New HIV-1 Drug Resistance Genotyping Assay of the Protease, Reverse Transcriptase and Integrase Gene Regions in Major Group-M Subtypes

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INTRODUCTION

To meet the UNAIDS 95-95-95 Fast-Track Targets by year 2030, robust drug resistance (DR) genotyping solutions for HIV-1 are urgently needed. Presented here is the development and performance of a novel HIV-1 genotyping assay to address this market need. The assay is designed to aid in detecting genomic mutations(in the protease (PR), reverse transcriptase (RT), and integrase (IN) regions of the pol gene) in HIV-1 viral RNA extracted from EDTA plasma and dried blood spots (DBS). The assay is part of a workflow that combines both targeted PCR amplification and Sanger sequencing technology to provide comprehensive drug resistance profiles in HIV-1 subtypes A, B, C, D, F, G, CRF01_AE, CRF02_AG, and CRF06_cpx.

METHODS

A combination of HIV-1 positive EDTA plasma clinical specimens and viral isolates were procured ranging across 9 different HIV-1 subtypes with varying viral loads. Clinical specimens and viral isolates were also spiked into healthy donor EDTA whole blood to create DBS. RNA was extracted from plasma and DBS samples using the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, for HIV-1 DBS (Cat# A53770) and the Thermo Scientific™ KingFisher™ Flex Purification System. The extracted RNA samples proceeded through the HIV-1 genotyping assay workflow, using the Applied Biosystems™ Veriti™ Dx 96-well Thermal Cycler and the Applied Biosystems™ 3500xL Dx Genetic Analyzer. Resulting data files were analyzed using the Exatype™ Platform by Hyrax Biosciences.

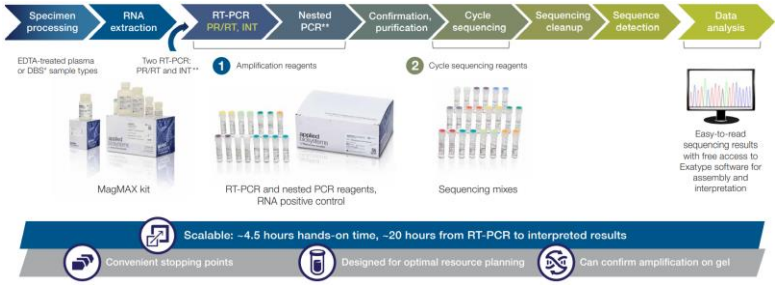

Figure 1: Complete sample testing workflow

RESULTS

RNA was extracted from 46 EDTA plasma and 46 DBS HIV-1-positive specimens. The specimens spanned 9 HIV-1 subtypes with a viral load of ≥ 1,000 cps/mL. RNA was tested with three lots of the HIV-1 Genotyping Kit.

