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QUALITY CONTROL for MOLECULAR DIAGNOSTICS

# Final Report

## QCMD 2010

## ENVA

## HIV Drug Resistance Typing

## EQA Programme

## Version 2

Rob Schuurman

on behalf of QCMD and its Scientific Council

December 2010

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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.



# 1. Programme Aims

The primary aims of this external quality assessment programme were to assess the performance of laboratories in the detection of drug resistance mutations in the HIV-1 protease and reverse transcriptase genes.

## 2. Programme details

**Table 1: Programme Details**

QCMD 2010 ENVA	
Date programme distributed	23/08/2010
Number of participants	122
Number of countries	42
Number of respondents	114 (93%)
Number of non-respondents	8 (7%)
Number of datasets submitted	118

All participants who had not submitted results prior to the closing date for submission were contacted by e-mail. Two participants withdrew from the programme citing "Technical issues" (n=2) while six did not respond or submit results. In the previous HIV Drug Resistance Typing Proficiency Programme (ENVA9) 12 % of participants (14 laboratories) were non-respondents (QCMD 2009).

## 3. Panel composition

The 2010 ENVA panel consisted of five well-characterised samples derived from clinical isolates (ENVA10-04, ENVA10-06) or cultured viruses (ENVA010-02, ENVA010-05, ENVA10-07). The sample materials were heat inactivated (30 minutes at 56 degrees centigrade) and aliquoted into 1ml aliquots. Viral loads of the material were determined before and after lyophilisation (Table 2).

**Table 2: Panel composition & viral load determination**

Sample	Matrix <sup>1</sup>	Subtype	Release testing <sup>2</sup>
			Lyophilised samples Viral load (copies/ml)
ENVA10-02	Citrate plasma	C	$7.16 \times 10^3$
ENVA10-04	Citrate plasma	A	$3.02 \times 10^3$
ENVA10-05	Citrate plasma	B	$1.21 \times 10^4$
ENVA10-06	Citrate plasma	B	$1.46 \times 10^4$
ENVA10-07	Citrate plasma	AG	$1.34 \times 10^4$

1. All samples were diluted in HIV, HBV and HCV negative citrate plasma and lyophilised prior to distribution.

2. Release testing was performed using the Roche COBAS TaqMan HIV-1 Test.

## 4. Programme results

### 4a. Analysis of the Sequence Data

Table 3a: Summary of submitted results by individual dataset

Lab. Code <sup>2</sup>	Method	ENVA10-02		ENVA10-04		ENVA10-05		ENVA10-06		ENVA10-07	
		PR2 <sup>3</sup>	RT2 <sup>3</sup>	PR4 <sup>3</sup>	RT4 <sup>3</sup>	PR5 <sup>3</sup>	RT5 <sup>3</sup>	PR6 <sup>3</sup>	RT6 <sup>3</sup>	PR7 <sup>3</sup>	RT7 <sup>3</sup>
11_CD	IH										
1200.SP	IH										
1217_CD	IH										
1703.SP	IH										
2263_CD	IH										
31_CD	IH										
1333_CD	IH										
156_CD	IH										
1320_CD	IH										
1330_CD	IH										
2403_CD	IH										
2987_CD	IH										
2153.SP	IH										
23.SP	IH										
24_CD	IH										
1294.SP	IH										
2436.SP	IH										
2696.SP	IH										
113_CD	IH										
1917_CD	IH										
117_CD	IH										
120_CD	IH										
126_CD	IH										
129_1_CD	IH										
129_2_CD	IH										
129_3_CD	IH										
1920_CD	IH										
2082_CD	IH										
2313_CD/SP	IH										
1413.SP	IH										
3308.SP	IH										
2261_CD/SP	IH										
1484_CD	IH										
94_CD	IH										
1532_CD	IH										
53_CD	IH										
2421_CD	IH										
1667_CD	IH										
76.SP	IH										
1609_1_CD	IH										
2280.SP	IH										
2227_CD	IH										
3090.SP	IH										
1968_CD	IH										
66_CD	IH										
1619_CD	IH										
2_CD	TG										
1152.SP	TG										
5.SP	TG										
6_CD	TG										
9_CD	TG										
131_CD	TG										
2011_CD	TG										
1185_CD	TG										
1209.SP	TG										
1346_CD	TG										
1321.SP	TG										
1630_CD	TG										
1635.SP	TG										
2005_CD	TG										
1650_CD	TG										
1651_CD	TG										
2398_CD	TG										
2622_CD	TG										
1313_CD	TG										
2262_2_CD	TG										
2246_CD	TG										
40_CD	TG										
139.SP	TG										
2439.SP	TG										
2991_CD	TG										
2253_CD	TG										
1554_CD	TG										
84_CD	TG										
2229_CD	TG										
2631.SP	TG										
274.SP	TG										
1971_CD	TG										
64.SP	TG										
69_CD	TG										
2044_CD	TG										
1160_CD	VS										
2260_CD	VS										
14_CD	VS										
29_CD	VS										
32_CD	VS										
248_CD	VS										
1352_CD	VS										
2307_CD/SP	VS										
1238_CD	VS										
1264_CD	VS										
111_CD	VS										
119_CD	VS										
124_CD	VS										
168_CD	VS										
2262_1_CD	VS										
2006_CD	VS										
250_CD	VS										
1460_CD	VS										
1476_CD	VS										
2428_CD	VS										
2533_CD	VS										
50_CD	VS										
51_CD	VS										
1512_CD	VS										
89_CD	VS										
90_CD	VS										
95_CD	VS										
257_CD	VS										
1751_CD	VS										
59_CD	VS										
1563_CD	VS										
137_CD	VS										
2264_CD	VS										
1608_2_CD	VS										
1961_CD	VS										
2179_CD	VS										
3147_CD	VS										

Please see legend on page 4.

