Bio QControl

HIV-RNA reference panels

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

The HIV-RNA reference panels provide a consistent standard across nucleic acid amplification technology (NAT) methods, enabling blood screening laboratories and *in vitro* Diagnostics (IVD) manufacturers to assess the analytical sensitivity and quantification limits of molecular diagnostic test methods for the qualitative and quantitative detection of human immunodeficiency virus (HIV)-RNA in blood samples. This product can be used with amplification methods, including (real time) polymerase chain reaction (PCR) and transcription mediated amplification (TMA) assays. The HIV-RNA reference panels are useful for establishing the lower limit of detection (LOD), limit of quantification (LOQ), NAT reagent batch acceptance, NAT system validation and training. The products are for research use only and not for diagnostic use.

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 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 25 (15-41) 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 at -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 (table 1 and 2)[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.

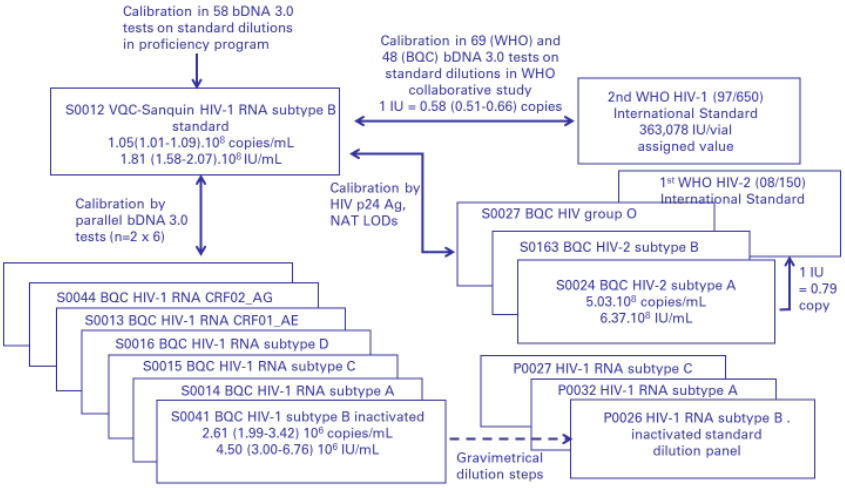
Originally HIV-2 subtypes and group O standards had been calibrated against the HIV-1 subtype B standard in parallel line p24 antigen assays but later by probit analysis in Ultrio Elite and cobas MPX assays[7].

Of all these HIV standards reference panels of (approximately) 3000, 1000, 300, 100, 30, 10, 3, 1, 0.3 and 0.1 copies/mL were tested in multiple replicate tests in different NAT blood screening assays in order to determine the 95% and 50% LOD by probit analysis[8-12]. More recently 8 member dilution panels of the same HIV subtype standards are manufactured starting at 300 copies/mL. The available replicate test data on the series of HIV-RNA reference panels (a total of 12,000 test results) are presented in this package insert. The proportion of reactive results and the 95% and 50% LODs estimated by probit analysis can be used for comparison.

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 subtype reference panels, the Bio Quality Control (BQC) subtype standards, the primary 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 HIV-RNA reference panels, BQC and VQC-Sanquin standards and 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[4,5]. It was decided to use the Siemens bDNA 3.0 assay as the reference method for quantification and assign the value of 1.05 (1.01-1.09).10^8 copies/mL to the undiluted S0012 VQC-Sanquin standard[4,5].

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)

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[5]. 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[6]. 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 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)[5].

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.

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)

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[5] (table 4). 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.

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)

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[13]. The conversion factor of 0.31 (0.21-0.63) copies/IU of the VQC/Sanquin standard when compared with the 3rd WHO 10/152 standard (table 4) was confirmed by the 50% LODs found in the cobas MPX assay on these two standards [50% LOD of 1.3 (1.0-1.6) copies/mL (n=48) versus 50% LOD of 3.8 IU/mL][14]

Calibration of HIV-1 subtype A to H standards in copies/mL

A series of HIV-1 subtype standards have been calibrated against the VQC-Sanquin HIV-1 subtype B standard in replicate bDNA 3.0 assays (table 3)[7]. The standard dilutions used for the calibration experiment fell well in the dynamic range of the bDNA 3.0 assay (table 5).

Table 5. Calibration of HIV-1 RNA standards of different genotypes against the primary S0012 VQC-Sanquin HIV-1 subtype B standard containing $1.05.10^8$ copies/mL according to original quantification in bDNA 3.0 assay. Dilutions of the HIV-1 subtype A to H standards in the concentration range of 20.000 – 200.000 copies/mL were tested in parallel in replicate bDNA tests (VR4026).

HIV-1 RNA standard	subtype	n	copies/mL (95% CI)#	(95% CI)%
S0012 VQC-Sanquin	B	58	$1.05\ (1.01\text{-}1.09).10^8$	(96-104%)
S0041 BQC inactivated	B	6	$2.61\ (1.99\text{-}3.42).10^6$	(76-131)%
S0014 BQC	A	6	$5.31\ (4.03\text{-}7.00).10^8$	(76-132)%
S0015 BQC	C	6	$1.44\ (0.97\text{-}2.14).10^8$	(67-129)%
S0016 BQC	D	6	$6.35\ (4.09\text{-}9.84).10^8$	(65-155)%
S0013 BQC (1)	CRF01_AE	6	$3.18\ (2.13\text{-}4.75).10^8$	(67-149)%
S0045 Thailand (2)	CRF01_AE	3	$6.09\ (5.24\text{-}7.50).10^8$	(86-123)%
S0044 Ghana	CRF02_AG	3	$7.58\ (7.43\text{-}9.18).10^8$	(95-117)%
S0046 Brazil (1)	F	3	$7.86\ (5.01\text{-}12.5).10^6$	(64-159)%
S0047 Romania (2)	F	3	$7.98\ (5.75\text{-}11.3).10^8$	(72-142)%
S0048 Zaire (1)	G	3	$1.46\ (1.13\text{-}1.96).10^9$	(77-134)%
S0049 Kenya(2)	G	3	$1.32\ (0.96\text{-}1.88).10^8$	(72-142)%
S0050 Zaire	H	3	$3.59\ (3.23\text{-}4.30).10^8$	(90-120)%

The same HIV-1 subtype A to H standard dilutions that were used for the bDNA 3.0 calibration experiments (table 5) were tested in triplicate Abbott RealTime PCR tests and the concentrations in copies/mL reported by the Abbott assay (0.58 copy/IU) were adjusted for the copy numbers assigned to the primary VQC-Sanquin standard. Table 6 compares the calibration results against the subtype B standard in Abbott RealTime assay when concentrations were adjusted to 1000 copies/mL according to the original calibration in the bDNA 3.0 assay.

