External Quality Assessment for HIV Co-receptor Tropism Testing

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INTRODUCTION

Chemokine receptor 5 [CCR5] inhibitors such as Maraviroc target the interaction between the HIV-1 virus and the CCR5 [R5] co-receptor and are appropriate for treatment of individuals infected with R5 strains. These agents are not active against CXCR4 [X4]-using strains. HIV-1 co-receptor tropism determination is advised to optimise successful treatment outcomes and minimise the use of ineffective drug therapy.

From the outcomes of clinical trials [MOTIVATE; MERIT] and the deliberations of expert international committees recommendations have been formulated to guide clinical tropism testing [1, 2, 3, 5, 8]. Coupled with differences that exist between the guidelines and use of in-house genotypic protocols by most testing laboratories potential exists for variation in test outcome. External Quality Assessment Schemes [EQAS] can be utilised to improve testing proficiency and standardise test outcomes [4, 7].

OBJECTIVES

This EQAS aims to investigate protocols and outcomes of laboratories performing tropism testing; the long term objectives are to assist in standardisation of testing outcomes and develop a collaborative international testing network.

METHOD

When testing EQAS panels, laboratories were asked to follow their standard protocol and reported tropism and False Positive Rate values [FPRs]. Details regarding protocols were gathered by questionnaire.

Panel I samples [n=12] were chromatograms of the HIV-1 env gene [V3 loop]. All samples were sourced from HIV-1 infected individuals; seven samples were X4, four were R5 and one [Sample 5] was considered of suboptimal quality for tropism prediction as determined by a reference laboratory. Participation was at no cost.

Panel II required tropism determination from 10 DNA samples shipped ambient to participants. Two samples were sourced from clones; reference tropism X4. Tropism of the eight samples sourced from HIV-1 infected individuals was as determined by the majority of participants called the *majority consensus*: five samples were X4, one was R5 and two were considered to have had mixed populations of X4 and R5 sequences since the X4/R5 breakdown between participants was approximately equal [Table 3 and Figure 1b]. Participation was charged at cost recovery of AUD 515.

RESULTS

Parameters of testing protocols

- Laboratories from 15 countries participated in the EQAS; 23 laboratories tested Panel I; 17 tested Panel II.
- Most [16 of 24] routinely performed triplicate testing; most [14 of 16] determined tropism from the lowest FPR derived from triplicate testing [data not shown]. FPRs varied between 5% and 20%. Various software programs were used to assemble and edit sequences. All but one laboratory used the online algorithm geno2pheno [g2P] to determine tropism from viral sequence.

RESULTS [continued]

Table 1. Parameters of laboratories' HIV tropism testing protocols [self-reported].

Lab ID	Software	Triplicate testing		FPR [%]		Bioinformatic algorithm	
		Panel I	Panel II	Panel I	Panel II	Panel I	Panel II
A	SeqScape			10	10	g2P	g2P
B	Not given			10		g2P	
C	ReCALL			20	20	g2P	g2P
D	ChromasPro			20		g2P	
E	ReCALL			5.75	5.75	g2P	g2P
F	ReCALL			5 - 15 [1]	5 - 15 [1]	g2P	g2P
G	Staden Package			10	10	g2P	g2P
H	Staden Package			5 - 15 [1]	5 - 15 [1]	g2P	g2P
I	OpenGene			10		g2P	
J	ChromasPro			15	20	g2P	g2P
K	SeqScape			10		g2P	
L	Chromaspro			5 - 15 [1]		g2P	
M	SeqScape			10	10	g2P	g2P
N	Conexio Assign			20	5 - 15 [1]	g2P	g2P
O	ReCALL			10	10	g2P	g2P
P	ReCALL			10	10	g2P	g2P
Q	DNASTAR SeqMan			5 - 15 [1]	10	g2P	g2P
R	Staden Package			5 - 15 [1]	15	g2p	g2p
S	DNASTAR SeqMan			6	6	g2p	g2p
T	OpenGene			20	10	g2P	TruGene
U	ABI; Sequencher			15		g2P	
V	DNASTAR SeqMan			5		g2P	
W	ReCALL			20	20	g2P	g2P
X	ABI3500				10		g2P

Blue = triplicate testing; Orange = single replicate testing; Grey = did not participate; g2P at http://coreceptor.bioinf.mpi-inf.mpg.de/index.php.
1. According to German Guidelines FPR values: X4<5% - Dual tropic - >15% R5.

