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Final Report

QCMD 2008 ENVA8 HIV Drug Resistance Typing EQA Programme

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on behalf of QCMD and its Scientific Advisory Board
December 2008

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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Registered Office: 39 Castle Street, Edinburgh EH2 3BH

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 in plasma samples containing HIV and to assess the performance of laboratories in the analysis of HIV-1 negative samples.

2. Programme details

Table 1: Programme Details

QCMD ENVA8	
Date programme distributed	11/08/2008
Number of participants	113
Number of countries	42
Number of respondents	104 (92%)
Number of non-respondents	9 (8%)
Number of datasets submitted	108

All participants who had not submitted results prior to the closing date for submission were contacted by e-mail. Five participants withdrew from the programme and four did not respond or submit results. In the previous HIV Drug Resistance Typing Proficiency Programme (ENVA7) 14 % of participants (16 laboratories) were non-respondents (QCMD 2007).

3. Panel composition

The ENVA8 panel consisted of five well-characterised samples derived from clinical isolates (ENVA8-01, -02 and -05) or cultured viruses (ENVA8-03). The ENVA8-04 sample negative for HIV-1. 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[1]	Subtype	Release testing[2] Lyophilised samples Viral load (copies/ml)
ENVA8-01	Citrate Plasma	G	6.57×10^3
ENVA8-02	Citrate Plasma	C	2.63×10^4
ENVA8-03	Citrate Plasma	B	4.68×10^3
ENVA8-04	Citrate Plasma	HIV Negative	
ENVA8-05*	Citrate Plasma	C	1.51×10^4

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 HIV-1 Monitor Test.

* Two insertions of 3 base pairs between codon 67 and 69 in Reverse transcriptase.

4. Programme results

4a. Analysis of the Sequence Data

Table 3a: Summary of submitted results by individual dataset

		ENVA8-01		ENVA8-02		ENVA8-03		ENVA8-04		ENVA8-05	
Lab. Code	Method	PR1	RT1	PR2	RT2	PR3	RT3	PR4	RT4	PR5	RT5
AU008.SP	IH										
BE007.SP	IH										
BE037.SP	IH										
BE049.2.CD	IH										
CA008.CD	IH										
CH020.SP	IH										
CY002.CD	IH										
DE006.CD	IH										
DE012.CD	IH										
DE026.CD	IH										
DE034.SP	IH										
DE093.1.CD	IH										
DE093.2.CD	IH										
ES036.CD	IH										
FR002.CD	IH										
FR003.CD	IH										
FR039.SP	IH										
GB003.CD	IH										
GB006.CD	IH										
GB007.CD	IH										
GB010.CD	IH										
GB019.CD	IH										
GB064.CD	IH										
GB098.CD	IH										
HK004.CD	IH										
HK020.CD	IH										
HU008.SP	IH										
IL003.2.SP	IH										
IT012.CD	IH										
IT043.CD	IH										
NO010.CD	IH										
NZ001.CD	IH										
PK001.SP	IH										
SE001.CD	IH										
SE002.SP	IH										
SK005.SP	IH										
UG002.CD	IH										
US048.CD	IH										
ZA018.CD	IH										
AT002.CD	TG										
AU012.1.SP	TG										
BE001.SP	TG										
BE002.CD	TG										
BE005.CD	TG										
BE010.CD	TG										
BE020.CD	TG										
BE026.CD	TG										
BG002.SP	TG										
DE003.CD	TG										
DE027.SP	TG										
ES018.CD	TG										
ES028.CD	TG										
ES031.CD	TG										
ES032.CD	TG										
ES044.CD	TG										
FR053.CD	TG										
GB061.CD	TG										
GR013.CD	TG										
HR004.CD	TG										
IE002.CD	TG										
IL003.1.SP	TG										
IT057.SP	TG										
LV005.CD	TG										
PL010.CD	TG										
TH001.SP	TG										
US009.SP	TG										
US016.CD	TG										
US051.CD	TG										
ZA006.CD	TG										
AT012.CD	VS										
AU012.2.CD	VS										
BE049.1.CD	VS										
CZ001.CD	VS										
DE004.CD	VS										
DE007.CD	VS										
DE020.CD	VS										
DE023.SP	VS										
DE084.CD	VS										
DK009.CD	VS										
FI001.CD	VS										
GB001.CD	VS										
GB009.CD	VS										
GB011.CD	VS										
GB014.CD	VS										
GB016.CD	VS										
GB020.CD	VS										
HK001.CD	VS										
IT011.CD	VS										
IT027.CD	VS										
IT037.CD	VS										
LT001.CD	VS										
LU001.CD	VS										
NL003.CD	VS										
NL008.CD	VS										
NL009.CD	VS										
NL022.CD	VS										
NL037.CD	VS										
NO006.CD	VS										
PT008.CD	VS										
PT020.CD	VS										
RO001.CD	VS										
SE003.CD	VS										
SI006.CD	VS										
US008.CD	VS										
US054.CD	VS										
US076.CD	VS										
ZA009.CD	VS										
ZA030.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 4.

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 system and VS = Celera Diagnostics ViroSeq HIV-1 genotyping system.

