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Simultaneous and Sensitive Detection of Human Immunodeficiency Virus Type 1 (HIV) Drug Resistant Genotypes by Multiplex Oligonucleotide Ligation Assay

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Abstract

Oligonucleotide ligation assay (OLA) is a highly specific and relatively simple method to detect point mutations encoding HIV-1 drug-resistance, which can detect mutants comprising 2–5% of the viral population. Nevirapine (NVP), tenofovir (TDF) and lamivudine (3TC) are antiretroviral drugs (ARV) used worldwide for treatment of HIV infection and prevention of mother-to-child-transmission. Adapting the OLA to detect multiple mutations associated with HIV resistance to these ARV simultaneously would provide an efficient tool to monitor drug resistance in resource-limited settings. Known proportions of mutant and wild-type plasmids were used to optimize a multiplex OLA for detection of K103N, Y181C, K65R, and M184V in HIV subtypes B and C, and V106M and G190A in subtype C. Simultaneous detection of two mutations was impaired if probes annealed to overlapping regions of the viral template, but was sensitive to 2–5% when testing codons using non-overlapping probes. PCR products from HIV-subtype B and C-infected individuals were tested by multiplex-OLA and compared to results of single-codon OLA. Multiplex-OLA detected mutations at codon pairs 103/181, 106/190 and 65/184 reliably when compared to singleplex-OLA in clinical specimens. The multiplex-OLA is sensitive and specific and reduces the cost of screening for NVP, TDF and/or 3TC resistance.

Keywords

HIV; antiretroviral drug resistance; oligonucleotide ligation assay; minority genotypes

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1. Introduction

Human immunodeficiency virus type-1 (HIV) treatment guidelines in the United States and Europe

(Thompson, Aberg, Cahn, Montaner, Rizzardini, Telenti, Gatell, Gunthard, Hammer Hirsch Jacobsen Reiss Richman VolberdingYeni Schooley, 2010; World Health Organization, 2009) recommend that persons infected with HIV be tested for antiretroviral (ARV) drug-resistance prior to initiation or modification of antiretroviral therapy (ART). Access to ART has increased in low-resource communities during the past decade (Price, Wallis, Lakhi, Karita, Kamali, Anzala, Sanders, Bekker, Twesigye, Hunter, Kaleebu, Kayitenkore, Allen, Ruzagira, Mwangome, Mutua, Amornkul, Stevens, Pond, Schaefer, Papathanasopoulos, Stevens, Gilmour, 2011); however, low-cost assays to monitor ART, including viral load and resistance assays, have not kept pace. Transmission of ARV-resistant HIV appears to be increasing in these communities (Price, Wallis, Lakhi, Karita, Kamali, Anzala, Sanders, Bekker, Twesigye, Hunter, Kaleebu, Kayitenkore, Allen, Ruzagira, Mwangome, Mutua, Amornkul, Stevens, Pond, Schaefer, Papathanasopoulos, Stevens, Gilmour, 2011). If economical assays were available to guide the use of ARV offered by government-sponsored programs, ART outcomes could be improved and the spread of drug-resistant viruses limited.

HIV drug-resistance is assessed routinely by consensus sequencing of plasma HIV RNA. Consensus sequencing provides a comprehensive evaluation of the HIV genome that encodes ARV-resistance. However, sequencing is expensive, technically complex, and its limit of detection for drug-resistant mutants is between 25–50% (Schuurman, Brambillade, Groot, Huang, Land, Bremer, Benders, Boucher, 2002). Oligonucleotide ligation assay (OLA) is an alternative method for the sensitive detection of point mutations associated with HIV drug-resistance (Beck, Crowell, Kittoe, Bredell, Machaba, Willamson, Janssens, Jallow, van der Groen, Shao, Jacob, Samuelde Rivera, Ngo-Giang-Huong, Cassol, Alemnji, Frenkel, 2008; Edelstein, Nickerson, Tobe, Manns-Arcuino, Frenkel, 1998; Micek, Blanco, Beck, Dross, Matunha, Montoya, Seidel, Gantt, Matediane, Jamisse, Gloyd, Frenkel, 2010). OLA is especially well suited for use in resource-limited areas due to its low cost, utilization of relatively inexpensive laboratory equipment, and because only a limited number of drug-resistance mutations need to be assessed. To decrease the cost of the OLA further, the simultaneous detection of two different HIV drug-resistance mutations was evaluated and compared to the detection of only a single mutation.

