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**REPORT OF AN INTERNATIONAL COLLABORATIVE
STUDY TO EVALUATE A WHO HIV-1 RNA GENOTYPE
REFERENCE PANEL**

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Summary

Twenty-eight laboratories from 16 countries participated in a collaborative study to evaluate an HIV-1 RNA Genotype Reference Panel (NIBSC Code 01/466) for use with nucleic acid-based tests (NAT). The Reference Panel consisted of 11 coded samples representing different HIV-1 genotypes (subtypes A, B, C, D, AE, F, G, AG-GH, groups N and O) as well as a negative diluent control. Each laboratory assayed the eleven panel members concurrently with the 1st International Standard for HIV-1 RNA (NIBSC Code 97/656) on at least three separate occasions and the data collated and analysed at NIBSC. Twenty-nine sets of data were received, 19 from quantitative and 10 from qualitative assays, with six different commercial assays and five “in-house” assays represented. The results showed that subtypes A-E were detected consistently by most assays, although there were minor discrepancies between assays. However, a variety of problems occurred with the detection and quantitation of the other subtypes and groups. In particular the Nuclisens assay had difficulty in the detection of subtypes F, G, AG-GH and group N. Most assays failed to detect the group O representative. The study highlighted the shortcomings of some molecular assays particularly in detection of some of the non-B genotypes, which are important viruses in the global AIDS pandemic and illustrated the value of a well-characterized genotype panel. It was proposed that the HIV-1 RNA Genotype Reference Panel be established as a WHO Reference Reagent.

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Introduction

HIV-1 exhibits substantial genetic diversity and several different genotypes of HIV-1 exist. There is a major group (group M), consisting of subtypes A-K, and a more diverse collection of outliers have been referred to as groups N and O. Many of the early nucleic acid-based tests (NAT) had a fairly narrow band of specificity targeted mainly at subtype B viruses, as these predominated in the developed world. Greater awareness of HIV genetic diversity and the desire to detect as broad a range of HIV strains as possible has led to a number of improvements in assay design. However, it has been recognised that some assays are still poor at detecting certain subtypes/groups, occasionally giving sub-optimal or even negative results for samples that are clearly positive in other assays.

Representatives of the WHO Collaborating Centres involved in the Working Group on Reference Preparations for testing HBsAg, HCV and HIV Diagnostic Kits as well as the WHO International Working Group on Standardisation of Gene Amplification Techniques for the Virological Safety Testing of Blood and Blood Products (SoGAT) agreed that there is a need for a well characterised reference panel of different HIV-1 subtypes/groups. Such a reference panel would be of particular use in regions of the world where non-B genotypes of HIV-1 predominate or are frequently encountered by laboratories involved in molecular (NAT) diagnosis and patient monitoring as well as by kit manufacturers. NIBSC agreed to formulate a candidate reference panel and evaluate it in an international collaborative study.

Participants

A total of twenty-eight expert laboratories from around the world took part in the collaborative study and are listed in Table 1. The summary below shows the geographical spread of the participants.

Australia	2
Austria	1
France	1
Brazil	1
Belgium	1
Germany	5
Japan	3
The Netherlands	1
UK	2
USA	4
South Africa	1
Ivory Coast	1
Uganda	1
Finland	1
Sweden	1
Spain	2

Materials

Table 2 shows the viral strains and genotypes chosen for inclusion in the candidate reference panel, their corresponding study code, geographical origin and source through which they were obtained. Where possible viral isolates with a published full-length genomic sequence were chosen (accession numbers given where known), thus establishing that they were non-recombinant, representative viruses. An exception was 92TH001, which is an AE recombinant (no pure subtype E virus has ever been isolated), and V1525, which was subsequently shown to be a complex mixture of two recombinant viruses, one a GH and the other an AG recombinant.

Where possible, viral isolates were obtained at low passage. All viruses were propagated on human PBMC's and, after clarification by centrifugation, a stock of cell-free culture supernatant was stored under liquid nitrogen vapour as 1ml aliquots.

