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Specific, Sensitive, and Rapid Assay for Human Immunodeficiency Virus Type 1 *pol* Mutations Associated with Resistance to Zidovudine and Didanosine

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The effectiveness of antiretroviral therapy may be limited by the development of human immunodeficiency virus type 1 (HIV-1) resistance. Monitoring for resistance will perhaps allow changes in therapy prior to deterioration in the patient's clinical or immunologic status. Our objective was to develop a rapid, specific, and sensitive genotypic assay for HIV-1 resistance to zidovudine (ZDV) and didanosine (ddI) which is simple to perform. In our assay the DNA of HIV-1 *pol* was amplified by PCR using two sets of nested oligonucleotide primers. Mutations of reverse transcriptase (RT) encoding amino acids (aa) 74 and 41, 70, and 215 which have been associated with HIV-1 resistance to ddI and ZDV, respectively, were detected with a ligase detection reaction (LDR) and indicated colorimetrically. The RT genotypes of 35 patient specimens (140 codons) blindly assessed for these mutations were in agreement by PCR-LDR and by dideoxynucleotide sequencing. To evaluate the limits of the assay, other specimens with mutations close to the ligation site were evaluated by PCR-LDR. The assay was sensitive and specific for all specimens except when mutations occurred within 2 bases on either side of the ligation site. In summary, this PCR-LDR assay specifically, sensitively, and rapidly detected *pol* mutations (RT aa 74, 41, 70, and 215) associated with HIV-1 resistance to ddI and ZDV.

Antiretroviral agents appear to favorably impact the course of human immunodeficiency virus type 1 (HIV-1) disease progression (3, 4, 27); however, their effects appear to be of limited duration (2, 9). While patients receive antiretroviral therapy, HIV-1 clinical disease progression has been found to correlate with the development of resistance to the antiretroviral agent (11, 14, 21, 26). Specific mutations in the reverse transcriptase (RT) portion of HIV-1 *pol* which confer resistance in vitro to nucleoside and nonnucleoside antiretroviral agents have been identified (13, 17, 22, 25). Monitoring for resistance perhaps will allow physicians to make changes in therapeutic agents prior to significant deterioration in the patient's clinical or immunologic status. In the case of HIV-1-infected pregnant women, treatment with an antiretroviral agent to which the virus remains susceptible may be important in preventing vertical transmission of HIV-1 (1). We have developed a rapid, specific, and sensitive assay for mutations in HIV-1 associated with resistance to zidovudine (ZDV) and didanosine (ddI). Our assay detects mutations in the DNA encoding RT amino acids (aa) 41, 70, and 215 associated with resistance to ZDV and aa 74 associated with resistance to ddI.

Several assays have been used to evaluate HIV-1 resistance to antiretroviral agents by both phenotypic (10) and genotypic (7, 8, 12, 16, 23) techniques. Phenotypic assays evaluate the ability of HIV-1 to replicate in various concentrations of the antiretroviral agent, and genotypic assays detect mutations of *pol* associated with phenotypic resistance. The phenotypic assays are labor-intensive, taking several weeks to perform, and thus are expensive. Another disadvantage of phenotypic assays is that viral selection may occur during repeated culturing of the HIV-1 isolate being evaluated (24). In contrast, genotypic assays can be used directly on patient specimens and are rapid and relatively inexpensive. Nonetheless, important disadvantages of genotypic assays include the inability to detect and/or interpret mutations not previously correlated with phenotypic assays, utilization of radiolabled reagents, and limitations in specificity and sensitivity related to the molecular techniques utilized. Our assay uses a ligase reaction to specifically identify the DNA base of interest as wild type (WT) or mutant and a colorimetric streptavidin-horseradish peroxidase (SA-HRP)-based detection system.