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

Sample		Number of full datasets (PR and RT) [1, 2]		
	Cumulative (%)	In-house (%)	TruGene (%)	ViroSeq (%)
ENVA8-01	101/108 (93.51 %)	35/39 (89.74 %)	29/30 (96.67 %)	37/39 (94.87 %)
ENVA8-02	99/108 (91.67 %)	33/39 (84.62 %)	29/30 (96.67 %)	37/39 (94.87 %)
ENVA8-03	98/108 (90.74 %)	33/39 (84.62 %)	28/30 (93.33 %)	37/39 (94.87 %)
ENVA8-05	104/108 (96.29 %)	36/39 (92.31 %)	29/30 (96.67 %)	39/39 (100 %)
ALL SAMPLES*	91/108 (84.26 %)	29/39 (74.36 %)	29/30 (96.67 %)	33/39 (84.62 %)

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

* Panel sample ENVA8-04 was not considered in this analysis as it was a panel sample negative for HIV-1.

Calculation of the consensus sequences

The ENVA8 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 resistance codons involved was 38 for Protease and 28 for Reverse transcriptase (plus two codons for the 6 base pair insertion sequence in ENVA8-05). These were according to the resistance codons listed under :
Johnson VA et al. Update of the Drug Resistance Mutations in HIV-1: Spring 2008. Topics in HIV Medicine 2008: 16(1); 62 – 68.
Therefore the maximum performance score that could be achieved was 66 points for panel members ENVA8-01, ENVA8-02 and ENVA8-03 and 68 points for ENVA8-05. This resulted in an overall maximum score achievable of 266 points.

Panel sample ENVA8-04 was not considered in this analysis as it was a panel sample negative for HIV-1.

Table 4: Genotypic composition of the ENVA8 samples at each of the IAS defined resistance codons

Amino Acid position[1]	WT[2]	ENVA8			
		-01	-02	-03	-05
PR-10	CTC	CTA	TTT	ATC	
PR-11	GTC				
PR-13	ATA	GTA		GTA	
PR-16	GGG		GAG		
PR-20	AAG	ATA			AAA
PR-24	TTA				
PR-30	GAT		AAC		
PR-32	GTA				
PR-33	TTA	TTG	CTA		
PR-34	GAA				
PR-35	GAA	GAC	GAC	GAC	
PR-36	ATG	ATA	GTA	ATA	ATA
PR-43	AAA				
PR-46	ATG			ATA	
PR-47	ATA				
PR-48	GGG		GGA		GGA
PR-50	ATT				ATA
PR-53	TTT				
PR-54	ATC			GTC	
PR-58	CAG				CAA
PR-60	GAT				GAG
PR-62	ATA		GTA	GTA	
PR-63	CTC	CTT	CTT	CCC	CCT
PR-64	ATA				
PR-69	CAT	AAA	AAA		AAA
PR-71	GCT			ACT	GCA
PR-73	GGT	GGG	GGC		
PR-74	ACA		TCA		
PR-76	TTA				
PR-77	GTA	RTA			GTG
PR-82	GTC	ATC		GCC	
PR-83	AAC				
PR-84	ATA				
PR-85	ATT				
PR-88	AAT		GAT		AAC
PR-89	CTG	ATG	ATG		ATG
PR-90	TTG			ATG	
PR-93	ATT		CTT	CTT	CTT

Amino Acid position[1]	WT[2]	ENVA8			
		-01	-02	-03	-05
RT-41	ATG		TTG		
RT-62	GCC				
RT-65	AAA		AAG		AAG
RT-67	GAC		AAC		AAC
67-68	INSERT				TCT
68-69	INSERT				GAG
RT-69	ACT		GAT		TCT
RT-70	AAA		AAG		AAG
RT-74	TTA				
RT-75	GTA				
RT-77	TTC	TTT	TTT		
RT-90	GTT	GTC			
RT-98	GCA	GCG	GGA		
RT-100	TTA				
RT-101	AAA				GAG
RT-103	AAA				
RT-106	GTA		GTG		ATG
RT-108	GTA				
RT-115	TAT				
RT-116	TTT				
RT-151	CAG				
RT-179	GTC	GTG	GTT	GTT	
RT-181	TAT	TAC			
RT-184	ATG		GTA		
RT-188	TAT				
RT-190	GGA				GCA
RT-210	TTG		TGG		TGG
RT-215	ACC		TAC		TAC
RT-219	AAA		AAG		AAG
RT-225	CCT		CCC		CCC

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* . Update of the Drug Resistance Mutations in HIV-1: Spring 2008. Topics in HIV Medicine 2008: 16(1); 62 – 68.

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.

Panel sample ENVA8-04 was not considered in this analysis as it was a panel sample negative for HIV-1.

Additional legend information for Figures 1 to 5*

The consensus sequences are presented at the top of figures 1,2, 3 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 4 represents the Panel Sample ENVA8-04 which was panel sample negative for HIV-1. The submitted participant sequences for this panel sample are illustrated here, while the other participants sequences are represented by "---".