2. Materials and methods

2.1. OLA testing of single resistance mutations (singleplex, SPX)

Single-codon OLA was performed as previously described (Edelstein, Nickerson, Tobe, Manns-Arcuino, Frenkel, 1998; Ellis, Mahalanabis, Beck, Pepper, Wright, Hamilton, Holte, Naugler, Pawluk, Li, Frenkel, 2004). Briefly, mutations at single codons were detected using 3 oligonucleotide probes: one labeled with fluorescein to detect the mutant genotype, one labeled with digoxigenin to detect the wild-type genotype, and a third labeled with biotin that captured the ligated product for subsequent detection. The three probes were added to a ligation reaction containing a PCR amplicon generated from the subjects' specimen. Following the ligation reaction, the products were bound to a streptavidin-coated microtiter plate and an ELISA performed using alkaline phosphatase (AP) and horseradish peroxidase (POD) labeled antibodies to develop color for the mutant and wild-type genotypes, respectively. The protocol was modified by developing the AP reaction with AP Yellow Liquid Substrate System (Sigma-Aldrich, P7998) instead of the ELISA Amplification System (Invitrogen, 19589–019).

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OLA probes used detected mutations associated with high-level drug-resistance in HIV *pol* encoding reverse transcriptase, including K65R and M184V selected by nucleoside reverse transcriptase inhibitors (NRTI), and K103N, V106M, Y181C, and G190A selected by non-nucleoside reverse transcriptase inhibitors (NNRTI) (Table 1). Resistance mutations were semi-quantified in subjects' specimens by comparing to a standard curve of mutant (0%, 2%, 5%, 10%, and 100%) mixed with wild-type plasmids assayed in the same 96-well microtiter plate. Control plasmids were prepared as previously described (Beck, Mahalanabis, Pepper, Wright, Hamilton, Langston, Frenkel, 2002) with detailed information available in the OLA manual (http://depts.washington.edu/idimmweb/faculty/frenkel/OLAmanual1305april04.pdf). The lower-limit of detection at each codon was defined as the concentration of the mutant control plasmid with an optical density (OD) more than twice the OD value of the control with 0% mutant. The lower limit of detection of mutations across all codons tested was at a concentration of 2% except for subtype B K65R and Y181C, which were sensitive to a concentration of 5% mutant.

Ligation of the genotype-specific to the common oligonucleotide probe requires complimentary bases in the DNA template within 2 bases of the ligation site. This requirement of the ligase enzyme confers high specificity for detection of the mutant base of interest. However, the ligation reaction fails when polymorphisms in the template are present within this 4-base region, causing a negative OLA result. Indeterminate results in SPX-OLA were defined in this study as reactions with a mutant OD less than the 2% mutant control (or 5% mutant control for subtype B K65R and Y181C) and a wild-type result less than 0.5 OD. If a specimen tested indeterminate in SPX-OLA, a consensus sequence of the HIV amplicon was generated to identify interfering polymorphisms (Ellis, Mahalanabis, Beck, Pepper, Wright, Hamilton, Holte, Naugler, Pawluk, Li, Frenkel, 2004).