Prior to formulating the Reference Panel, an estimate of the RNA concentration of the undiluted viral stocks was obtained using four diverse NAT systems, Roche Amplicor HIV-1 Monitor Version 1.5 (Monitor), Biomerieux Nuclisens HIV-1 QT (Nuclisens), Bayer Versant bDNA V3 (bDNA) and an "in-house" assay based on the LTR region of the viral genome (Cleland et al, 2001). Based on these results, dilutions were estimated for each viral stock to give a final concentration for each panel member in the region of 2000-10,000 copies/ml. In order to prepare the reference panel, viral stocks were spun at 10,000g for 20mins to remove cellular debris and diluted by the pre-determined amount in defibrinated plasma (Base Matrix, Boston Biomedica Inc, USA) which had been pre-screened for HBsAg, Anti-HCV and Anti-HIV-1/2 and found to be negative. A total of 550ml of each panel member was produced and these were distributed in aliquots of 1.1ml. Each panel member was prepared on a separate occasion. A virus-negative diluent control was included. A total of 500 Genotype Reference Panels (NIBSC Code 01/466) were prepared each containing 11 panel members.

For the collaborative study, 100 Reference Panels were coded such that each panel member was assigned a random code from ST01 – ST11, as shown in table 2. The remaining 400 vials were labelled according to their strain designation. All vials were batch frozen at -70°C .

The International Standard for HIV-1 RNA (NIBSC Code 97/656) was included in the study (Holmes et al, 2001).

Organization of the International Collaborative Study

All twenty-eight participating laboratories were assigned a code number, allocated at random, for the duration of the study and are referred to in this report by their code number.

Each participant was sent 3 sets of the Genotype Reference Panel (NIBSC Code 01/466) containing the 10 coded HIV-1 RNA genotypes (subtypes A, B, C, D, AE, F, G, AG-GH, groups N and O) and negative control and three vials of the International Standard for HIV-1 RNA (NIBSC code 97/656). All samples were shipped on dry ice and stored at -70°C to -85°C until required. The use of vials after a freeze thaw cycle was not recommended.

Participants were asked to thaw the liquid preparations (genotype panel) at 37⁰C in a water bath. The International Standard is a lyophilised preparation and participants were instructed to reconstitute the vial by removing the tear-off crimp and loosening the rubber seal and adding 1.0ml of de-ionised RNase-free water. The vial was gently agitated for 20minutes to fully dissolve the contents before use.

Participating laboratories were requested to assay the eleven panel samples concurrently with the International Standard on at least three separate occasions. Laboratories performing quantitative assays were requested to assay each panel member and the international standard undiluted. Laboratories performing qualitative assays were requested to perform an initial assay using 1.0 log₁₀ dilution steps followed by at least two further assays using a dilution series of 0.5 log₁₀ steps selected to cover the anticipated end-point.

Stability

The stability of the Genotype Reference Panel at -70⁰C has not been established, but samples will be assayed periodically in order to determine the real time stability of the frozen panel. No loss of potency has been observed with a number of HIV-1 RNA Working Standards consisting of cultured HIV diluted in human cryo-poor plasma and stored at -70⁰C for periods of up to two years.

Statistical methods

a. Quantitative assays

Where laboratories returned quantitative results, a single estimate was calculated for each sample as the geometric mean over all assays performed by the individual laboratory (arithmetic mean of log₁₀ estimates).

b. Qualitative assays

Where laboratories returned results from qualitative assays, a single estimate was calculated for each laboratory using methods based on the Poisson model, as described in the report of the collaborative study to establish the International Standard (Holmes et al, 2001).

Results

Data were received from 28 laboratories with one laboratory reporting data from two different assays. These comprised data sets from 10 (34%) qualitative assays and 19 (66%) quantitative assays. A variety of assay methods were used. Of the 10 sets of qualitative assays, 5 were from “in-house” methods, 4 from Roche Ampliscreen, and 1 TMA. Of the 19 quantitative, 12 were versions of the Roche Amplicor Monitor assay v1.5 (data from the standard and Ultrasensitive techniques as well as from the automated COBAS system have been combined), 4 were Biomerieux Nuclisens HIV-1 QT, 2 were Bayer Versant bDNA V3, and 1 Abbott LCx HIV quantitative assay. Details of assay methods used by individual laboratories and a summary of the codes used for the assays in this report are given in table 3. For the purposes of analysis all 5 “in house” assay data sets have been grouped together, although a variety of different methods were reported.