Table 2. HIV tropism and FPRs reported by laboratories testing Panel I [chromatograms].

Lab ID	Ref Lab	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Panel I																								
1			0.7		1.7		1.7	1.7	0.7	0.7	0.7		0.7	0.7	0.7	0.7	0.7	1.7	1.7	1.1	0.7		0.7	0.7	0.7	0.7
2			1.7	9.2	1.7	1.7	1.7	1.7	1.7	1.7	4.2	4.2	9.2	0.1	1.7	1.7	1.7	1.7	1.7	1.7	1.7	0.5	1.7	1.5	1.7
3			1.3	25.6	1.7	1.3	1.7	1.7	1.3	1.3	1.3	1.3	1.3	28.1	1.7	1.3	1.7	1.7	1.7	1.1	1.3	1.3	1.3	0.7	1.3
4			20.4	27.3	42.2	20.4	41.6	1.7	30.1	28.8	19.1	25.3	37.3	49.6	27.3	20.4			21.4	28.8	20.4	20.4	30.1	41.3	31.6
5							NS							4.7	3.9										
6			67.6	87.8	55.3	60.8	34.9	17.9	17.9	49.9	43.6	70.8	64.0	66.5	78.8	42.6	18.9	35.3	30.1	45.7	17.9	43.8	42.8	47.0	17.9
7			1.3	16.2	1.8	1.8	1.8	1.8	1.8	1.7	1.8	6.7	2.8	2.2	2.2	1.8	1.7	1.8	1.8	2.1	1.8	1.8		4.8	1.8
8			1.5	12.8	1.3	1.5	1.3	1.2	21.5	1.7	1.5	1.5	1.5	28.8	1.7	1.7	1.3	1.3	1.7	1.7	1.5	1.3		2.7	1.1
9			68.9	68.9	23.3	53.5	22.6	2.5	28.8	68.9	68.9	68.9	68.9	68.9	68.9	41.3	30.1	23.3	2.8	68.9	4.0	53.5	68.9	68.9	9.2
10			0.7	2.5	0.8		0.7	1.4	5.0	5.0	0.8	2.9	2.7		0.7	1.1	0.5	0.8		0.7	5.0	0.8	0.7	1.7	5.0
11			1.7	35.3	35.3	35.3	35.1	18.8	35.3	35.3	35.2	34.1	1.7	35.3	35.3	1.8	35.3	35.3	11.7	31.7		2.1	35.3	35.3	35.3
12			1.7	1.3	1.7	1.7	1.7	1.7	1.7	1.7	2.9	1.7	0.2	1.3	1.7	1.7	1.7	1.7	1.7	1.7	1.7	0.1	1.7	1.7	1.3

Table 3. HIV tropism and FPRs reported by laboratories testing Panel II [DNA from clones and clinical samples].

Lab ID		A	C	E	F	G	H	J	M	N	O	P	Q	R	S	T	W	X
Lab FPR		10	20	5.75	5-15	10	5-15	20	10	5-15	10	10	10	15	8	10	20	10
Panel II	Majority consensus																	
Clone 1		0.5	0.5	1.7	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Clone 2		0.5	0.5	1.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.5
1		1.7		24	24.8	24.8	1.3	24.8	24.8	21.2	2.2	24.8	17.3	1.7	1.7	24.8	1.1	24.8
2		1.1	2.8	1.7	1.7	1.1	1.1	1.7	1.7	1.1	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.5
3		0.2	1.1	1.7	1.1	1.1	0.7		0.7	0.7	0.7	0.7	0.7	1.3	1.1		1.1	0.2
4		13.5	20.2	13	16.4	13	20.2	20.2	20.2	16.4		13	16.4	49	16.4	20.2	20.2	18.5
5		1.8	1.7	1.7	1.8	3.2	3.2	1.2	5.3	2.8	1.7	3.2	11.7	1.2	3.2	1.8	3.2	0.2
6		9.8	9	7.9	4.7	1.7	7.4	1.2	9.8	9.8	9	9	7.4	9.8	9	9.8	9.8	4
7		1.7	21.2	1.7	21.2	5.3	5.8	28.5	1.7	1.7	16.4		1.7	1.7	6.8	8.5	3.1	1.7
8		2.5	2.6	2.6	2.6	2.6	2.5	2.6	2.6	2.6	2.5	2.6	2.5	2.6	3.7	2.6	2.6	2.5