2.2. OLA testing of two resistance mutations (multiplex, MPX)

Codon-specific OLA probes were paired in a single microtiter well for the MPX-OLA. The ligation reaction included the two mutant probes labeled with fluorescein or digoxigenin, the biotinylated common probes for each codon, and unlabeled wild-type probes to decrease background signal. Following the ligation reaction, the fluorescein and digoxigenin-labeled ligated products were detected using label-specific antibodies followed by sequential colorimetric reactions with AP and POD, respectively. The concentrations and ratios of the probes in each paired codon reaction (Table 1) were optimized using HIV subtype-specific standard curves, described in section 2.1. The lower limit of detection at each mutant codon was defined as the concentration of the mutant control plasmid with an optical density (OD) more than twice the OD value of the control with 0% mutant. Mutant was detected at 2% for all codons tested except for K103N and Y181C, which had a lower limit of detection of 5% mutant.

Indeterminate reactions at one (or both) codons assessed by the MPX-OLA cannot be detected because the wild-type reaction is not measured. A negative MPX-OLA indicated that a specimen either had no mutant detected at the codon tested or that the ligation reaction failed at that codon due to interfering polymorphisms.

2.3. Clinical specimens

PCR amplicons derived from persons infected with HIV were selected for evaluation based on availability of data and specimens from prior testing by SPX-OLA, under protocols approved by Seattle Children's Hospital Institutional Review Board. Amplicons for OLA were generated by nested PCR of HIV *pol* (Ellis, Mahalanabis, Beck, Pepper, Wright, Hamilton, Holte, Naugler, Pawluk, Li, Frenkel, 2004) using plasma from individuals infected with HIV subtype B, and whole blood collected on FTA™ filter paper (Whatman,

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Piscataway, NJ) or plasma from individuals with HIV subtype C infection. Subjects may or may not have taken NVP, TDF, and/or 3TC.

2.4. Comparison of clinical specimens by SPX- and MPX-OLA

Amplicons from plasmid controls and specimens were tested in duplicate by SPX- and MPX-OLA using validated HIV subtype B- and subtype C-specific probes for detection of drug-resistance mutations K65R, M184V, K103N, V106M, Y181C, and G190A. SPX-OLA results were compiled from their original dates of testing spanning from 2006 to 2009. MPX-OLA was performed from 2009 to 2010. The same subtype B amplicons were tested in the SPX- and MPX-OLA; however, some subtype C first round PCR amplicons were re-amplified for MPX- testing if the second round amplicon had been depleted by SPX-OLA testing.

2.5. Statistical comparisons

McNemar's exact tests were used to compare the number of specimens with drug resistance mutations detected by SPX- to MPX-OLA.

3. Results

Comparison of MPX- to SPX-OLA showed decreased detection of one or both mutations when codons in close proximity on the HIV genome were evaluated using overlapping OLA probes (data not shown). When non-overlapping probes were used, MPX-OLA consistently detected mutant plasmids at concentration 2–5% in codon pairs 103/181, 106/190, and 65/184 (data not shown). Therefore, these codon pairs were selected for further optimization in MPX-OLA and used to analyze amplicons from clinical specimens. The analysis of plasmid mixtures showed that the limit of detection for HIV subtype B mutants in MPX-OLA was 5% for 103/181 and 2% for 65/184. The limit of detection for HIV subtype C mutants in MPX-OLA was 5% for 103/181, 2% for 106/190, and 2% for 65/184.

A total of 215 specimens were evaluated at three to six codons for a total of 690 SPX-OLA codons tested, including 242 subtype B and 448 subtype C. The number of subjects evaluated at each codon varied by whether the subject had potentially been treated with the antiretroviral selecting resistance at the codon, and in rare cases when a specimen had been exhausted. In the SPX-OLA, indeterminate reactions occurred at a rate of 0% to 9% across codons (Table 2). Because the MPX-OLA cannot detect indeterminate reactions, these were excluded from further comparison of the two assays. Genotyping results for a total of 657 codons (228 subtype B and 429 subtype C) were compared in the SPX- and MPX-OLA.

