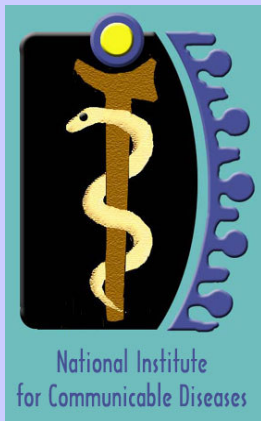




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# SA NICD Final Report

## QCMD 2008 Human Immunodeficiency Virus (HIVRNA08B) EQA Programme B

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on behalf of QCMD and its Scientific Advisory Board  
March 2009

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The QCMD programme is organised  
in collaboration with the European  
Society for Clinical Virology and the  
European Society for Clinical  
Microbiology & Infectious Diseases.



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# 1. Programme aims

The primary aims of this External Quality Assessment Programme were:

- 1) To determine the proficiency of laboratories in the detection of HIV-1 RNA.
- 2) To determine the proficiency of laboratories in the quantification of HIV-1 RNA.

# 2. Programme details

**Table 1: Programme Details**

<b>HIVRNA08B</b>	
Date of panel distribution	30/09/2008
Number of participants	26
Number of countries	4
Number of respondents	22 (85%)
Number of datasets submitted	28
Number of qualitative datasets submitted	0 (0%)
Number of qualitative and quantitative datasets submitted	28 (100%)

Four participants did not return results of which two withdrew officially.

# 3. Panel composition

This EQA panel for the detection of HIV-1 RNA consisted of eight samples containing various concentrations and types of HIV-1 and two samples negative for HIV-1. All panel materials were subjected to independent testing by laboratories recognised as expert in the detection of these targets.

This panel contained cultured HIV-1 virus of Group M Subtype A/G (ID: POC44951); Group M Subtype C (ID: DJ256) and Subtype B (ID: 8E5/LAV).

**Table 2: Panel composition**

Sample	Sample content	Sample * matrix	Sample conc. † Copies/ml	Sample ‡ status
HIVRNA08B-01	HIV-1 Type A/G	Plasma	66	Weak Positive
HIVRNA08B-02	HIV-1 Type C	Plasma	177	Positive
HIVRNA08B-03	HIV-1 Type B	Plasma	123	Positive
HIVRNA08B-04	HIV-1 Type B	Plasma	492	Strong Positive
HIVRNA08B-05	HIV-1 Neg Plasma	Plasma		Negative
HIVRNA08B-06	HIV-1 Type C	Plasma	1845	Strong Positive
HIVRNA08B-07	HIV-1 Type A/G	Plasma	310	Positive
HIVRNA08B-08	HIV-1 Neg Plasma	Plasma		Negative
HIVRNA08B-09	HIV-1 Type C	Plasma	182	Positive
HIVRNA08B-10	HIV-1 Type C	Plasma	76	Weak Positive

Samples HIVRNA08B-02 and -09 were duplicate samples.

\* Human plasma negative for HIV-1 RNA.

† Consensus values calculated from all of the data returned by participants in the full EQA programme, once outliers had been removed. The values are not technology specific and should not be used by participants for method comparison or as a target for individual laboratory assessment.

‡ A sample status was assigned to each panel sample and consisted of 'Strong positive', 'Positive', 'Weak positive' and 'Negative'.

## 4. Programme results

### 4a. Qualitative analysis of the EQA data

The number (percentage) of correct qualitative results are presented in Table 3. Qualitative data were returned by participants as 'positive', 'negative' or 'not determined'. Not determined results were counted as incorrect for all panel samples (positive or negative).

QCMD organises datasets according to commercial and in-house technology groups, which are Conventional PCR, Real time PCR, NASBA, SDA, TMA and bDNA. Where datasets were reported as 'other' for a technology or kit method this was reviewed by the QCMD Neutral Office and assigned to an appropriate group where possible.