For Tables 2 and 3. Green = R5; Red = X4 [reported as X4 or dual tropic]; Grey= not reported; poor quality sequence. In this analysis a determination of dual tropism by a laboratory was considered to be in agreement with a majority consensus X4.

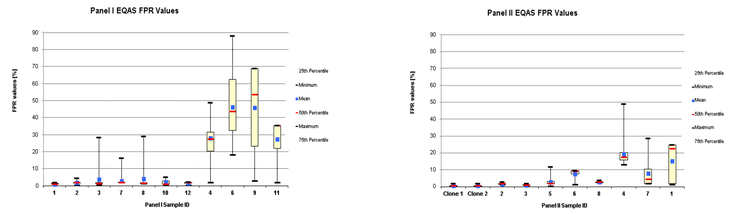
RESULTS (continued)

Panel I [Table 2 and Figure 1a.]

- Reference tropism was reported by laboratories for 226 of 243 [93%] tropism determinations.
- Of 23 laboratories three reported X4-using virus as R5 [Lab B: Samples 3, 7 and 8; Lab G: Sample 8 and Lab L: Samples 3 and 8]; eight reported R5-using virus as X4; 21 did not predict tropism from suboptimal sequence.
- Most discordant tropism calls were not a consequence of inter-laboratory difference in FPRs; they were more likely due to differences in sequence interpretation.
- Of 154 FPRs reported by all the laboratories testing the seven X4 samples [Samples 1, 2, 3, 7, 8, 10 and 12] six [3.9%] were > FPR 10 (i.e., more likely to be R5); of the 89 FPRs reported for the four R5 samples [Samples 4, 6, 9, and 11] , nine [10.1%] were < FPR 10 (i.e. more likely to be X4).

Panel II [Table 3 and Figure 1b.]

- All laboratories [n=17] agreed with reference tropism [X4] for cloned samples.
- Majority consensus tropism was reported for 96 of 100 [96%] clinical samples.
- Three reported X4-using virus as R5 [Lab Q: Sample 5; Labs E and S: Sample 6]; none reported R5-using virus as X4.
- Samples 1 and 7 appeared to have had mixed populations of X4 and R5 sequences. Laboratories that performed single replicate testing [e.g. Labs F and J] can fail to detect the X4 that others doing triplicate testing detect.
- Of the 83 FPRs reported by all the laboratories testing the five X4 samples [Samples 2, 3, 5, 6 and 8] one [1.2%] was > FPR 10; no laboratory [n=16] reported FPR <10 for Sample 4 [R5 virus].



Figures 1a and b. Inter-laboratory variation in FPRs.

DISCUSSION

The results of this international EQAS highlighted that

- There were common elements and differences in parameters between laboratories' testing protocols including variation in the FPRs.
- Although laboratories applied different FPRs depending upon the guidelines they followed, by and large, the tropism interpretations were consistent.
- Most laboratories did not attempt to interpret tropism from poor quality sequence.
- Failure to generate good-quality sequence may indicate a problem with PCR.
- Single replicate testing may contribute to the failure to detect X4 sequence in mixed virus populations [6].
- Participation in EQAS may be cost sensitive.
- The EQAS is open to all laboratories worldwide that perform genotypic HIV tropism testing.

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