Figure 1: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA8-01

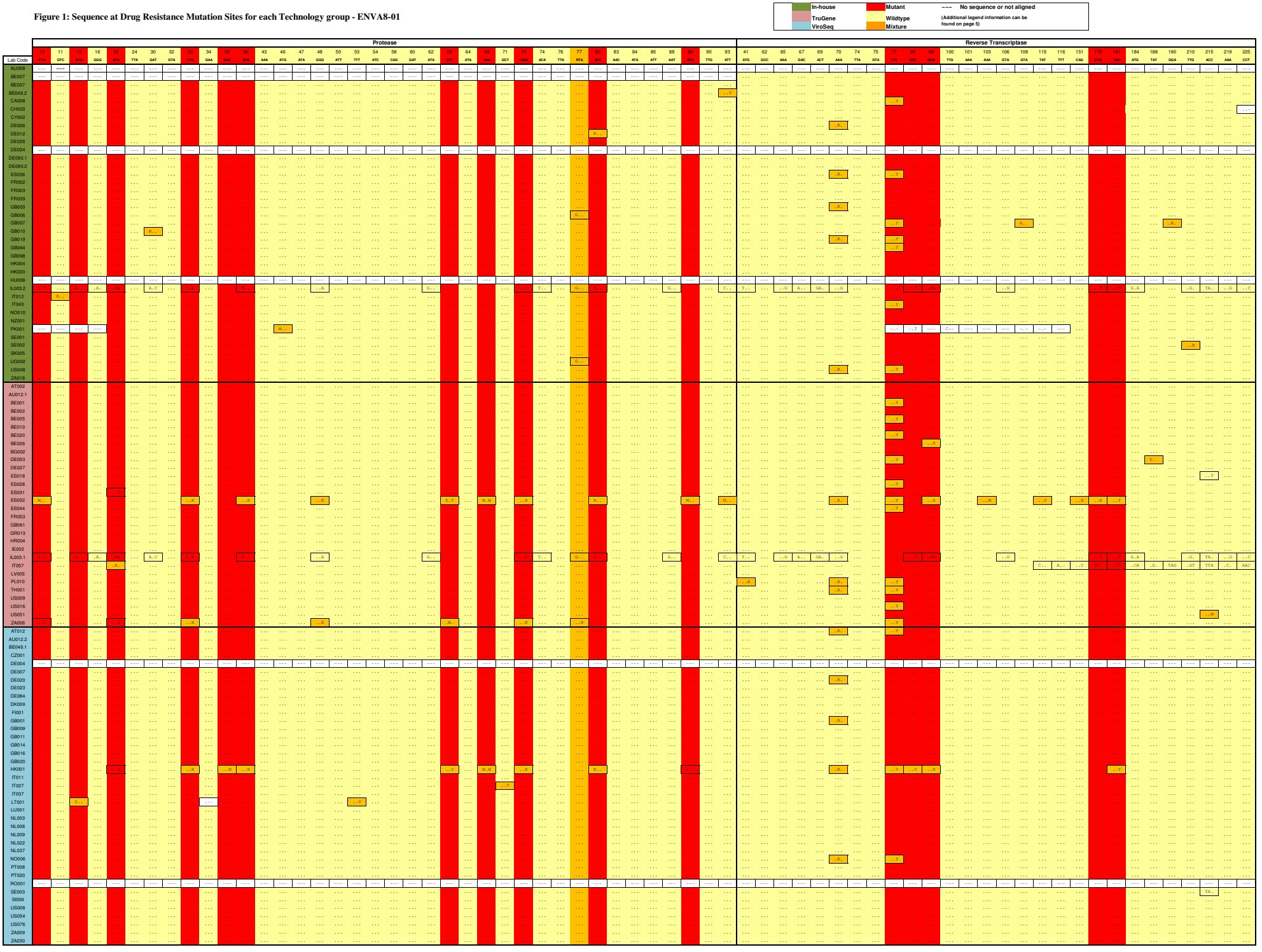

Figure 2: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA8-02

Figure 3: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA8-03

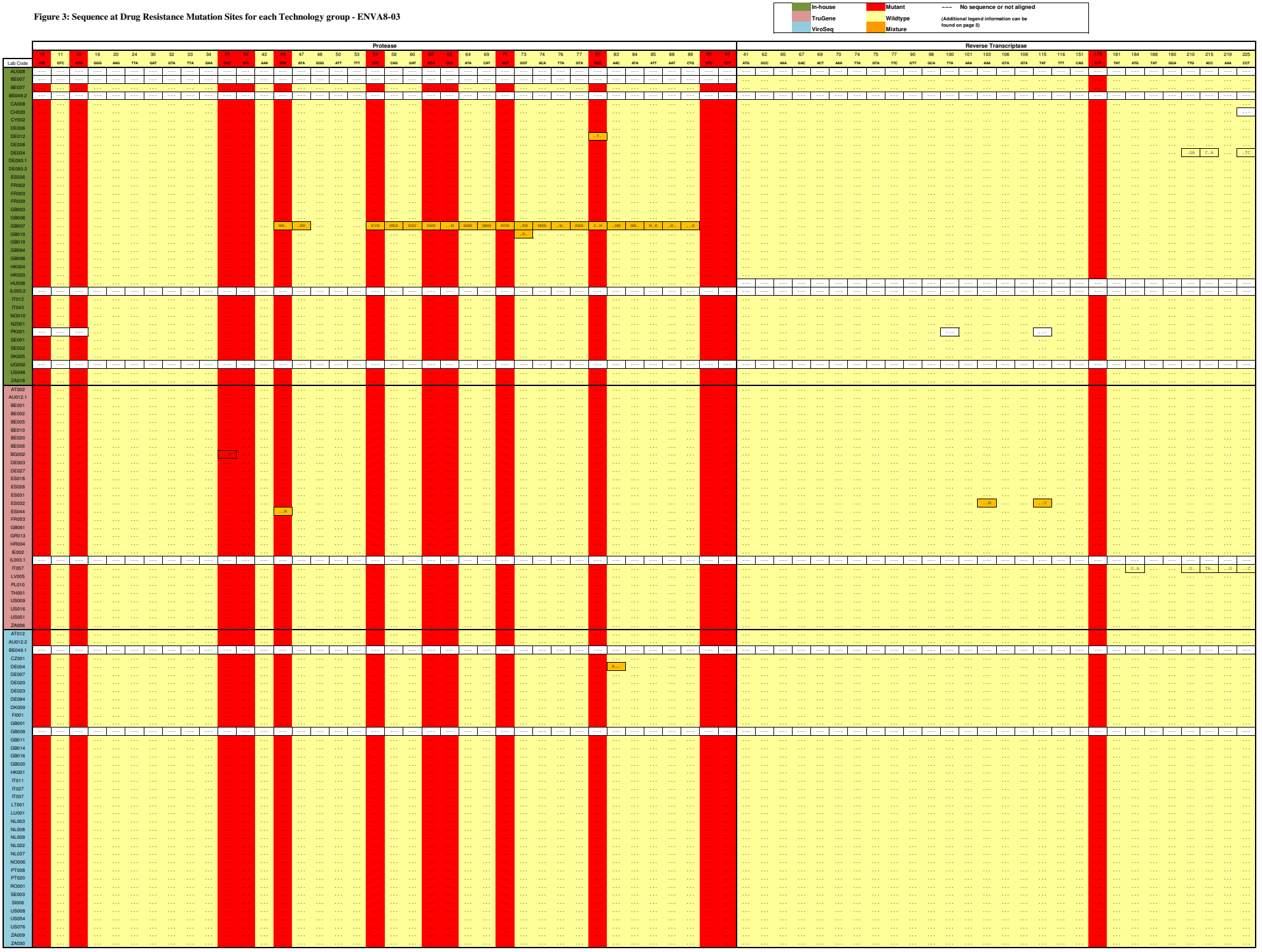

Figure 4: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA8-04*

In-house
TruGene
ViroSeq
--- No sequence submitted
(Additional legend information can be found on page 5)

Protease

Lab Code	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
AU008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE007	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE037	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE049.2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
CA008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
CH020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
CY002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE012	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE026	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE034	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN	NNN
DE093.1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE093.2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ES036	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FR002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FR003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FR039	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB007	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB019	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB064	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB098	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HK004	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HK020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HU008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IL003.2	CTC	GTC	ATA	GGG	AAA	TTA	GAT	GTA	TTA	GAA	GAA	ATA	AAA	ATG	ATA	GGA	ATA	TTT	ATC	CAR	GAG	ATA	CCT	ATA	AAA	GCW	GGT	ACA	TTA	GTG	GTC	AAC	ATA	ATT	AAC	ATG	TTG	CTT