**Table 3: Number of correct qualitative results per panel member and technology type**

Sample	Sample content	Sample conc. Copies/ml	Total datasets n=28		PCR						NASBA <sup>e</sup>	
					Conventional		Real time				n=14	
					Commercial <sup>a</sup> n=7		Commercial <sup>c</sup> n=6		In-house <sup>d</sup> n=1			
n	%	n	%	n	%	n	%	n	%			
HIVRNAR8B-07	HIV-1 Type A/G	310	14	50.0	6	85.7	5	83.3	0	0.0	3	21.4
HIVRNA08B-01	HIV-1 Type A/G	66	5	17.9	2	28.6	2	33.3	0	0.0	1	7.1
HIVRNA08B-04	HIV-1 Type B	492	26	92.9	6	85.7	6	100.0	1	100.0	13	92.9
HIVRNA08B-03	HIV-1 Type B	123	15	53.6	4	57.1	6	100.0	0	0.0	5	35.7
HIVRNA08B-06	HIV-1 Type C	1845	27	96.4	7	100.0	6	100.0	1	100.0	13	92.9
HIVRNA08B-02	HIV-1 Type C	177	18	64.3	4	57.1	5	83.3	1	100.0	8	57.1
HIVRNA08B-09	HIV-1 Type C	182	18	64.3	5	71.4	6	100.0	0	0.0	7	50.0
HIVRNA08B-10	HIV-1 Type C	76	8	28.6	2	28.6	5	83.3	0	0.0	1	7.1
HIVRNA08B-05	HIV-1 Neg Plasma		25	89.3	7	100.0	6	100.0	0	0.0	12	85.7
HIVRNA08B-08	HIV-1 Neg Plasma		25	89.3	7	100.0	6	100.0	0	0.0	12	85.7

a: Roche Amplicor HIV-1 Monitor (n=1), Roche COBAS Amplicor HIV-1 Monitor (n=6).

c: Abbott Real time HIV-1 (n=1), Biocentric Generic HIV Charge Virale (n=1), Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test (n=3), Roche COBAS TaqMan HIV-1 Test (n=1).

d: Details not specified.

e: bioMerieux NucliSens EasyQ HIV-1 (n=13), bioMerieux NucliSens HIV-1 QT Assay (n=1).

QCMD uses a colour-coded scheme for scoring based on the classification of results in relation to expected or consensus results. A more detailed explanation of the scoring system is provided in the appendix to this report and on the QCMD website ([http://www.qcmd.org/QCMD\\_Report\\_Key.pdf](http://www.qcmd.org/QCMD_Report_Key.pdf))

## 4b. Qualitative performance scores

Table 4: Qualitative performance scores per technology type

Sample	Sample Status	Total				PCR								NASBA <sup>e</sup>							
		All technologies				Conventional				Real time											
		n=28				Commercial <sup>a</sup>				Commercial <sup>c</sup>				In-house <sup>d</sup>				n=14			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
HIVRNAR8B-07	Positive	14	0	14	0	6	0	1	0	5	0	1	0	0	0	1	0	3	0	11	0
HIVRNA08B-01	Weak Positive	5	23	0	0	2	5	0	0	2	4	0	0	0	1	0	0	1	13	0	0
HIVRNA08B-04	Strong Positive	26	0	0	2	6	0	0	1	6	0	0	0	1	0	0	0	13	0	0	1
HIVRNA08B-03	Positive	15	0	13	0	4	0	3	0	6	0	0	0	0	0	1	0	5	0	9	0
HIVRNA08B-06	Strong Positive	27	0	0	1	7	0	0	0	6	0	0	0	1	0	0	0	13	0	0	1
HIVRNA08B-02	Positive	18	0	10	0	4	0	3	0	5	0	1	0	1	0	0	0	8	0	6	0
HIVRNA08B-09	Positive	18	0	10	0	5	0	2	0	6	0	0	0	0	0	1	0	7	0	7	0
HIVRNA08B-10	Weak Positive	8	20	0	0	2	5	0	0	5	1	0	0	0	1	0	0	1	13	0	0
HIVRNA08B-05	Negative	25	0	0	3	7	0	0	0	6	0	0	0	0	0	0	1	12	0	0	2
HIVRNA08B-08	Negative	25	0	0	3	7	0	0	0	6	0	0	0	0	0	0	1	12	0	0	2

