P0140 HIV 1000 copies/mL subtype reference panel

RUO

REF P0140

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

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Intended Use

P0140 HIV 1000 copies/mL subtype reference panel is composed of a series of cross calibrated HIV-RNA standard dilutions covering most of the genetic variants of the virus, i.e. HIV-1 group M subtypes A-H, circulating recombinant forms (CRF's) AE and AG, some group O isolates and HIV-2 subtypes A and B. The panel can be used to investigate the accuracy and relative sensitivity of quantitative nucleic acid amplification technology (NAT) methods for detection of different subtypes of Human Immunodeficiency Virus (HIV) RNA type I and 2 and group O in plasma samples. The product can be used for evaluation of HIV-1 (and HIV-2) nucleic acid amplification technology (NAT) methods, including real-time PCR and TMA assays. This product is not for diagnostic use but for research only.

Key to Symbols Used


Manufacturer

Lot number

Catalogue number

Store below -65°C

Biological substance Category B

Research use only


Expiry date

Contents


Caution


Read instructions for use

Summary and Explanation

The P0140 HIV 1000 copies/mL subtype reference panel has been designed to evaluate the accuracy and relative sensitivity of quantitative HIV-RNA assays. The WHO recommends using a 1000 copies/mL threshold level as indication of lack of virological control in patients receiving anti-viral therapy (ART)[1]. However there is considerable variability in quantitative values reported by different viral load (VL) assays on our HIV-1 subtype B standard[2] and there are no studies comparing the quantification of multiple HIV subtypes by different quantitative NAT methods.

Since the mid1990s a series of tissue culture derived HIV-1, HIV-2 and group O standards of different subtypes and circulating recombinant forms (CRFs) have been established and used for preparation of reference panels for the VQC proficiency program and NAT validation studies[3-8].

The HIV-1 subtype B standard in the reference panel can best be used for evaluating the accuracy of quantitative NAT methods to report VL in copies/mL or International Units/mL. 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[3,9] and used as candidate material in WHO collaborative studies to establish the 1st and 2nd International HIV-1 RNA standards[10]. We used the bDNA 3.0 assay as reference method for calibration in copies/mL[11] and the data from this method in the WHO collaborative study[10] 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[11]. 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[12]. 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[13]. In the period between 1998 and 2004 the quantitative methods reported similar copy numbers on the VQC-Sanquin standard as in 2018. 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 RNA standards of different subtypes in the reference panel 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[14]. The latter method was also used for calibration of HIV group O standards.

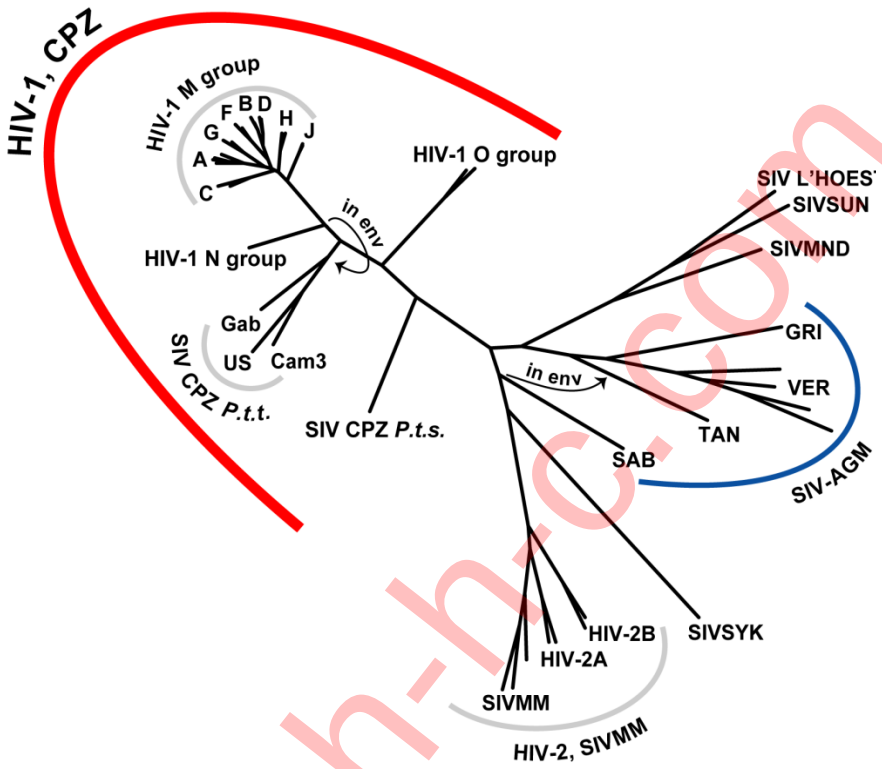
Originally HIV-2 subtypes and group O standards in the reference panel have been calibrated against the HIV-1 subtype B standard in parallel line p24 antigen assays but later by probit analysis in Ultrio Elite and cobas MPX assays[14].

