 (34.22 - 34.41)		
	(blank)	10	34.16 (33.89 - 34.44)		
All	**All**	**2765**	**34.26 (34.25 - 34.28)**	**2765**	**34.26 (34.25 - 34.28)**

Table 9. Comparison of mean HBV-DNA Ct values (and 95% CI) on P0273 ViraQ Multi-Marker Check 75 Control batches and between consecutive cobas MPX reagent lots

P0273 ViraQ run control batch	cobas MPX reagent lot	n	Average HBV Ct (95% CI) on MPX reagent lot	n	Average HBV Ct (95% CI) on run control batch
B4264-001	J11224	13	34.33 (33.30 - 35.36)	**13**	34.33 (34.09 - 34.57)
B4264-002	284023	23	34.21 (34.04 - 34.38)	**185**	**34.30 (34.24 - 34.37)**
	296869	45	34.36 (34.24 - 34.49)		
	312109	68	34.37 (34.28 - 34.47)		
	E08514	19	34.46 (34.19 - 34.72)		
	(blank)	30	34.04 (33.89 - 34.19)		
B4264-003	E08514	50	34.27 (34.17 - 34.38)	**673**	**34.08 (34.04 - 34.11)**
	E16887	93	34.42 (34.33 - 34.50)		
	E24868	50	34.12 (34.02 - 34.22)		
	E29921	81	34.05 (33.95 - 34.14)		
	E31293	20	34.38 (34.18 - 34.57)		
	E31294	78	33.88 (33.78 - 33.99)		
	E31351	56	33.87 (33.77 - 33.96)		
	F23147	89	33.86 (33.77 - 33.94)		
	G15705	91	34.13 (34.05 - 34.21)		
	G30218	55	33.95 (33.83 - 34.06)		
	Y28593	10	34.32 (34.05 - 34.59)		
B4264-004	*F23147*	*7*	*33.77 (33.49 - 34.04)*	**222**	**34.30 (34.23 - 34.37)**
	G15705	99	34.39 (34.30 - 34.49)		
	G30218	71	34.41 (34.29 - 34.53)		
	H09363	45	34.01 (33.88 - 34.14)		
B4264-005	G15705	38	34.24 (34.03 - 34.44)	**227**	**33.98 (33.91 - 34.05)**
	H09363	47	34.17 (34.02 - 34.31)		
	H14558	134	33.85 (33.77 - 33.92)		
	H28856	*5*	*34.02 (33.37 - 34.67)*		
	H33238	*3*	*33.71 (32.54 - 34.89)*		
B4264-006	H09363	27	34.02 (33.92 - 34.13)	**115**	**34.04 (33.97 - 34.10)**
	H14558	12	34.09 (33.79 - 34.39)		
	H29158	57	34.06 (33.96 - 34.16)		
	H33238	19	33.96 (33.78 - 34.14)		

(continued)

Table 9 *(continued)*

P0273 ViraQ run control batch	cobas MPX reagent lot	n	Average HBV Ct (95% CI) on MPX reagent lot	n	Average HBV Ct (95% CI) on run control batch
B4264-007	H09363	55	34.33 (33.35 - 35.30)	**55**	**34.33 (34.21 - 34.44)**
B4264-008	H29158	41	34.08 (33.95 - 34.22)	**189**	**34.13 (34.07 - 34.18)**
	H33238	14	34.35 (34.13 - 34.58)		
	J11224	12	34.23 (34.07 - 34.40)		
	J23629	122	34.11 (34.04 - 34.18)		
B4264-009	J18176	65	34.31 (34.21 - 34.42)	**724**	**34.12 (34.09 - 34.14)**
	J23629	247	34.11 (34.06 - 34.16)		
	K00715	325	34.05 (34.00 - 34.09)		
	(blank)	87	34.23 (34.15 - 34.32)		
B4264-010	*J23629*	*4*	*34.13 (33.11 - 35.15)*	**370**	**34.06 (34.02 - 34.10)**
	J30027	14	34.11 (33.80 - 34.43)		
	K00715	79	34.04 (33.95 - 34.12)		
	K05368	15	33.82 (33.57 - 34.06)		
	K07140	188	34.12 (34.07 - 34.17)		
	K20606	61	33.96 (33.86 - 34.07)		
	(blank)	10	34.03 (33.80 - 34.27)		
All	**All**	**2774**	**34.12 (34.10 - 34.13)**	**2774**	**34.12 (34.10 - 34.13)**

Figure 7. Average HBV-DNA Ct values (and 95% confidence interval (CI)) in cobas MPX assay reagent lots on P0273 ViraQ Multi-Marker Check 75 run control batches over a period of seven years (data of table 9).