IT012	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IT043	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NO010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NZ001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PK001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SE001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SE002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SK005	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
UG002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US048	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA018	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AT002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AU012.1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
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BE002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
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ES018	CTC	GTC	ATA	GGG	AAG	TTA	GAT	GTA	TTA	GAA	GAC	ATA	AAA	ATG	ACA	GGG	ATT	TTT	ATC	CAG	GAT	GTA	TCC	ATA	CAT	GTT	GGT	ACA	TTA	ATA	GTC	AAC	ATA	ATT	AAT	CTG	TTG	CTT
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ES032	ATT	GTC	GTA	GGG	AAG	TTA	GAT	GTA	TTA	GAA	GAA	ATG	AAA	ATG	ATA	GGA	ATT	TTT	ATC	CAG	GAT	ATA	GCC	ATA	CAT	GCC	GGT	RCA	TTA	ATA	GTC	AAC	ATA	ATT	AAT	CTG	TTG	CTT
ES044	CTM	GTC	GTA	GGG	ATA	TTA	GAC	GTG	TTA	CAG	GAT	ATT	AAA	ATG	ATA	GGA	ATT	TTT	ATC	CAG	GAG	ATA	CYT	ATA	AAA	GCT	GGG	ACA	TTA	GTA	ATT	AAC	ATA	ATT	AAT	ATG	TTA	CTT
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IL003.1	CTC	GTC	ATA	GGG	AAA	TTA	GAT	GTA	TTA	GAA	GAA	ATA	AAA	ATG	ATA	GGA	ATA	TTT	ATC	CAA	GAG	ATA	CCT	ATA	AAA	GCW	GGT	ACA	TTA	GTG	GTC	AAC	ATA	ATT	AAC	ATG	TTG	CTT
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LV005	CTT	GTC	GTA	GGA	AAA	TTA	GAT	GTA	TTA	GAA	GAT	ATA	AAA	ATG	ATA	GGG	ATA	TTT	ATC	CAG	GAT	ATA	CYT	ATA	AAA	GCT	GGT	ACR	TTA	GTA	GTC	AAC	ATA	ATT	AAT	ATG	TTG	CTT
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TH001	GTT	GTC	GTA	GAA	AAG	TTA	GAT	GTA	TTA	GAA	GAT	ATA	AAA	ATG	ATA	GGG	ATT	TTT	ATC	CAA	GAT	ATA	CTT	ATA	AAA	GCT	GGT	ACA	TTA	GTA	GTC	AAC	ATA	ATT	AAT	ATG	TTG	ATT
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Reverse Transcriptase