Mean estimates of \log_{10} copies/ml or \log_{10} detectable units/ml were obtained for each sample and laboratory, using the methods described above, and these are shown in table 4. They are also shown in histogram form, in figures 1a – 1k, where each box represents the mean estimate from one laboratory, and is labelled with the laboratory code and a code representing the assay method. The different types of assay kit are also colour coded, with all variants of the Monitor assay being coloured the same. For convenience the “in-house” methods are coloured the same, but all represent different methodologies. The histograms specifically include completely negative (or below detection limit) results for laboratories and samples. Negative results are plotted on the extreme left of the horizontal axis, to the left of the label for 0.0. (On the log scale (\log_{10} copies/ml) 0.0 represents 1 copy/ml, and not a negative). The mean estimated RNA copies/ml (or detectable units/ml for qualitative assays) are shown in table 5 for the different assay types used. Again, all variants of Monitor have been grouped as a single assay method, as have the “in-house” methods. The minimum, maximum, standard deviation and range of estimates from the different laboratories are also shown and the means are summarised in table 6.

Analysis of the data shows that estimates from laboratories using qualitative assays are generally lower than those from quantitative assays, as expected. Furthermore, it can be seen that most assays detect subtypes A-E satisfactorily, but that difficulties were experienced by some assays in the detection of subtypes F-H and groups N and O.

International Standard: There is reasonable agreement between the majority of laboratories using quantitative assays and the results are in line with that obtained in previous studies (Holmes et al 2001; Davis et al 2002). The two laboratories using the bDNA assay obtained estimates that were slightly lower than those laboratories that used Monitor or Nuclisens assays. The single laboratory using the Abbott LCx assay obtained an estimate slightly lower than the Monitor estimates. One laboratory (lab code 12) had a particularly low estimate with an “in-house” qualitative assay, being around 1.0-1.5 \log_{10} lower than other laboratories.

Subtypes A-E: Overall there was good agreement between the majority of laboratories using quantitative assays and the performance of the laboratories and assays for these genotypes were similar to the performance of the international standard. Laboratories using bDNA assays obtained estimates that were slightly lower than laboratories using other assays for the subtype A and C samples. Whilst commercial qualitative assays often gave values that fell within the range given by quantitative assays, the “in house” qualitative assays showed somewhat lower levels of detection.

Subtype F: Of the four laboratories that used the Nuclisens assay, two obtained negative results and two gave results much lower than the other quantitative assays. There was good agreement between the other quantitative assay methods, but with the bDNA and Abbott LCx assays giving results slightly lower than the Monitor assays. The “in-house” qualitative assays gave lower and more variable results.

Subtype G: All laboratories using the Nuclisens assays obtained negative (below lower limit of detection) results for this sample. The “in-house” qualitative assays from laboratories 12, 67 and 54 gave estimates that were lower than other qualitative assays, particularly for laboratory 12. The laboratories using the bDNA and Abbott LCx assays obtained estimates that were slightly lower than those laboratories that used Monitor assays.

Subtype AG-GH: There was considerable variation between results from the different assay methods with this complex recombinant virus mixture. Within laboratories using the same assay method, however, there was good agreement. The Nuclisens assays detected the sample, but gave estimates around $2 \log_{10}$ lower than the Monitor assay. The “in-house” and Ampliscreen qualitative assays were also lower than the Monitor, although the TMA assay gave results that were in-line with Monitor. Results from the bDNA and Abbott assays were also slightly lower than Monitor for this sample.

Group N: All laboratories using the Nuclisens assays obtained values below the lower limit of detection for this sample, as did the some of the “in-house” assays. The remaining “in-house” qualitative assays gave estimates that were lower than commercial qualitative assays. The two laboratories using the bDNA assay and the one laboratory using the Abbott LCx obtained estimates that were around 1 log lower than those laboratories that used Monitor assays. The performance of the qualitative TMA assay was similar to the quantitative bDNA and Abbott assays, while the qualitative Ampliscreen assays gave higher estimates, similar to the quantitative Monitor results.

Group O: This sample was found to be negative or below detection limit by all but 3 laboratories. The exceptions were the qualitative TMA assay, a qualitative “in-house” assay and the Abbott LCx assay. It should be noted that negative results were obtained with the Abbott LCx assay with the initial assays, however extra data was submitted from an additional assay using a larger sample volume (1ml), which was positive. The positive estimate in the table and histogram is based solely on this one assay.