The HIV subtype standards in the P0140 1000 copies/mL reference panel have also been used in analytical sensitivity studies of NAT blood screening assays[3-8] and the 95% and 50% lower limits of detection (LODs) are given for reference in this package insert.

HIV genetic variants

The Human Immunodeficiency Virus type 1 is characterized by extensive genetic heterogeneity (figure 1). Due to this variability, HIV-1 variants are classified in three major phylogenetic groups: group M (major), a group O (outlier) as well as a group N (new)[15-17]. The group M, responsible for the majority of infections in the HIV-1 worldwide epidemic, can be further subdivided into 10 recognized phylogenetic subtypes (A – K)[18-24], which are approximately equidistant from one another. Within the group M, the average inter-subtype genetic variability is 15%, for the gag gene, and 25% for the env gene. However, with the increasing number of viral isolates available worldwide and improvement of sequencing methods, HIV-1 phylogenetic classifications are based either on nucleotide sequences derived from multiple sub-genomic regions (gag, pol and env) of the same isolates or on full-length genome sequence analysis. This approach has revealed virus isolates in which phylogenetic relations with different subtypes switch along their genomes. These inter-subtype recombinant forms are thought to have originated in multiply infected individuals. When an identical recombinant virus is identified in at least three epidemiologically unlinked people it can be designated as circulating recombinant forms (CRFs)[25-31]. More than 20 CRFs have been reported. Nowadays the CRF's do account for 18 % of new infections while CRF01_AE and CRF02_AG are dominant in respectively South-east Asia and West-Central Africa. Group O and group N viruses appear to be related to monkey viruses or a recombination event between a simian immunodeficiency virus (SIV)-like and an HIV-1-like virus. These more distant genetic variants are rarely found in humans and likely result from more recent zoonotic cross-species transmission as the origin of the HIV-1 group M pandemic[32]. The sequences of HIV-1 group O virus isolates are the most distant from HIV-1 group M and highly variable[33] and therefore challenging for HIV-1 diagnostic assays. HIV type 2 is phylogenetically the closest to SIV and therefore classified as a different virus from HIV-1. HIV-2 infections are mainly restricted to West Africa and two subtypes A and B are relevant for HIV-2 viral load assays[34].

Figure 1. Genetic diversity of HIV-1 in humans and several monkey related viruses.



Panel composition

Table 1 gives the composition of P0140 HIV 1000 copies/mL subtype reference panel. The details of the calibration of the different subtype standards in copies and IUs are described in the next chapter. The HIV-RNA standards have been diluted in a pool of plasma units that tested individually negative for HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV 1 and 2, HIV-1-RNA, HCV-RNA and HIV-2 RNA. The HIV-1 group M subtypes and CRFs are the most prevalent around the world and important for evaluation of subtype detection efficiency of HIV-1 VL assays. Of each group M subtype and the most prevalent CRF one sample of 1000 copies/mL is present in the panel. Four group O isolates have been included in the panel to challenge the HIV-1 VL assays (because HIV-1 group O sequences are known to be highly variable[33] and phylogenetically the most distant from HIV-1 group M).

Table 1. Composition P0140 HIV subtype reference panel.