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MATERIALS AND METHODS

Patient specimens. Specimens collected (after informed consent in accordance with guidelines established by the Human Subject's Institutional Review Board of the University of Rochester) from 35 HIV-1-infected persons were chosen for analysis on the basis of varied antiretroviral treatment regimens. Uncultured patient peripheral blood mononuclear cells (PBMCs) and PBMCs from first-passage HIV-1 cultures were analyzed for mutations associated with HIV-1 resistance to ZDV and ddI by our assay employing PCR followed by a ligase detection reaction (LDR), and these results were compared, in a blinded fashion, with those obtained by dideoxynucleotide sequencing of the same PCR product. At a later date, all sequencing data generated in our laboratory (284 sequences from 54 subjects) were reviewed and specimens noted to have mutations within 5 bases of the ligation site were assayed by PCR-LDR to define the limitations of the assay.

PCR. DNA from patient or cultured PBMCs was obtained with a lysis buffer (28). Nested sets of oligonucleotide primers (outer pair, 5′-GTTGACTCAGATTGGTTGCAC-3′ and 5′-GTATGTCATTGACAGTCCAGC-3′; inner pair, 5′-TATCAGGATGGAGTTCATAAC-3′ and 5′-GGATGGCCCAAAAGTTAAAC-3′) were used to amplify a 665-bp region of *pol* encoding aa 17 to 237. The reaction mixture (100 μl) contained 10 μl of the cell pellet lysate (equivalent to 150,000 patient PBMCs) or 5 μl of first-round PCR product, 10 μl of 10× PCR buffer (Promega, Madison, Wis.), 1 μl (2 to 5 U) of *Taq* (Promega), 1 μl of 100× (100 mM) deoxynucleoside triphosphates (Promega), 68 μl or (for nested PCR) 73 μl of sterile H_2O, and 5 μl of each primer (20 pM/μl). Thirty cycles of amplification consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min were used for both primer pairs. CEM-SS (5) and 8E5 (6) cells served, respectively, as HIV-1-negative and WT positive controls, and HXBRT L74V (gift of J. Louise Martin and Philip A. Furman, Burroughs Wellcome Co.) was used as

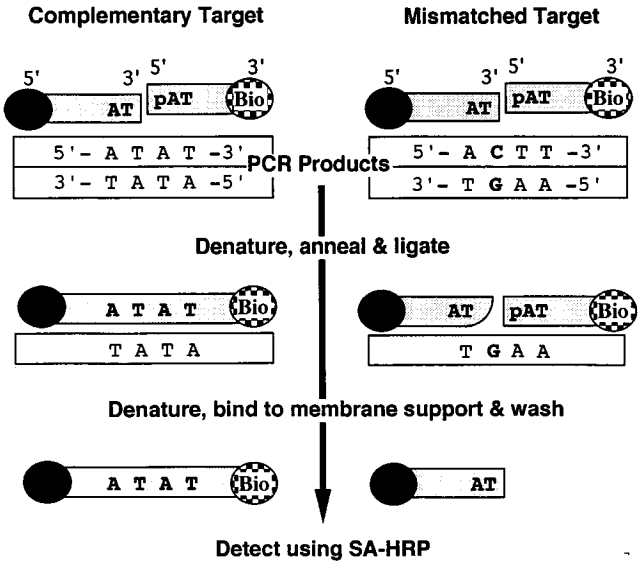

FIG. 1. LDR occurs by annealing and subsequent ligation of two oligonucleotides. The probe oligonucleotide is attached to a 1-μm latex bead (●) on the 5′ end, and its 3′ end is complementary to either the WT or mutant genome. When this probe is complementary to the template (compare left to right schema at top), a second oligonucleotide, the detector, can be attached by ligation. The detector is biotinylated (Bio). Ligation of the probe and detector occurs only when the 2 bases on either side of the ligation site are complementary to the template. The probe and detector oligonucleotides are denatured from the template by heating. The denatured mixture is passed through a nylon membrane which traps the latex bead. When ligation has occurred, the biotin moiety is trapped in the membrane. The biotin is detected by a reaction with SA-HRP, followed by colorimetric detection.

a known ddI-resistant mutant control. Amplified *pol* DNA from each specimen was used for both dideoxynucleotide sequencing and PCR-LDR to identify mutations associated with ZDV and ddI resistance.

To avoid contamination of specimens with PCR-generated product, the patients' specimens prior to PCR were processed in a tissue culture hood situated within a biohazard level 3 laboratory. The analysis of specimens after PCR was done in a separate laboratory. Precautions were taken to limit the contamination of the post-PCR laboratory and personnel.