Lab Code	41	62	65	67	69	70	74	75	77	90	98	100	101	103	106	108	115	116	151	179	181	184	188	190	210	215	219	225
AU008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
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IL003.2	WTG	GCC	AAG	AAC	AGT	GAG	AGA	AAA	GTA	TGG	CAC	GCA	GGG	GAG	AAA	ATG	GAT	GCA	CTT	GAA	GTC	CAA	GAC	TAT	CAC	GGA	CCA	AAA
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BE001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE005	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE026	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BG002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE027	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ES018	ATG	GCC	AAA	GAC	ACT	AAA	TTA	GTA	TTC	GTT	GCA	CTA	AAA	AAA	GTA	GTA	TAT	TTC	CAG	GTT	TAT	ATG	TAT	GGA	TTG	ACC	AAA	CCT
ES028	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ES031	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ES032	ATG	GCC	AAA	GAY	ACT	AAA	TTA	GTA	TTT	GTT	GCA	TTA	AAA	AAC	GTA	GTA	TAC	TTT	CAA	GTT	TAT	ATG	TAT	GGA	TTG	ACC	AAA	CCT
ES044	ATG	GCC	AAA	GAC	ACT	AAA	TTG	GTA	TTT	GTC	GCG	CTA	AAA	AAA	ATA	GTA	TAC	TTT	CAG	GTR	TAC	ATG	TAT	GGA	CTG	ACC	AAA	CCT
FR053	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB061	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GR013	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HR004	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IE002	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IL003.1	ATG	GCC	AAG	AAC	AGT	GAG	AGA	AAA	GTA	TGG	CAC	GCA	GGG	GAG	AAA	ATG	GAT	GCA	CTT	GAA	GTC	CAA	GAC	TAT	CAC	GGA	CCA	AAA
IT057	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
LV005	ATG	GCT	AAA	GAC	ACT	AAR	TTA	GTA	TTC	GTT	GCG	TTA	AAA	AAA	GTA	GTA	TAT	TTT	CAG	GTT	TAT	ATG	TAT	GGC	TTK	ACT	AAA	CCT
PL010	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
TH001	CTG	GCT	AAA	GAC	ACC	AAA	TTA	GTA	TTC	GTT	GGA	CTA	AAA	AAC	GTA	GTA	TAT	TTT	CAG	GTT	TAT	GTA	TAT	GGA	TTG	TAT	AAA	CCT
US009	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US016	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US051	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AT012	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AU012.2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BE049.1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
CZ001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE004	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE007	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE023	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DE084	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DK009	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FI001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB009	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB011	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB014	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB016	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
GB020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
HK001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IT011	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IT027	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
IT037	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
LT001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
LU001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NL003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NL008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NL009	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NL022	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NL037	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
NO006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PT008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PT020	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
RO001	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SE003	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SI006	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US008	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US054	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
US076	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA009	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
ZA030	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Figure 5: Sequence at Drug Resistance Mutation Sites for each Technology group - ENVA8-05