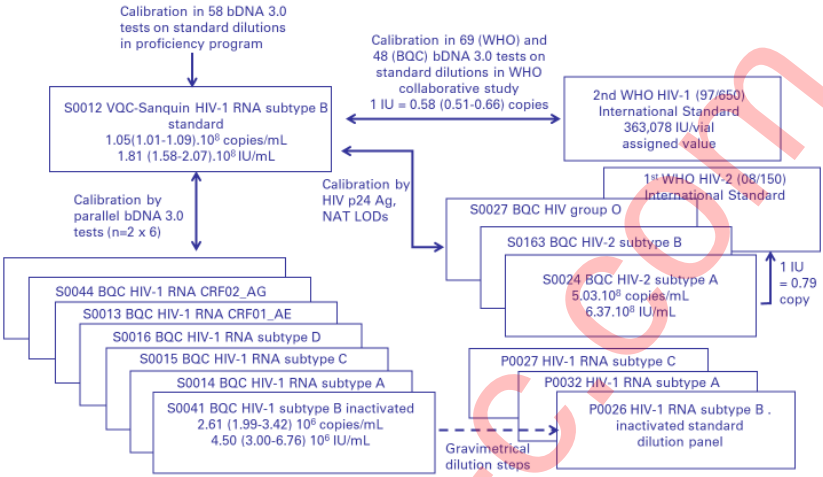
Panel member no	HIV clade/subtype	copies/mL (95% C.I.) #	Country of origin
1	HIV-1 Group M, subtype A	1000 (758-1319)	
2	HIV-1 Group M, subtype B	1000 (962-1041)	
3	HIV-1 Group M, subtype C	1000 (722-1386)	
4	HIV-1 Group M, subtype D	1000 (694-1442)	
5	HIV-1 Group M, subtype F	1000 (616-1623)	Roemenia
6	HIV-1 Group M, subtype F	1000 (703-1422)	Brazil
7	HIV-1 Group M, subtype G	1000 (494-2024)	Zaire
8	HIV-1 Group M, subtype G	1000 (707-1414)	Kenya
9	HIV-1 Group M, subtype H	1000 (886-1129)	Zaire
10	HIV-1 Group M, CRF01_AE	1000 (717-1395)	
11	HIV-1 Group M, CRF01_AE	1000 (798-1253)	Indonesia
12	HIV-1 Group M, CRF02_AG	1000 (497-2022)	Cameroon
13	HIV-1 Group O	4614	USA
14	HIV-1 Group O	3991	Cameroon
15	HIV-1 Group O	4266	Spain
16	HIV-1 Group O	4102	Cameroon
17	HIV-2-RNA subtype A	1720	
18	HIV-2-RNA subtype B	1000	
19	Negative human plasma		
20	Negative human plasma		

HIV-1 group M subtype standards in panel member 1-12 were cross calibrated against S0012 HIV-1 subtype B standard in bDNA 3.0 assay. Group O and HIV-2 standard were calibrated by other methods

Traceability to HIV-RNA copies and International Units

Figure 2 shows the traceability chain between the HIV subtype reference panels, the Bio Quality Control (BioQ) HIV subtype standards, the primary VQC-Sanquin HIV-1 subtype B standard and the 2nd WHO International Standard.

Figure 2. Traceability chain between HIV-RNA reference panels, BioQ 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 2 compares the geometric mean values in copies/mL as reported by different quantitative NAT methods when adjusted to 1000 copies/mL values. 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[12].

Table 2: 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[2]. When comparing the quantitative results obtained two decades later (table 3) with those in the early days of NAT (table 2) 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[13]. 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 3. 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).

Assay	n	geomean cp/mL	(95% CI) cp/mL
Abbott m2000 RealTime Assay m2000	24	1084	(784-1572)
Hologic Aptima	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[10] in which the 1st and 2nd WHO standard were compared with the VQC-Sanquin standard. The data in table 4 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[12].

Table 4. 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[10]

	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)

More recently the VQC-Sanquin subtype B standard was recalibrated against the 3rd and 4th WHO standard in three dilutions varying between 1.000 and 10.000 copies/mL (6 replicate Abbott RealTime VL tests per dilution) and the results of parallel line analysis

indicate that the conversion factor nowadays is 0.25 copies/IU[1]. 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 5).

Table 5. 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 subtype B	1000 copies/mL	18	944 (698-1276)	
2nd WHO standard 97/650	1000 IU/mL	6	392 (266-577)	0.41 (0.27-0.63)
3rd WHO standard 10/152	1000 IU/mL	18	291 (220-577)	0.31 (0.21-0.45)
4th WHO standard 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 69-89% lower copy numbers were reported on the 4th than on the 3rd WHO standard by the quantitative NAT methods used in the participating laboratories[35]. 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][36]

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 6)[14]. The standard dilutions used for the calibration experiment fell well in the dynamic range of the bDNA 3.0 assay.

Table 6. 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 BioQ inactivated	B	6	$2.61\ (1.99\text{-}3.42).10^6$	(76-131)%
S0014 BioQ	A	6	$5.31\ (4.03\text{-}7.00).10^8$	(76-132)%
S0015 BioQ	C	6	$1.44\ (0.97\text{-}2.14).10^8$	(67-129)%
S0016 BioQ	D	6	$6.35\ (4.09\text{-}9.84).10^8$	(65-155)%
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)%
S0013 BioQ (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)%

The same HIV-1 subtype A to H standard dilutions that were used for the bDNA 3.0 calibration experiments (table 6) 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 7 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 7 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 7. 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[14].