Data were also received from an additional laboratory using a novel quantitative viral load assay based on the detection of viral reverse transcriptase. The assay consisted of an initial viral lysis stage during which inhibitory factors were removed, followed by a micro-RT assay in which BrdUTP incorporation was quantified through an alkaline phosphatase-substrate reaction. Results indicated that the assay was capable of detecting all the genotypes in the panel with a negative reaction for the diluent control. However, the study protocol was not followed and the results were therefore not included in the analysis.

Discussion/Conclusions

A total of 28 laboratories participated in the collaborative study submitting data sets from 19 quantitative and 10 qualitative assays. Despite the extensive nature of the study, it is important to exercise caution in making general comments regarding the comparative performance of the different assay methods, as methods other than the Monitor have had limited representation in the study. However, from the tables and figures it is clear that the results from laboratories using qualitative assays, particularly the “in-house” versions, were generally lower than those from quantitative assays. This was to be expected, as the data from qualitative assays were treated as dilution series and used to provide single estimates of detectable units per ml in the undiluted samples using the method of maximum likelihood that takes account of the Poisson distribution (Collet 1991). This method assumes that the presence of at least one ‘detectable unit’ in the sample volume tested will lead to a positive result and makes no correction for assay inefficiencies, such as lysis and extraction effects and those that occur during reverse transcription. The estimated ‘detectable units per ml’ cannot therefore be directly equated to an estimate of RNA copies per ml.

There was good agreement between all laboratories using the Monitor assay. The results from the bDNA assays were generally slightly lower than those from the Monitor for the majority of the liquid samples, as well as for the freeze-dried International Standard. There was a difference of around 0.3-0.4 log₁₀ between the overall means (table 6) in most cases. The exceptions were subtypes B & D where no difference was apparent and group N, where the bDNA was 1.0 log₁₀ lower than Monitor.

The results from the Nuclisens assay appear less consistent across samples than the other assay kits. Subtypes G and group N were not detected by the Nuclisens assay, and subtype F was either not detected or gave much lower estimates than other assays types. The Nuclisens assay also had much lower estimates for the recombinant mixture subtype AG-GH than other assay types. For other samples, the results from the Nuclisens assay were similar or higher than from the Monitor assay.

The Abbot LCx, a *pol*-based PCR assay, was the only quantitative assay to detect group O, although this was only when the assay was repeated using a larger sample volume. This sample was found negative by all other assays except the qualitative TMA and one of the qualitative “in-house” methods.

The performance of the “in-house” methods was more variable than the commercial assays, with some methods giving much lower estimates than other qualitative assays. The “in-house” assay results from laboratory 59 were consistent with many of the commercial assay results.

The diluent control sample was found to be negative by all laboratories and assay types.

This study has highlighted the shortcomings of a number of commercial and “in-house” assays with regard to their ability to detect and, in the case of quantitative assays, to quantitate HIV-1 RNA, particularly some of the less frequently encountered non-subtype B HIV-1 genotypes. The 1st International Standard for HIV-1 RNA was included in the study and showed good correlation with the results of two previous studies (Holmes et al 2001; Davis et al 2002). However, the sub-optimal detection of some genotypes with some assays makes the determination of potencies relative to the International Standard inappropriate for these subtypes. We have therefore not attempted to calibrate the Genotype Reference Panel members in terms of international units.

Proposal

An interim oral report was given to a meeting of the representatives of the WHO Collaborating Centres involved in the Working Group on Reference Preparations for testing HBsAg, HCV and HIV Diagnostic Kits held in Geneva in December 2001. A report on the collaborative study was also circulated to members of the SoGAT International Working Group and a presentation given to the most recent meeting of the SoGAT Working Group (Athens, June 2002). It was agreed that the HIV-1 Genotype Reference Panel (NIBSC Code 01/466) would provide a valuable set of well characterised reagents for HIV-1 NAT for use in regions of the world where non-B subtypes of HIV-1 predominate or are frequently encountered by laboratories involved in molecular (NAT) diagnosis and patient monitoring as well as by kit manufacturers. It was further agreed that the panel should be proposed as a WHO Reference Reagent, although no assignment of potency was recommended.

