Chemokine Receptor CCR5 Δ32 Genetic Analysis Using Multiple Specimen Types and the NucliSens Basic Kit†

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Resistance to HIV-1 infection and delayed disease progression have been associated with a 32-bp deletion (Δ32) in the gene encoding the CCR5 chemokine receptor. In the present study we describe the modification of a nucleic acid sequence-based amplification (NASBA)-based CCR5 genotyping assay for a NucliSens Basic Kit (Organon Teknika, Durham, N.C.) format using a new target-specific sandwich oligonucleotide detection methodology. The new method permitted the use of generic electrochemiluminescent probes supplied in the NucliSens Basic Kit, whereas the original NASBA method required expensive target-specific ruthenium detection probes. The Basic Kit CCR5 Δ32 genotypic analysis was in 100% concordance with both the original NASBA assay and DNA PCR results. This study also evaluated the use of multiple specimen types, including peripheral blood mononuclear cells (PBMC), whole blood, dried blood spots, buccal scrapings, and plasma, for CCR5 genotype analysis. The sensitivities of the three assays were comparable when PBMC or whole blood was the specimen source. In contrast, when dried blood spots, buccal scrapings, or plasma was used as the sample source, the sensitivity of DNA PCR was 80.95, 42.8, or 0%, respectively, compared to 100% sensitivity obtained with the original NASBA and Basic Kit NASBA assays. Our study indicates that the NucliSens Basic Kit NASBA assay is very sensitive and specific for CCR5 Δ32 genotyping using multiple sample types.

The beta chemokine receptor CCR5 has been identified as the major coreceptor that, in conjunction with CD4, facilitates the viral fusion and infection of human host cells by macrophage-tropic human immunodeficiency virus type 1 (HIV-1) strains (7, 8). Studies have identified a genetic polymorphism in the CCR5 gene consisting of a 32-bp deletion (Δ32) which has been linked to resistance to HIV-1 infection in certain individuals homozygous for the mutation (11, 15, 16). Overall, individuals heterozygous for this mutation have been shown to exhibit slower rates of HIV-1 disease progression than matched cohorts of persons homozygous for the wild-type (WT) genotype (6, 9). In addition to CD4+ T-cell counts, HIV-1 viral load, and viral phenotype, the CCR5 Δ32 genotype may serve as an important marker for assessing the rate of disease progression, provide deeper insight into disease pathogenesis, and contribute to the development of new therapeutic strategies aimed at suppressing or blocking HIV-1 infection (13).

Recent collaborative studies from our laboratories have described the development and clinical use of an isothermal nucleic acid sequence-based amplification (NASBA) assay for CCR5 Δ32 genotyping using peripheral blood mononuclear cells (PBMC) (14). The single NASBA assay targets the mRNA transcribed from both the WT and Δ32 mutant (Mut) CCR5 alleles. Amplification of the CCR5 mRNA target sequence is performed by using a single oligonucleotide set which flanks the 32-bp deletion region and three enzymes (avian myeloblastosis virus reverse transcriptase [AMV-RT], RNase H, and T7 RNA polymerase). Detection is achieved by use of two target-specific ruthenium (Ru²⁺)-labeled nucleic acid probes, one whose sequence is internal to the 32-bp deletion region (WT probe) and one that spans the deletion junction site (Mut probe).

The present studies were designed to modify the original NASBA-based CCR5 genotyping assay (referred to as the standard assay) (14) for a NucliSens Basic Kit (Organon Teknika, Durham, N.C.) format, using a sandwich hybridization target-specific detection oligonucleotide methodology. The CCR5 Δ32 genotypes determined by use of the Basic Kit assay were validated by comparison to the standard NASBA assay and a DNA PCR-based assay described previously (9). In addition, we determined if additional specimen types, including whole blood, dried whole-blood spots, plasma, and buccal scrapings, were suitable for CCR5 genotyping by the NucliSens Basic Kit assay. The ability to use alternative specimen types, particularly dried whole-blood spots, would provide an easy and convenient method for the processing and safe shipping of large numbers of specimens from sites where immediate sample processing and freezing are not possible. Buccal scrapings are a simpler alternative specimen type for CCR5 genotyping, since they can be obtained by a completely noninvasive method.

MATERIALS AND METHODS

Study population. The study population consisted of 83 HIV-1-infected adults and 1 HIV-1-seronegative female with high-risk exposure to HIV-1 (HRSNF)

† Dedicated to the memory of Suryakumari Tetali, who died tragically before the submission of this paper. This work is a tribute to her scientific excellence and dedication to HIV research.
who attend the North Shore University Hospital Center for AIDS Research and Treatment and 20 HIV-1-teronegative volunteer donors. Institutional Review Board approval and informed consent were obtained prior to collection of blood and/or buccal scrapings.

Sample collection and processing. All whole-blood samples were collected in VACUTAINER EDTA anticoagulant tubes (Becton Dickinson, Franklin Lakes, N.J.). If indicated, whole-blood samples were centrifuged for 20 min at 1,200 × g in a swinging bucket rotor (RT6000B; Sorvall) and processed as described below within 8 h of specimen collection. Cell-free plasma was removed and immediately frozen in cryovials (Nalgene, Nunc International, Roskilde, Denmark) at −70°C. The remaining cells were used for PBMC separation as described below.

(i) Whole blood. Whole blood was collected from 21 individuals, including 15 HIV-1-infected and 6 uninfected individuals. Next, 50.0- and 100.0-μl aliquots of whole blood from each patient were independently lysed in 0.9 ml of NucliSens lyss buffer (Organon Teknika) containing guanidine thiocyanate and Triton X for 30 min at room temperature and then stored at −70°C until further testing.