Table 3(a): This table summarises the datasets submitted by participants, depending on whether combined or separate analysis of the Protease and Reverse transcriptase genes was performed. Details of the alignments are provided in Figures 1 to 5.

1. Shaded Cell = sequence submitted and non-shaded cell = sequence NOT submitted.
2. CD = combined amplification for Protease and Reverse transcriptase and SP = separate amplification for Protease and Reverse transcriptase.
3. PR = Protease and RT = Reverse transcriptase.

These data are presented by panel sample and method of analysis. IH = In-house techniques, TG = Siemens TRUGENE HIV-1 Genotyping Test and VS = Celeria Diagnostics ViroSeq HIV-1 Genotyping System

**Table 3(b): Summary of the number of reported datasets per sample and technology**

Sample	Cumulative (%)	Number of full datasets (PR and RT) <sup>1,2</sup>		
		In-house (%)	TruGene (%)	ViroSeq (%)
<b>ENVA10-02</b>	86/118 (72.88 %)	35/46 (76.01 %)	35/35 (100 %)	16/37 (43.24 %)
<b>ENVA10-04</b>	93/118 (78.81 %)	38/46 (82.61 %)	19/35 (54.29 %)	36/37 (97.30 %)
<b>ENVA10-05</b>	111/118 (94.07 %)	42/46 (91.30 %)	33/35 (94.29 %)	36/37 (97.30 %)
<b>ENVA10-06</b>	114/118 (96.61 %)	43/46 (93.48 %)	34/35 (97.14 %)	37/37 (100 %)
<b>ENVA10-07</b>	102/118 (86.44 %)	38/46 (82.61 %)	28/35 (80 %)	36/37 (97.30 %)
<b>ALL SAMPLES</b>	62/118 (52.54 %)	30/46 (65.22 %)	16/35 (45.71 %)	16/37 (43.24 %)

1. PR = Protease and RT = Reverse transcriptase.

2. Datasets were counted only if data were submitted for both the Protease and Reverse transcriptase genes.

## Calculation of the consensus sequences

The 2010 ENVA consensus sequences were prepared by aligning the sequences submitted by all participants in the programme. The sequences returned for each codon were then analysed. The majority result (observed in >60 % of the sequences) was recorded as the consensus sequence for each codon. The consensus sequences calculated from all the sequences submitted by the participants were in substantial agreement with the consensus sequences calculated from the independent testing results.

## The ENVA Scoring system

In order to compare the results of participants, a scoring system was applied in which the correct detection of a codon genotype (i.e. identical to the codon genotype of the consensus sequence) was given 1 point and an incorrect codon (not matching the consensus sequence) was given 0 points. For codons containing a mixture of 2 or 3 nucleotides at a certain position, 1 point was given if the mixture was reported or if the correct mutation was reported.

**Table 3(c): Scoring Table**

Expected Result \ Participants Result	Wild Type	Mutant Type	Mixed Type
Wild Type	1	0	0
Mutant Type	0	1	1
Mixed Type	0	1	1

The number of codons involved was 38 for Protease and 30 for Reverse transcriptase. Therefore the maximum performance score that could be achieved was 68 points for all panel members. This resulted in an overall maximum score achievable of 340 points.

**Table 4: Genotypic composition of the 2010 ENVA samples at each of the IAS defined resistance codons**

Amino Acid position <sup>1</sup>	WT <sup>2</sup>	ENVA10				
		-02	-04	-05	-06	-07
PR-10	CTC	CTT	TTA			ATA
PR-11	GTC					
PR-13	ATA		GTA			GTA
PR-16	GGG				GAG	GAG
PR-20	AAG		ATA			ATA
PR-24	TTA					
PR-30	GAT					
PR-32	GTA					
PR-33	TTA					
PR-34	GAA					
PR-35	GAA		GAC			
PR-36	ATG	ATA	ATA		ATA	ATA
PR-43	AAA					
PR-46	ATG					
PR-47	ATA					
PR-48	GGG	GGA				
PR-50	ATT					
PR-53	TTT					
PR-54	ATC					
PR-58	CAG		CAA			
PR-60	GAT					
PR-62	ATA					RTA
PR-63	CTC		CCT		GCC	YCT
PR-64	ATA					MTA
PR-69	CAT	AAA	AAA		AAA	AAA
PR-71	GCT		GCC			GCC
PR-73	GGT					
PR-74	ACA					
PR-76	TTA					
PR-77	GTA		GTG		GTG	
PR-82	GTC					
PR-83	AAC					
PR-84	ATA					
PR-85	ATT					
PR-88	AAT					
PR-89	CTG	ATG	ATG		TTG	ATG
PR-90	TTG					
PR-93	ATT	CTT			CTT	