Dideoxynucleotide sequencing. The PCR product was purified with the Magic PCR Preps DNA Purification System (Promega) and sequenced directly via the *fmol* DNA Sequencing System (Promega) under conditions recommended by the supplier. The reaction products were denatured by heating to 70°C for 2 min before loading onto a 5.7% polyacrylamide–urea gel (Sequagel; National Diagnostics, Atlanta, Ga.) for separation by electrophoresis. The gel was dried and exposed for 18 to 24 h to X-ray film (X-Omat; Eastman Kodak, Rochester, N.Y.). Sequences were read by the investigators, and when distinct bands for two different nucleotides were seen for a single-base locus, this was interpreted as a mixed genotype.

PCR-LDR (Fig. 1). Mutations associated with ZDV or ddI resistance were detected by DNA probing of the PCR-amplified HIV-1 *pol* DNA. For each amino acid codon of interest, a set of 3 or 4 oligonucleotides was designed and purified by high-pressure liquid chromatography prior to use (Table 1). Each set contained one oligonucleotide probe for the WT genome and one or two probes for the mutant or mutants of interest. These probes had a latex bead on the 5′ end, approximately 20 bases from the base of interest, which was on the 3′ end. The 3′ bases of the probes were complementary to either the WT or mutant base. Each oligonucleotide set also contained a detector. The 5′ end of the detector oligonucleotide was phosphorylated and was designed to anneal to the bases of HIV-1 *pol* gene immediately adjacent to the 3′ end of those bound by the probe oligonucleotide. The detector had a biotin on the 3′ end. The probe and detector oligonucleotides annealed to the PCR product and were ligated if the two 3′-most bases of the probe and the two 5′-most bases of the detector were complementary to the PCR product. The ligated product was detected colorimetrically with SA-HRP.

The ligation reaction of the PCR-LDR assay was carried out in a 100 μl screw-top PCR tube (Sarstedt Inc., Newton, N.C.). Five microliters of the PCR product was combined with 2.5 μl of 4% latex beads with the probe and 10 μl of 10 μM detector oligonucleotides in sterile water, 2.5 μl (12.5 U) of DNA ligase (Ampligase; Epicentre Technologies, Madison, Wis.), 10 μl of 10× ligation buffer (Ampligase; Epicentre Technologies), and 70 μl of sterile water to total 100 μl without employing an overlay of mineral oil or wax. The reaction mixture was then transferred to a thermocycler at 94°C for 2 min to denature the DNA and then 55°C for 5 min to allow annealing of the oligonucleotides to the PCR product and ligation of the two oligonucleotides when complementary to the PCR product at the ligation junction. Separate reactions were conducted for the detection of mutant and WT genomes.

A colorimetric assay developed by Eastman Kodak Co. was modified to detect the ligated oligonucleotides for the mutant and WT genomes. This technology is simple to perform, is suitable for small numbers of samples, and does not require sophisticated laboratory equipment or radioisotopes. To perform the detection assay, the reaction mixtures were heated to 95°C in a heating block for 5 min to ensure denaturation of the DNA and were then passed through a membrane which trapped the oligonucleotides by the latex beads. The membrane was washed with 80°C buffer, incubated for 2 min with SA-HRP, and then washed with 80°C buffer. The ligated oligonucleotides were then colorimetrically detected following the conversion of dye precursor to dye by a reaction with hydrogen peroxide and an electron transfer agent. The reaction product was visually scored 0 to 10 by comparison with a color chart (Fig. 2).