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Table 1: Characteristics of the Genotype Reference Panel

Subtype /Group	Strain	Study code	Origin	Source	Full length Sequence	Accession Number
A	92UG037	ST04	Uganda	UNAIDS	Yes	U51190
B	92TH014	ST07	Thailand	UNAIDS	Yes	U86572
C	98TZ017	ST10	Tanzania	UNAIDS	Yes	AF286235
D	94UG114	ST03	Uganda	UNAIDS	Yes	U88824
AE	92TH001	ST01	Thailand	UNAIDS	Near full length	U86565
F	93BR020	ST08	Brazil	UNAIDS	Yes	AF005494
G	RU570	ST02	Russia	CFAR (Dr. A Bobkov)	No	U08368
AG-GH	VI525	ST05	Gabon	CFAR (Dr. v.d.Groen)	No	L11792 U09665 AJ277822
N	YBF30	ST09	Cameroon	CFAR (Dr. F Simon)	Yes	AJ006022
O	MVP5180	ST06	Cameroon	CFAR (Dr. L Gurtler)	Yes	L20571

Table 2: Laboratory Assay Methods and Codes

<u>Laboratory Assay Methods and Codes</u>		
Lab Code	Assay Code	Assay Method
Quantitative		
04	N	Nuclisens
05	CM	COBAS Monitor v1.5
09	CM	COBAS Monitor v1.5
14	B3	bDNA v3
17	M	Monitor v1.5
22	M	Monitor v1.5
27	UCM	COBAS Monitor v1.5 (Ultrasensitive)
45	AL	Abbot LCx
48	N	Nuclisens
56	M	Monitor v1.5
57	CM	COBAS Monitor v1.5
58	B3	bDNA v3
60	M	Monitor v1.5
61	M	Monitor v1.5
63	M*	Modified Monitor v1.5
64	N	Nuclisens
68	N	Nuclisens
69	CM	COBAS Monitor v1.5
72	M	Monitor v1.5
Qualitative		
12	IH	In-House
17	T	TMA
19	CAS	COBAS Ampliscreen
30	CAS	COBAS Ampliscreen
52	IH	In-House
54	IH	In-House
59	IH	In-House
65	AS	Ampliscreen
66	AS	Ampliscreen
67	IH	In-House

Summary of Assay Codes:

M	Monitor	AS	Ampliscreen
M*	Monitor (modified)	T	TMA
CM	COBAS Monitor v1.5	IH	In-House
UM	Monitor v1.5 (Ultrasensitive)	AL	Abbot LCx
N	Nuclisens	B3	bDNA v3.0
	CAS		COBAS Ampliscreen

Table 3: Laboratory Mean Estimates
RNA Copies/ml or PCR Detectable Units (log₁₀/ml)

Lab	Assay	IS	A	B	C	D	AE	F	G	AG GH	N	O
<i>Quantitative</i>												
04	N	4.93	3.43	3.24	3.27	3.47	3.46	-	-	2.06	-	-
05	CM	4.97	3.19	3.05	3.38	3.38	3.48	3.50	3.79	4.27	3.67	-
09	CM	4.65	3.39	3.19	3.30	3.61	3.62	3.63	3.73	3.84	3.40	-
14	B3	4.51	3.05	3.17	3.04	3.50	3.27	3.41	3.23	3.57	2.58	-
17	M	4.85	3.62	3.04	3.62	3.66	3.68	3.74	3.89	4.27	3.73	-
22	M	4.81	3.42	3.19	3.35	3.44	3.49	3.54	3.61	4.12	3.62	-
27	UCM	4.66	3.18	2.99	3.25	3.22	3.47	3.48	3.61	3.95	3.53	-
45	AL	4.51	3.57	3.40	3.44	3.71	3.64	3.53	3.34	3.62	2.48	2.18
48	N	5.26	3.68	3.64	3.78	3.98	3.69	2.12	-	2.34	-	-
56	M	5.07	3.52	3.26	3.54	3.41	3.71	3.70	3.87	4.26	3.81	-
57	CM	5.14	3.57	3.40	3.61	3.67	3.97	3.81	3.89	4.26	3.75	-
58	B3	4.65	3.07	3.23	3.11	3.48	3.39	3.53	3.28	3.58	2.65	-
60	M	4.75	3.29	2.89	3.40	3.45	3.59	3.84	3.51	4.11	3.87	-
61	M	4.93	3.23	3.06	3.33	3.18	3.58	3.46	3.73	4.06	3.24	-
63	M*	4.99	3.62	3.37	3.37	3.68	3.80	3.92	4.01	4.20	3.46	-
64	N	4.95	3.49	3.54	3.65	3.73	3.50	2.54	-	2.14	-	-
68	N	5.26	3.61	3.68	3.65	4.06	3.49	-	-	2.18	-	-
69	CM	4.94	3.26	3.27	3.54	3.76	3.62	3.77	3.78	4.10	3.73	-
72	M	4.57	3.32	3.11	3.46	3.57	3.54	3.60	3.76	3.92	3.47	-
<i>Qualitative</i>												
12	IH	3.26	2.39	1.99	1.75	1.83	1.65	1.90	1.34	2.88	0.75	-
17	TMA	4.52	3.16	3.04	3.52	3.33	3.00	3.80	3.07	3.97	2.78	1.64
19	CAS	4.10	3.21	2.69	3.21	3.08	3.13	3.84	3.68	3.42	3.42	-
30	CAS	4.47	3.11	2.66	2.90	2.95	3.52	3.98	3.17	3.57	3.17	-
52	IH	4.24	2.68	2.57	3.15	3.21	2.68	1.47	3.68	2.77	-	-
54	IH	4.30	2.00	3.13	2.42	2.99	2.58	2.42	2.81	3.00	-	-
59	IH	4.34	3.21	2.72	3.39	3.45	3.21	3.21	3.45	3.21	-	-
65	AS	4.74	3.38	2.54	3.61	3.21	3.51	2.77	3.11	3.21	3.38	-
66	AS	4.64	3.06	3.23	3.82	3.27	3.61	3.51	3.75	4.05	3.79	-
67	IH	4.53	2.20	2.61	2.47	2.47	1.99	2.59	2.25	2.46	2.10	1.08

