P0043 HIV-1 RNA subtype B Quant (50 to 250,000 copies/mL)

RUO

REF P0043

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

P0043 HIV-1 RNA subtype B Quant (50 to 250,000 copies/mL) provides a consistent standard across nucleic acid amplification technology (NAT) methods, enabling diagnostic laboratories and *in vitro* Diagnostics (IVD) manufacturers to assess the linearity and accuracy of NAT systems for quantitative detection of human immunodeficiency virus type 1 (HIV-1) RNA in plasma samples. This product can be used with amplification methods, including (real time) polymerase chain reaction (PCR) and transcription mediated amplification (TMA) assays. It also can be used as an independent calibration panel for quantification of HIV-1 RNA concentrations in donor or patient samples. This product is for research use only.

Key to Symbols Used


Manufacturer

Lot number

Catalogue number

Store below -65°C

Research use only

Biological substance category B


Date of manufacturing

Contents


Caution


Read instructions for use

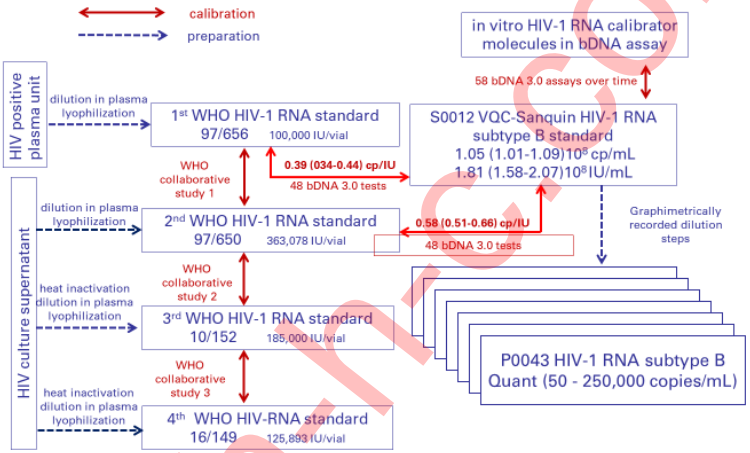
Summary and explanation

In the late 1990s the liquid frozen VQC-Sanquin HIV-1 subtype B standard was among the first reference materials for evaluation of NAT methods[1,2] and used as candidate material in WHO collaborative studies to establish the 1st and 2nd International HIV-1 standards[3]. We used the bDNA 3.0 assay as reference method[4] for calibration in copies/mL and the data from this method in the WHO collaborative study showed a drift in the amount of virus per International Unit (IU) from 0.39 (0.34-0.44) to 0.58 (0.51-0.66) copies/IU when the 1st WHO HIV-1 97/656 standard was replaced by the 2nd WHO HIV-1 97/650 standard[5]. Later the 3rd and 4th WHO HIV-1 subtype standards have been introduced and recent calibration studies against the VQC-Sanquin standard indicate that currently the conversion factor is 0.25 (0.15-0.41) copies/IU when the Abbott RealTime assay was used[6]. Thorough stability studies have demonstrated that the primary VQC-Sanquin HIV-1 subtype B standard is completely stable for more than two decades when stored below at -65˚C[7]. In the period between 1998 and 2004 the quantitative methods reported similar copy numbers on the VQC-Sanquin standard as in 2018 (table 1 and 2)[6]. Hence the liquid frozen primary S0012 HIV-1 subtype B standard calibrated in copies/mL can function as a second anchor in addition to the WHO standards calibrated in IU/mL. The S0012 VQC-Sanquin HIV-1 RNA subtype B standard was used for preparation of the P0043 linearity panel composed of dilutions ranging from 250,000 to 50 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 this S0012 standard has been extensively calibrated in both copies and IUs[5] it can be used as an independent linearity panel for testing the accuracy and precision of quantitative HIV-1 NAT methods.

Traceability to HIV-1 RNA copies and International Units

Figure 1 shows the traceability chain between the HIV-1 RNA subtype B linearity panel, the primary S0012 VQC-Sanquin subtype B standard and the 1st HIV-1 97/656 standard and 2nd WHO HIV-1 97/650 International Standards.

Figure 1. Traceability chain between P0043 HIV-1 RNA subtype B linearity panel, the S0012 VQC-Sanquin HIV-1 standard and the WHO International Standards



Calibration of S0012 VQC-Sanquin HIV-1 subtype B standard in copies/mL

The viral concentration in the S0012 VQC-Sanquin HIV-1 RNA subtype B standard was established by laboratories testing dilutions of these standards in the VQC proficiency program organized between 1996 and 2004. Table 1 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 method[4] for quantification and assign the value of 1.05 (1.01-1.09).10^8 copies/mL to the undiluted S0012 VQC-Sanquin standard[5].