RESULTS

The PCR-LDR was able to evaluate the genotypes of codons 74, 41, 70, and 215 of all 35 patient and culture PBMCs, the 8E5 WT control specimen, and the CEM-SS negative controls, (selected data shown in Tables 2 to 6). When assessed with the mutant probe, a specimen which scored up to 2 above the WT control was classified as WT and those which scored 3 or more than the negative control were classified as mutant. Low levels of background color occurred occasionally in the colorimetric

TABLE 1. Probe and dectector oligonucleotides used to determine the genotype at four HIV-1 RT codons

aa	Probe or detector	Type	Sequence[a]
74	Probe	WT	5′-AC-AGT-ACT-AAA-TGG-AGA-AAA-**T**-3′
		Mutant	5′-AC-AGT-ACT-AAA-TGG-AGA-AAA-**G**-3′
	Detector		5′-TA-GTA-GAT-TTC-AGA-GAA-CTT-3′
41	Probe	WT	5′-GTA-GAA-ATT-TGT-ACA-GAA-**A**-3′
		Mutant	5′-GTA-GAA-ATT-TGT-ACA-GAA-**T**-3′
		Mutant	5′-GTA-GAA-ATT-TGT-ACA-GAA-**C**-3′
	Detector		5′-TG-GAA-AAG-GAA-GGG-AAA-A-3′
70	Probe	WT	5′-A-AAG-AAA-AAA-GAC-AGT-ACT-A**A**-3′
		Mutant	5′-A-AAG-AAA-AAA-GAC-AGT-ACT-A**G**- -3′
	Detector		5′-A-TGG-AGA-AAA-TTA-GTA-GAT-TT-3′
215	Probe	WT	5′-CTG-TTG-AGG-TGG-GGA-TTT-**AC**-3′
		Mutant	5′-CTG-TTG-AGG-TGG-GGA-TTT-**TT**-3′
		Mutant	5′-CTG-TTG-AGG-TGG-GGA-TTT-**TA**-3′
	Detector		5′-C-ACA-CCA-GAC-AAA-AAA-CA-3′[b]

[a] Boldface type indicates the mutated nucleotide(s).
[b] Codon 219 is WT.

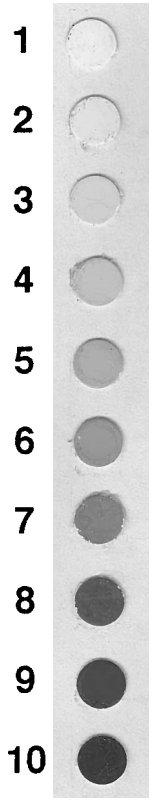

FIG. 2. Color chart used to read the PCR-LDR assay.

detection, usually when the SA-HRP used was greater than 3 months old.

With these parameters there was agreement of the genotype by PCR-LDR and sequencing for all codons assayed under blinded conditions (n = 140) (selected samples shown in Tables 2 to 5). A number of these specimens demonstrated genetic variability from the North American consensus sequence for HIV-1. In several cases, mutations were detected in *pol* regions corresponding to the LDR oligonucleotides. The distances of point mutations from the ligation site were as short as 11 bases for codon 41, 3 bases for codon 70, and 5 bases for codons 215 and 74. Several specimens also had multiple mutations within the span of either the probe or detector oligonucleotides. For example, three patients, a mother and her two children, had three mutations within close proximity to codon 70 (3, 10, and 11 bases towards the 5′ end from the aa 70 ligation site); nevertheless, PCR-LDR assay evaluated all three accurately.

From all patient specimens sequenced in our laboratory (n = 71 from 54 patients), in 10 of the 284 codons analyzed a mutation occurred at or within 5 bases of the ligation site (Table 6). None of these 10 appeared to have mixed genotypes by dideoxynucleotide sequencing. The PCR-LDR assay was able to evaluate the codon of interest if a mutation was 3 or more bases from the ligation site. In 2 of these 10 instances (0.7% of 284 sites evaluated), the PCR-LDR assay gave incorrect or no results. These included one specimen with a muta-

TABLE 2. HIV sequence encoding RT aa 74 as determined by sequencing and PCR-LDR

Specimen[a]	ZDV/ddI treatment[b]	Dideoxy sequence	PCR-LDR score[c]		PCR-LDR interpretation
			TTA	**G**TA[d]	
CEM-SS	NA		0	0	Negative
8E5	NA	TTA	6	0	WT
HXBRT-L74V	NA	**G**TA	1	7	Mutant
1L	+/−	TTA	8	0	WT
2L	+/−	TTA	6	0	WT
3C	−/−	TTA	8	0	WT
4C	+/−	TTA	6	0	WT
5C	+/−	TTA	6	0	WT
6C	−/−	TTA	7	1	WT
7L	+/−	TTA	7	0	WT
8L	−/+	**G**TA and TTA	5	7	Mixture
9L	−/+	**G**TA	2	5	Mutant
10L	−/+	**G**TA	2	6	Mutant
11C	+/+	**G**TA	2	5	Mutant