Plasma ID#	Plasma HIV-1 Subtype	Plasma VL (cps/mL)	Plasma (PRRT)	Plasma (IN)	DBS ID#	DBS HIV-1 Subtype	DBS VL (cps/mL)	DBS (PRRT)	DBS (IN)
1	A	9,000	3/3	3/3	1	A	7,780	3/3	3/3
2	A	38,900	3/3	3/3	2	A	18,500	3/3	3/3
3	A	88,700	3/3	3/3	3	A	30,350	3/3	3/3
4	A	303,497	3/3	3/3	4	A	37,875	3/3	3/3
5	B	6,508	3/3	3/3	5	B	2,380	2/3	1/3
6	B	10,847	3/3	3/3	6	B	7,541	3/3	3/3
7	B	23,800	3/3	3/3	7	B	7,730	3/3	2/3
8	B	37,703	3/3	3/3	8	B	8,650	3/3	3/3
9	B	54,100	3/3	3/3	9	B	10,350	2/3	3/3
10	B	91,300	3/3	3/3	10	B	10,411	3/3	3/3
11	B	104,111	3/3	3/3	11	B	62,500	3/3	3/3
12	C	2,368	3/3	3/3	12	C	5,704	3/3	3/3
13	C	8,854	3/3	3/3	13	C	10,000	3/3	3/3
14	C	26,800	3/3	3/3	14	C	10,000	3/3	3/3
15	C	27,374	3/3	3/3	15	C	10,000	3/3	3/3
16	C	101,787	3/3	3/3	16	C	10,179	3/3	3/3
17	CRF01_AE	6,840	3/3	3/3	17	C	13,400	3/3	3/3
18	CRF01_AE	26,400	3/3	3/3	18	CRF01_AE	10,000	2/3	3/3
19	CRF01_AE	36,957	3/3	3/3	19	CRF01_AE	12,998	3/3	3/3
20	CRF01_AE	99,500	3/3	3/3	20	CRF01_AE	31,125	3/3	3/3
21	CRF01_AE	141,000	3/3	3/3	21	CRF01_AE	63,375	3/3	3/3
22	CRF02_AG	11,291	3/3	3/3	22	CRF02_AG	5,646	3/3	3/3
23	CRF02_AG	75,600	3/3	3/3	23	CRF02_AG	30,402	3/3	3/3
24	CRF02_AG	125,577	3/3	3/3	24	CRF02_AG	42,480	3/3	3/3
25	CRF02_AG	424,798	3/3	3/3	25	CRF02_AG	44,218	3/3	2/3
26	CRF02_AG	442,180	3/3	3/3	26	CRF02_AG	105,160	3/3	3/3
27	CRF06_cpx	1,050	3/3	3/3	27	CRF06_cpx	3,078	3/3	3/3
28	CRF06_cpx	3,078	3/3	3/3	28	CRF06_cpx	7,230	3/3	3/3
29	CRF06_cpx	16,300	3/3	3/3	29	CRF06_cpx	8,150	3/3	3/3
30	CRF06_cpx	72,300	3/3	3/3	30	CRF06_cpx	9,050	3/3	3/3
31	D	21,000	3/3	3/3	31	D	9,273	3/3	3/3
32	D	33,061	3/3	3/3	32	D	10,500	3/3	3/3
33	D	42,600	3/3	3/3	33	D	19,935	3/3	3/3
34	D	48,200	3/3	3/3	34	D	143,000	3/3	3/3
35	D	199,348	3/3	3/3	35	F	4,702	3/3	3/3
36	F1	6,260	3/3	3/3	36	F2	9,403	3/3	3/3
37	F2	4,653	3/3	3/3	37	F1	10,000	3/3	3/3
38	F2	47,016	3/3	3/3	38	F2	10,000	3/3	3/3
39	F2	526,872	3/3	3/3	39	F	45,625	3/3	3/3
40	G	20,983	3/3	3/3	40	F	52,687	3/3	3/3
41	G	29,599	3/3	3/3	41	F	107,375	3/3	3/3
42	G	33,800	3/3	3/3	42	G	2,960	3/3	3/3
43	G	58,008	3/3	3/3	43	G	10,492	3/3	3/3
44	G	60,500	3/3	3/3	44	G	12,443	3/3	3/3
45	G	124,427	3/3	3/3	45	G	40,504	3/3	3/3
46	G	405,035	3/3	3/3	46	G	220,251	3/3	3/3

Table 1: Summary of amplification results using 3 different lots of reagents

RESULTS

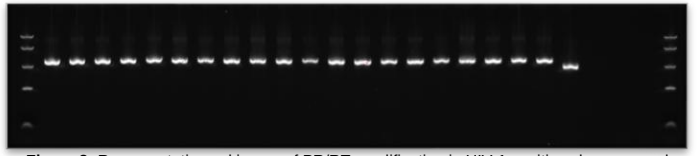

Figure 2: Representative gel image of PR/RT amplification in HIV-1 positive plasma samples.

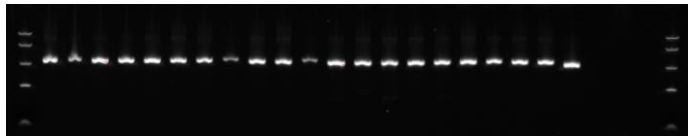

Figure 3: Representative gel image of IN amplification in HIV-1 positive plasma samples.

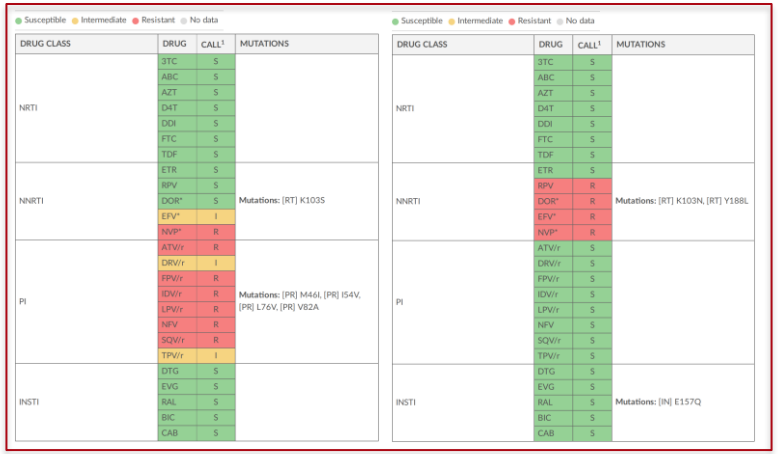

Figure 4: Representative examples of the data output from the Exatype™ Platform by Hyrax Biosciences.

CONCLUSIONS

Overall, the new HIV-1 genotyping assay presented here provides a robust Sanger sequencing-based assay alternative to existing on-market solutions for HIV-1 DR surveillance.

Analyses of reproducibility and precision indicated amean of 99.7% nucleotide alignment for the PR/RT gene region, and 99.8% nucleotide alignment for the IN gene region.