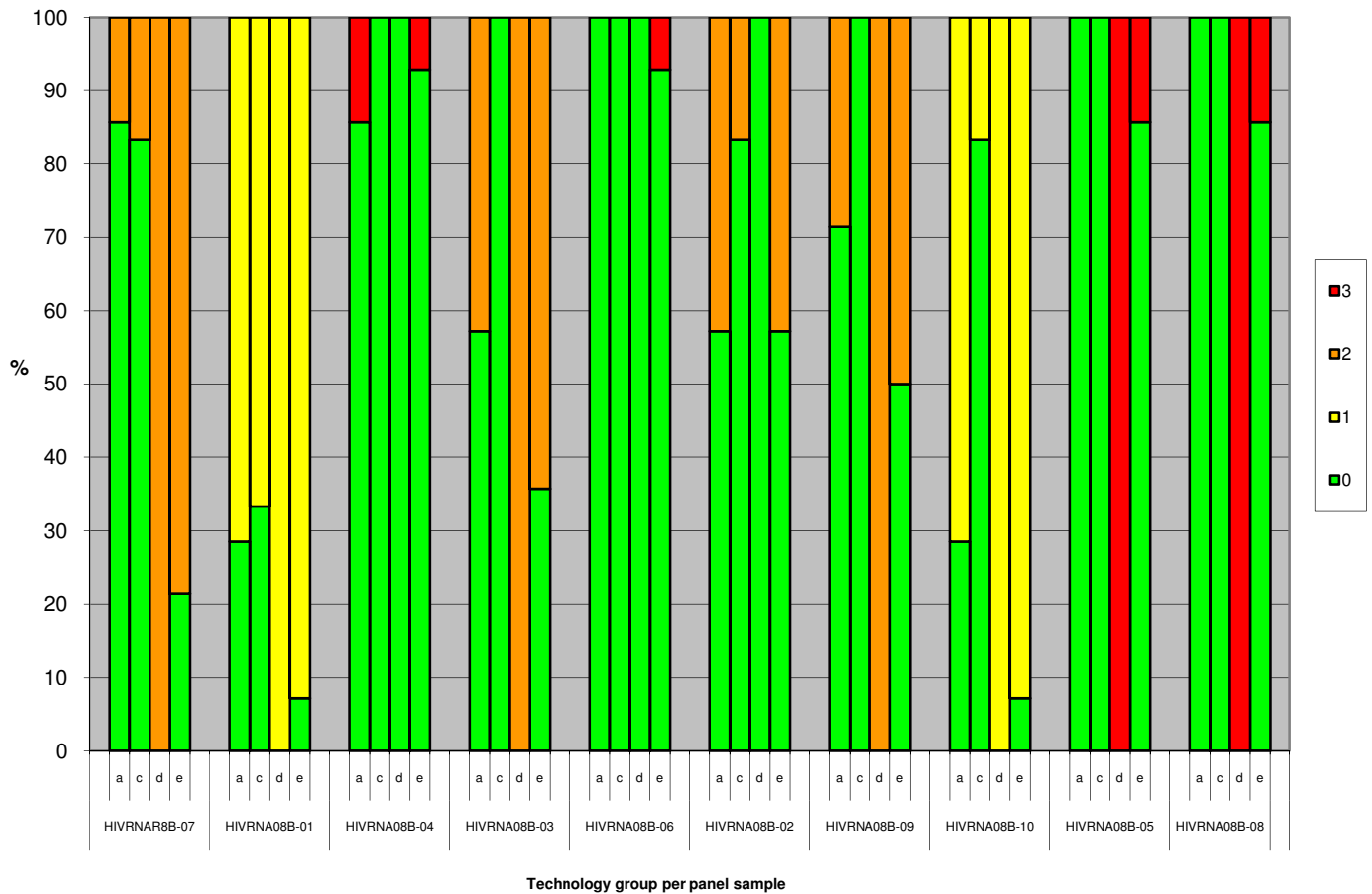
a: Roche Amplicor HIV-1 Monitor (n=1), Roche COBAS Amplicor HIV-1 Monitor (n=6).

c: Abbott Real time HIV-1 (n=1), Biocentric Generic HIV Charge Virale (n=1), Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test (n=3), Roche COBAS TaqMan HIV-1 Test (n=1).

d: Details not specified.

e: bioMerieux NucliSens EasyQ HIV-1 (n=13), bioMerieux NucliSens HIV-1 QT Assay (n=1).