[a] L, patient PBMCs; C, cultured pool of patient and donor PBMCs.
[b] +, treatment; −, no treatment; NA, not applicable.
[c] The cutoff for negative is ≤2, and the cutoff for positive is >2.
[d] Boldface type indicates mutations associated with HIV-1 resistance.

TABLE 3. HIV sequence encoding RT aa 41 as determined by sequencing and PCR-LDR[a]

Specimen	ZDV/ddI treatment	DNA sequence	PCR-LDR score			PCR-LDR interpretation
			ATG	**T**TG	**C**TG	
CEM-SS	NA		0	0	0	Negative
8E5	NA	ATG	7	0	0	WT
1L	+/−	ATG	7	0	0	WT
2L	+/−	ATG	6	0	0	WT
3C	−/−	ATG	5	0	0	WT
4L	+/−	ATG	8	0	0	WT
5L	+/−	ATG	6	1	0	WT
6C	−/+	ATG	7	1	0	WT
7L	+/−	ATG	7	1	0	WT
8L	+/−	**C**TG and ATG	4	1	4	Mixture
9L	+/−	**C**TG	0	0	7	Mutant
10P[b]	+/−	**C**TG	0	0	4	Mutant
11L	+/+	**T**TG	0	8	0	Mutant
12P	+/−	**T**TG	0	4	0	Mutant
13L	+/−	**T**TG	0	7	0	Mutant
14C	+/−	**C**TG and **T**TG	0	3	5	Mutant

[a] For definitions, see Table 2, footnotes *a*, *b*, *c*, and *d*.
[b] P, plasma.

TABLE 4. HIV sequence encoding RT aa 70 as determined by sequencing and PCR-LDR[a]

Specimen	ZDV/ddI treatment	DNA sequence	PCR-LDR score[b]		PCR-LDR interpretation
			AAA	A**G**A	
CEM-SS	NA		1	1	Negative
8E5	NA	AAA	4	1	WT
1L	+/−	AAA	5	1	WT
2L	+/−	AAA	6	1	WT
3L	−/−	AAA	6	1	WT
4L	−/+	AAA	4	1	WT
5C	−/−	AAA	5	1	WT
6L	+/−	A**G**A and AAA	4	4	Mixture
7L	+/−	A**G**A and AAA	4	4	Mixture
8C	+/−	A**G**A	1	6	Mutant
9C	+/−	A**G**A	1	7	Mutant
10L	+/−	A**G**A	1	5	Mutant

[a] For definitions, see Table 2, footnotes *a*, *b*, and *d*.
[b] The cutoff for negative is ≤3, and the cutoff for positive is >3.

TABLE 5. HIV sequence encoding RT aa 215 as determined by sequencing and PCR-LDR[a]

Specimen	ZDV/ddI treatment	DNA sequence	PCR-LDR score			PCR-LDR interpretation
			ACC	**TTC**	**TAC**	
CEM-SS	NA		0	0	0	Negative
8E5	NA	ACC	7	0	0	WT
1C	−/−	ACC	5	0	0	WT
2C	−/−	ACC	5	0	0	WT
3L	−/−	ACC	7	0	0	WT
4C	+/−	ACC	7	0	0	WT
5C	+/−	ACC	7	0	0	WT
6L	+/−	**TAC** and ACC	8	0	8	Mixture
7L	+/−	**TAC** and ACC	8	1	8	Mixture
8C	+/−	**TAC**	0	0	6	Mutant
9C	+/−	**TAC**	0	0	5	Mutant
10C	+/+	**TAC**	0	0	6	Mutant
11L	+/−	**TAC**	1	1	5	Mutant
12C	+/−	**TAC**	0	0	6	Mutant
13L	+/−	**TAC**	1	1	4	Mutant
14L	+/−	**TAC**	2	1	6	Mutant
15L	+/−	**TAC**	2	1	6	Mutant