The data in table 6 show 1.7 to 4.9 fold higher geometric mean values in Abbott Real Time assay on the HIV-1 subtype A-H standards.

Table 6. Comparison of HIV-1 RNA quantification of different genotypes against the primary S0012 VQC-Sanquin HIV-1 subtype B standard in bDNA 3.0 assay and in Abbott RealTime assay. Samples in concentration range of 20.000 to 200.000 copies/mL were tested in both assays and values were adjusted to 1000 copies/mL concentrations according to bDNA 3.0 calibration for comparison in this table. The values in Abbott Real Time assay were adjusted to 1.336 fold higher values because of calibration against the value assigned to the primary S0012 VQC-Sanquin subtype B standard.

Dilution tested from HIV-1 RNA standards:	subtype	n	bDNA 3.0 copies/mL (95% CI)	n	Abbott RealTime copies/mL (range)
S0012 VQC-Sanquin	B	58	1000 (960-1040)	3	1000 (870-1065)
S0041 BQC inactivated	B	6	1000 (760-1310)	6	920 (713-1208)$
S0014 BQC	A	6	1000 (760-1320)	3	4948 (4535-5259)
S0015 BQC	C	6	1000 (670-1290)	2	2549 (2309-2788)
S0016 BQC	D	6	1000 (650-1550)	3	3715 (3502-3822)
S0013 BQC (1)	CRF01_AE	6	1000 (670-1490)	3	4221 (4107-4423)
S0045 Thailand (2)	CRF01_AE	3	1000 (860-1230)	3	2703 (2536-3021)
S0044 Ghana	CRF02_AG	3	1000 (950-1170)	3	3669 (3417-3962)
S0046 Brazil (1)	F	3	1000 (640-1590)	3	1736 (1511-1991)
S0047 Romania (2)	F	3	1000 (720-1420)	3	2645 (2567-2746)
S0048 Zaire (1)	G	3	1000 (770-1340)	3	3210 (2963-3367)
S0049 Kenya(2)	G	3	1000 (720-1420)	3	4106 (3852-4262)
S0050 Zaire	H	3	1000 (900-1200)	3	3873 (3734-4021)

$ separate experiment 2018

Calibration of HIV group O standards in copies/mL

The calibration of group O samples was originally based on parallel line p24 antigen testing using the Murex HIV-Ag assay and later also on NAT methods that were sensitive for both HIV-1 group M and group O detection. Table 7 compares the calibration of 5 group O standards against the S0012 HIV-1 subtype B standard and the Abbott RealTime assay7. The final calibration was based on additional NAT tests and is described in validation report VR4026[7].

Table 7. Calibration of HIV group O samples against S0012 VQC-Sanquin HIV-1 subtype B standard in Murex HIV p24 antigen assay and triplicate tests on Abbott RealTime assay (values corrected for established concentration of 1.05 (1.01-1.09).10^8 copies/mL in S0012 standard). The details for the final calibration are described in VR4026[7].

HIV-RNA group O standard	copies/mL p24 Ag calibration in Murex ELISA	copies/mL HIV-RNA calibration in Abbott RealTime assay n=3	Final calibration[7]
S0017 BQC (1)	$1.78.10^7$		$3.16.10^7$
S0051 USA (2)	$3.06.\ 10^8$	$1.41.10^9$	$1.41.10^9$
S0067 Cameroon (3)	$6.34.10^7$	$2.74.10^9$	$2.53.10^8$
S0068 Spain (3)	$6.04.10^7$	$2.98.10^8$	$2.58.10^8$
S0069 Cameroon (4)	$8.30..10^7$	$3.79.10^8$	$3.41.10^8$

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 8)[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 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 8. 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

described in Supplemental material in Ultrio Elite validation study of Grabarczyk et al [ref].

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 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[6]. Hence, it can be guaranteed that the reference panels are stable when stored below -65˚C.[6]

Overview HIV-RNA subtype reference panels

Table 9 gives an overview of the HIV-RNA reference panels that are available for establishing the analytical sensitivity or validation of NAT methods