Amino Acid position <sup>1</sup>	WT <sup>2</sup>	ENVA10				
		-02	-04	-05	-06	-07
RT-41	ATG	TTG				
RT-62	GCC					
RT-65	AAA	AAG				
RT-67	GAC	GAT	GAT	GAY		GAT
RT-69	ACT		ACC			
RT-70	AAA	AAG				
RT-74	TTA			CTA		
RT-75	GTA	ACA				GTR
RT-77	TTC				TTT	
RT-90	GTT	ATT	GTC		GTC	GTC
RT-98	GCA		GCG			
RT-100	TTA					
RT-101	AAA					
RT-103	AAA					
RT-106	GTA	ATG				
RT-108	GTA					
RT-115	TAT					
RT-116	TTT					
RT-138	GAG	GAA				
RT-151	CAG		CAA			
RT-179	GTC	GAC	GTG	GTT	GTT	GTG
RT-181	TAC	TAT		TAT	TAT	
RT-184	ATG					
RT-188	TAT					
RT-190	GGA					
RT-210	TTG	TTA	CTG			CTG
RT-215	ACC					
RT-219	AAA					
RT-225	CCT	CCC	CCC			
RT-230	ATG					

**Key: Genotypic mixture using the IUB nucleotide ambiguity system**

IUB Code	K	M	R	S	W	Y	B	D	H	V	N
Mixture of	G or T	A or C	A or G	G or C	A or T	C or T	C, G or T	A, G or T	A, C or T	A, C or G	Any Base

Cornish-Bowden A. IUPAC-IUB Symbols for Nucleotide Nomenclature. *Nucleic Acids Research*. 1985; 13: 3021-3030.

The Protease and Reverse transcriptase gene codons presented are those associated with clinical drug resistance, according to Johnson VA *et al.* (2009). Update of the Drug Resistance Mutations in HIV-1: December 2009. *Topics in HIV Medicine*, 17(5):138-145.

1. PR = Protease and RT = Reverse transcriptase.
2. WT = Wild type sequence pNL4-3.

An empty box signifies that the consensus sequence was in agreement with the wild type sequence pNL4-3.

**Additional legend information for Figures 1 to 5**

The consensus sequences are presented at the top of figures 1, 2, 3, 4 and figure 5 and were determined by analysing all sequences submitted by participants for the Protease (PR) and Reverse transcriptase (RT) genes.

A period (.) indicates agreement with the consensus sequence.

A nucleotide letter or IUB code indicates a difference in sequence composition compared with the consensus sequence.

Figure 1: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA10-02

Lab Code	Protease																																																		Reverse Transcriptase																						
	10	11	13	16	20	24	30	32	33	34	35	36	43	46	47	48	50	53	54	58	60	62	63	64	69	71	73	74	76	77	82	83	84	85	88	89	90	93	41	62	65	67	69	70	74	75	77	80	88	100	101	103	105	108	115	116	138	151	173	181	184	188	190	210	215	219	220	230					
Genotype	GTC	ATA	GGG	AAG	TTA	GAT	GTA	TTA	GAA	GAA	ATA	AAA	ATG	ATA	GGA	ATT	TTT	ATC	CAG	GAT	ATA	CTC	ATA	AAA	GCT	GGT	ACA	TTA	GTA	GTC	AAC	ATA	ATT	AAT	ATG	TTG	GTT	TTG	GCC	AAG	GAT	ACT	AAG	TTA	ACA	TTC	ATT	GCA	TTA	AAA	AAA	ATG	GTA	TAT	TTT	GAA	CAG	GAG	TAT	ATG	TAT	GGA	TTA	ACC	AAA	CCC	ATG						
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64																																																																									
69																																																																									
2044																																																																									

■ In-house

■ Mutant

TrueGene

■ Wildtype

■ Mixture

--- No sequence or not aligned  
(Additional legend information can be found on page 9)



Figure 2: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA10-04

<span style="background-color: #90EE90; border: 1px solid black; padding: 2px;"> </span> In-house	<span style="background-color: #FF0000; border: 1px solid black; padding: 2px;"> </span> Mutant	---
<span style="background-color: #ADD8E6; border: 1px solid black; padding: 2px;"> </span> TruGene	<span style="background-color: #FFA500; border: 1px solid black; padding: 2px;"> </span> Wildtype	---
<span style="background-color: #ADD8E6; border: 1px solid black; padding: 2px;"> </span> ViroSeq	<span style="background-color: #FFA500; border: 1px solid black; padding: 2px;"> </span> Mixture	---
		--- No sequence or not aligned (Additional legend information can be found on page 9)

