P0319 HIV-2 RNA subtype A Quant (10 to 100,000 copies/mL)

RUO

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

P0319 HIV-2 RNA subtype A Quant (100 to 100,000 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 2 (HIV-2) 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-2 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 mid1990s we established a series of tissue culture derived HIV-1 RNA standards and reference panels of different subtypes and circulating recombinant forms (CRFs) that have been used for comparison of the analytical sensitivity of NAT methods in the VQC proficiency studies. In addition reference panels for HIV-2 subtypes and HIV group O have been developed and used for NAT validation studies. These analytical sensitivity and linearity panels help ensure that NAT methods for detection of HIV-RNA are properly validated and that test results are consistent across IVD manufacturers, laboratories, operators, NAT platforms and assay versions.

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 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[4]. 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[5]. 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 -65°C[6]. In the period between 1998 and 2004 the quantitative methods reported similar copy numbers on the VQC-Sanquin standard as in 2018[5]. 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 HIV-1 standards of different subtypes have been calibrated in copies/mL against the primary VQC-Sanquin HIV-1 subtype B standard using multiple replicate bDNA 3.0 tests but when the Abbott Real Time assay was used for calibration against this subtype B standard the quantitative values were somewhat higher[7]. The latter method was also used for calibration of HIV goup O standards.

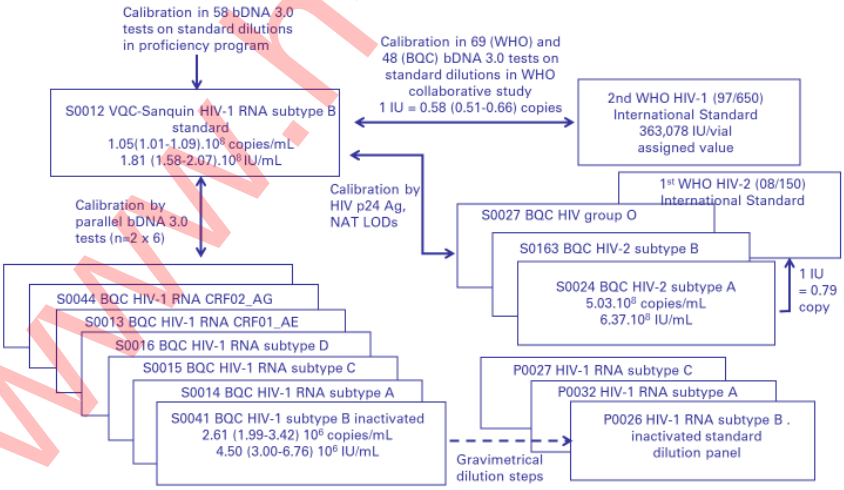
Originally HIV-2 subtypes had been calibrated against the HIV-1 subtype B standard in parallel line p24 antigen assays but later by probit analysis in Ultrio Elite and cobas MPX assays[7].

The S0024 HIV-2 RNA subtype A standard was used for preparation of the P0319 linearity panel composed of dilutions ranging from 100,000 to 10 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 S0024 standard has been calibrated in both copies and IUs it can be used as an independent linearity panel for testing the accuracy and precision of quantitative HIV-2 NAT methods.

Traceability to HIV-RNA copies and International Units

Figure 1 shows the traceability chain between the HIV-1 group M, group O and HIV-1 as well as HIV-2 subtype reference panels prepared from BioQControl (BQC) standards, the primary VQC-Sanquin HIV-1 subtype B standard and the first WHO HIV-1 and HIV-2 International Standards.

Figure 1. Traceability chain between HIV-RNA reference panels, BQC and VQC-Sanquin standards and WHO International Standards



Calibration of HIV-2 subtypes in copies/mL and IU/mL

The original quantification of the HIV-2 subtype A standard in copies/mL was based on comparison with the S0012 HIV-1 subtype B standard in parallel line p24 antigen testing using the Murex HIV-Ag assay. Later we adjusted the concentration based on probit analysis in the TaqScreen 1.0 and Ultrio Elite assay (table 1)[7]. In these assays we estimated a conversion factor of 0.79 copies/IU against the 1st WHO HIV-2 08/150 standard. It must be emphasized that we have not checked the conversion factor against the later WHO HIV-2 16/296 replacement standard in the current cobas MPX and Ultrio Elite assays. The S0163 HIV-2 subtype B standard was calibrated against the S0024 HIV-2 subtype A standard by replicate testing and comparison of Ct values in the cobas MPX assay[7].