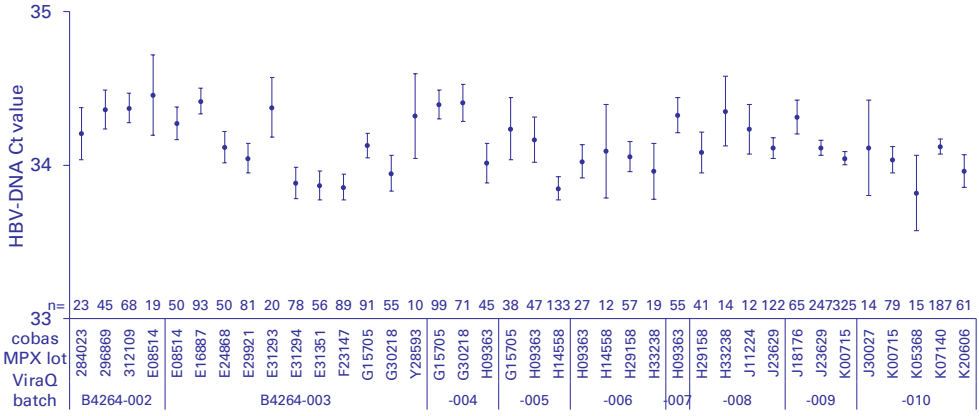


Table 10. Comparison of mean HCV-RNA Ct values (and 95% CI) on P0273 ViraQ Multi-Marker Check 75 Control batches and between consecutive cobas MPX reagent lots

P0273 ViraQ run control batch	cobas MPX reagent lot	n	Average HCV Ct (95% CI) on MPX reagent lot	n	Average HCV Ct (95% CI) on run control batch
B4264-006	H09363	27	36.62 (36.46 - 36.79)	**114**	**36.73 (36.64 - 36.82)**
	H14558	12	36.43 (36.24 - 36.63)		
	H29158	57	36.90 (36.77 - 37.03)		
	H33238	19	36.55 (36.29 - 36.80)		
B4264-007	H09363	55	36.43 (36.32 - 36.53)	**55**	**36.43 (36.32 - 36.53)**
B4264-008	H29158	41	36.99 (36.84 - 37.13)	**191**	**36.83 (36.76 - 36.90)**
	H33238	14	36.78 (36.51 - 37.05)		
	J11224	12	37.30 (36.81 - 37.80)		
	J23629	122	36.74 (36.65 - 36.82)		
B4264-009	J18176	65	37.02 (36.88 - 37.15)	**723**	**36.90 (36.87 - 36.94)**
	J23629	247	36.98 (36.92 - 37.04)		
	K00715	324	36.88 (36.83 - 36.94)		
	(blank)	87	36.68 (36.59 - 36.77)		
B4264-010	*J23629*	*4*	*36.96 (36.14 - 37.78)*	**377**	**36.80 (36.76 - 36.85)**
	J30027	14	36.49 (36.26 - 36.72)		
	K00715	79	36.65 (36.56 - 36.74)		
	K05368	17	36.88 (36.72 - 37.04)		
	K07140	194	36.84 (36.77 - 36.90)		
	K20606	61	36.99 (36.86 - 37.11)		
	(blank)	*9*	*36.43 (36.07 - 36.79)*		
All	**All**	**1460**	**36.84 (36.81 - 36.86)**	**1460**	**36.84 (36.81 - 36.86)**

Figure 8. Comparison of distribution of HCV Ct values in cobas MPX assay on P0273 ViraQ Multi-Marker Check 75 run control batches containing 45 and 75 copies/mL of HCV-RNA