More recently in 2018 a dilution of 1000 copies/mL of this VQC Sanquin subtype B standard (P0327 ViraQ HIV-1 Quant 1000 run control) was tested in 4 runs of 6 replicate viral load (VL) measurements by 5 laboratories using different quantitative methods[6]. When comparing the quantitative results obtained two decades later (table 2) with those in the early days of NAT (table 1) the results were comparable as was predicted by our stability studies of the liquid frozen S0012 HIV-1 subtype B standard stored at -80°C[7]. However there were still significant differences in the copy numbers reported by the currrent VL assays with geometric mean values varying between 1084 to 2505 copies/mL (table 2).

Table 1: Quantification of S0012 VQC-Sanquin HIV-1 RNA subtype B 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
Abbott LCx	18	1819	(1752-1895)
Chiron bDNA 1.0	13	449	(188-1067)
Bayer bDNA 2.0	57	1038	(1000-1086)
Siemens bDNA 3.0	**58**	**1000**	**(962-1038)**
Organon Teknika NucliSens	119	2295	(2171-2419)
Organon Teknika QT-NASBA	366	3162	(3057-3267)
Roche Amplicor Monitor V1.0	437	2143	(2095-2181)
Roche Amplicor. Monitor mixed primers	63	1457	(1390-1514)
Roche Amplicor Monitor V1.5	316	1295	(1238-1352)
Roche Amplicor Monitor Ultra	142	1181	(1124-1229)

Table 2. Quantification of 1000 copies/mL samples of S0012 VQC-Sanquin HIV-1 RNA subtype B standard (P0327 ViraQ HIV-1 Quant 1000) by different laboratories (Viral load assays performed in 2018)[6].

Assay	n	geomean cp/mL	(95% CI) cp/mL
Abbott m2000 RealTime Assay m2000	24	1084	(784-1572)
Hologic Aprima	24	1616	(1324-1973)
Roche CAP/CTM	24	1277	(892-1828)
Cepheid Xpert	24	2502	(1333-3465)
BioMerieux NucliSens EasyQ	24	1110	(690-1900)

Calibration of S0012 VQC-Sanquin HIV-1 subtype B standard in IU/mL

Dr. H. Holmes (NIBSC, Potterbar, UK) kindly shared the raw data of the laboratories that participated in the first WHO collaborative study[3] in which the 1st and 2nd WHO standard were compared with the VQC-Sanquin standard. The data in table 3 show that the calibration results are dependent on the quantitative NAT method. When using the bDNA 3.0 assay as reference method there was a shift in the conversion factor from 0.39 (0.34-0.44) copies/IU to 0.58 (0.51-0.66) copies/IU when the 1st WHO WHO 97/656 standard was replaced by the 2nd WHO 97/650 standard, which may be due to under-detection of the 2nd WHO standard by the Organon Teknika NucliSens method used at that time.

More recently the VQC-Sanquin standard was recalibrated against the 3rd and 4th WHO standard in three dilutions varying between 1000 and 10.000 copies/mL (6 replicate Abbott RealTime VL tests per dilution) and the results from the parallel line analysis indicate that the conversion factor nowadays is 0.25 copies/IU (table 4)[6]. With the replacement of the 2nd and 3rd WHO standard there seems to have been a drift to a 40% lower amount of virus per IU. When analysing quantitative data in the WHO collaborative study report of the 4th WHO HIV-1 standard also lower copy numbers (69-89%) were reported on the 4th than on the 3rd WHO standard by the quantitative NAT methods used in the participating laboratories[8].

Table 3. Calibration of VQC-Sanquin HIV-RNA subtype B standard on the first (97/656) and second (97/650) WHO HIV-1 RNA subtype B standards (containing 100,000 and 363,078 IU per ampoule respectively) as calculated from individual quantitative assays on standard dilutions with five methods as reported by the laboratories participating in the first WHO collaborative study[3]

	n assays			copies/IU on 1st WHO (97/656) standard		copies/IU on 2nd WHO (97/650) standard	
	1st WHO	2nd WHO	VQC-Sanquin	mean	(95%CI)	mean	(95%CI)
Abbott LCx	14	15	14	0.76	(0.60-0.96)	0.69	(0.56-0.86)
Roche Amplicor Monitor	125	134	112	0.70	(0.60-0.81)	0.93	(0.80-1.08)
Siemens bDNA 3.0	64	69	48	0.39	(0.34-0.44)	0.58	(0.51-0.66)
Organon Teknika NucliSens	46	51	36	0.80	(0.69-0.92)	0.43	(0.36-0.50)
Roche Amplicor Monitor Ultra	16	15	11	0.51	(0.27-0.95)	0.86	(0.49-1.51)

Table 4. Recalibration of VQC-Sanquin standard against 3rd and 4th WHO standard in Abbott realTime assay.