Figure 1: Percentage of qualitative performance scores per technology type



a: Conventional commercial PCR, c: Real time commercial PCR, d: Real time in-house PCR, e: NASBA.

## 4c. Quantitative analysis of the EQA data and performance scores

### Consensus concentration score

The consensus concentration score relates to scoring on the basis of a consensus mean for each positive panel sample.

**Table 5: Quantitative scores by technology type in comparison to the consensus concentration**

Sample	Consensus Log <sub>10</sub> virus concentration		Total														NASBA <sup>®</sup>										
			All technologies					Conventional				PCR					Real time				NASBA <sup>®</sup>						
			n=28					Commercial <sup>a</sup>				Commercial <sup>c</sup>			In-house <sup>d</sup>		n=14										
			Mean	SD	0	1	2	3	LOD/NR	0	1	2	3	LOD/NR	0	1	2	3	LOD/NR	0	1	2	3	LOD/NR			
HIVRNAR8B-07	2.466	0.163	4	7	1	2	14	2	3	1	0	1	2	3	0	0	1	0	0	0	0	1	0	1	0	2	11
HIVRNA08B-01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HIVRNA08B-04	2.605	0.405	22	3	2	0	1	4	1	1	0	1	5	0	1	0	0	1	0	0	0	0	12	2	0	0	0
HIVRNA08B-03	2.167	0.379	12	2	1	0	13	3	1	0	0	3	6	0	0	0	0	0	0	0	0	1	3	1	1	0	9
HIVRNA08B-06	3.181	0.262	12	14	2	0	0	2	3	2	0	0	1	5	0	0	0	0	1	0	0	0	9	5	0	0	0
HIVRNA08B-02	2.230	0.258	8	9	0	0	11	2	2	0	0	3	2	2	0	0	2	1	0	0	0	0	3	5	0	0	6
HIVRNA08B-09	2.177	0.241	5	11	2	0	10	2	3	0	0	2	1	4	1	0	0	0	0	0	0	1	2	4	1	0	7
HIVRNA08B-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a: Roche AmpliCor HIV-1 Monitor (n=1), Roche COBAS AmpliCor HIV-1 Monitor (n=6).

c: Abbott Real time HIV-1 (n=1), Biocentric Generic HIV Charge Virale (n=1), Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test (n=3), Roche COBAS TaqMan HIV-1 Test (n=1).

d: Details not specified.