Table 9 product overview. 28 HIV-RNA reference panels

Cat. No	Source/Standard	HIV-RNA subtype reference panels$	Quantity	range copies/mL$
P0030	2nd WHO 97/650	P0030 HIV-1 RNA subtype B	10 x 4 mL^	0.07 - 2081
P0022		P0022 HIV-1 RNA subtype B	8 x 5.6 mL	0 - 348
P0350	4th WHO 16/149	P0350 HIV-1 RNA subtype B	7 x 4 mL	0.1 - 116
P0025	VQC-Sanquin§	P0025 HIV-1 RNA subtype B	10 x 4 mL^	0.1 - 2590
P0290		P0290 HIV-1 RNA subtype B	8 x 4 mL	0.1 - 300
P0272		P0272 Multi-Marker HBV/HCV/HIV-1	8 x 1.5 mL	0.78 - 50
P0026	BQC inactivated	P0026 HIV-1 RNA subtype B inact.	10 x 4 mL^	0.08 - 2446
P0291		P0291 HIV-1 RNA subtype B inact.	8 x 4 mL	0.1 - 300
P0032	BQC	P0032 HIV-1 RNA subtype A	10 x 4 mL^	0.17 - 5020
P0296		P0296 HIV-1 RNA subtype A	8 x 4 mL	0.1 - 300
P0027	BQC	P0027 HIV-1 RNA subtype C	10 x 4 mL^	0.1 - 2883
P0292		P0292 HIV-1 RNA subtype C	8 x 4 mL	0.1 - 300
P0033	BQC	P0033 HIV-1 RNA subtype D	10 x 4 mL^	0.19 - 5610
P0297		P0297 HIV-1 RNA subtype D	8 x 4 mL	0.1 - 300
P0028	BQC	P0028 HIV-1 RNA CRF01_AE (1)	10 x 4 mL^	0.1 - 3075
P0293		P0293 HIV-1 RNA CRF01_AE (1)	8 x 4 mL	0.1 - 300
P0052	Thailand	P0052 HIV-1 RNA CRF01_AE (2)	8 x 4 mL	0.1 - 300
P0053	Brazil	P0053 HIV-1 RNA subtype F (1)	8 x 4 mL	0.1 - 300
P0054	Romania	P0054 HIV-1 RNA subtype F (2)	8 x 4 mL	0.1 - 300
P0098	Zaire	P0098 HIV-1 RNA subtype G (1)	8 x 4 mL	0.1 - 300
P0099	Kenya	P0099 HIV-1 RNA subtype G (2)	8 x 4 mL	0.1 - 300
P0051	Ghana	P0051 HIV-1 RNA CRF02_AG	8 x 4 mL	0.1 - 300
P0100	Zaire	P0100 HIV-1 RNA subtype H	8 x 4 mL	0.1 - 300
P0354	2nd WHO 16/296	P0354 HIV-2 RNA subtype A	8 x 4 mL	0.08 - 237
P0034	BQC	P0034 HIV-2 RNA subtype A	10 x 4 mL^	0.17 - 5021
P0298		P0298 HIV-2 RNA subtype A	8 x 4 mL	0.1 - 300
P0212	Belgium	P0212 HIV-2 RNA subtype B	8 x 4 mL	0.1 - 300
P0015	BQC	P0015 HIV RNA group O (1)	10 x 4 mL^	0.1 - 2580
P0287		P0287 HIV-RNA group O (1)	8 x 4 mL	0.1 - 300
P0101	USA	P0101 HIV-RNA group O (2)	8 x 4 mL	0.46- 1382
P0102	Cameroon	P0102 HIV-RNA group O (3)	8 x 4 mL	0.40 - 1197
P0103	Spain	P0103 HIV-RNA group O (4)	8 x 4 mL	0.43 - 1281
P0104	Cameroon	P0104 HIV-RNA group O (5)	8 x 4 mL	0.41 - 1233

$ HIV-1 1 IU = 0.58 copies HIV-2 1 IU = 0.79 copies ^10 x 4 mL format will be phased out and replaced by 8 x 4 mL format

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 VQC-Sanquin subtype B (and the secondary HIV subtype 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.

Materials Provided

Table 10.1 to table 10.31 gives the composition of the HIV-RNA reference panels and the quantification of the panel members, listed in table 9. Either ten or eight member panels are filled off in 4.0 mL volumes in polypropylene tubes (10 mL) with screw caps. The quantification in copies/mL and the uncertainty expressed by the 95% confidence interval (CI) was based on calibration experiments in the bDNA 3.0 assay (table 2). The quantification in IU/mL is obtained using the same conversion factor of 0.58 copies per IU for all subtyped standards. A confidence interval was not given since the uncertainty for calibration of WHO replacement standards in IUs ignored[3].
The tube identification is Byyyy-xxx-number, where yyyy is product specific and xxx the sequential batch number. The identification is present on the bar-code and further explained on the tube label.

Materials not provided

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

Storage Instructions

It is recommended that the panels are 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 (except for P0026 that is prepared from a heat inactivated standard)[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 markers also the reference panel members of P0025 (inactivated HIV standard) 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 proportion of reactive test results in NAT assays

The historically observed proportion of reactive results in replicate tests on the HBV-DNA reference panels in Procleix Ultrio (Grifols) or cobas MPX (Roche) assay versions are presented in tables 10.1 to 4.31. These data can be used to select panel members to be tested in multiple replicates and for comparison of the proportion of reactive test results. It is recommended to test the panels in at least in 12 replicates (and preferably 24 to 48 times) for estimating the 50% and 95% LODs by probit analysis[18]. If HBV-DNA reference panels are used for NAT reagent batch acceptance testing the panel members could be tested in 4-6 replicates.

Table 10. Composition HIV-1 standard dilution panels of different subtypes, circulating recombinant forms (CRFs), HIV-2 and HIV group O with the proportion of NAT reactive results in Ultrio and cobas MPX assay versions

Table 10.1. P0030 HIV-1 RNA subtype B (WHO 97/650 standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio Plus
B4033-xxx-01	701.8	1210	24/24 (100%)
B4033-xxx-02	210.5	363	54/54 (100%)
B4033-xxx-03	70.2	121	55/55 (100%)
B4033-xxx-04	20.9	36	55/55 (100%)
B4033-xxx-05	7.0	12	50/55 (91%)
B4033-xxx-06	2.1	3.6	24/55 (44%)
B4033-xxx-07	0.70	1.2	7/50 (14%)
B4033-xxx-08	0.21	0.36	4/55 (7%)
B4033-xxx-09	0.07	0.12	0/56 (0%)
B4033-xxx-10	0.02	0.04	0/24 (0%)

Table 10.2. P0022 HIV-1 RNA subtype B (WHO 97/650 standard)

WHO standard dilution panel previously manufactured for Grifols

Sample-id	cp/mL	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite	cobas 6800 MPX
B4089-xxx-01	348	600	32/32 (100%)	286/286 (100%)	228/228 (100%)	12/12 (100%)
B4089-xxx-02	116	200	32/32 (100%)	280/280 (100%)	229/229 (100%)	12/12 (100%)
B4089-xxx-03	34.8	60	40/40 (100%)	294/294 (100%)	229/229 (100%)	12/12 (100%)
B4089-xxx-04	11.6	20	39/40 (98%)	273/291 (94%)	215/230 (94%)	12/12 (100%)
B4089-xxx-05	3.48	6	22/40 (55%)	177/288 (62%)	126/229 (55%)	9/12 (75%)
B4089-xxx-06	1.16	2	8/40 (20%)	68/288 (24%)	36/120 (30%)	0/12 (0%)
B4089-xxx-07	0.35	0.6	1/40 (3%)	8/140 (6%)	16/114 (14%)	0/12 (0%)
B4089-xxx-08	0.00	0	0/8 (0%)	0/256 (0%)	0/216 (0%)	0/12 (0%)