Table 4: Mean Estimated RNA Copies/ml or PCR Detectable Units/ml (Log₁₀)

Sample	N* / N	Mean	Min	Max	SD	Range
<u>International Standard</u>						
<i>Quantitative:</i>						
Monitor	12/12	4.86	4.57	5.14	0.18	0.57
Nuclisens	4/ 4	5.10	4.93	5.26	0.19	0.34
bDNA	2/ 2	4.58	4.51	4.65	0.10	0.15
Abbot LCx	1/ 1	4.51				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	4.49	4.10	4.74	0.28	0.65
TMA	1/ 1	4.52				
In-House	5/ 5	4.14	3.26	4.53	0.50	1.27
<u>Subtype A</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.38	3.18	3.62	0.16	0.44
Nuclisens	4/ 4	3.55	3.43	3.68	0.11	0.25
bDNA	2/ 2	3.06	3.05	3.07	0.01	0.02
Abbot LCx	1/ 1	3.57				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.19	3.06	3.38	0.14	0.32
TMA	1/ 1	3.16				
In-House	5/ 5	2.50	2.00	3.21	0.47	1.21
<u>Subtype B</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.15	2.89	3.40	0.16	0.51
Nuclisens	4/ 4	3.53	3.24	3.68	0.20	0.44
bDNA	2/ 2	3.20	3.17	3.23	0.04	0.06
Abbot LCx	1/ 1	3.40				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	2.78	2.54	3.23	0.31	0.70
TMA	1/ 1	3.04				
In-House	5/ 5	2.60	1.99	3.13	0.41	1.14

Table 4 (continued)

Mean Estimated RNA Copies/ml or PCR Detectable Units/ml (Log₁₀)

Sample	N*/ N	Mean	Min	Max	SD	Range
<u>Subtype C</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.43	3.25	3.62	0.12	0.37
Nuclisens	4/ 4	3.59	3.27	3.78	0.22	0.51
bDNA	2/ 2	3.07	3.04	3.11	0.05	0.07
Abbot LCx	1/ 1	3.44				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.38	2.90	3.82	0.41	0.92
TMA	1/ 1	3.52				
In-House	5/ 5	2.64	1.75	3.39	0.65	1.64
<u>Subtype D</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.50	3.18	3.76	0.19	0.59
Nuclisens	4/ 4	3.81	3.47	4.06	0.27	0.59
bDNA	2/ 2	3.49	3.48	3.50	0.02	0.02
Abbot LCx	1/ 1	3.71				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.13	2.95	3.27	0.14	0.33
TMA	1/ 1	3.33				
In-House	5/ 5	2.79	1.83	3.45	0.65	1.62
<u>Subtype AE</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.63	3.47	3.97	0.14	0.49
Nuclisens	4/ 4	3.53	3.46	3.69	0.10	0.23
bDNA	2/ 2	3.33	3.27	3.39	0.09	0.12
Abbot LCx	1/ 1	3.64				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.44	3.13	3.61	0.21	0.48
TMA	1/ 1	3.00				
In-House	5/ 5	2.42	1.65	3.21	0.61	1.56

