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ACHIEV$_2$E
A Collaboration on HIV-2 infection

Quality control assessment of HIV-2 viral load quantification assays
Results from an international collaboration on HIV-2 infection, 2006

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BACKGROUND

There is currently no gold standard for the quantification of plasma HIV-2-RNA. At the patient level, difficulties arise for the interpretation of results and may yield inappropriate clinical decision; at the population level, this may explain controversial evaluation of response to treatment among different cohorts.

We evaluated the validity of HIV-2 subtype A RNA quantification assays performed in reference laboratories located in 9 countries (Belgium, France, Gambia, Germany, Portugal, Spain, Sweden, Switzerland and UK) within the ACHIEV$_2$E study group.

MATERIAL and METHODS

In a double blind experimental design, each of the nine participating laboratories quantified:

- 3 series of 10 aliquots of NIHZ HIV-2 subtype A supernatant counted by electronic microscopy (ABI technologies) previously diluted in HIV negative human plasma and stored at -80°C. Each series was diluted at a different theoretical viral load: 1.7, 2.3 and 3 $\log_{10}$ copies/ml.
- 5 HIV-2 negative aliquots.

Laboratories used mainly real PCR assays, with primers and probes located on various regions of HIV-2 genome and different HIV-2 standards.

Accuracy and precision of assays were estimated for the 3 theoretical concentration levels. A quantification assay was defined as accurate if at least 9/10 of the quantifications were in the expected interval:

[(theoretical viral load)÷3 - (theoretical viral load)×3].

Precision of assays was evaluated using the intralaboratory coherence coefficient (ILCC) and the coefficient of variation (CV) at each of the 3 theoretical concentration viral loads.

$$ILCC_{ij} = \frac{s_{ij}\sqrt{p_j}}{\sqrt{\Sigma s_{ij}^2}}$$

where i = laboratory
j = concentration level
p = number of laboratory
s = standard deviation
s^2 = variance

A quantification heterogeneity exists when ILCC > L
(L extracted from a reference table for p laboratories and n repetitions)

$$CV_{ij} = \frac{s_{ij}}{m_{ij}}$$

where s_{ij} = standard deviation of the laboratory i at the concentration level j
m_{ij} = mean of the quantifications of the laboratory i at the concentration level j

RESULTS

Characteristics of each evaluated quantification assays are shown in table 1.
No false positive result was reported.

Table 1. Characteristics of quantification assays assessed in the ACHIEV$_2$E Collaboration, 2006.

Laboratories	System	primers and probe's localization	RNA extraction	Standard used	Threshold ($\log_{10}$ copies/mL)	subtypes tested	Primer supplier	Probe supplier
1	Light cycler 2.0	LTR region (SYBR Green detection)	Nuclisens-MiniMag	external, synthetic RNA (ROD seq)	1.7	A,B	Eurogentec	No probe used
2	Light cycler	Gag gene	Magnapure	external, NIHZ quantified by electronic microscopy	2.0	A,B,H	MWG, proligo and applied	Proligo
3	In-house PCR + ELONA	LTR	Boom	CBL23 + internal control	2.0	A,B	MWG	MWG
4	nested PCR	LTR-gag, gag, pol-RT, pol-IN	Qiagen, viral RNA	external/MVP-11971	2.7	no preference	TIB MolBiol	TIB MolBiol
5	In house QcRT-PCR	Env gene and biotin / digoxigenin	easyMag Biomérieux	Internal control	2.3	A,B	Invitrogen	Invitrogen
6	Nuclisens EasyQ v1.1	Gag gene	Nuclisens EasyMAG	internal	2.3	A,B	Provided in the Nuclisens EasyQ kit (bioMérieux)	
7	ExaVir Load Analyzer v1.61	teh Exavir RT test from Cavidi			2.6	A, SIVsm	-	-
8	TaqMan	gag leader	HIV Monitor	external, ST isolate, only relative quantification	not yet evaluated	A,B	Microsynth	Microsynth
9	ABI Prism 7000 TaqMan probe	LTR	Qiagen viral RNA	CBL22 external & BMV internal	2.0	A,B	MWG	MWG

At the concentration level of 3 $\log_{10}$ copies/ml, five laboratories reported accurate estimations (n°2, 4, 5, 6, 9 ; figure 1) and seven reported precise estimations (CV= 3 to 6%; ILCC= 0.5 to 0.9) (n°1, 2, 3, 6, 7, 8, 9 ; table 2).