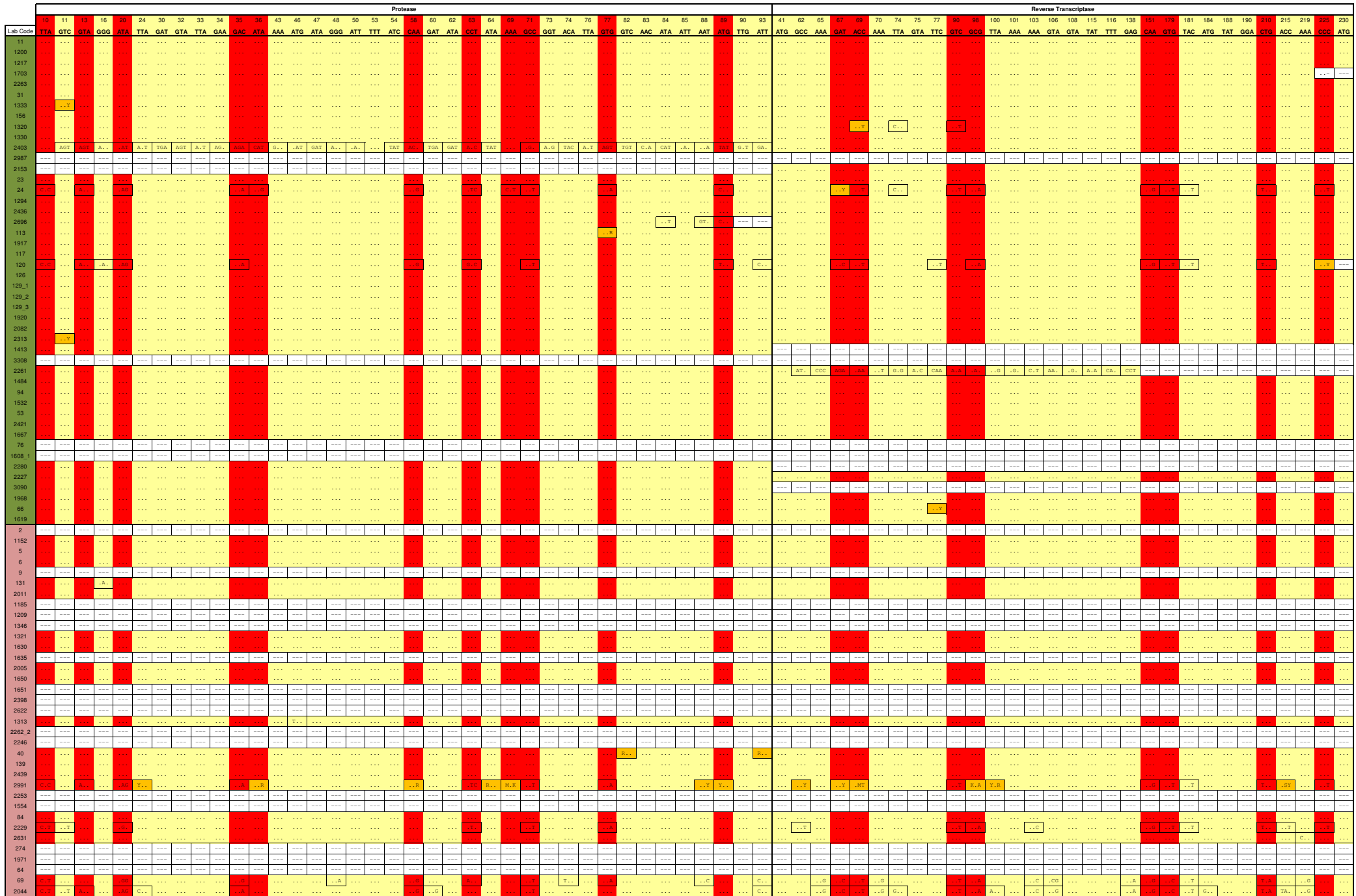






Figure 3: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA10-05

<span style="background-color: #90EE90; border: 1px solid black; padding: 2px;"> </span> In-house	<span style="background-color: #FF0000; border: 1px solid black; padding: 2px;"> </span> Mutant	<span style="border-bottom: 1px dashed black; padding: 2px;"> </span> No sequence or not aligned
<span style="background-color: #ADD8E6; border: 1px solid black; padding: 2px;"> </span> TruGene	<span style="background-color: #FFD700; border: 1px solid black; padding: 2px;"> </span> Wildtype	(Additional legend information can be found on page 5)
<span style="background-color: #FFB6C1; border: 1px solid black; padding: 2px;"> </span> ViroSeq	<span style="background-color: #FFA500; border: 1px solid black; padding: 2px;"> </span> Mixture	

Lab Code	Protease																																																																																													Reverse Transcriptase																			
	10	11	13	16	20	24	30	32	33	34	35	36	43	46	47	48	50	53	54	58	60	62	63	64	69	71	73	74	76	77	82	83	84	85	88	89	90	93	41	62	65	67	69	70	74	75	77	90	98	100	101	103	106	108	115	116	138	151	179	181	184	188	190	210	215	219	225	230																																													
11	CTC	GTC	ATA	GGG	AAG	TTA	GAT	GTA	TTA	GAA	GAA	ATG	AAA	ATG	ATA	GGG	ATT	TTT	ATC	CAG	GAT	ATA	CTC	ATA	CAT	GCT	GGT	ACA	TTA	GTA	GTC	AAC	ATA	ATT	AAT	CTG	TTG	ATT	ATG	GCC	AAA	GAY	ACT	AAA	CTA	GTA	TTC	GTT	GCA	TTA	AAA	AAA	GTA	GTA	TAT	TTT	GAG	CAG	GTT	TAT	ATG	TAT	GGA	TTG	ACC	AAA	CCT	ATG																																													



Figure 4: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA10-06

<span style="background-color: #c8e6c9; border: 1px solid black; padding: 2px;">In-house</span>	<span style="background-color: #ffcccc; border: 1px solid black; padding: 2px;">Mutant</span>	<span style="border-bottom: 1px solid black; padding: 0 5px;">---</span> No sequence or not aligned
<span style="background-color: #e8f5e9; border: 1px solid black; padding: 2px;">TruGene</span>	<span style="background-color: #fff9c4; border: 1px solid black; padding: 2px;">Wildtype</span>	(Additional legend information can be found on page 5)
<span style="background-color: #e2efda; border: 1px solid black; padding: 2px;">ViroSeq</span>	<span style="background-color: #fff176; border: 1px solid black; padding: 2px;">Mixture</span>	