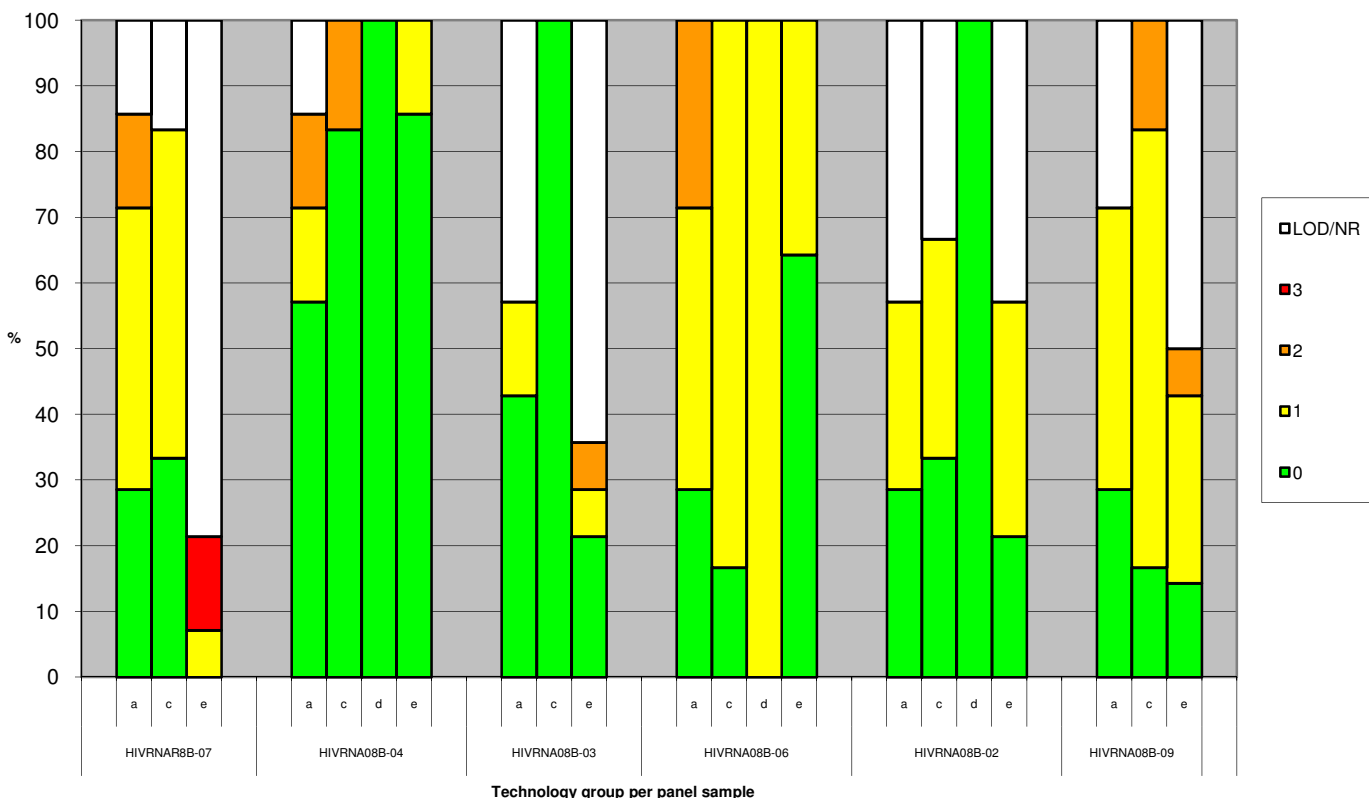
e: bioMerieux NucliSens EasyQ HIV-1 (n=13), bioMerieux NucliSens HIV-1 QT Assay (n=1).

SD: Standard Deviation.

LOD/NR: Result reported as lower limit of detection or upper limit of detection / no value or no result reported. These were excluded from the scoring.

-: Less than five quantitative values were received. Analysis of these data was not possible.

**Figure 2: Percentage of quantitative performance scores in comparison to the consensus concentration**



a: Conventional commercial PCR, c: Real time commercial PCR, d: Real time in-house PCR, e: NASBA.

## Technology consensus score

The technology consensus score relates to scoring on the basis of a technology group consensus mean per positive panel sample.

**Table 6: Quantitative scores by technology type in comparison to the technology consensus concentration**

Sample	Consensus Log <sub>10</sub> virus concentration		Conventional Commercial <sup>a</sup> n=7				
	Mean	SD	0	1	2	3	LOD/NR
HIVRNAR8B-07	2.632	0.192	4	2	0	0	1
HIVRNA08B-01	-	-	-	-	-	-	-
HIVRNA08B-04	2.612	0.498	5	1	0	0	1
HIVRNA08B-03	2.064	0.275	0	0	0	0	7
HIVRNA08B-06	3.238	0.410	4	3	0	0	0
HIVRNA08B-02	2.466	0.175	0	0	0	0	7
HIVRNA08B-09	2.330	0.325	4	1	0	0	2
HIVRNA08B-10	-	-	-	-	-	-	-

Sample	Consensus Log <sub>10</sub> virus concentration		Real time Commercial <sup>c</sup> n=6				
	Mean	SD	0	1	2	3	LOD/NR
HIVRNAR8B-07	2.617	0.116	3	2	0	0	1
HIVRNA08B-01	-	-	-	-	-	-	-
HIVRNA08B-04	2.557	0.461	5	1	0	0	0
HIVRNA08B-03	2.146	0.138	4	2	0	0	0
HIVRNA08B-06	3.452	0.224	5	1	0	0	0
HIVRNA08B-02	2.515	0.049	0	0	0	0	6
HIVRNA08B-09	2.438	0.236	5	1	0	0	0
HIVRNA08B-10	-	-	-	-	-	-	-