Table 10.3. P0350 HIV-1 RNA subtype B (4th WHO 16/149 standard)

Replacement of P0022 WHO standard dilution panel above (table 10.2)

Sample-id	cp/mL (95% CI)	IU/mL
B4299-xxx-01	116	200
B4299-xxx-02	34.8	60
B4299-xxx-03	11.6	20
B4299-xxx-04	3.48	6
B4299-xxx-05	1.16	2
B4299-xxx-06	0.348	0.6
B4299-xxx-07	0.116	0.2

Table 10.4. P0025 HIV-1 RNA subtype B (VQC-Sanquin standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite
B4010-xxx-01	2590 (2491-2689)	4466	24/24 (100%)	24/24 (100%)	
B4010-xxx-02	863 (830-896)	1488	24/24 (100%)	24/24 (100%)	
B4010-xxx-03	259 (249-269)	447	60/60 (100%)	47/47 (100%)	9/9 (100%)
B4010-xxx-04	86.3 (83.0-89.6)	149	60/60 (100%)	48/48 (100%)	16/16 (100%)
B4010-xxx-05	25.9 (24.9-26.9)	44.7	60/60 (100%)	47/47 (100%)	15/15 (100%)
B4010-xxx-06	8.63 (8.30-8.96)	14.9	59/60 (98%)	43/48 (90%)	29/30 (97%)
B4010-xxx-07	2.59 (2.49-2.69)	4.5	35/60 (58%)	27/48 (56%)	12/24 (50%)
B4010-xxx-08	0.86 (0.83-0.90)	1.5	17/60 (28%)	12/48 (25%)	5/24 (21%)
B4010-xxx-09	0.26 (0.25-0.27)	0.45	4/60 (7%)	7/48 (15%)	0/11 (0%)
B4010-xxx-10	0.09 (0.08-0.090)	0.15	3/48 (6%)	0/36 (0%)	0/9 (0%)

Table 10.5. P0290 HIV-1 RNA subtype B (VQC-Sanquin standard)

Replacement of P0025 VQC-Sanquin standard dilution panel above (table 10.4)

Sample-id	cp/mL (95% CI)	IU/mL
B4281-xxx-01	300 (289-311)	517
B4281-xxx-02	100 (96-104)	172
B4281-xxx-03	30 (28.9-31.1)	52
B4281-xxx-04	10 (9.6-10.4)	17
B4281-xxx-05	3 (2.89-3.11)	5.2
B4281-xxx-06	1 (0.96-1.04)	1.7
B4281-xxx-07	0.3 (0.29-0.31)	0.52
B4281-xxx-08	0.1 (0.096-0.104)	0.17

Table 10.6. P0272 HIV-1 RNA subtype B (VQC-Sanquin standard)

VQC-Sanquin standard dilutions in HBV/HCV/HIV-1 panel manufactured for Roche

Sample-id	cp/mL (95% CI)	IU/mL	cobas 6800 MPX
B4263-xxx-01	50 (48.1-51.9)	86.00	48/48 (100%)
B4263-xxx-02	25 (24.0-26.0)	43.00	48/48 (100%)
B4263-xxx-03	12.5 (12.0-13.0)	21.50	46/46 (100%)
B4263-xxx-04	6.25 (6.0-6.5)	10.75	45/48 (94%)
B4263-xxx-05	3.12 (3.0-3.2)	5.37	36/48 (75%)
B4263-xxx-06	1.56 (1.50-1.62)	2.68	26/48 (54%)
B4263-xxx-07	0.78 (0.75-0.81)	1.34	17/46 (37%)

Table 10.7. P0026 HIV-1 RNA subtype B (heat inactivated VQC-Sanquin standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	cobas 6800 MPX
B4011-xxx-01	2446 (1865-3205)	4217	52/52 (100%)	
B4011-xxx-02	816 (622-1069)	1406	52/52 (100%)	
B4011-xxx-03	245 (187-321)	422	52/52 (100%)	
B4011-xxx-04	81.6 (62.2-107)	141	52/52 (100%)	12/12 (100%)
B4011-xxx-05	24.5 (18.6-32.0)	42.2	51/52 (98%)	12/12 (100%)
B4011-xxx-06	8.16 (6.22-10.7)	14.1	43/52 (83%)	12/12 (100%)
B4011-xxx-07	2.45 (1.86-3.20)	4.2	17/52 (33%)	9/12 (75%)
B4011-xxx-08	0.82 (0.62-1.07)	1.4	7/52 (13%)	5/12 (42%)
B4011-xxx-09	0.25 (0.19-0.32)	0.42	2/52 (4%)	2/12 (17%)
B4011-xxx-10	0.082 (0.062-0.11)	0.14	0/52 (0%)	0/12 (0%)

Table 10.8. P0291 HIV-1 RNA subtype B (heat inactivated VQC-Sanquin standard)

Replacement of P0026 heat inactivated BQC standard dilution panel above (table 10.7)

Sample-id	cp/mL (95% CI)	IU/mL
B4282-xxx-01	300 (229-393)	517
B4282-xxx-02	100 (76-131)	172
B4282-xxx-03	30 (22.9-39.3)	52
B4282-xxx-04	10 (7.6-13.1)	17
B4282-xxx-05	3 (2.29-3.93)	5.2
B4282-xxx-06	1 (0.76-1.31)	1.7
B4282-xxx-07	0.3 (0.23-0.39)	0.52
B4282-xxx-08	0.1 (0.076-0.131)	0.17

Table 10.9. P0032 HIV-1 RNA subtype A (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio Plus	Panther Ultrio Elite
B4009-xxx-01	5020 (3810-6618)	8655		
B4009-xxx-02	1680 (1275-2215)	2897		
B4009-xxx-03	502 (381-662)	866		8/8 (100%)
B4009-xxx-04	168 (128-221)	290	24/24 (100%)	27/27 (100%)
B4009-xxx-05	50.2 (38.1-66.2)	87	24/24 (100%)	43/51 (84%)
B4009-xxx-06	16.8 (12.8-22.1)	29	18/24 (75%)	27/51 (53%)
B4009-xxx-07	5.02 (3.81-6.62)	8.7	7/24 (29%)	12/51 (24%)
B4009-xxx-08	1.68 (1.28-2.21)	2.9	2/24 (8%)	2/27 (2%)
B4009-xxx-09	0.50 (0.38-0.66)	0.87	3/24 (13%)	2/27 (2%)
B4009-xxx-10	0.17 (0.13-0.22)	0.29	1/24 (4%)	0/8 (0%)