The detection of mutant genotypes was similar by MPX- and SPX-OLA (Table 2). The MPX- and SPX-OLA of HIV subtype B specimens were rarely discordant, occurring at 2/242 (<1%) codons evaluated. MPX-OLA detected the same number of mutant genotypes as SPX-OLA at codons 103 (14/46) and 184 (14/67), one additional specimen had a mutant detected at codon 65 (1/68 vs. 0/68), and one less at codon 181 (3/47 vs. 4/47). The mutant OD of the specimen with discordant results at codon 65 was between 2% and 5% in both assays, but the cutoff for SPX-OLA was set at 5% while the MPX-OLA cutoff was 2%. The discordant result at codon 181 had an OD value just below the 2% control by MPX-OLA and between 2–5% by SPX-OLA.

Discordant results for the HIV subtype C specimens were similarly rare (7/448, 1.6%) between the MPX- and SPX-OLA (Table 2). The subtype C specimens included both whole blood collected on FTA™ filter paper and plasma, therefore detection by specimen type was examined (Table 3). Slightly more V106M, Y181C, and G190A mutants were detected by MPX- compared to SPX-OLA across the filter paper and plasma specimens. The specimens

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giving discordant results had OD values just below the limit of mutant detection in SPX-OLA, and just above the limit in MPX-OLA. Conversely, one additional 103 mutant was detected by SPX- compared to MPX-OLA for both sample types. This specimen had an OD just above the lower limit of detection of mutant in SPX-OLA, and just below the lower limit in MPX-OLA.

4. Discussion

This study demonstrates that OLA can be adapted to simultaneously detect HIV resistance genotypes at two codons conferring resistance to NVP, TDF and/or 3TC. The lower limit of detection in the MPX-OLA was similar to the SPX-OLA, ranging between concentrations of 2% to 5% mutants in the subject's viral population. In NVP-exposed women treated subsequently with NVP-based ART, the detection of 5% mutant in DNA of their peripheral blood mononuclear cells from the day that ART was initiated correlates with a greater risk of virologic failure (Jourdain, Wagner, Ngo-Giang-Huong, Sirirungsi, Klinbuayaem, Fregonese, Nantasen, Techapornroong, Halue, Nilmanat, Wittayapraparat, Chalermpolprapa, Pathipvanich, Yuthavisuthi, Frenkel, Lallemant, 2010). Furthermore, detection of 5% mutant in these women's HIV DNA appeared to capture all the risk conferred by past single-dose-NVP, as the rate of virologic failure in NVP-exposed women without mutations was similar to the rate in women who had received placebo instead of NVP. This suggests that an OLA with a sensitivity of 5% mutant detected all clinically relevant resistance.

The detection of mutations by SPX- and MPX-OLA was not significantly different. In fact, the rates varied by <2% and were attributed to slight differences in the mutant cutoff used for the SPX- and MPX-OLA for both plasma and filter paper specimens. This occurred when the 2% mutant control result was: 1) below 0.1 OD and therefore too low for interpretation, or 2) not twice the OD of the control with 0% mutant, resulting in a sensitivity cut-off of 5% mutant. The specimens collected on filter paper were re-amplified prior to MPX-OLA, which may have resulted in slight differences in the concentration of mutant genotype in the amplicon.

Both MPX- and SPX-OLA are relatively simple to perform compared to consensus sequencing. Specimens are ready for OLA once the nucleic acid has been extracted, PCR-amplified, and confirmed positive by agarose gel electrophoresis. Either OLA assay takes 6–8 hours to complete and one OLA plate can assess one or two resistance mutations for about 40 specimens, depending on the number of controls included on the plate. More than one OLA plate can be run at a time if multiple thermocyclers are available for the ligation reaction. Results are available as soon as the assay is completed because the OD of an individual's specimen is directly compared to that of the controls run on the plate. The only equipment necessary to run OLA is a 96-well thermocycler and a plate spectrophotometer, although access to a computer eases data collection, interpretation, and management.