HIV-1 Standard	Nominal value	n	copies/mL	copies/IU (95% CI)
VQC-Sanquin	1000 copies/mL	18	944 (698-1276)	
2nd WHO 97/650	1000 IU/mL	6	392 (266-577)	0.41 (0.27-0.63)
3rd WHO 10/152	1000 IU/mL	18	291 (220-577)	0.31 (0.21-0.45)
4th WHO 16/149	1000 IU/mL	18	236 (156-356)	0.25 (0.15-0.41)

Stability of HIV standards and reference panels

The long term stability of the liquid frozen HIV-1 subtype B standard stored at ≤65˚C has been firmly established[7]; hence the stock solutions from which the reference panels are prepared have shown to be stable for more than two decades in the BQC storage facilities. Real time stability experiments using quantitative NAT assays showed no degradation of HIV-RNA reference panels and controls when stored below -65˚C[7]. Hence, it can be guaranteed that the reference panels are stable when stored below -65˚C.

Materials Provided

Seven (7) panel members; polypropylene tubes (7 mL) with screw caps, each containing 1.2 mL of plasma. The composition is given in table 5

Table 5. Composition of P0043 HIV-1 RNA subtype B linearity panel. Quantification in copies/mL and calibration in IU/mL against the 2nd WHO standard 97/650) was performed on S0012 standard dilutions in multiple replicate bDNA assays (table 1, table 3, figure 1).

Panel member	HIV-1 RNA copies/mL (95 % CI)	HIV-1 RNA IU/mL (95% CI)	Quantity (mL per vial)
1	2.50 (2.41-2.59).10^5	4.31 (3.77-4.93).10^5	1 x 1.2 mL
2	2.50 (2.41-2.59).10^4	4.31 (3.77-4.93).10^4	1 x 1.2 mL
3	1.00 (0.96-1.04).10^4	1.72 (1.50-1.97).10^4	1 x 1.2 mL
4	2.50 (2.41-2.59).10^3	4.31 (3.77-4.93).10^3	1 x 1.2 mL
5	1.00 (0.96-1.04).10^3	1.72 (1.50-1.97).10^3	1 x 1.2 mL
6	2.50 (2.41-2.59).10^2	4.31 (3.77-4.93).10^2	1 x 1.2 mL
7	5.00 (4.81-5.19).10^1	1.72 (1.50-1.97).10^2	1 x 1.2 mL

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage instructions

The run controls should be stored at or below -65˚C. Once thawed the panel members should be used within 8 hours. During this period, when not in use, store sample at 2-8 ˚C [7]. Do not refreeze the panel member after thawing to prevent formation of cryoprecipitates. Any panel member that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

P0043 HIV-1 RNA subtype B Quant (50 to 250,000 copies/mL) contains infectious HIV-1 virions and is infectious to humans. 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-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 reference panel is handled.
- Disinfect liquids, materials or spills with a 0.5% sodium hypochlorite solution or equivalent.
- Dispose of all materials and liquids used in the procedure as if they contained pathogenic agents.

Test procedure

- *Thaw the panel members quickly in a water bath at 37°C.*
- *Mix gently during thawing until contents are just thawed.*
- *Immediately after thawing remove the panel member tube from the water bath.*
- *Mix the panel member(s).*
- *Give a short spin in a centrifuge before releasing screw cap from vial.*
- *Minimise the time period from thawing until usage of the members.*
- *The panel member should be handled and tested in a manner identical to that of clinical specimens in the test procedure being evaluated.*
- *Do not refreeze panel members after thawing. When a panel member is tested multiple times it should be organized within 8 hours. When not placed in the robot store at 2-8°C.*

Interpretation of Results

Precision

Quantitative HIV-1 NAT methods report Ct values and/or HIV-1 RNA concentration in either copies/mL or IU/mL. The dilution factor between the subsequent panel members is exact (with less than 0.5% variation as gravimetrically recorded). As a consequence the distance in Ct value between panel members should be ^{2}log(dilution factor). For other quantitative results one should apply log transformation. On the log transformed results one can calculate precision assuming a normal distribution.

Accuracy

The panel members are quantified in copies/mL using previously used bDNA 3.0 assay as reference method[4]. The IU/mL values are directly traceable to the 2nd International Standard (97/650)[3,5]. The accuracy and precision of copy numbers reported by quantitative HIV-1-NAT methods used two decades ago is given in the table 1 and of those used nowadays in table 2.

Limitations

- P0043 HIV-1 RNA subtype B Quant (50 to 250,000 copies/mL) must not be substituted for the mandatory controls or calibrators provided with quantitative NAT test kits for calculating the lower limit of quantification, the HIV-1 RNA concentrations and/or criteria for releasing test results.
- The assigned quantification in IU/mL is traceable to the 2nd WHO HIV-1 97/650 standard and was found to be different in calibration experiments against the 1st, 3rd and 4th WHO standards. Therefore the P0043 HIV-1 RNA subtype B (50 to 250,000 copies/mL) linearity panel should not be used for establishing accuracy of quantitative NAT results expressed in IU/mL assigned to the current 4th WHO standard. For this purpose only dilutions of the WHO 16/149 International Standard can be used.
- The panel is for research use only

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