Table 10.10. P0296 HIV-1 RNA subtype A (BQC standard)

Replacement of P0032 BQC standard dilution panel above (table 10.9)

Sample-id	cp/mL (95% CI)	IU/mL
B4287-xxx-01	300 (228-395)	517
B4287-xxx-02	100 (76-132)	172
B4287-xxx-03	30 (22.8-39.5)	52
B4287-xxx-04	10 (7.6-13.2)	17
B4287-xxx-05	3 (2.28-3.95)	5.2
B4287-xxx-06	1 (0.76-1.32)	1.7
B4287-xxx-07	0.3 (0.23-0.40)	0.52
B4287-xxx-08	0.1 (0.076-0.13)	0.17

Table 10.11. P0027 HIV-1 RNA subtype C (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite	cobas 6800 MPX
B4012-xxx-01	2883 (2083-4004)	4971				
B4012-xxx-02	961 (694-1335)	1658				
B4012-xxx-03	288 (208-400)	497	36/36 (100%)	42/42 (100%)	24/24 (100%)	24/24 (100%)
B4012-xxx-04	96.1 (69.4-134)	166	36/36 (100%)	53/53 (100%)	24/24 (100%)	24/24 (100%)
B4012-xxx-05	28.8 (20.8-40.0)	50	36/36 (100%)	52/52 (100%)	42/42 (100%)	24/24 (100%)
B4012-xxx-06	9.61 (6.94-13.4)	17	36/36 (100%)	53/54 (98%)	42/42 (100%)	24/24 (100%)
B4012-xxx-07	2.88 (2.08-4.00)	5	30/36 (83%)	47/52 (90%)	39/42 (93%)	24/24 (100%)
B4012-xxx-08	0.96 (0.69-1.34)	1.7	13/36 (36%)	28/54 (52%)	18/42 (43%)	12/24 (50%)
B4012-xxx-09	0.29 (0.21-0.40)	0.5	11/36 (31%)	12/52 (23%)	6/42 (14%)	4/24 (17%)
B4012-xxx-10	0.10 (0.07-0.13)	0.17	2/24 (8%)	4/36 (11%)	0/24 (0%)	2/24 (8%)

Table 10.12. P0292 HIV-1 RNA subtype C (BQC standard)

Replacement of P0027 BQC standard dilution panel above (table 10.11)

Sample-id	cp/mL (95% CI)	IU/mL
B4283-xxx-01	300 (217-417)	517
B4283-xxx-02	100 (72-139)	172
B4283-xxx-03	30 (21.7-41.7)	52
B4283-xxx-04	10 (7.2-13.9)	17
B4283-xxx-05	3 (2.17-4.17)	5.2
B4283-xxx-06	1 (0.72-1.39)	1.7
B4283-xxx-07	0.3 (0.22-0.42)	0.52
B4283-xxx-08	0.1 (0.072-0.14)	0.17

Table 10.13. P0033 HIV-1 RNA subtype D (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite
B4013-xxx-01	5610 (3896-8075)	9672			
B4013-xxx-02	1870 (1299-2692)	3224			
B4013-xxx-03	561 (390-807)	967	12/12 (100%)	12/12 (100%)	
B4013-xxx-04	187 (130-269)	322	12/12 (100%)	24/24 (100%)	
B4013-xxx-05	56.1 (39.0-80.7)	97	12/12 (100%)	24/24 (100%)	18/18 (100%)
B4013-xxx-06	18.7 (13.0-26.9)	32	12/12 (100%)	24/24 (100%)	18/18 (100%)
B4013-xxx-07	5.61 (3.90-8.07)	9.7	10/12 (83%)	23/24 (96%)	16/18 (89%)
B4013-xxx-08	1.87 (1.30-2.69)	3.2	4/12 (33%)	12/24 (50%)	12/18 (67%)
B4013-xxx-09	0.56 (0.39-0.81)	0.97	2/12 (17%)	8/24 (33%)	6/18 (33%)
B4013-xxx-10	0.19 (0.13-0.27)	0.32			

Table 10.14. P0297 HIV-1 RNA subtype D (BQC standard)

Replacement of P0033 BQC standard dilution panel above (table 10.13)

Sample-id	cp/mL (95% CI)	IU/mL
B4288-xxx-01	300 (208-432)	517
B4288-xxx-02	100 (69-144)	172
B4288-xxx-03	30 (20.8-43.2)	52
B4288-xxx-04	10 (6.9-14.4)	17
B4288-xxx-05	3 (2.1-4.3)	5.2
B4288-xxx-06	1 (0.69-1.44)	1.7
B4288-xxx-07	0.3 (0.21-0.43)	0.52
B4288-xxx-08	0.1 (0.069-0.14)	0.17

Table 10.15. P0028 HIV-1 RNA CRF01_AE (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite
B4014-xxx-01	3075 (2205-4283)	5301			
B4014-xxx-02	1022 (732-1423)	1761			
B4014-xxx-03	307 (220-428)	530	12/12 (100%)	11/11 (100%)	
B4014-xxx-04	102 (73.2-142)	176	12/12 (100%)	21/21 (100%)	
B4014-xxx-05	30.7 (22.0-42.8)	53	12/12 (100%)	24/24 (100%)	18/18 (100%)
B4014-xxx-06	10.2 (7.32-14.2)	18	12/12 (100%)	24/24 (100%)	18/18 (100%)
B4014-xxx-07	3.07 (2.20-4.28)	5	9/12 (75%)	20/23 (87%)	16/18 (89%)
B4014-xxx-08	1.02 (0.73-1.42)	1.8	10/12 (83%)	8/24 (33%)	9/18 (50%)
B4014-xxx-09	0.31 (0.22-0.43)	0.5	4/12 (33%)	5/24 (21%)	5/18 (28%)
B4014-xxx-10	0.10 (0.073-0.14)	0.18		2/12 (17%)	