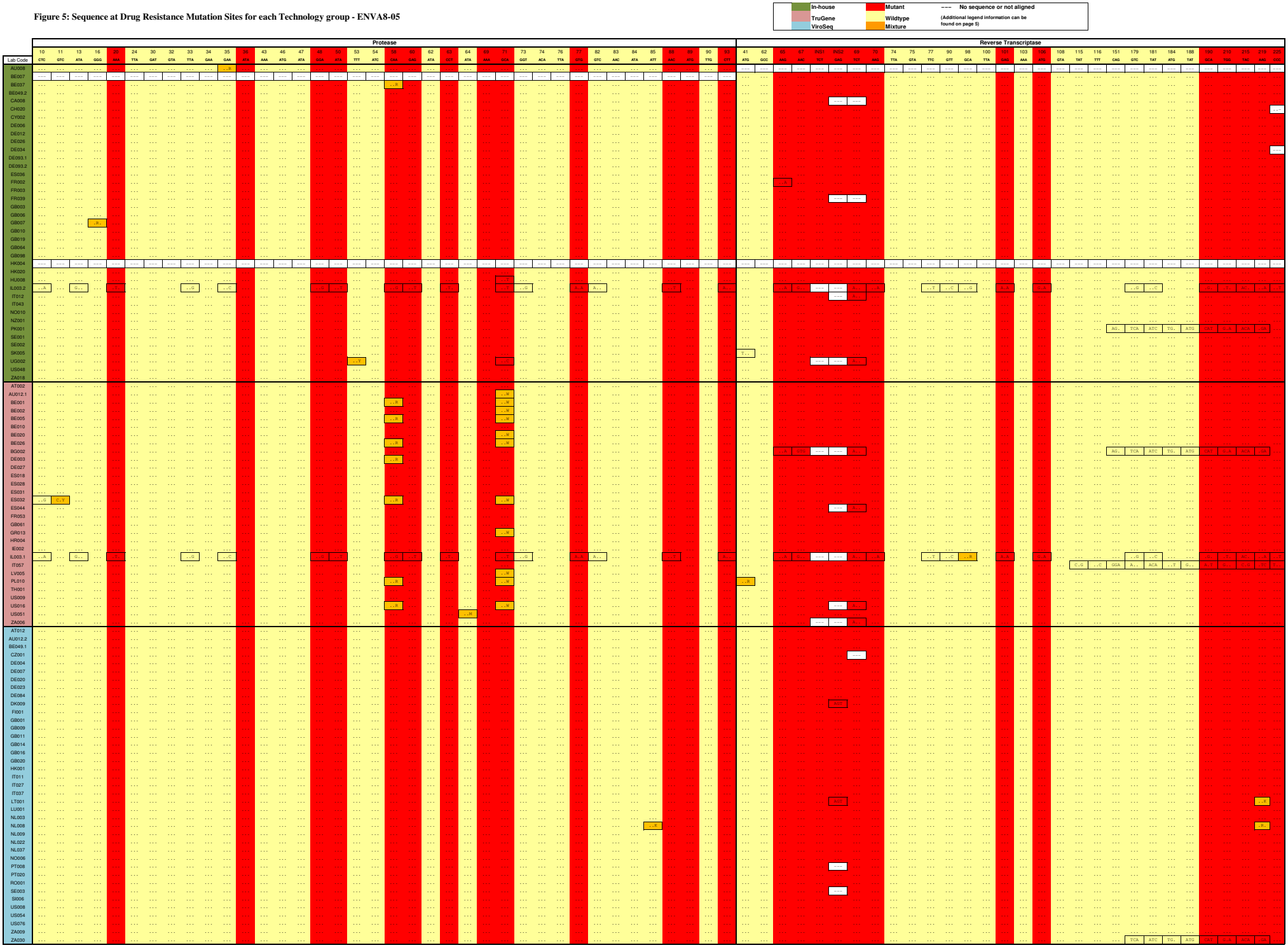
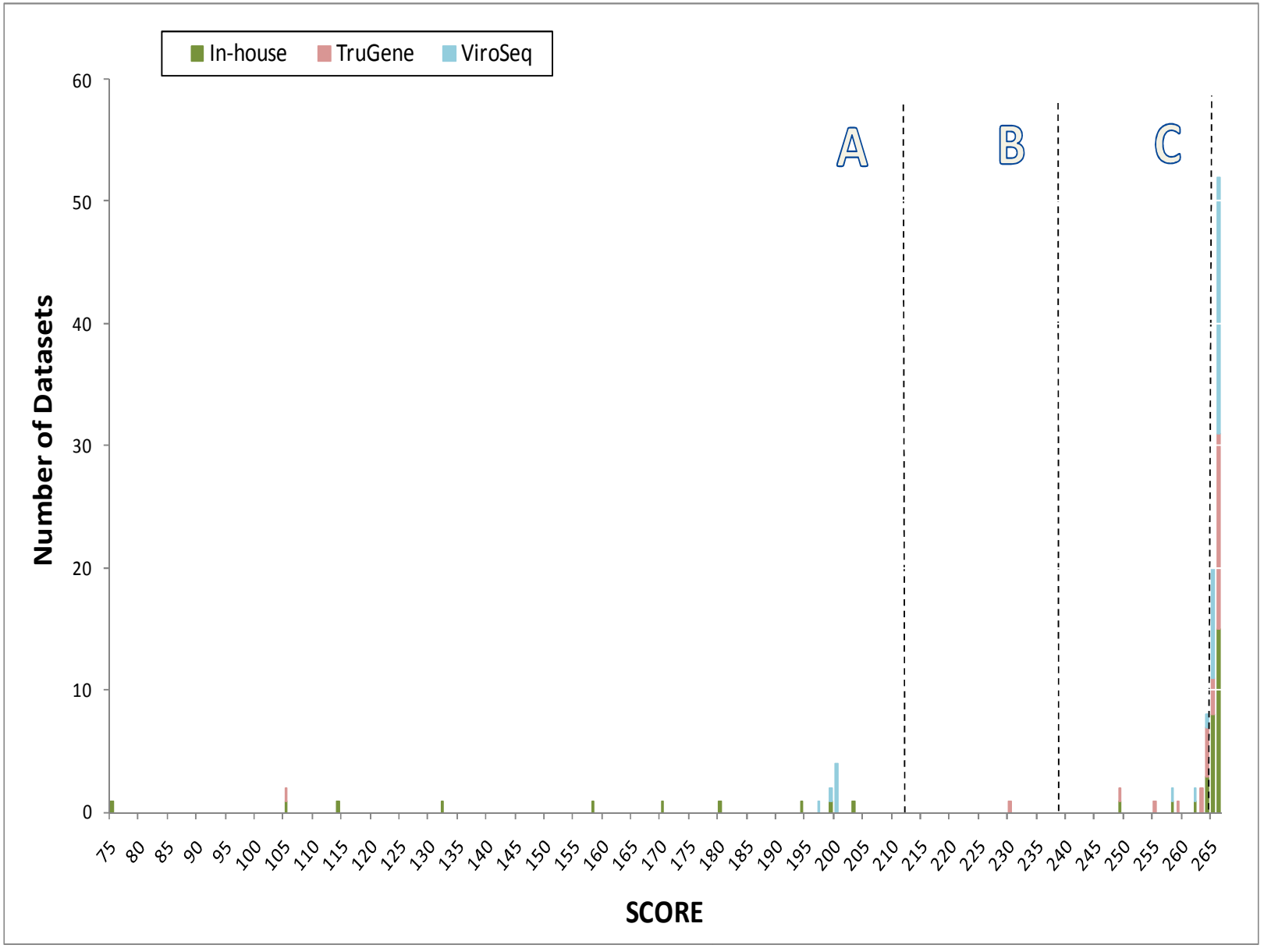