Sample	Consensus Log <sub>10</sub> virus concentration		NASBA <sup>e</sup> n=14				
	Mean	SD	0	1	2	3	LOD/NR
HIVRNAR8B-07	1.880	0.752	0	0	0	0	14
HIVRNA08B-01	-	-	-	-	-	-	-
HIVRNA08B-04	2.622	0.336	9	4	1	0	0
HIVRNA08B-03	2.276	0.547	4	1	0	0	9
HIVRNA08B-06	3.037	0.174	9	5	0	0	0
HIVRNA08B-02	2.005	0.258	6	2	0	0	6
HIVRNA08B-09	1.843	0.169	5	2	0	0	7
HIVRNA08B-10	-	-	-	-	-	-	-

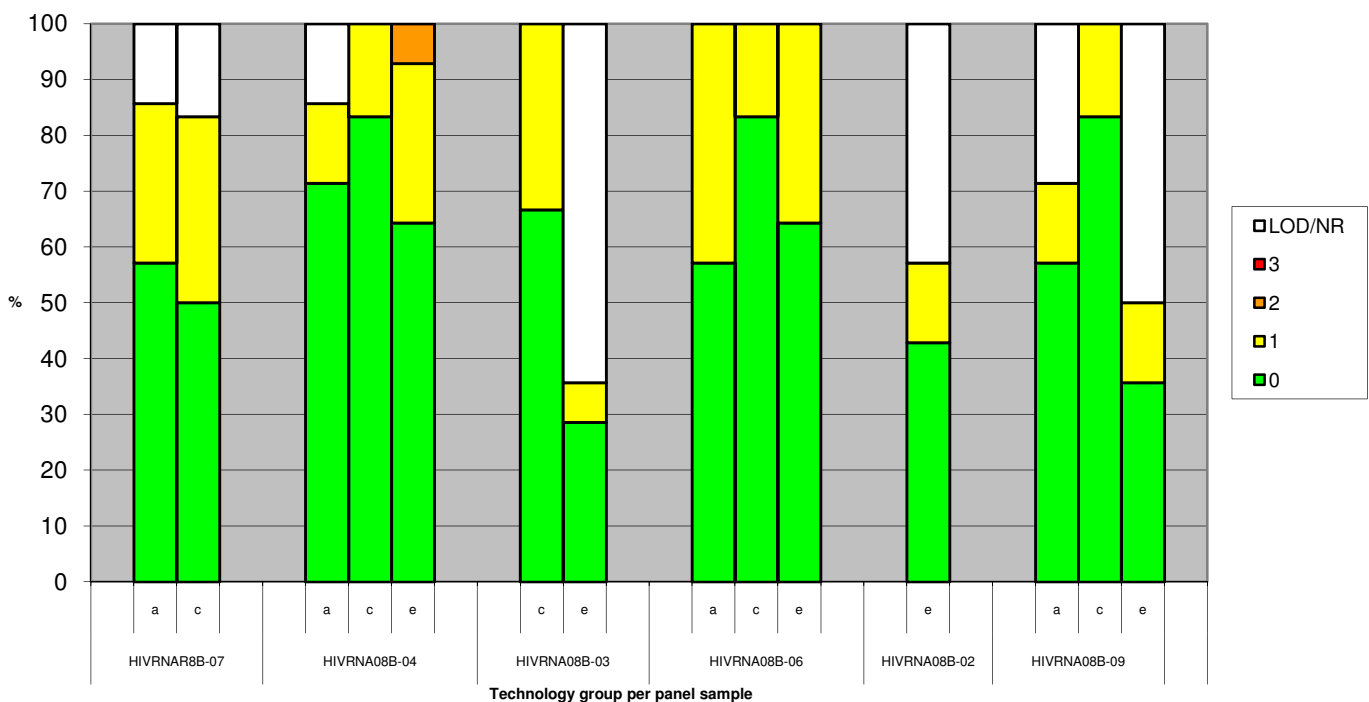
a: Roche Amplificor HIV-1 Monitor (n=1), Roche COBAS Amplificor HIV-1 Monitor (n=6).

c: Abbott Real time HIV-1 (n=1), Biocentric Generic HIV Charge Virale (n=1), Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test (n=3), Roche COBAS TaqMan HIV-1 Test (n=1).

e: bioMerieux NucliSens EasyQ HIV-1 (n=13), bioMerieux NucliSens HIV-1 QT Assay (n=1).