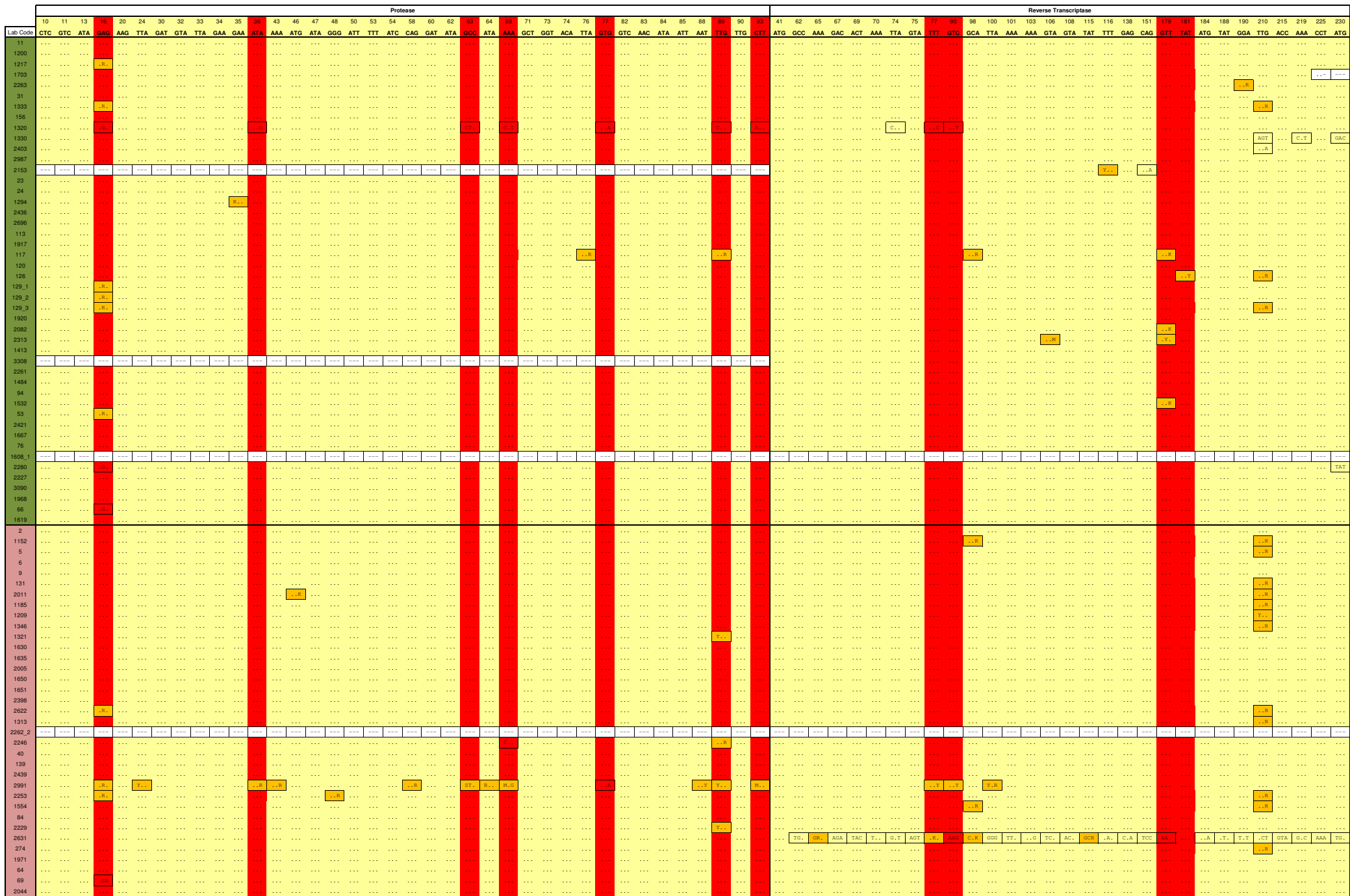
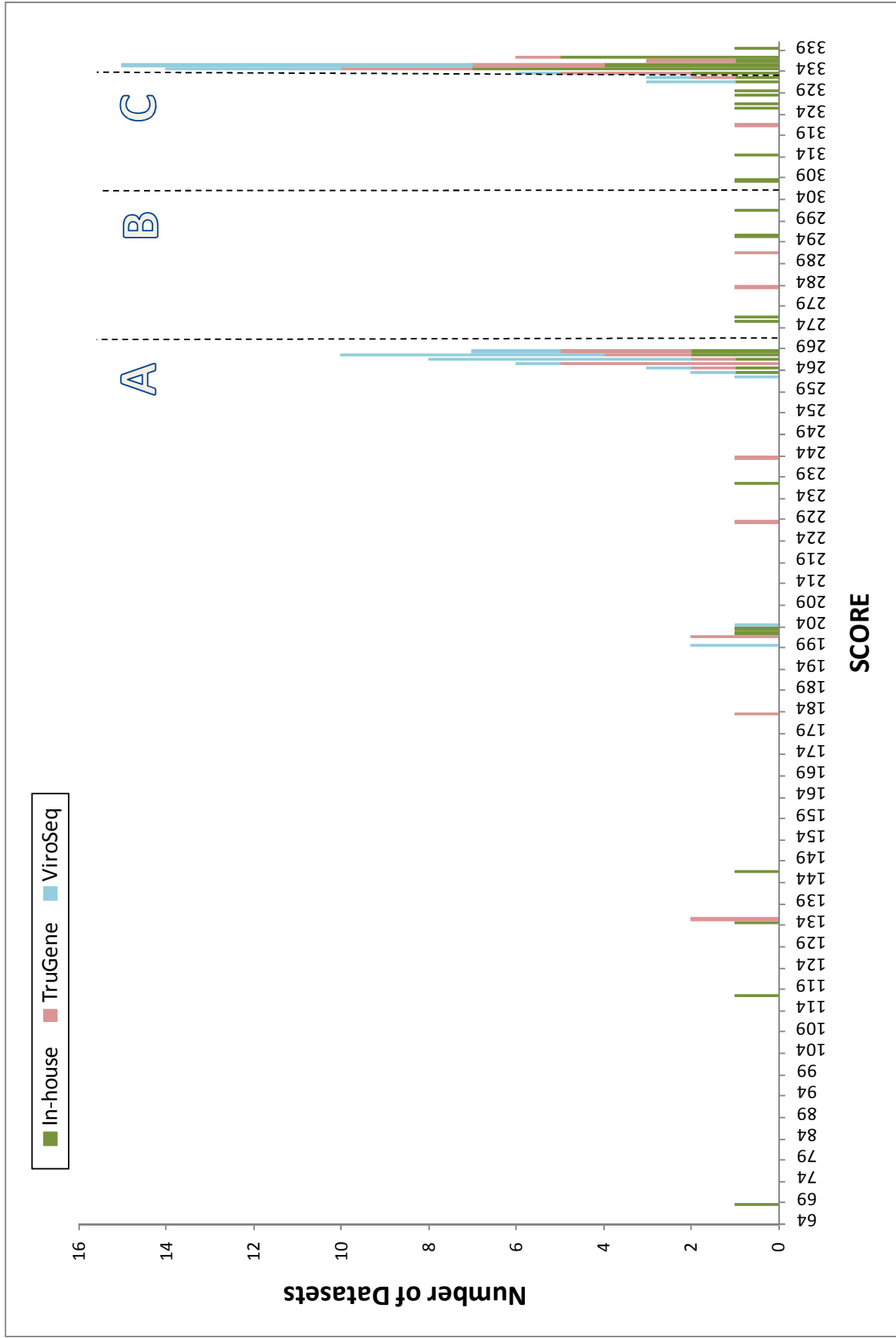








