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**ABSTRACT**

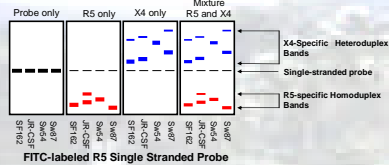
Antiretroviral therapeutic regimens that contain CCR5 antagonists can lead to various shifts in HIV co-receptor usage within HIV patients. Therefore, a diagnostic method that can simultaneously detect and quantify the amount of both CXCR4-tropic and CCR5-tropic HIV will be useful (1) in identifying candidates for specific therapies (e.g. CCR5 antagonist treatment); (2) for monitoring changes in HIV viral co-receptor tropism in response to antiretroviral treatments and (3) for further defining the role of CXCR4-tropic HIV in disease progression.

The qualitative form of the SensiTrop assay uses heteroduplex tracking technology to detect CXCR4-tropic HIV with numerous mutations in the V3 loop by forming heteroduplexes with CCR5 V3 probes. We have modified this technique by generating fluorescently labeled single-stranded CCR5 V3 probes, which allow the simultaneous separation, detection and quantitation of both CCR5/CCR5 homoduplex DNA hybrids and CXCR4/CCR5 heteroduplex DNA hybrids. Using the SensiTrop QT assay format, we have determined the precision and the limit of quantification of this assay using different X4/R5 DNA mixtures with ratio ranges from 0% to 100% CXCR4/CCR5. The data show SensiTrop QT assay can reproducibly quantify X4/R5 ratios down to 5% with acceptable inter-assay CV values ranging from 1.8%- 16.9%. We have also tested the SensiTrop QT assay using spiked plasma containing X4/R5 viral mixtures with ratio ranges from 1% to 50%. This data clearly demonstrates that the SensiTrop QT assay can also similarly quantify X4/R5 ratios at the level of viral RNA when the HIV viral load is >1000 HIV copies/ml. Lastly, in random HIV patient specimens, we have used the quantitative form of the SensiTrop QT assay to confirm that the qualitative form of the SensiTrop assay can accurately detect CXCR4-tropic HIV when it is present at only 1% of the amount of CCR5-tropic HIV.

We believe that the ability to rapidly and sensitively quantify co-receptor tropism usage in HIV patients will be a valuable new diagnostic tool to help physicians, researchers and pharmaceutical companies monitor how changes in antiretroviral therapy cause positive or negative shifts in the HIV co-receptor status.

**METHODOLOGY**

**Quantitative HTA Analysis using Single stranded DNA probe Composition of Patient HIV-1 Quasispecies**



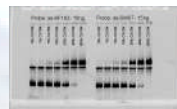
**Usage of quantitative assay for HIV viral tropism**

1. Quantitative assay  
CCR5 and CXCR4 HIV virus content can be easily quantified using this approach. It is informative to use this method to follow patients who are on antiretroviral drugs to monitor shifts in virus coreceptor usage in patients.
2. Better Discrimination  
CCR5 can hybridize to the probe and then move much faster than original probe, giving a distinct CCR5 band.
3. Avoid potential false negative

We can easily tell when the sample loses DNA during gel extraction procedure because the single stranded probe will not move.

**METHOD VALIDATION**

Standard Curve Construction for Sensi-Trop QT assay on spiked CXCR4/CCR5 DNA mixtures



Standard Curve Construction for Sensi-Trop QT Assay CXCR4 vs. CCR5 Ratios

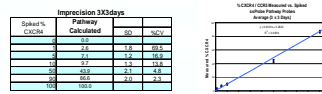


Intra-day and Inter-day imprecision of Sensi-Trop assay on spiked CXCR4/CCR5 DNA mixtures

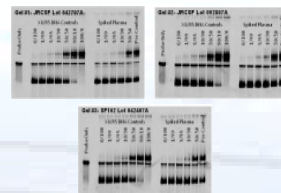
Spiked % CXCR4	Intra-day Imprecision		Inter-day Imprecision	
	CV (%)	SD	CV (%)	SD
0	0.0	0.0	0.0	0.0
1	1.4	0.2	1.9	0.3
5	1.8	0.3	2.5	0.4
10	1.9	0.3	3.1	0.5
50	2.2	0.4	3.8	0.6
100	2.5	0.5	4.5	0.7

Spiked % CXCR4	Intra-day Imprecision		Inter-day Imprecision	
	CV (%)	SD	CV (%)	SD
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100	2.5	0.5	4.5	0.7

**Summary of Imprecision Study**

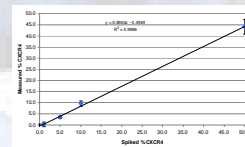


**Sensi-Trop QT assay on spiked CXCR4/CCR5 viral mixtures**

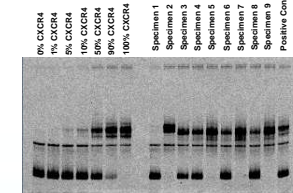


**Spiked viral mixtures, theoretical vs. measured**

Theoretical %CXCR4	JRCFSF 042707A	JRCFSF 092807A	SP162 042407A	Mean	Std Dev	%CV
0	0.0	0.0	0.0	0.0	0.0	NA
1	1.4	2.2	2.8	2.1	0.7	33.3
5	5.2	6.0	6.7	5.9	0.7	12.5
10	11.2	10.0	12.0	11.4	0.6	5.1
50	52.3	52.0	55.0	53.4	1.4	2.6
90	91.2	91.0	93.1	91.8	1.9	2.1
100	100.0	100.0	100.0	100.0	0.0	NA



**Sensi-Trop QT Assay on Patient Specimens**



ssJRCFSF Standard Curve

Sample	Spiked CXCR4 % Ratio	Measured CXCR4 % Ratio
0% X4 Control	0	0.0
1% X4 Control	1	1.3
5% X4 Control	5	5.9
10% X4 Control	10	11.6
50% X4 Control	50	49.5
90% X4 Control	90	88.1
100% X4 Control	100	100.0
Specimen 1		1.2
Specimen 2		92.5
Specimen 3		54.0
Specimen 4		46.8
Specimen 5		89.3
Specimen 6		36.9
Specimen 7		95.8
Specimen 8		34.6
Specimen 9		95.1
Positive Control		45.5

**Discussion:**

We have developed the new method, SensiTrop QT, to detect and quantify simultaneously HIV viral co-receptor tropism. In order to validate this method, we have reconstructed experiments to evaluate the detection sensitivity and quantification quality of the method. We concluded that SensiTrop QT can detect CXCR4-tropic HIV down to 1% in the CXCR4/CCR5 HIV virus mixture. In addition, SensiTrop QT method can reproducibly quantify X4/R5 ratios down to 5%. SensiTrop QT can rapidly and sensitively quantify co-receptor tropism usage in HIV patients, which will be a valuable new diagnostic tool to HIV research and drug development community.