The reagents for SPX-OLA are inexpensive (US$2/codon) and MPX-OLA queries two codons using the same reagents for only slightly greater cost (US$2.25/codon). Standard consensus sequencing of one specimen identifies about 39 codons associated with major drug-resistance for approximately US$16 in reagents. The number of codons tested determines the labor cost differential between the two assays, while the cost of equipment to perform consensus sequencing is significantly greater compared to OLA.

MPX-OLA was successful when probes for the two codons tested did not overlap on the HIV genome. Multiplexing of probes for additional resistance mutations and HIV subtypes could improve the utility and cost-effectiveness of the OLA even further as a tool to detect drug-resistance in resource-limited settings. The evaluation of three or more codons was not performed in this study because the development of the colorimetric reaction is currently

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limited to AP and POD; thus, testing of 3 or more codons would not allow us to discriminate the positive mutation. However, multiple probes for one drug, or several probes for different drugs, could be combined in a single assay for screening followed by further testing of specimens positive for mutant.

One drawback of MPX-OLA is that it does not discriminate between failed ligation reactions caused by interfering polymorphisms and those lacking the mutant genotype. Additionally, the probes often need to be adapted to regional HIV variants to limit indeterminate reactions by OLA. Previous studies have reported rates of indeterminate reactions between 5.5% and 7% (Chi, Ellis, Chintu, Cantrell, Sinkala, Aldrovandi, Warrier, Mbewe, Nakamura, Stringer, Frenkel, Stringer, 2009; Ellis, Page, Burman, Buskin, Frenkel, 2009). Importantly, when the MPX-OLA queries codons conferring resistance to the same ARV, such as the 103/181 pair, detection of a mutant at either codon provides clinically relevant drug-resistance information.

OLA is one of several sensitive methods for the detection of single nucleotide mutations that can be used to assess HIV drug-resistance. Real-time allele-specific PCR (Paredes, Marconi, Campbell, Kuritzkes, 2007), pyrosequencing (Wang, Mitsuya, Gharizadeh, Ronaghi, Shafer, 2007), radiolabeled (Troyer, Lalonde, Fraundorf, Demers, Kyeyune, Mugyenyi, Syed, Whalen, Bajunirwe, Arts, 2008) and Luminex-based OLA (Bruse, Moreau, Azaro, Zimmerman, Brzustowicz, 2008), and real-time ligation (Fogel, Hoover, Sun, Mofenson, Fowler, Taylor, Kumwenda, Taha, Eshleman, 2011) also offer highly sensitive detection of mutants, but compared to the OLA are technically more complex, expensive, and/or utilize equipment often not available in resource-limited areas.

In summary, this study demonstrated that MPX-OLA was as sensitive and specific as SPX-OLA in the detection of HIV drug-resistance in participants' samples. The utility of the MPX-OLA to screen patients in resource-limited settings for NVP, TDF, and/or 3TC resistant minority genotypes prior to initiation of ART requires further evaluation.

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Highlights

- Oligonucleotide ligation assay (OLA) detects point mutations in HIV-1.
- It is a simple and cost-effective method for use in resource-limited settings.
- Here OLA is adapted to detect simultaneously two HIV drug resistance mutations.
- Multiplexed OLA reliably detects minority genotypes when compared to singleplex-OLA.
- Multiplex-OLA could be used as a screening tool for detection of HIV drug resistance.