Figure 6: Summary of overall performance scores by technology

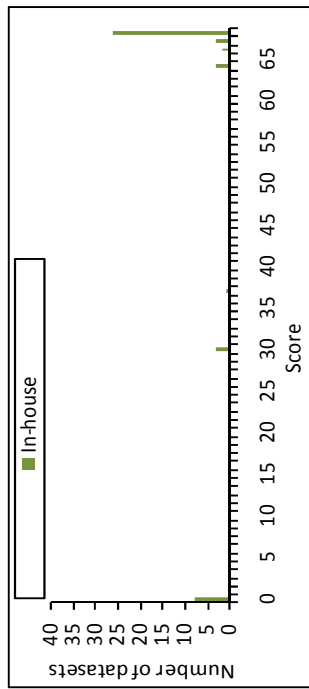


A: 80% (272) of maximum achievable score (340), B: 90% (306) of maximum achievable score (340), C: 99% (336) of maximum achievable score (340).

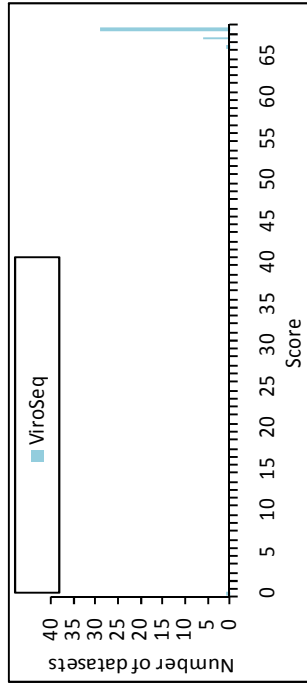
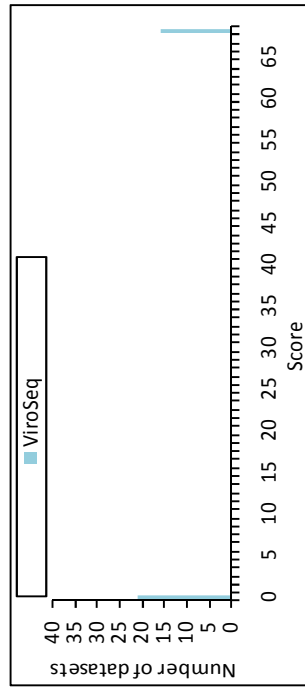
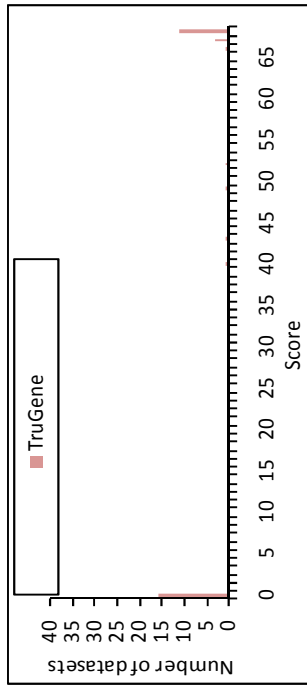
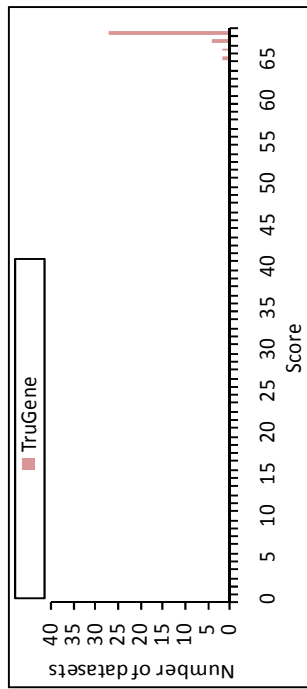
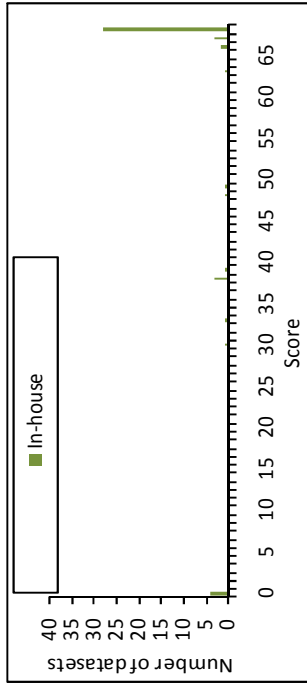