Figure 6: Summary of overall performance scores by technology

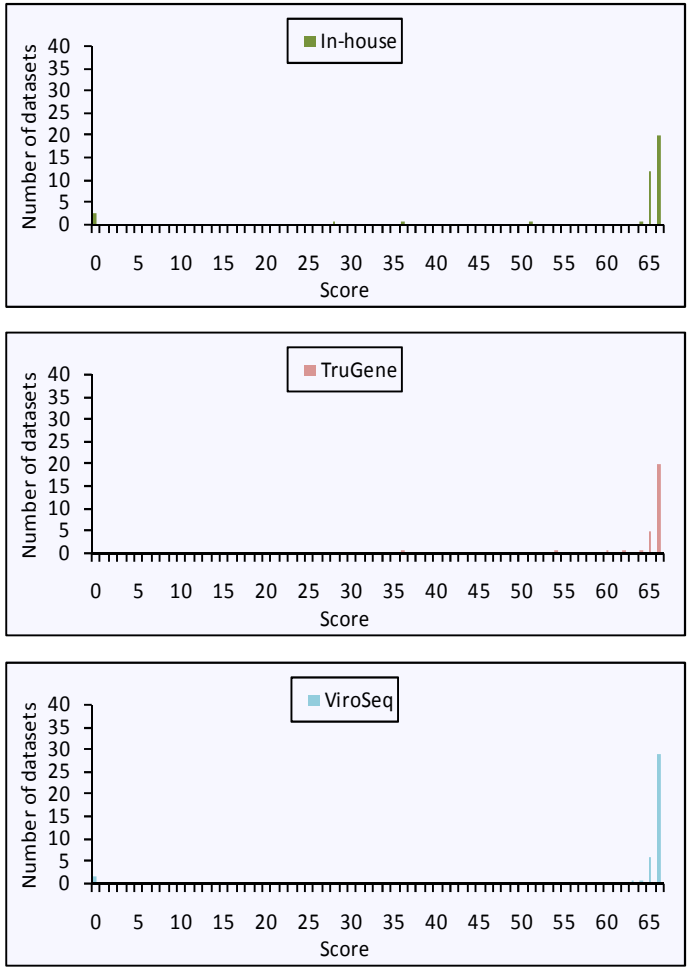


A: 80% (213) of maximum achievable score (266), B: 90% (239) of maximum achievable score (266), C: 99% (263) of maximum achievable score (266).

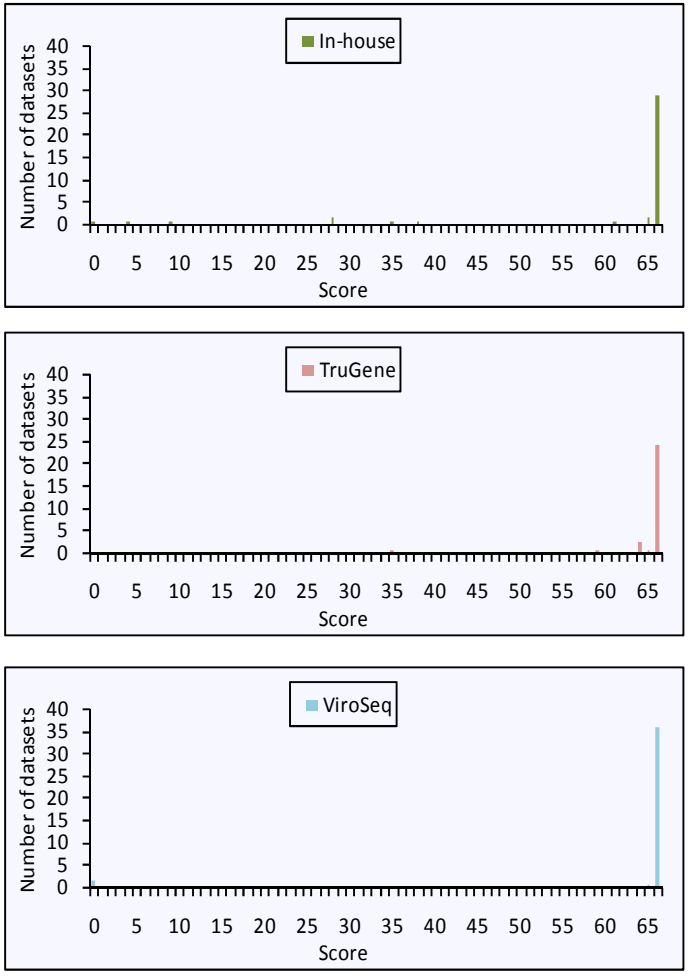
Panel sample ENVA8-04 was panel sample negative for HIV-1, and was not scored.

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

ENVA8-01



ENVA8-02



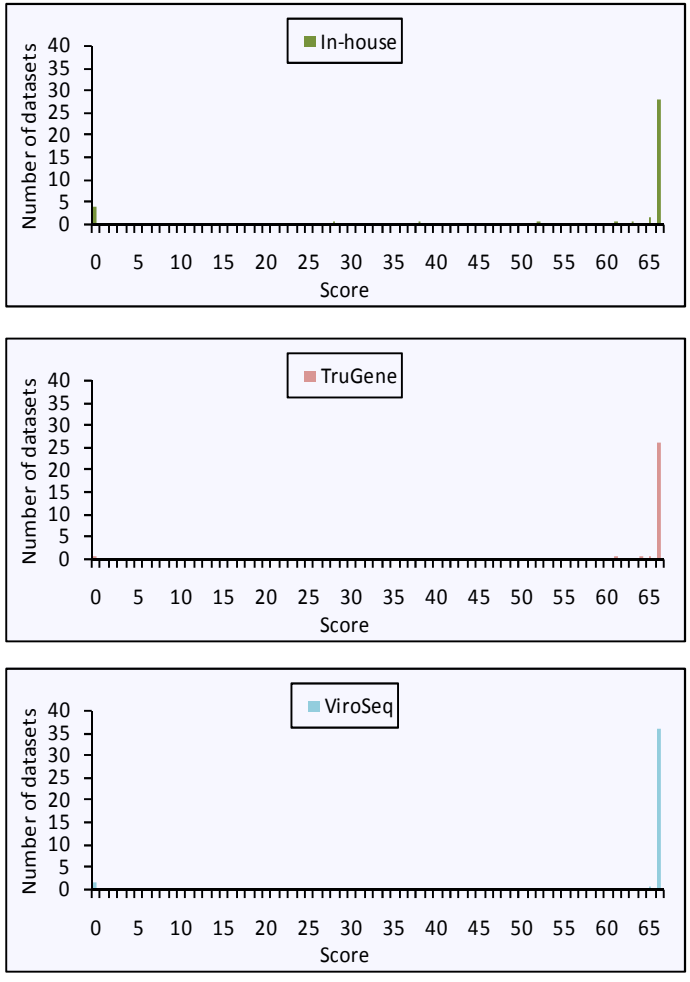
The maximum score achievable for ENVA8-01, ENVA8-02 and ENVA8-03 was 66 points each.
The maximum score achievable for ENVA8-05 was 68.