-. Less than five quantitative values were received. Analysis of these data was not possible.

**Figure 3: Percentage of overall quantitative performance scores in comparison to the technology consensus concentration**



a: Conventional commercial PCR, c: Real time commercial PCR, e: NASBA.

## Appendix A

### Scoring system for qualitative EQA data

The scores awarded for qualitative EQA data were based on the sample status (see Section 3). The scoring system is represented in the following table, where 0 is 'highly satisfactory' and 3 is 'highly unsatisfactory'. Colour has been included as an extra visual aid.

#### Scoring system based on the assigned sample status

Sample status	Participant's result		
	Negative	Not determined	Positive
Strong Positive	3	3	0
Positive	2	2	0
Weak Positive	1	1	0
Negative	0	3	3

### Scoring system for quantitative EQA data

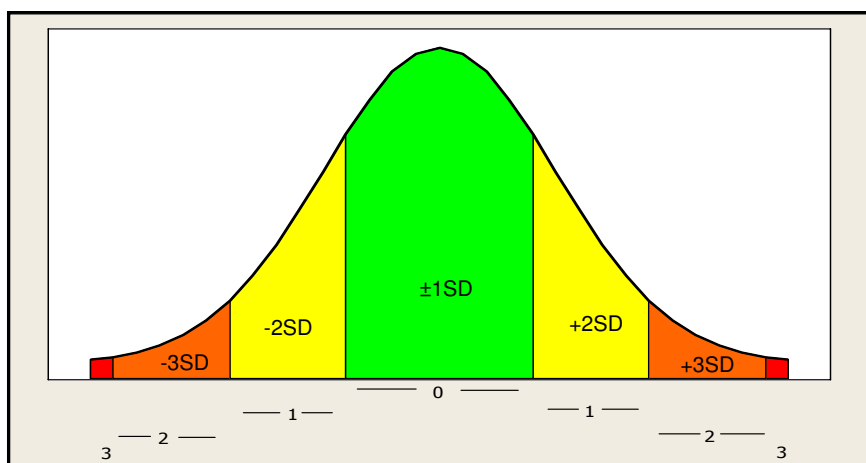
In order to compare the participants results within specific technologies or kit methods, where sufficient datasets were reported (5 or more) methods or kits were assigned to a technology group e.g. Real time, bDNA or NASBA etc. Where datasets were reported as 'other' for a technology or kit method this was reviewed by the QCMD Neutral Office and assigned to an appropriate group where possible. The HIV-1 negative panel sample (HIVRNA08B-05 and -08) were not included in these analysis.

The normal distribution was estimated from the log of the datasets submitted by participants for each panel sample. Scores were assigned based on the distance from the mean value for each panel sample. The scoring system used for quantitative EQA data ranged from 0 (highly satisfactory) to 3 (highly unsatisfactory).

A quantitative mean value was calculated for each panel sample using two methods. These were:

1. Consensus concentration - the mean of the participants' results once outliers had been removed.
2. Technology consensus concentration - the mean of the participants' results per technology group once outliers had been removed.

Scores were awarded based on the distance from the calculated mean value for each panel sample. Zero points was awarded if the quantitative value returned was within one standard deviation from the mean. One point was awarded if the quantitative value was between one and two standard deviations, two points if the value was within two and three standard deviations and three points for quantitative values more than three standard deviations from the mean.



Each coloured division represents one standard deviation (SD) from the mean, so that zero points were awarded for quantitative values that were within one standard deviation and three points for quantitative values that were more than three standard deviations from the mean.