Table 1. Calibration of HIV-2 subtype A and B standards in copies/mL[7]

HIV-2 RNA standards	subtype	copies/ mL	IU/vial	Calibration procedure
S0024 BQC	A	5.03 .10^8		Potency comparison against S0012 HIV-1 subtype B standard by: - 50% LODs in TaqScreen 1.0 - 50% LODs in Ultrio Elite - Murex p24 antigen parallel line ELISA
S0163 BQC	B	3.40. 10^8		Potency comparison against S0024 HIV-2 subtype A standard based on Ct values in cobas MPX assay (n=12 per standard)
2nd WHO 16/296	A		144200	WHO collaborative study

For preparation of the HIV-RNA reference panels, the HIV-RNA standards were diluted in a pool of plasma units that tested negative for viral markers by NAT and serology testing. Lot-to-lot consistency of the viral concentrations in the reference panel is ensured during manufacturing by gravimetrically recorded dilutions from calibrated viral stock solutions, stored below –65°C. The accurate calibration of the primary HIV-1 and 2 standards in copies/mL has been confirmed in analytical sensitivity studies of the Grifols Procleix TMA and Roche cobas MPX assay versions[8-12]. The BQC manufacturing and quality control procedures guarantee consistent virus concentrations in consecutive batches of the HIV-RNA reference panels.

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[6]; hence the stock solutions from which the reference panels are prepared have shown to be stable for more than two decades in the BioQControl 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[6]. Hence, it can be guaranteed that the HIV-1 and HIV-2 reference panels are stable when stored below -65˚C.[6]

Materials Provided

Five (5) panel members in 2mL polystyrene vials with screw caps, each containing 1.2 mL of plasma. The composition of the P0319 HIV-2 RNA subtype A Quant panel is given in table 2

Table 2. Composition of P0319 HIV-2 RNA subtype A linearity panel.

Panel member	HIV-2 RNA copies/mL	HIV-1 RNA IU/mL	Quantity (mL per vial)
B4312-001	100,000	127,000	1 x 1.2 mL
B4312-002	10,000	12,700	1 x 1.2 mL
B4312-003	1000	1270	1 x 1.2 mL
B4312-004	100	127	1 x 1.2 mL
B4312-005	10	12.7	1 x 1.2 mL

Materials not provided

Test kit and pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

It is recommended that the panel is stored below 65°C to ensure highest quality. At this temperature the panel is stable. Discard any unused material after the first use. Any panel members that appear cloudy or contain precipitates after thawing should be discarded.

Warning and precautions

Warning: The HIV-RNA reference panel members contain infectious virus and are potentially bio-hazardous[15]. Apply the universal precautions for prevention of transmission of infectious agents when handling these materials[16,17]. Although the normal human plasma used in the production of this panel was negative for blood borne infectious disease should be handled as if capable of transmitting (unknown) infectious agents.

- 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

Expected Lower limit of detection (LOD)

The historically observed proportion of reactive results in replicate tests on S0024 HIV-2 RNA standard dilutions in Procleix Ultrio (Grifols) assay versions are presented in table 3a and the 95% and 50% LOD in table 3b.

Table 3a P0034 HIV-2 RNA subtype A (BioQControl S0024 standard dilutions)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4015-xxx-01	5021	6356	
B4015-xxx-02	1687	2135	
B4015-xxx-03	502	635	36/36 (100%)
B4015-xxx-04	169	214	36/36 (100%)
B4015-xxx-05	50.2	63.5	42/42 (100%)
B4015-xxx-06	16.9	21.4	42/42 (100%)
B4015-xxx-07	5.02	6.35	33/42 (79%)
B4015-xxx-08	1.69	2.14	18/42 (43%)
B4015-xxx-09	0.5	0.63	1/42 (2%)
B4015-xxx-10	0.17	0.22	

Table 3b. Detection limits on S0024 HIV-2 subtype A standard dilution panels in Procleix Ultrio assay versions calculated by probit analysis[18]

HIV-2 RNA standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0024 HIV-2 subtype A	P0034	Ultrio Elite	42	2.2 (1.7-2.8)	9.3 (6.7-16.2)

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 indirectly quantified in copies/mL as described above. The IU/mL values are directly traceable to the 1st International Standard (08/150).

Limitations

The cross calibration of the HIV-2 reference panels in copies/mL was based on testing of HIV-1 subtype standards in the previous bDNA 3.0 assay and p24 antigen quantification. Therefore there is a considerable uncertainty attached to the copy numbers assigned to the panel members. The IU values assigned to the reference panels were based on the 1st WHO HIV-2 08/150 standard and a conversion factor of 0.79 copy/IU. However cross calibration of different HIV standards is known to be dependent on the quantitative NAT method used[5]. Therefore viral loads and LODs expressed in IU/mL, and copy/IU conversion factors may change when the 2nd WHO 16/296 standard is used.