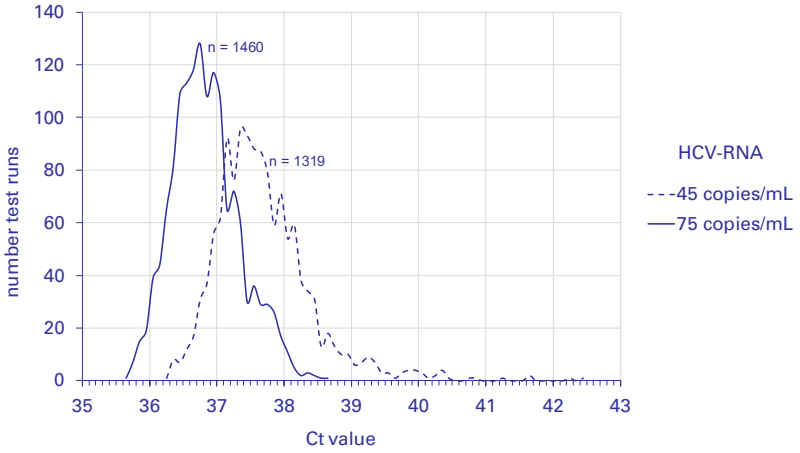
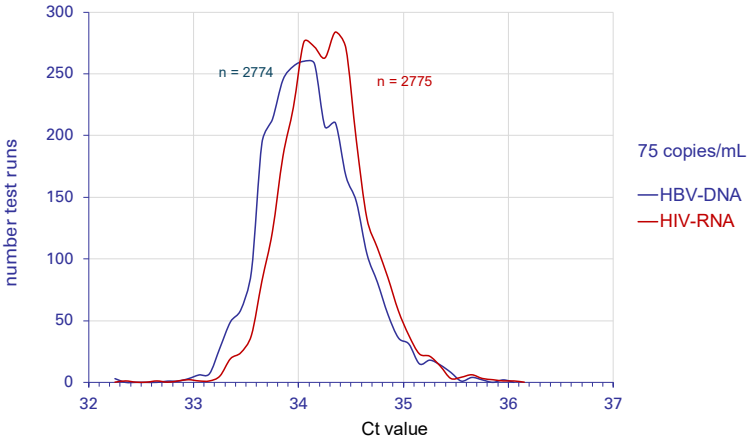


Figure 9. Distribution of HBV-DNA and HIV-1 RNA Ct values in cobas MPX assay on P0273 ViraQ Multi-Marker Check 75 run control batches



Limitations

- P0273 ViraQ Multi-Marker Check 75 Control cannot be used to evaluate the analytical or diagnostic sensitivity of the cobas MPX assay or other multi-dye real time PCR methods (although a significant reduction of analytical sensitivity of the NAT system can become apparent with repeated occurrence of non-reactive results or an upward shift in Ct values).
- P0273 ViraQ Multi-Marker Check 75 Control must not be substituted for the mandatory controls or calibrators provided with NAT test kits for calculating the cut-off and/or criteria for releasing test results.
- The Poisson distribution in samples with low virus concentrations cannot guarantee that 100% reactive results will always be found on P0273 ViraQ Multi-Marker Check 75 Control in NAT blood screening assays. Therefore, the response values on the run controls should not be used for a decision to accept or reject the test run.
- The expected distributions of Ct values on P0273 ViraQ Multi-Marker Check 75 Control that are presented in this package insert were based on performance evaluation studies involving a limited number of run control batches and NAT reagent lots. It cannot be guaranteed that slightly different results will not be found on other run control batches, cobas MPX reagent lots or cobas instruments.
- It cannot be excluded that some variation exists between the performance of consecutive batches of the P0273 ViraQ Multi-Marker Check 75 control due to matrix effects or other manufacturing variables.

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Revision history

Date	Version	Description of change
August 2022	V9.0	Performance evaluation data added, including data on the new composition following the recalibration of the HCV-RNA standard.
August 2022	V9.0	Changed UDI-DI code of P0273/02 in Table 2.
June 2024	V10.0	Post-market performance follow-up data and expected assay response values added. Traceability figures (Figures 1-3) updated. Tables 2-4 updated with detection limits on viral standards in TaqScreen 1.0. Updated section for Reagent preparation. Added limitation regarding batch performance variation due to matrix effects or other manufacturing variables.
December 2024	V11.0	Tables 2-4 updated with detection limits on viral standards in cobas MPX. Traceability figures (Figures 1-3) updated. Corrections in calculations of post-market performance follow-up data, including Figure 7.
April 2025	V11.1	Corrected UDI-DI code of P0273/02 in Table 2.
January 2026	V11.2	Figures 1-3 updated to align with production optimization. Corrections in calculation methods description.
July 2026	V11.3	Contact information added for reporting serious incidents in chapter “Warning and precautions”. Revision history table added. Symbol for positive control added. Expected assay response values updated.

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