Panel sample ENVA8-04 was panel sample negative for HIV-1, and was not scored.

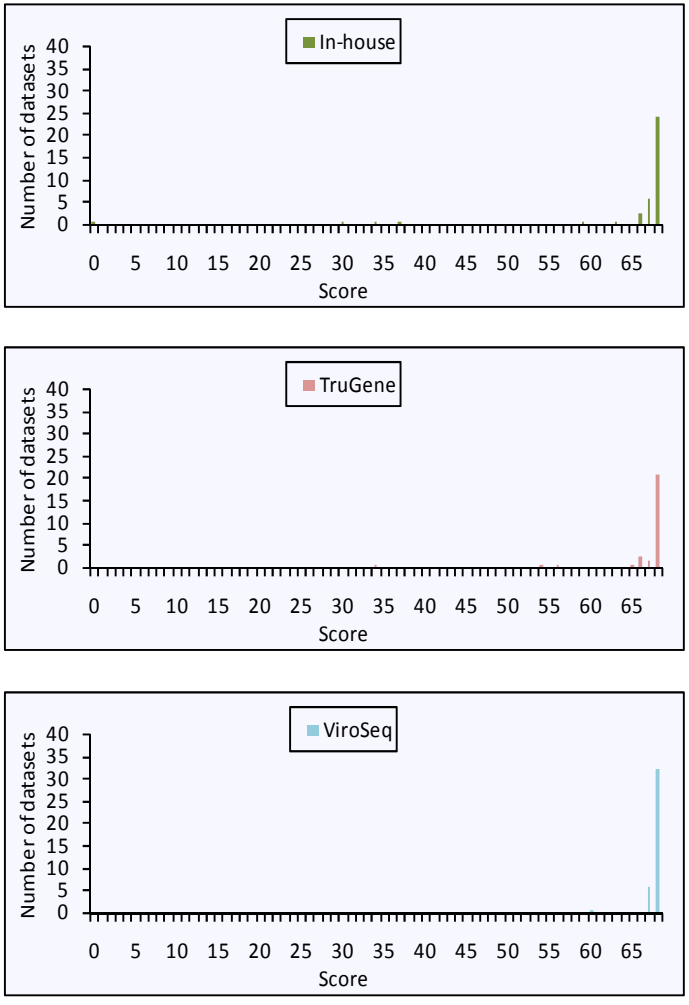
Datasets containing either a frame-shift or missing sequences resulted in a low performance score.

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

ENVA8-03



ENVA8-05



The maximum score achievable for ENVA8-01, ENVA8-02 and ENVA8-03 was 66 points each.
The maximum score achievable for ENVA8-05 was 68.

Panel sample ENVA8-04 was panel sample negative for HIV-1, and was not scored.

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

Comments

The ENVA8 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 B,C or G. One panel was sample negative for HIV-1.

The consensus sequences calculated from the independent 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: 39 For Viroseq [36.1%] and 30 for TruGene [27.8%]). The remaining 39 datasets [36.1%] were generated with in-house genotyping assays (Table 3b). All technologies were successful in the sequence analysis of the panel samples. There were no systematic negative results with any of the positive panel samples. For each of the positive panel samples the number of complete datasets (covering both the Protease and Reverse Transcriptase genes) was comparably high (84.26%; compared to 82% in ENVA7 (QCMD, 2007).

The number of full datasets reported to QCMD was lower for the in-house assays (74.4%), compared to TruGene (96.7%) or ViroSeq (84.6%): a trend that was also observed in ENVA7, where it was attributed to difficulties sequencing an HIV-1 subtype-F strain. No obvious trends were observed in the ENVA8 distribution to account for the lower percentage of complete datasets generated using in-house assays (Table 3b).

This was the first year that an ENVA EQA panel has included an HIV-1 negative panel sample. There were eight datasets submitted containing full sequencing data for both PR and RT. This represents a false positivity of 7.4% (Figure 4). A pairwise alignment of these data was performed. The outcomes of this analysis were that:

1. None of the submitted results showed complete identity with any of the other ENVA8 panel samples.
2. Highest homology (99%) was found with panel sample ENVA8-05 by one participant, but this was not found with the other participants reporting false positive results.
3. Future ENVA distributions may contain negative panel samples so that trends in false identifications can be monitored.

Performance scores for each of the positive panel samples were high as well as scores for the overall panel (Figures 6, 7a & 7b) Overall, the percentage of datasets recording over 99% of the maximum achievable score (266) was 75.9% (n=82/108) (Figure 6). The range of scores reported for those datasets that did not achieve 99% (of the maximum achievable score or greater) ranged from 75 to 262 points. Most of the lower scoring datasets were due to missing results for complete genes (either PR or RT) or complete panel samples (Table 3a and Figure 6). Scoring was not performed on the HIV-1 negative panel sample (ENVA8-04) but future distrubutions will consider this action in order to properly reflect the importance of false positive results.

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