References

1. Lelie PN, Van Drimmelen AAJ, Cuypers HTM, Best SJ, Stramer Hyland SL C, J.- Allain P, Moncharmont P, Defer C, Nubling CM, Glauser A, da Silva Cardoso M, -F. Viret J, Lankinen M, Grillner L, Wirthmuller U, Coste J, Schottstedt V, Masecar B. and E.M. Dax. Sensitivity of HCV-RNA and HIV-RNA blood screening assays. Transfusion. 2002,42:527-36.
2. C. Davis, A. Heath, S. Best, I. Hewlett, N. Lelie, R. Schuurman, H. Holmes Calibration of HIV-1 working reagents for nucleic acid amplification techniques against the 1st international standard for HIV-1 RNA. J of Virol Methods 2003;107:37-44.
3. Holmes H, Davis C, Heath A, Hewlett I and Lelie PN. An international collaborative study to establish the 1st International Standard for HIV-1-RNA for use in Nucleic Acid-Based Techniques. J. Virol. Methods 2001, 92: 141-150
4. Lelie PN, Van Drimmelen AAJ. Calibration of native and inactivated viral standards and traceability to viral nucleic acid copies and International Units. VR4060, www.bioqcontrol.com
5. Lelie N, Van Drimmelen H. Accuracy of quantitative HIV-1 RNA test methods at 1000 copies/mL and the potential impact of differences in assay calibration on therapy monitoring of patients. J Med Virology 2020;92:3246-3253
6. Van Drimmelen AAJ, Lelie PN. Stability of ViraQ run controls for NAT. VR4058. www.bioqcontrol.com
7. Van Drimmelen AAJ, Lelie PN. Quantification of HBV, HCV and HIV genotype standards. VR4026.
8. Assal A, Barlet V, Deschaseaux M, Dupont I, Gallian P, Guitton C, Morel P, David B, and De Micco P. Comparison of the analytical and operational performance of two viral nucleic acid test blood screening systems: Procleix Tigris and cobas s 201. Transfusion 2009; 49:289-300.
9. Koppelman M, Assal A, Chudy M, Torres P, de Villaescusa RG, Reesink HW, Lelie PN, Cuypers HT. Multi-center performance evaluation of a transcription-mediated amplification assay for screening of human immunodeficiency virus-1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA in blood donations. Transfusion 2005; 45:1258-66.
10. Grabarczyk P, van Drimmelen H, Kopacz A, Gdowska J, Liszewski G, Piotrowski D, Górska J, Kuśmierczyk J, Candotti D, Lętowska M, Lelie N, Brojer E. Head-to-head comparison of two transcription-mediated amplification assay versions for detection of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus Type 1 in blood donors. Transfusion. 2013; 53:2512-2524.
11. Grabarczyk P, Koppelman M, Boland F, Sauleda S, Fabra C, Cambie G, O'Riordan K, Van Drimmelen H, Vermeulen M, O'Riordan J, Lelie N. Inclusion of human immunodeficiency virus Type 2 (HIV-2) in a multiplex transcription-mediated amplification assay does not affect detection of HIV-1 and hepatitis B and C virus genotypes: a multicenter performance evaluation study. Transfusion 2015; 55:2246-55.
12. Vermeulen M, Coleman C, Mitchel J, Reddy R, Van Drimmelen H, Ficket T, Busch M, Lelie N Comparison of HIV assays in window phase and elite controller samples: viral load distribution and implications for transmission risk. Transfusion 2013;53:2384-2398
13. WHO/BS/2017.2314 EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION. Prescott G, Hockley J, Atkinson E, Rigsby P and Morris C. International Collaborative Study to Establish the 4th WHO International Standard for HIV-1 NAT Assays

14. Galel AG, Simon TL, Williamson PC, AuBuchon JP, Waxman DA, Erickson Y, Bertuzis R, Duncan JR, Malhotra K, Vaks J, Huynh N, Pate LL. Sensitivity and specificity of a new automated system for the detection of hepatitis B virus, hepatitis C virus and human immunodeficiency virus nucleic acid in blood and plasma donations. Transfusion. 2018;58:649-659.
15. Lelie PN. Van Drimmelen AAJ. Preparation of inactivated secondary viral standards: Safety assessment of quality control samples for viral serology and NAT assays in blood screening laboratories.CE4006.
16. Centers for Disease Control (CDC). Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. MMWR 1988; 37:377-388.
17. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.
18. Probit Analysis. [2nd ed.] by D. J. Finney

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