Figure 7(a): Summary of performance scores by panel sample

ENVA10-02



ENVA10-04

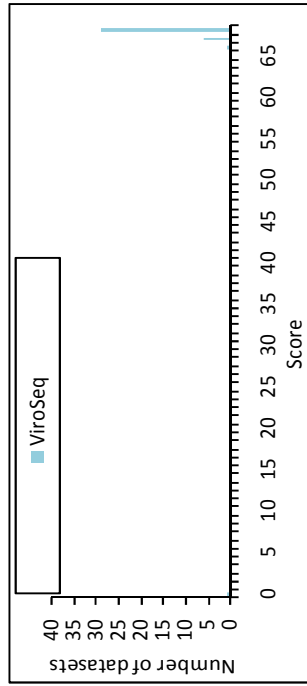
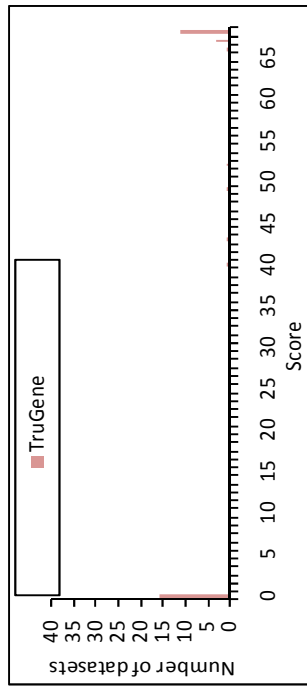
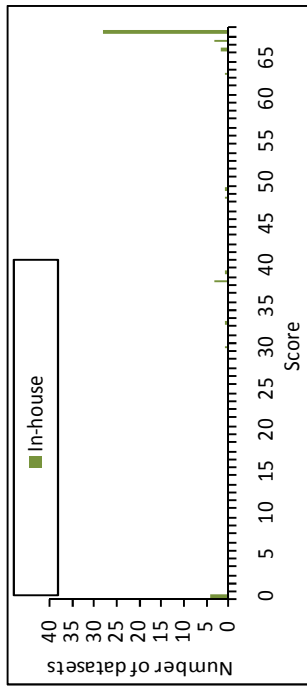


The maximum score achievable for all samples was 68.

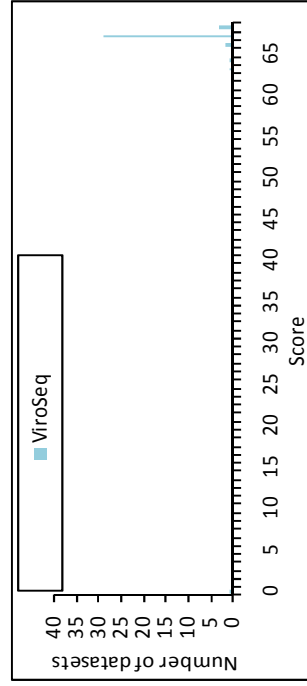
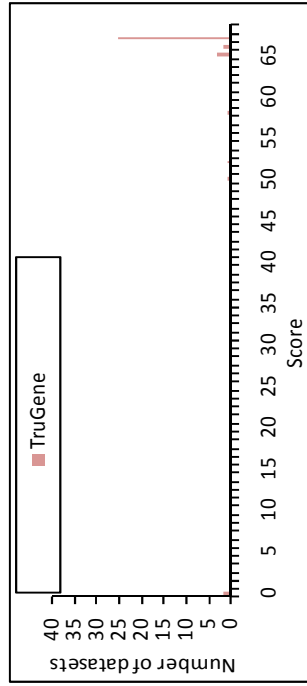
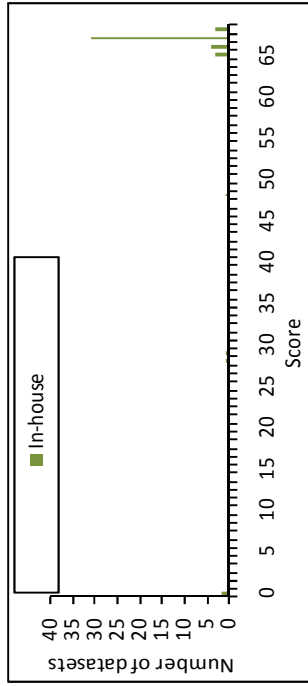
Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