Table 10.16. P0293 HIV-1 RNA CRF01_AE (BQC standard)

Replacement of P0028 BQC standard dilution panel above (table 10.15)

Sample-id	cp/mL (95% CI)	IU/mL
B4284-xxx-01	300 (215-418)	517
B4284-xxx-02	100 (72-139	172
B4284-xxx-03	30 (21.5-41.8)	52
B4284-xxx-04	10 (7.2-13.9)	17
B4284-xxx-05	3 (2.15-4.18)	5.2
B4284-xxx-06	1 (0.72-1.39)	1.7
B4284-xxx-07	0.3 (0.22-0.42)	0.52
B4284-xxx-08	0.1 (0.072-0.14)	0.17

Table 10.17. P0052 HIV-1 RNA CRF01_AE (Thailand)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4047-xxx-01	300 (239-376)	517	
B4047-xxx-02	100 (79.8-125)	172	7/7 (100%)
B4047-xxx-03	30.0 (23.9-37.6)	52	7/7 (100%)
B4047-xxx-04	10.0 (7.98-12.5)	17	7/7 (100%)
B4047-xxx-05	3.00 (2.39-3.76)	5.2	7/7 (100%)
B4047-xxx-06	1.00 (0.80-1.25)	1.7	2/7 (29%)
B4047-xxx-07	0.30 (0.24-0.38)	0.52	1/7 (14%)
B4047-xxx-08	0.10 (0.080-0.13)	0.17	0/7 (0%)

Table 10.18. P0053 HIV-1 RNA subtype F (Brazil)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4048-xxx-01	300 (185-489)	517	6/6 (100%)
B4048-xxx-02	100 (61.6-163)	172	6/6 (100%)
B4048-xxx-03	30.0 (18.5-48.9)	52	6/6 (100%)
B4048-xxx-04	10.0 (6.16-16.3)	17	4/6 (67%)
B4048-xxx-05	3.00 (1.85-4.89)	5.2	2/6 (33%)
B4048-xxx-06	1.00 (0.62-1.63)	1.7	2/6 (33%)
B4048-xxx-07	0.30 (0.19-0.49)	0.52	2/6 (33%)
B4048-xxx-08	0.10 (0.062-0.16)	0.17	0/6 (0%)

Table 10.19. P0054 HIV-1 RNA subtype F (Romania)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4049-xxx-01	300 (211-425)	517	
B4049-xxx-02	100 (70.3-142)	172	18/18 (100%)
B4049-xxx-03	30.0 (21.1-42.5)	52	18/18 (100%)
B4049-xxx-04	10.0 (7.03-14.2)	17	18/18 (100%)
B4049-xxx-05	3.00 (2.11-4.25)	5.2	15/18 (83%)
B4049-xxx-06	1.00 (0.70-1.42)	1.7	5/18 (28%)
B4049-xxx-07	0.30 (0.22-0.43)	0.52	3/18 (17%)
B4049-xxx-08	0.10 (0.070-0.14)	0.17	

Table 10.20. P0098 HIV-1 RNA subtype G (Zaire)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4098-xxx-01	300 (148-607)	517	
B4098-xxx-02	100 (49.4-202)	172	39/39 (100%)
B4098-xxx-03	30.0 (14.8-60.7)	52	37/37 (100%)
B4098-xxx-04	10.0 (4.93-20.2)	17	37/37 (100%)
B4098-xxx-05	3.00 (1.48-6.07)	5.2	29/37 (78%)
B4098-xxx-06	1.00 (0.49-2.02)	1.7	14/37 (38%)
B4098-xxx-07	0.30 (0.15-0.61)	0.52	6/37 (16%)
B4098-xxx-08	0.10 (0.05-0.20)	0.17	1/7 (14%)

Table 10.21. P0099 HIV-1 RNA subtype G (Kenya)

Sample-id	cp/mL (95% CI)	IU/mL
B4099-xxx-01	300 (212-427)	517
B4099-xxx-02	100 (70.7-142)	172
B4099-xxx-03	30.0 (21.2-42.7)	52
B4099-xxx-04	10.0 (7.06-14.2)	17
B4099-xxx-05	3.00 (2.12-4.27)	5.2
B4099-xxx-06	1.00 (0.71-1.42)	1.7
B4099-xxx-07	0.30 (0.21-0.43)	0.52
B4099-xxx-08	0.10 (0.071-0.14)	0.17

Table 10.22. P0051 HIV-1 RNA CRF02_AG (Ghana)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4046-xxx-01	300 (277-325)	517	
B4046-xxx-02	100 (92.2-108)	172	7/7 (100%)
B4046-xxx-03	30.0 (27.7-32.5)	52	7/7 (100%)
B4046-xxx-04	10.0 (9.22-10.8)	17	7/7 (100%)
B4046-xxx-05	3.00 (2.77-3.25)	5.2	7/7 (100%)
B4046-xxx-06	1.00 (0.92-1.08)	1.7	6/7 (86%)
B4046-xxx-07	0.30 (0.28-0.33)	0.52	4/7 (57%)
B4046-xxx-08	0.10 (0.092-0.11)	0.17	0/7 (0%)

Table 10.23. P0100 HIV-1 RNA subtype H (Zaire)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4100-xxx-01	300 (266-339)	517	
B4100-xxx-02	100 (88.6-113)	172	37/37 (100%)
B4100-xxx-03	30.0 (26.8-33.9)	52	37/37 (100%)
B4100-xxx-04	10.0 (8.86-11.3)	17	37/37 (100%)
B4100-xxx-05	3.00 (2.66-3.39)	5.2	32/37 (86%)
B4100-xxx-06	1.00 (0.89-1.13)	1.7	13/37 (35%)
B4100-xxx-07	0.30 (0.27-0.34)	0.52	3/37 (8%)
B4100-xxx-08	0.10 (0.089-0.11)	0.17	1/7 (14%)

Table 10.24. P0207 HIV-2 RNA subtype A (WHO 08/150 standard)
WHO standard dilution panel previously manufactured for Grifols