[a] For definitions, see Table 2, footnotes *a*, *b*, *c*, and *d*.

tion 2 bases from the ligation site of the probe, as well as a second mutation 4 bases away in the detector, for codon 215 (Table 6, aa 215, specimen 2). This specimen on sequencing had faint bands suggestive of WT and scored WT by the PCR-LDR. This is the only specimen for which results differed by the two assays. The other specimen, which could not be evaluated by PCR-LDR, was the only specimen with a mutation of the most 5′-base of the detector, at the ligation site for codon 70 (Table 6, aa 70, specimen 5). Since ligation of this base on the 5′ detector was necessary for product formation, the PCR-LDR for this specimen scored negative for both WT and the mutant genomes.

When known quantities of cloned codon 74 mutant DNA (clone HXBRT L74V) were mixed into a background of cells containing a single copy of HIV-1 (8E5 cells) (6), the color score increased above baseline when 5,000 to 10,000 copies of mutant DNA were mixed with 100,000 copies of WT DNA. Assessment of 14 coded mixtures of diminishing quantities of a cloned aa 215 mutant mixed into WT HIV-1, as part of an interlaboratory comparison of the limitations of detection by the ACTG 215 SWAT team, showed that PCR-LDR could reliably detect mutant virus when present as ≥10% (n = 7 of 7 [data not shown]). LDR-PCR assessed samples containing 99 to 100% WT HIV as WT (n = 7 of 7 [data not shown]). Thus, the threshold for detecting mutations associated with HIV-1 resistance by the PCR-LDR occurred when between 2 and 9% of the HIV-1 genome was mutant. In addition, cDNA generated from the plasma of 13 pregnant women treated with ZDV were assayed by the PCR-LDR. There were no differences in ZDV-associated mutations between each woman's plasma and PBMCs obtained on the same date, (data not shown).

DISCUSSION

The use of genotypic assays rather than phenotypic assays for the detection of HIV-1 resistance to antiviral agents is favored because of their rapidity, ease of use, and reduced cost. Several methods of assessing genotypic resistance which use PCR to amplify a portion of *pol* in conjunction with diverse methods to detect the WT or mutant genome have been described (7, 8, 12, 16, 23). The methods for detection include Southern blotting with radiolabeled oligonucleotide probes (23), self-sustained sequence replication and Southern blotting with a bead-based sandwich detection system (8), primer-specific PCR for the WT or mutant base (16), RNase A mismatch cleavage (7), and an assay in which an oligonucleotide which spans a portion of *pol* up to the base of interest was annealed to the PCR-amplified patient specimen, after which a single radiolabeled nucleotide was added to the oligonucleotide if it complemented the specimen's genome (12). Similarly, our PCR-LDR assay first uses PCR to amplify the region of interest. Our detection method was inspired by that used by U. Landegren and colleagues to detect the single-base change encoding the hemoglobin gene causing sickle cell disease (15). In their assay T4 DNA ligase was found to ligate oligonucleotides only when the two terminal bases, at and adjacent to the ligation site on both oligonucleotides, were complementary to the template DNA (15).

TABLE 6. DNA sequences and PCR-LDR scores of all HIV-1 specimens with mutations within 5 bases of the ligation site[a] of the PCR-LDR assay

aa	Specimen	Codon					PCR-LDR score			PCR-LDR interpretation
41		39	40	41	42	43	ATG	**T**TG	**C**TG	
	1	ACA	GAA	AT-G	GA**G**	AAG	5	0	0	WT
70		68	69	70	71	72	AAA	A**G**A		
	2	AGT	A**A**T	A**G**-A	TGG	AGA	1	5		Mutant
	3	AGT	A**A**T	A**G**-A	TGG	AGA	0	5		Mutant
	4	AGT	AC**A**	AA-A	TGG	AGA	4	1		WT
	5	AGT	AC**C**	AA-**G**	TGG	AGA	1	1		Negative[b]
	6	AGT	ACT	AA-A	TC**G**	GGA	5	1		WT
215		213	214	215	216	217	ACC	**TT**C	**TA**C	
	2	GGA	TTT	**GT**-C	AC**C**	CCA	4	0	0	WT[c]
	3	GGA	**C**TT	AC-C	ACA	CCA	6	0	0	WT
	5	GGA	TTT	AC-C	AC**G**	CCA	8	0	0	WT
74		72	73	74	75	76	TTA	**G**TA		
	7	AGA	AAA	T-TA	GT**C**	GAT	4	1		WT
	8	AG**G**	AAA	T-TA	GTA	GAT	5	1		WT