Figure 7(b): Summary of performance scores by panel sample

ENVA10-05



ENVA10-06

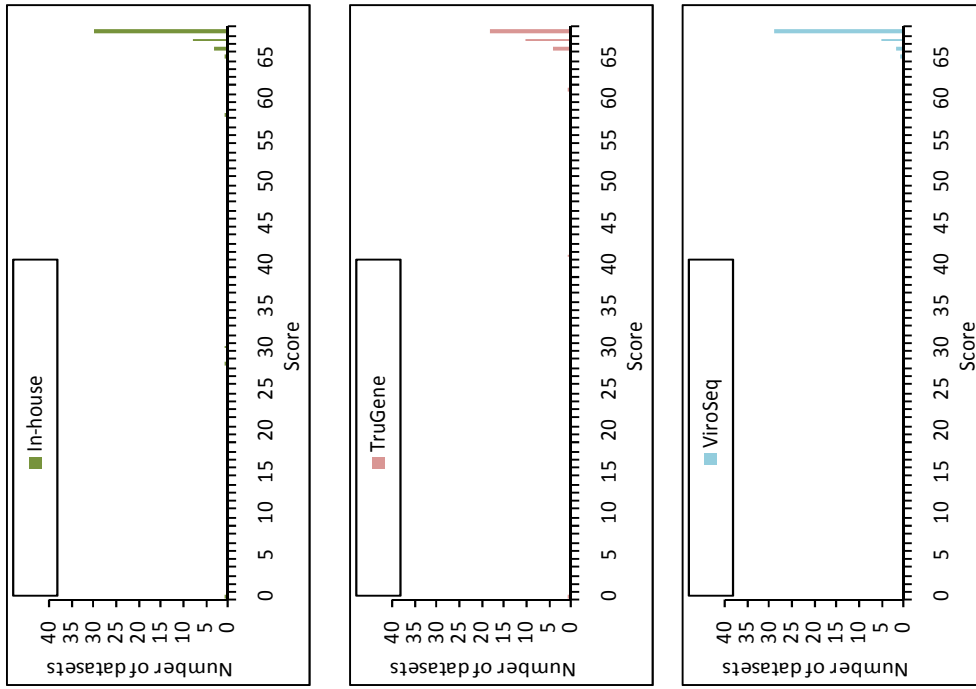


The maximum score achievable for all samples was 68.

Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

Figure 7(c): Summary of performance scores by panel sample

ENVA10-07



The maximum score achievable for all samples was 68.

Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

## Comments

The 2010 ENVA EQA panel was chosen by the Europe HIV Resistance (EHR) working group on quality control. The panel consisted of human plasma samples spiked with cultured patient isolates or recombinant viruses. The viruses present in the panel were of HIV-1 subtype A, B, C or AG.

- The consensus sequences calculated from the reference testing results were in agreement with the consensus sequences calculated from the results of all participant datasets submitted during the actual distribution.
- The majority of datasets were generated using commercial HIV drug resistance genotyping kits: 37 For ViroSeq (31.4%) and 35 for TruGene (29.7%). The remaining 46 datasets (38.9%) were generated with in-house genotyping assays (Table 3b).
- The number of complete datasets (covering both the Protease and Reverse transcriptase genes) for all samples was 52.5%. This was lower compared to the success rate in ENVA9 (72.2%). This difference maybe due to the different composition of the 2010 ENVA panel. In order to provide state of the art panels in each distribution, the ENVA panels are composed differently for each round. Therefore it is only possible to draw general conclusions on variations in success rates between distributions.
- All technologies were successful in the sequence analysis of the panel samples. There were no systematic negative results with any of the panel samples.
  - o The success rate for genotyping of all samples using Viroseq ranged from 97.3% - 100 %, except for ENVA10-02 (43.24%) which harboured an HIV-1 subtype C strain and had a lower success rate as compared to other technologies.
  - o The success rate for genotyping of all samples using TruGene ranged from 80% - 100%, except for ENVA10-04 (54.29%) which harboured an HIV-1 subtype A strain and had a lower success rate as compared to other technologies.
  - o The success rate for genotyping of all samples using In-house technologies ranged from 76.01% - 93.48%.
- The number of full datasets reported to QCMD was higher for the in-house assays (65.2%), compared to TruGene (45.7%) or ViroSeq (43.2%) (Table 3b). The lower rate for Trugene was due to the lower success rate for sample ENVA10-04. The lower rate for ViroSeq was due to the lower success rate for sample ENVA10-02.
- Overall, the percentage of datasets recording over 90.0% of the maximum achievable score (340) was 49.1% (n=58/118) (Figure 6). The range of scores reported for those datasets that did not achieve 90.0% (of the maximum achievable score or greater) ranged from 68 to 305 points. Most of the lower scoring datasets were due to:
  - o Missing results for complete genes (either PR or RT) or complete panel samples (Table 3a and Figure 6).
  - o Reporting of mixed genotypes at various WT positions . It is of note that there is a tendency for increased mixed base calling for TruGene users, in particular for codon 210 of sample ENVA10-06 (in addition to mixed base calling at disperse codons by some labs).
  - o A higher frequency of mixed base calls for all technologies at codon 151 of sample ENVA10-07 was observed. This panel sample contained HIV-1 type A/G.

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