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4207-xxx-001	237.00	300	
B4207-xxx-002	79.00	100	272/272 (100%)
B4207-xxx-003	23.70	30	276/276 (100%)
B4207-xxx-004	7.90	10	227/272 (83%)
B4207-xxx-005	2.37	3	124/278 (45%)
B4207-xxx-006	0.79	1	51/278 (18%)
B4207-xxx-007	0.24	0.3	26/278 (9%)
B4207-xxx-008	0.00	0	0/278 (0%)

Table 10.25 P0354 HIV-2 RNA subtype A (2nd WHO 16/296 standard)
Replacement of P0207 WHO standard dilution panel above (table 10.24)

Sample-id	cp/mL (95% CI)	IU/mL
B4299-xxx-01	237	300
B4299-xxx-02	79	100
B4299-xxx-03	23.7	30
B4299-xxx-04	7.9	10
B4299-xxx-05	2.37	3
B4299-xxx-06	0.79	1
B4299-xxx-07	0.237	0.3

Table 10.26. P0034 HIV-2 RNA subtype A (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite	Cobas MPX
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%)	10/10 (100%)
B4015-xxx-05	50.2	63.5	42/42 (100%)	10/10 (100%)
B4015-xxx-06	16.9	21.4	42/42 (100%)	10/10 (100%)
B4015-xxx-07	5.02	6.35	33/42 (79%)	10/10 (100%)
B4015-xxx-08	1.69	2.14	18/42 (43%)	7/10 (70%)
B4015-xxx-09	0.5	0.63	1/42 (2%)	3/10 (30%)
B4015-xxx-10	0.17	0.22		2/10 (20%)

Table 10.27. P0298 HIV-2 RNA subtype A (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite	Cobas MPX
B4289-xxx-01	300	488		
B4289-xxx-02	100	127		
B4289-xxx-03	30	49		
B4289-xxx-04	10	13		
B4289-xxx-05	3	4.9		
B4289-xxx-06	1	1.3		
B4289-xxx-07	0.3	0.49		
B4289-xxx-08	0.1	0.13		

Table 10.28. P0212 HIV-2 RNA subtype B (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL
B4212-xxx-01	300	379.75
B4212-xxx-02	100	126.58
B4212-xxx-03	30	37.97
B4212-xxx-04	10	12.66
B4212-xxx-05	3	3.80
B4212-xxx-06	1	1.27
B4212-xxx-07	0.3	0.38
B4212-xxx-08	0.1	0.13

Table 10.29. P0015 HIV group O (BQC standard)

Sample-id	cp/mL (95% CI)	IU/mL	Tigris Ultrio	Tigris Ultrio Plus	Panther Ultrio Elite
B4034-xxx-01	2580	4448			
B4034-xxx-02	861	1484			
B4034-xxx-03	258	445	12/12 (100%)	11/11 (100%)	
B4034-xxx-04	86.1	148	12/12 (100%)	23/23 (100%)	30/30 (100%)
B4034-xxx-05	25.8	44.5	12/12 (100%)	22/22 (100%)	30/30 (100%)
B4034-xxx-06	8.61	14.8	12/12 (100%)	23/24 (96%)	30/30 (100%)
B4034-xxx-07	2.58	4.4	6/12 (50%)	16/24 (67%)	17/26 (65%)
B4034-xxx-08	0.861	1.48	5/12 (42%)	7/24 (29%)	5/22 (23%)
B4034-xxx-09	0.258	0.44	0/12 (0%)	6/23 (26%)	4/22 (18%)
B4034-xxx-10	0.09	0.15		1/12 (8%)	

Table 10.30. P0101 HIV RNA group O (USA)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4101-xxx-01	1382	2383	
B4101-xxx-02	461	794	19/19 (100%)
B4101-xxx-03	138	238	19/19 (100%)
B4101-xxx-04	46.1	79	19/19 (100%)
B4101-xxx-05	13.8	24	18/19 (95%)
B4101-xxx-06	4.6	7.9	16/19 (84%)
B4101-xxx-07	1.38	2.4	3/19 (16%)
B4101-xxx-08	0.46	0.79	1/7 (14%)

Table 10.31. P0102 HIV RNA group O (Cameroon)

Sample-id	cp/mL (95% CI)	IU/mL
B4102-xxx-01	1197	2064
B4102-xxx-02	399	688
B4102-xxx-03	120	206
B4102-xxx-04	39.9	69
B4102-xxx-05	12.0	21
B4102-xxx-06	4.0	6.9
B4102-xxx-07	1.20	2.1
B4102-xxx-08	0.40	0.69

Table 10.32. P0103 HIV RNA group O (Spain)

Sample-id	cp/mL (95% CI)	IU/mL	Panther Ultrio Elite
B4103-xxx-01	1281	2209	6/6 (100%)
B4103-xxx-02	427	736	6/6 (100%)
B4103-xxx-03	128	221	6/6 (100%)
B4103-xxx-04	42.7	74	6/6 (100%)
B4103-xxx-05	12.8	22.1	6/6 (100%)
B4103-xxx-06	4.3	7.4	5/6 (83%)
B4103-xxx-07	1.28	2.21	4/6 (67%)
B4103-xxx-08	0.43	0.74	1/6 (17%)

Table 10.33. P0104 HIV RNA group O (Cameroon)

Sample-id	cp/mL (95% CI)	IU/mL
B4104-xxx-01	1233	2125
B4104-xxx-02	411	708
B4104-xxx-03	123	213
B4104-xxx-04	41.1	71
B4104-xxx-05	12.3	21.3
B4104-xxx-06	4.1	7.1
B4104-xxx-07	1.23	2.13
B4104-xxx-08	0.41	0.71

Expected Lower limit of detection (LOD)

For establishing the 95% and 50% LOD by probit analysis[18] it is recommend to test the panel members in at least 12 and preferably in 24 or 48 replicates. Panel members ranging from at least one concentration of 100% reactivity to at least one concentration below 50% reactivity should be used for a reliable probit analysis. Apply log transformation of the concentrations in copies or IU/mL before interpreting the number of reactive and nonreactive results by probit analysis. It is recommended to report both the 50% and 95% LOD and compare these with the historically established values presented in tables 11.1 and 11.2 for the different HIV-RNA subtype reference panels.