[a] Boldface indicates mutations; hyphens indicate ligation sites.
[b] Specimen did not score appropriately as WT because of a mutation at the 5′ end of the detector.
[c] Specimen did not score appropriately as mutant because of a mutation adjacent to the 3′ end of the probe. There were faint bands for WT on the dideoxy sequencing gel; however, it was not read as a mixture because these bands were very faint in comparison to the mutant genotype.

The main strength of the PCR-LDR is that its specificity is derived from the requirements of ligase and not the conditions for DNA annealing. Detection of single-base mutations based on the properties of DNA annealing generally require fine-tuning of the reaction conditions to achieve a high degree of specificity. The stringent conditions used by Richman et al. (23) and Gingeras et al. (8) detect the single-base mutations of interest also limited annealing of specimens containing additional mutations. Thus, mutations within the span of the oligonucleotides used in their assays, which occur frequently, may compromise their detection system.

Primer-specific PCR has become a popular method for the evaluation HIV-1 *pol* mutations associated with resistance. The properties of PCR priming are based on DNA annealing. Mispriming of PCR primer oligonucleotides, that is, priming in spite of base pair mismatches, may occur. This theoretical problem of the assay described by Larder et al. (16) has led some groups using this assay to repeat the differential primer amplification on diluted PCR product when both WT and mutant genomes appear to be present (11).

The conditions used in our PCR-LDR assay were not stringent in respect to DNA annealing. All specimens tested with mutations more than 2 bases from the ligation site performed well in the PCR-LDR assay. Variability in the genome complementary to the oligonucleotides used in the assay, even multiple mutations, did not appear to diminish the specificity or sensitivity of the assay. The use of nonstringent conditions was especially important for evaluating codons 70 and 215, as a high degree of genetic diversity occurred surrounding these codons among our patients treated with ZDV.

On the basis of the data of others, when a mutation occurs within 2 bases on either side of the ligation site, ligation of the oligonucleotides does not occur (15). In this case, the PCR-LDR assay should score negative for both mutant and WT viruses. Among HIV-1 *pol* DNA regions sequenced, genetic variability at or adjacent to the ligation site was exhibited in 2 of 284 (0.7%) of codons examined. One of these two specimens, with a mutation at the 5′ base of the detector, scored negative for both the WT and mutant genomes. In cases such as this, the negative PCR-LDR results indicate that ancillary tests such as phenotypic sensitivity testing or sequencing of *pol* are necessary to further evaluate the specimen. The second specimen, which also appeared to interfere with ligation, had a mutation 2 bases from the ligation site. However, in this case the genotype was misrepresented by the LDR-PCR as WT, most likely because of multiple genotypes, including WT, within the specimen. The use of a synthetic universal nucleoside at the sites adjacent to but not at the ligation site may further enhance the utility of this PCR-LDR assay (20).

In summary, the strengths of the PCR-LDR assay include its highly specific determination of WT and/or mutant sequences at codons for RT aa 41, 70, 215, and 74 associated with HIV-1 resistance to ZDV and ddI and its sensitivity for detecting these mutant genomes even when present as a minority population and/or in conjunction with other mutations in close proximity. The assay is also simple and rapid, and it does not use radioisotopes. PCR-LDR will not, however, identify novel mutations associated with phenotypic resistance. This property is shared with other genotypic assays. We believe that this PCR-LDR assay may prove particularly useful when used prior to the initiation of ZDV or ddI therapy, to establish whether the WT genome is present, and then periodically during monotherapy with ZDV or ddI to detect expected mutations associated with HIV-1 resistance.

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