Table 4 (continued)**Mean Estimated RNA Copies/ml or PCR Detectable Units/ml (Log₁₀)**

Sample	N* / N	Mean	Min	Max	SD	Range
<u>Subtype F</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.67	3.46	3.92	0.15	0.46
Nuclisens	2/ 4	2.33	2.12	2.54	0.30	0.43
bDNA	2/ 2	3.47	3.41	3.53	0.09	0.12
Abbot LCx	1/ 1	3.53				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.53	2.77	3.98	0.54	1.21
TMA	1/ 1	3.80				
In-House	5/ 5	2.32	1.47	3.21	0.66	1.74
<u>Subtype G</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.76	3.51	4.01	0.14	0.50
Nuclisens	0/ 4	-				
bDNA	2/ 2	3.25	3.23	3.28	0.03	0.04
Abbot LCx	1/ 1	3.34				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.43	3.11	3.75	0.33	0.64
TMA	1/ 1	3.07				
In-House	5/ 5	2.71	1.34	3.68	0.95	2.34
<u>Subtype AG-GH</u>						
<i>Quantitative:</i>						
Monitor	12/12	4.11	3.84	4.27	0.15	0.44
Nuclisens	4/ 4	2.18	2.06	2.34	0.11	0.27
bDNA	2/ 2	3.58	3.57	3.58	0.00	0.00
Abbot LCx	1/ 1	3.62				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.56	3.21	4.05	0.36	0.84
TMA	1/ 1	3.97				
In-House	5/ 5	2.87	2.46	3.21	0.28	0.75

Table 4 (continued)

Mean Estimated RNA Copies/ml or PCR Detectable Units/ml (Log₁₀)

Sample	N* / N	Mean	Min	Max	SD	Range
<u>Group N</u>						
<i>Quantitative:</i>						
Monitor	12/12	3.61	3.24	3.87	0.19	0.64
Nuclisens	0/ 4	-				
bDNA	2/ 2	2.62	2.58	2.65	0.05	0.08
Abbot LCx	1/ 1	2.48				
<i>Qualitative:</i>						
Ampliscreen	4/ 4	3.44	3.17	3.79	0.26	0.62
TMA	1/ 1	2.78				
In-House	2/ 5	1.43	0.75	2.10	0.96	1.35

Group O

<i>Quantitative:</i>						
Monitor	0/12					
Nuclisens	0/ 4					
bDNA	0/ 2					
Abbot LCx	1/ 1	2.18				
<i>Qualitative:</i>						
Ampliscreen	0/ 4					
TMA	1/ 1	1.64				
In-House	1/ 5	1.08				

N* / N - Number of laboratories with positive results / Number of laboratories performing assays.

Summary statistics calculated from positive results only, and will therefore overestimate the mean and underestimate the spread for those samples and assay types where some of the laboratories obtained negative results.

Table 5: Estimated RNA Copies/ml or PCR Detectable Units/ml (Log₁₀)

Means by Assay Type

	Assay Type	IS	A	B	C	D	AE	F	G	AG-GH	N	O
Quantitative Assays	Monitor	4.86	3.38	3.15	3.43	3.50	3.63	3.67	3.76	4.11	3.61	-
	Nuclisens	5.10	3.55	3.53	3.59	3.81	3.53	2.33*	-	2.18	-	-
	bDNA	4.58	3.06	3.20	3.07	3.49	3.33	3.47	3.25	3.58	2.62	-
	Abbot LCx	4.51	3.57	3.40	3.44	3.71	3.64	3.53	3.34	3.62	2.48	2.18
Qualitative Assays	Ampliscreen	4.49	3.19	2.78	3.38	3.13	3.44	3.53	3.43	3.56	3.44	-
	TMA	4.52	3.16	3.04	3.52	3.33	3.00	3.80	3.07	3.97	2.78	1.64
	In-House	4.14	2.50	2.60	2.64	2.79	2.42	2.32	2.71	2.87	1.43*	1.08*

Based on laboratories with positive results only.