

HIV-1 PHENOSCRIP[®] ENV: A Sensitive Assay for the Detection of HIV X4 Minority Species and Determination of Non-B Subtype Viral Tropism

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BACKGROUND

Entry inhibitors that block the attachment of HIV gp120 to CCR5 or CXCR4 coreceptors are currently in clinical development and represent a new generation of antivirals for the treatment of HIV infection. The determination of patients' viral population tropism before the initiation and during therapy with such antagonists may be critical to optimize treatment strategies. PHENOSCRIP[®] ENV is a sensitive phenotypic assay for the evaluation of viral tropism. Its sensitivity has been established by: 1) detecting X4-tropic minority species, 2) evaluating the success rate for plasma samples with different viral loads, and 3) determining viral tropism of the most prevalent European HIV-1 non-B subtypes.

METHODOLOGY

Following viral RNA extraction from patients' plasma samples (1), viral envelope sequences were PCR-amplified (2). Two different envelope regions can be used for amplification: 1) gp120-gp41-tectodomain which allows the determination of both susceptibility to entry inhibitors and viral tropism, and/or 2) the V1-V3 region for determination of viral tropism only. The PCR products are cotransfected (3) into producer cells along with an HIV-1 plasmid, pNL4-3, deleted of the corresponding envelope region. The overlap between the sequences flanking the deletion in the plasmid and the PCR products allows homologous recombination to take place in transfected cells.

Supernatants containing recombinant virus particles are used to infect U373MG-CD4 indicator cells (4), expressing CCR5 or CXCR4 co-receptors, in the presence or absence of coreceptor inhibitors. Inhibition of infection in the presence of a coreceptor inhibitor is a reliable marker for the specificity of entry via this route.

The indicator cells carry the lacZ gene under the control of the HIV-1 LTR. The production of β -galactosidase by infected cells (5), induced by Tat activation, is quantified by a colorimetric test (CPRG) (6).

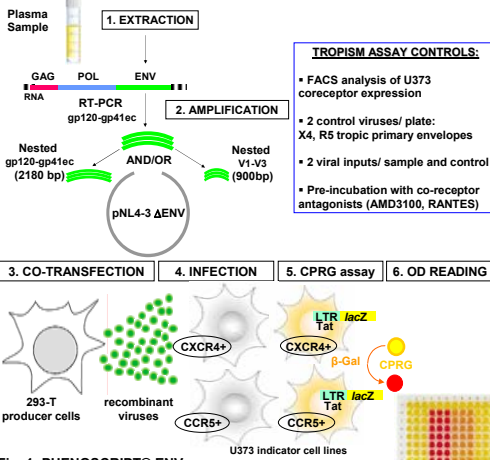


Fig. 1: PHENOSCRIP[®] ENV assay

METHODOLOGY

Detection of primary X4 tropic quaspecies:

We measured the sensitivity of the assay for detecting X4-tropic viruses using two different approaches:

- **Mixtures at different ratios of proviral plasmid carrying X4- and R5-tropic primary envelopes from patients' plasma samples.** PCR reactions (gp120-gp41ec and V1-V3) were carried out on each mixture of plasmids before analysis by PHENOSCRIP[®] (Fig.2).

- **Plasma samples harbouring different percentages of X4- and R5-tropic viruses.** These samples were generated by mixing equivalent amounts (determined by RNA copy number) of two tissue culture-derived stocks of an R5 virus (ASM 57) and an X4 virus (HC 4) into normal human plasma. The plasma stocks were mixed at different ratios and the RNA content of each mixture was determined to ensure that the total amount of RNA in each sample was approximately equal. Following viral RNA extraction, PCR amplification, tropism was determined by PHENOSCRIP[®] (Fig.3).

- **Tropism determination for different viral loads:** To document the sensitivity of the assay towards plasma samples with VL between 1000 and 10000 copies/ml, 4 characterized plasma samples with known viral load were diluted, and viral RNA was extracted. For each sample, the gp120-gp41ec region of the viral envelope was amplified in 4 independent RT-PCR and nested PCR. PCR products were analyzed in PHENOSCRIP[®] (Table 1). In addition, a retrospective analysis was performed on 85 plasma samples with different viral loads (Table 2).

Tropism determination for HIV-1 non-B subtypes:

To establish the success rate for tropism determination of HIV non-B subtypes, among a panel of 400 plasma samples representing the most prevalent European non-B subtypes, we amplified 187 samples with the gp120-gp41ec region and 268 with the V1V3 region and analyzed the PCR products in the PHENOSCRIP[®] (Table 3).