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Table 1

Oligonucleotide probes used in OLA to detect mutations in HIV *pol*

Subtype B Amplicons

SPX OLA			MPX OLA					
Codon	**Genotype**	**Oligonucleotide Sequence**	**Codon**	**Genotype**	**Oligonucleotide Sequence**	**Concentration per reaction [uM]**	**Ratio of Probe per codon set**	**Probe Mixture**
	wt[a]	dig[b]-CTCCAGTATTTGCCATAAAGAA		wt	CTCCAGTATTTGCCATAAARAA		1	
K65R	mutant	f[c]-CTCCAGTATTTGCCATAAAGAG	K65R	mutant	dig-CTCCAGTATTTGCCATAAAGAG	0.13	4	
	common	p[d]-RAAAGACRGTACTAAATGGAGAA-bio		common	p-RAAAGACRGTACTAAATGGAGAA-bio		2	
	wt	dig-AGACATAGTTATCTATCAATACA		wt	AGACATAGTTATCTATCAATACA		1	Sub B/C
M184V	mutant	f-AGACATAGTTATCTATCAATAYG	M184V	mutant	f-AGACATAGTTATCTATCAATAYG	0.03	4	Sub B/C
	common	p-TGGATGATTTGTATGTAGGATC-bio		common	p-TGGATGATTTGTATGTAGGATC-bio		4	
	wt	dig-ACATCCCGCAGGGTTAAAAAAGAAR		wt	ACATCCCGCAGGGTTAAAAAAGAAR		1	
K103N	mutant	f-ACATCCCGCAGGGTTAAAAAAGAAC	K103N	mutant	dig-ACATCCCGCAGGGTTAAAAAAGAAC	0.03	4	
	common	p-AAATCAGTAACAGTACTGGATGTGGGT-bio		common	p-AAATCAGTAACAGTACTGGATGTGGGT-bio		4	
	wt	dig-ACAAAATCCAGACATAGTTATCTA		wt	ACAAAATCCAGACATAGTTATCTA		1	
Y181C	mutant	f-ACAAAATCCAGACATAGTTATCTG	Y181C	mutant	f-ACAAAATCCAGACATAGTTATCTG	0.16	8	
	common	p-TCAATACATGGATGATTTGTATGTA-bio		common	p-TCAATACATGGATGATTTGTATGTA-bio		8	Sub B/C

Subtype C Amplicons

SPX OLA			MPX OLA					
Codon	**Genotype**	**Oligonucleotide Sequence**	**Codon**	**Genotype**	**Oligonucleotide Sequence**	**Concentration per reaction [uM]**	**Ratio of Probe per codon set**	**Probe Mixture**
	wt	dig-CTCCAGTATTTGCCATAAARAA		wt	CTCCAGTATTTGCCATAAARAA		1	
K65R	mutant	f-CTCCAGTATTTGCCATAAARAG	K65R	mutant	dig-CTCCAGTATTTGCCATAAARAG	0.06	4	
	common	p-RAARGACAGTACTAAATGGAGAA-bio		common	p-RAARGACAGTACTAAATGGAGAA-bio		4	Sub B/C