At the concentration level of 2.3 log10 copies/ml, three laboratories reported accurate estimations (n°2, 6, 9 ; figure 2) and six reported precise estimations (CV= 5 to 8%; ILCC= 0.5 to 1.1) (n°1, 2, 3, 7, 8, 9 ; table 3).

At the concentration level of 1.7 $\log_{10}$ copies/ml, two laboratories reported accurate estimations (n°2 and 9), but with lack of precision (CV=24% and 33%; ILCC=1.3 and 1.5 respectively), and three reported precise estimations (CV= 5% to 11%; ILCC= 0.4 to 0.8) (n°1, 3, 8).

Accuracy of quantification assays

Figure 1. Theoretical viral load = 3.0 $\log_{10}$ cp/ml

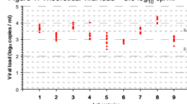


Figure 2. Theoretical viral load = 2.3 $\log_{10}$ cp/ml

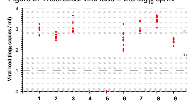
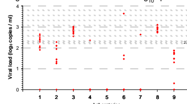


Figure 3. Theoretical viral load = 1.7 $\log_{10}$ cp/ml



Precision of quantification assays

Table 2. Theoretical viral load = 3.0 $\log_{10}$ cp/ml

Laboratories	Coefficient of variation (%)	Intra-laboratory coherence coefficient (*L = 1.35*)	*p*-value
1	4	0.71	NS
2	5	0.82	NS
3	4	0.74	NS
4	9	1.54	<0.01
4 (without the extreme value)	*4*	*(0.76)*	*(NS)*
5	10	1.63	<0.01
6	3	0.46	NS
7	4	0.76	NS
8	4	0.79	NS
9	6	0.93	NS

NS : Not Significant (p>0.05)

Table 3. Theoretical viral load = 2.3 $\log_{10}$ cp/ml

Laboratories	Coefficient of variation (%)	Intra-laboratory coherence coefficient (*L = 1.35*)	*p*-value
1	6	0.73	NS
2	5	0.58	NS
3	8	1.14	NS
4		-	
5		-	
6	15	1.70	<0.01
6 (without the extreme value)	*13*	*(1.50)*	*(0.01)*
7	8	1.03	NS
8	5	0.79	NS
9	5	0.49	NS

NS : Not Significant (p>0.05)

Table 4. Theoretical viral load = 1.7 $\log_{10}$ cp/ml

Laboratories	Coefficient of variation (%)	Intra-laboratory coherence coefficient (*L = 1.34*)	*p*-value
1	11	0.80	NS
2	24	1.26	NS
3	5	0.46	NS
4		-	-
5		-	-
6		-	-
7		-	
8	5	0.42	NS
9	33	1.55	<0.01
9 (without the extreme value)	*13*	*0.84*	*NS*

NS : Not Significant (p>0.05)

CONCLUSIONS

HIV-2 subtype A viral load quantifications varied considerably between laboratories. If the NIHZ HIV-2 supernatant counted by electronic microscopy is regarded as a standard, only 2 laboratories reported accurate and precise estimations at the viral loads of both 3 and 2.3 $\log_{10}$ copies/ml. The ACHIEV$_2$E network is working towards standardization of quantification assays to better interpret the response to treatment in HIV-2 infected patients and implement international clinical trials in the future.

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