RESULTS

Detection of primary X4 tropic quaspecies

Fig. 2: Mixtures of proviral plasmids carrying X4- and R5-tropic primary envelopes

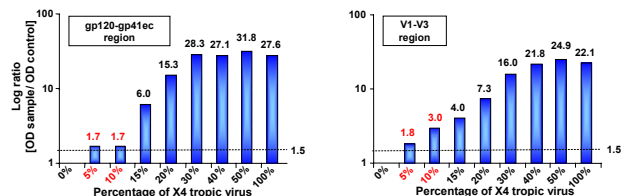
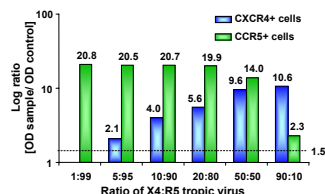


Fig. 3: Plasma samples harbouring different percentages of X4- and R5-tropic viruses



A sample is considered positive for the use of a specific co-receptor when the ratio [OD sample / OD control] equals or exceeds 1.5 (the control being the virus of the opposite tropism) and when the infection is inhibited by co-receptor antagonists. The complete inhibition by AMD3100 proves and confirms the specificity of the infection via CXCR4.

Both systems allowed the detection of as low as 5% of X4 tropic variants in a mixture, and the infection was completely inhibited by AMD3100.

RESULTS

Table 1: Tropism determination for subtype B samples with different viral loads

	Copy number per sample	PCR	RVA	Tropism
Patient 1	20020	4/4	4/4	R5
	12750	4/4	4/4	R5
	715	3/4	2/3	R5
	358	1/4	1/1	R5
Patient 2	8120	4/4	3/4	R5
	5220	4/4	4/4	R5
	290	4/4	4/4	R5
Patient 3	12600	4/4	4/4	X4
	8100	4/4	4/4	X4
	450	3/4	2/2	X4
	225	2/4	2/2	X4
Patient 4	44800	4/4	4/4	R5
	28800	4/4	4/4	R5
	2880	4/4	4/4	R5
	160	2/4	2/2	R5

Copies	Number of samples Tested	Success rate	
		PCR	RVA
>10000	5*	100%	100%
1000-10000	4*	100%	94%
<1000	6*	63%	100%

* 4 assays per sample

Table 2: Retrospective analysis of success rate

Number of samples tested	Viral Load	PCR	RVA	GLOBAL
74	>10 000	97%	96%	93%
11	1000-10000	82%	100%	82%
85		95%	97%	92%

* For plasma samples with viral loads above 10000 copies, all PCR and RVA were positive. Between 1000 and 10000 copies/ml, the success rate of the PHENOSCRIP[®] was 94% (one RVA was negative among the 16 tested), whereas below 1000 copies/ml it depended on the PCR success rate (63%).

* Furthermore, we retrospectively determined the success rate for 85 subtype B samples harbouring different viral loads. Between 1000 and 10000 copies of viral RNA/ml the global (PCR and RVA) success rate was 82% (9/11 samples), whereas it was 93% (69/74 samples) for viral loads above 10000 copies/ml.

The overall success rate for all 85 samples was 95% for PCR and 97% for RVA, resulting in an overall success rate of 92%.

Table 3: Tropism determination for HIV-1 non-B subtypes

Subtype	Prevalence in Europe	Number of samples available	Tested in PCR		Tested in RVA		%R5	%R5/X4	%X4
			gp120-gp41ec	PCR success rate	V1V3	PCR success rate			
C	37,5%	143	55	95%	95	75%	90	7.5	2.5
G	15,5%	59	38	87%	32	81%	40	70%	93
CRF02_AG	12,5%	61	30	87%	45	96%	46	87%	90
A	11,0%	39	21	57%	24	67%	19	42%	86
CRF01_AE	9,0%	59	19	89%	32	88%	41	56%	100
D	3,5%	21	12	83%	14	71%	15	73%	75
F	3,5%	26	8	63%	20	70%	16	75%	82
J	2,5%	9	4	100%	6	83%	6	67%	75

* The PCR success rate for the most prevalent European non-B subtypes with the 2 different cassettes was comparable, ranging from 57 to 100% for gp120-gp41ec, and from 67 to 96% for the V1V3 cassette.

* The RVA success rate for tropism determination using one or the other cassette ranged from 42 to 98%, depending on the subtype.

For samples that tested negative using gp120-gp41ec cassette, the use of the V1V3 region proved to be valuable alternative.

CONCLUSIONS

For subtype B viruses:

The limit of detection of PHENOSCRIP[®] for X4 tropic quaspecies in mixtures of R5 and X4 tropic viruses is 5%.

The determination of viral tropism for different viral loads confirms a good overall success rate between 1000 and 10000 copies. The success rate below 1000 copies is PCR dependent.

For non-B subtype viruses:

The success rate for tropism determination depends on the subtype.

Regarding the good PCR success rate for the gp120-gp41ec region on HIV-1 non-B subtypes, these samples are an appropriate source for the determination of baseline susceptibility to entry inhibitors.

Given the efficiency of PHENOSCRIP[®] ENV, our assay is suitable for the clinical management (selection and follow-up) of HIV infected individuals treated with coreceptor antagonists. Finally, it can be used to assign tropism of viral populations across diverse viral subtypes.