Table 11.1 Detection limits on HIV-1 RNA subtype A-H standard dilution panels in Procleix Ultrio assay versions and cobas MPX assay versions

HIV-1 RNA standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO HIV-1 RNA 97/650 WHO HIV-1	P0030	Ultrio Plus	55	2.2 (7.3-25.4)	11.7 (7.3-25.4)
	P0022	Ultrio	40	2.6 (2.1-3.3)	11.8 (8.2-20.7)
	P0022	Ultrio Plus	288	2.4 (2.2-2.6)	13.4 (11.4-16.3)
	P0022	Ultrio Elite	229	2.2 (1.4-3.2)	17.2 (10.3-40.1)
	P0022	cobas MPX	12	2.7 (1.7-3.9)	5.8 (3.9-24.9)
S0012 VQC-Sanquin HIV-1 RNA subtype B	P0025	Ultrio	60	1.5 (1.0-2.2)	11.2 (6.3-29.8)
	P0025	Ultrio Plus	48	1.7 (1.3-2.2)	15.1 (9.9-26.9)
	P0025	Ultrio Elite	24	2.1 (1.5-2.9)	9.0 (5.8-19.5)
	P0272	cobas MPX	48	1.3 (1.0-1.6)	7.3 (5.3-11.8)
S0041 BioQ HIV-1 RNA subtype B inactivated	P0026	Ultrio	52	3.1 (2.4-3.9)	20.2 (13.9-33.3)
	P0026	cobas MPX	12	1.0 (0.6-1.60)	5.8 (3.0-23.2)
	P0251	TaqScreen 2.0	12	2.0 (1.3-2.8)	7.6 (4.9-21.4)
S0014 BioQ HIV-1 RNA subtype A	P0032	Ultrio Plus	24	8.3 (5.7-12.1)^	78.8 (49.5-138)^
	P0032	Ultrio Elite	51	11.9 (8.1-17.5)^	143 (85-282)^
S0015 BioQ HIV-1 RNA subtype C	P0027	Ultrio	36	0.8 (0.6-1.1)	7.6 (4.7-16.1)
	P0027	Ultrio Plus	52	0.7 (0.5-0.9)	5.8 (3.9-10.2)
	P0027	Ultrio Elite	42	0.9 (0.731.2)	3.9 (2.8-6.7)
	P0027	cobas MPX	24	0.7 (0.5-0.9)	3.6 (2.2-8.0)
S0016 BioQ HIV-1 RNA subtype D	P0033	Ultrio	12	2.1 (1.3-3.5)	13.2 (7.6-28.1)
	P0033	Ultrio Plus	24	1.2 (0.9-1.8)	7.8 (5.0-14.8)
	P0033	Ultrio Elite	18	1.1 (0.7-1.7)	6.9 (4.2-13.0)
S0013 BioQ HIV-1 RNA CRF01_AE	P0028	Ultrio	12	0.6 (0.3-1.0)	4.8 (2.6-10.5)
	P0028	Ultrio Plus	24	0.9 (0.6-1.3)	8.0 (4.8-16.6)
	P0028	Ultrio Elite	18	0.7 (0.4-1.8)	6.4 (3.8-13.2)
S0047 subt F	P0054	Ultrio Elite	18	1.2 (0.8-1.8)	6.7 (3.8-17.8)
S0098 subt G	P0098	Ultrio Elite	37	1.1 (0.8-1.5)	7.8 (5.0-15.8)
S0050 subt H	P0100	Ultrio Elite	37	1.1 (0.8-1.6)	5.8 (3.6-13.4)

^ HIV-1 subtype A standard manufacturing traceability and calibration data need to be reassessed

Table 11.2 Detection limits on HIV-2 and HIV group O standard dilution panels in Procleix Ultrio assay versions and cobas MPX assay versions

HIV-RNA standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
WHO HIV-2 08/150	P0207	Ultrio Elite	278	2.2 (1.3-3.5)	18.3 (9.3-64.0)
S0024 HIV-2 subtype A	P0034	Ultrio Elite	37	2.2 (1.7-2.8)	9.3 (6.7-16.2)
S0017 HIV group O	P0015	Ultrio	12	1.6 (0.9-2.7)	13.0 (7.2-26.8)
	P0015	Ultrio Plus	24	1.1 (0.8-1.6)	8.9 (5.6-16.3)
	P0015	Ultrio Elite	30	1.3 (0.9-1.8)	10.4 (6.5-18.2)
S0051 HIV group O	P0101	Ultrio Elite	19	2.3 (1.4-3.7)	17.0 (9.7-62.1)

Limit of quantification (LOQ) of viral load assays
The HIV-RNA reference panels may also be used to check the LOQ of a viral load assay. The LOQ of a viral load assay is the lowest amount of nucleic acid in a sample which can be quantitatively determined with sufficient precision and accuracy.

- **Checking amplification efficiency**.
  For quantitative NAT methods the relation between ^{2}log(concentration) and ^{2}log(quantitative results) or Ct value can be judged using linear regression. Ideally the slope of the curve should be -1.00. If the result is different consider to remove lower concentrations with intermittent reactivity. The slope is accepted when the confidence interval on the slope overlaps -1.00
- **Calculation of precision**.
  The precision becomes less with lower concentrations approaching the LOQ and 95% LOD of the viral load assay. One could calculate the SD of replicate viral load tests for each concentration and compare this with the values in the package insert of the NAT method to be evaluated.

Accuracy
The P0025 or P0290 VQC-Sanquin subtype B standard dilution panels can best be used to examine the accuracy of a quantitative NAT method for reporting values in copies/mL in the lower viral load range. The P0030 or P0294 WHO 97/650 standard dilution panel can best be used for reporting accuracy of values reported in IU/mL. The accuracy is highest when the measured values are equal to the nominal values.

Limitations

The cross calibration of the reference panels in copies/mL was based on testing of HIV-1 subtype standards in the previous bDNA 3.0 assay as reference method. The 95% and 50% LODs will change when calibration would be based on the Abbott RealTime assay (table 6) or other quantitative NAT methods. The IU values assigned to the reference panels were based on the 2nd WHO HIV-1 97/650 standard and a conversion factor of 0.58 copy/IU. However cross calibration of different HIV-1 standards is known to be dependent on the quantitative NAT method used (data in this package insert)[19]. Therefore LODs and copy/IU conversion factors may change when the 3rd or 4th WHO (or other) standards are used.

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