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 BioQ inactivated	B	6	1000 (760-1310)	6	920 (713-1208)$
S0014 BioQ	A	6	1000 (760-1320)	3	4948 (4535-5259)
S0015 BioQ	C	6	1000 (670-1290)	2	2549 (2309-2788)
S0016 BioQ	D	6	1000 (650-1550)	3	3715 (3502-3822)
S0013 BioQ (1)	CRF01_AE (1)	6	1000 (670-1490)	3	4221 (4107-4423)
S0045 Thailand (2)	CRF01_AE (2)	3	1000 (860-1230)	3	2703 (2536-3021)
S0044 Ghana	CRF02_AG	3	1000 (950-1170)	3	3669 (3417-3962)
S0046 Brazil	F (1)	3	1000 (640-1590)	3	1736 (1511-1991)
S0047 Romania	F (2)	3	1000 (720-1420)	3	2645 (2567-2746)
S0048 Zaire	G (1)	3	1000 (770-1340)	3	3210 (2963-3367)
S0049 Kenya	G (2)	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 8 compares the calibration of 5 group O standards against the S0012 HIV-1 subtype B standard and the Abbott RealTime assay. The final calibration was based on additional NAT tests and is described in a validation report VR4026[14].

Table 8. 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[14].

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[14]
S0017 BioQ (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)	$8.30.10^7$	$3.79.10^8$	$3.41.10^8$

not present in this panel

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 9)[14]. 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[14].

Table 9. Calibration of HIV-2 subtype A and B standards in copies/mL[14]

HIV-2 RNA standards	subtype	copies/mL	Calibration procedure
S0024 BioQ	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 BioQ	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)

described in Supplemental material in Ultrio Elite validation study of Grabarczyk et al[7]

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[13]; 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. Hence, it can be guaranteed that the reference panels are stable when stored below -65˚C.[13]

NAT detection limits on HIV subtype standards

The HIV subtype standards in the reference panel have also been used in analytical sensitivity studies of NAT blood screening assays. Table 10 and 11 compare the 95% and 50% LODs of the Ultrio and cobas MPX versions on the different HIV subtype standards.

Table 11. 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
2nd WHO HIV-1 RNA standard 97/650	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)
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)
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)
BioQ HIV-1 RNA subtype A	P0032	Ultrio Plus	24	8.3 (5.7-12.1)	78.8 (49.5-138.4)
	P0032	Ultrio Elite	51	21.5(15.9-29.0)	250 (150-532)
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)
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)
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)
BioQ HIV-1 RNA subtype F	P0054	Ultrio Elite	18	1.2 (0.8-1.8)	6.7 (3.8-17.8)
BioQ HIV-1 RNA subtype G	P0098	Ultrio Elite	37	1.1 (0.8-1.5)	7.8 (5.0-15.8)
BioQ HIV-1 RNA Subtype H	P0100	Ultrio Elite	37	1.1 (0.8-1.6)	5.8 (3.6-13.4)

Table 1. 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
1st WHO HIV-2 RNA standard 08/150	P0207	Ultrio Elite	278	2.2 (1.3-3.5)	18.3 (9.3-64.0)
BioQ HIV-2 RNA subtype A	P0034	Ultrio Elite	37	2.2 (1.7-2.8)	9.3 (6.7-16.2)
BioQ HIV RNA 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)
BioQ HIV RNA group O	P0101	Ultrio Elite	19	2.3 (1.4-3.7)	17.0 (9.7-62.1)

not included in this panel

Storage Instructions

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

Kit contents (materials provided)

The run control contains human plasma without preservatives and is provided in two formats as detailed in Table 2.

Table 2. Description of kit formats and contents

Cat. Code	Description of contents	Primary packing	Secondary packing
P0140/01	20 x 4.0 mL panel member	10 mL vial	60 vial rack in box
P0140/02	20 x 1.2 mL run control	2 mL vial	Plastic zip bag

Materials required but not supplied

The test kits and liquid handling devices provided by the NAT manufacturer

Warning and precautions

The P0140 HIV 1000 copies/ml subtype reference panel members contain infectious HIV virions and are bio-hazardous. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[37,38,39]. 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 subtype 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.

- 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 P0140 HIV 1000 copies/ml subtype reference panel and specimens are 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 to avoid formation of cryo-precipitates.
- Mix gently during thawing until ice clot has disappeared.
- Immediately after thawing, vortex briefly, and give a short spin before releasing screw cap from the vials.
- The panel members should be handled and tested in a manner identical to that required for clinical specimens run in the test procedure being evaluated.
- Follow the manufacturers or testing laboratory instructions and recommendations for the handling and testing of clinical specimens.

Limitations

The P0140 HIV 1000 copies/ml subtype reference panel is not intended to replace the internal calibrators integral to in vitro diagnostic (IVD) test kits, but may be used as external, independent standards for the assessment of the performance of qualitative or quantitative NAT assays.

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