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Subtype C Amplicons

SPX OLA			MPX OLA					
Codon	**Genotype**	**Oligonucleotide Sequence**	**Codon**	**Genotype**	**Oligonucleotide Sequence**	**Concentration per reaction [uM]**	**Ratio of Probe per codon set**	**Probe Mixture**
	wt	dig-AGACATAGTTATCTATCAATATA		wt	AGACATAGTTATCTATCAATATA		1	
M184V	mutant	f- AGACATAGTTATCTATCAATATG	M184V	mutant	f-AGACATAGTTATCTATCAATAYG	0.06	8	Sub B/C
	common	p-TGGATGATTTGTATGTAGGATC-bio		common	p-TGGATGATTTGTATGTAGGATC-bio		8	
	wt	dig-ACATCCCGCAGGGTTAAAAAAGAAR		wt	ACATCCCGCAGGGTTAAAAAAGAAR		1	
	mutant	f-ACATCCCGCAGGGTTAAAAAAGAAC		mutant	f-ACATCCCGCAGGGTTAAAAAAGAAC		4	
K103N	common	p-AAATCAGRAACAGTACTGGATGTGGGGGAT-bio	K103N	common	p-AAATCAGTRACAGTACTGGATGTGGG-bio	0.06	4	
	common[f]	p-AAATCAGRAACAGTACTGGATGTGGG-bio						
	wt	dig-ACAAAATCCAGAAATAGTCATCTA		wt	ACAAAATCCAGAAATAGTCATCTA		1	
Y181C	mutant	f-AMAAAATCCAGAAATAGTCATCTG	Y181C	mutant	dig-AMAAAATCCAGAAATAGTCATCTG	0.20	4	
	common	p-TCAATACATGGATGATTTGTATGTA-bio		common	p-TCAATATATGGATGACTTGTATGTA-bio		8	Sub B/C
	wt	dig-CCAGCAGGGTTAAAAAAGAAAAAATCAG		wt	CCAGCAGGGTTAAAAAAGAAAAAATCAG		1	
V106M	mutant	f-CCAGCAGGGTTAAAAAAGAAAAAATCAA	V106M	mutant	f-CCAGCAGGGTTAAAAAAGAAAAAATCAA	0.06	4	
	common	p-TRACAGTACTGGATGTGGGGGATGCATAT-bio		common	p-TRACAGTACTGGATGTGGGGGATGCATAT-bio		4	
	wt	dig-CATGGATGACTTGTATGTAGG		wt	CATGGATGACTTGTATGTAGG		1	
G190A	mutant	f-CATGGATGACTTGTATGTAGC	G190A	mutant	dig-CATGGATGACTTGTATGTAGC	0.12	8	
	common	p-ATCTGAYTTAGAAATAGGGCAG-bio		common	p- ATCTGAYTTAGAAATAGGGCAG-bio		8	

[a] wt, wild-type;

[b] dig, digoxigenin;

[c] f, fluorescein;

[d] p, phosphate;

[e] bio, biotin;

[f] alternative probe

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Table 2

Detection of Mutant HIV Genotypes by Singleplex- versus Multiplex-OLA

	HIV-1 Subtype B Codons				HIV-1 Subtype C Codons			
	103 N (%)	**181 N (%)**	**65 N (%)**	**184 N (%)**	**103 N (%)**	**181 N (%)**	**106 N (%)**	**190 N (%)**
Total Specimens Tested	49	49	72	72	92	92	90	90
Indeterminate in SPX OLA	3 (6)	2 (4)	4 (6)	5 (7)	5 (5)	8 (9)	0 (0)	5 (6)
Total Compared in MPX vs. SPX	46	47	68	67	87	84	90	85
Mutants detected by SPX	14 (30)	4 (9)	0 (0)	14 (21)	16 (18)	22 (26)	8 (9)	12 (14)
Mutants detected by MPX	14 (30)	3 (6)	1 (1)	14 (21)	14 (16)	23 (27)	11 (12)	13 (15)
Difference in detection (p)[a]	1.00	1.00	1.00	1.00	0.50	1.00	0.25	1.00
Sensitivity of MPX	1.00	0.75	N/A[b]	1.00	0.88	0.98	1.00	1.00
Specificity of MPX	1.00	1.00	0.99	1.00	1.00	1.00	0.96	0.99

[a]P-values calculated by McNemar's exact test;

[b]Not applicable as no mutants detected by SPX-OLA.

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Table 3

Detection of Mutant HIV Genotype by Specimen Type

	HIV-1 Subtype C			
	Codon 103	**Codon 181**	**Codon 106**	**Codon 190**
SPX Mutants (N=)	16	22	8	12
Whole Blood on FTA[a]	5	19	7	5
Plasma[b]	11	3	1	7
MPX Mutants (N=)	14	23	11	13
Whole Blood on FTA[a]	4	19	8	6
Plasma[b]	10	4	3	7

[a]exposed to NVP; potentially exposed to AZT and 3TC;

[b]exposed to NVP; potentially exposed to AZT, TDF, FTC