(ii) Dried blood spots. Dried blood spots were prepared from whole blood collected from 21 individuals, including 15 HIV-1-infected and 6 uninfected individuals. Separate 50.0- and 100.0-μl aliquots of whole blood were spotted onto nitrocellulose blood collection cards (Schleicher & Schuell, Keene, N.H.). The spotted blood was allowed to dry completely for 3 to 4 h in a biohazard hood, and the dried cards were stored at room temperature in individual envelopes until testing. The dried whole-blood circles were then excised and placed in 9.0 ml of NucliSens lyss buffer. Whole blood was eluted from the filter cards by gentle rocking for 2 h at room temperature, after which the filter paper was removed from the lysis tubes and the lysis tubes were stored at −70°C until nucleic acid extraction. To determine the stability of CCR5 mRNA over time in dried blood spots, 50 μl of whole blood from five patients was spotted onto blood collection cards in quadruplicate and allowed to dry as described above. After 48 h, one sample from each patient was lysed and CCR5 Δ32 genotyping was performed. The three additional dried blood spots for each patient were processed and stored after testing on the blood collection cards at room temperature for 3, 6, and 12 months postcollection.

(iii) Separation of PBMC. PBMC were collected from a total of 21 subjects including 20 HIV-1-infected patients and 1 HRSNF. PBMC were isolated from whole blood by Ficoll-metrizoate (Lymphoprep; Nycomed Pharma, Oslo, Norway) density gradient centrifugation following standard protocols (4). Approximately 2 × 10^6 PBMC from each patient were lysed in 9.0 ml of NucliSens lyss buffer for 30 min at room temperature and stored at −70°C until testing.

(iv) Buccal scrapings. Buccal scraping samples were obtained from 20 healthy volunteers and 1 HRSNF using cytobrush cell collectors (Mediscand Inc., Holmwood, Fla.) by brushing the buccal surface for 5 s and then transferring the brush to 0.9 ml of NucliSens lyss buffer. The brushes were hand rotated vigorously in the lyss buffer tubes and removed. The eluted cellular material was lysed for 30 min at room temperature and stored at −70°C until testing.

NucliSens Basic Kit. The NucliSens Basic Kit was obtained from Organon Teknika. It is a complete kit for customized RNA amplification testing, containing all reagents necessary for nucleic acid release, Boom silica-based extraction of nucleic acids, NASBA, and electrochemiluminescence (ECL) detection. The kit contains a generic Ru2+−labeled detection probe (ECL probe). Using these standardized reagents, custom assays may be designed by analyzing allele-specific primers and probes. This home development is actively supported by a dedicated Basic Kit help desk. Alternatively, Basic Kit users may download Basic Kit protocols (including primer and probe sequences) from an application database hosted at an Extranet web site (http://www.basickit-support.com).

Nucleic acid isolation. Nucleic acids were isolated from all sample types by following the guanidine isothiocyanate-acetic acid silicone procedure of Boom et al. (2) using the NucliSens Basic Kit isolation reagents. After isolation, 5 μl of the nucleic acid extract was used in each NASBA and DNA PCR.

NASBA of CCR5 mRNA. NASBA of CCR5 RNA (Fig. 1A) was achieved by using either the NucliSens Basic Kit reagents as described by the manufacturer (Organon Teknika) or the procedure of Romano et al. (14). Briefly, NASBA was performed in a 20.0-μl reaction mixture containing 5 μl of the nucleic acid extract (described above) in 40 mM Tris (pH 8.5), 5 mM dithiothreitol, 12 mM MgCl2, 70 mM KCl, 2.0 mM (each) ATP, CTP, and UTP, 1.5 mM GTP, 0.5 mM ITP, 1.0 mM (each) dATP, dCTP, dGTP, and dITP, 0.1 μg of bovine serum albumin (BSA)/μl, 0.08 U of R.Nase H, 32 U of T7 RNA polymerase, 6.4 U of AMV-RT, 15% dimethyl sulfoxide (DMSO), and 0.2 μM each amplification oligonucleotide specific for CCR5. The P1A ( antisense) oligonucleotide sequence was 5′-AAAGTTTACGACTATGAGGGGAGCCAGCGCCAGCGGGA CCAGCCCCCA-3′; the P2B (sense) nucleotide sequence was 5′-TTTGGGTTTG TGTGACAAGTGTGTACCA-3′. The italicized portion of the P1A oligonucleotide designates the overhang encoding the T7 RNA polymerase promoter that is required for the transcription-based NASBA process.) Amplification reactions were conducted at 41 ± 0.5°C for 90 min. Included with each amplification run

![Figure 1](http://cvi.asm.org/)
was a set of controls consisting of in vitro-transcribed WT and Mut CCR5 RNA (14).

Analysis of CCR5 NASBA products. (i) Standard NASBA assay hybridization method. Analysis of CCR5 mRNA following amplification was performed by using a modified version of the differential probe hybridization procedure of Romano et al. (14). The Ru²⁺-labeled oligonucleotide probe specific for the WT amplification product (5'-AGATTCATTTGGAAGAATTTCA-3') anneals to the position corresponding to bases 557 to 581 (with base 1 corresponding to the A of the ATG initiator codon) and is located within the 32-base deleted portion of the Mut allele. The Ru²⁺-labeled oligonucleotide probe specific for the Mut allele (5'-TCCATACA:TTAAAGAT-3') corresponds to base positions 546 to 553 and 566 to 573. Importantly, this probe is a continuous oligonucleotide corresponding to these two distinct regions, with the colon designating the junction site that is present only in the Mut allele. A third probe, labeled with biotin at the 5' end and attached to streptavidin-coated beads (type 280; Dyon, Lake Success, N.Y.), was used for amplon capture. This capture probe (5'-AAAAGGTTCTATACCTGACAGC-3') was complementary to an iden-
tical sequence present in both alleles (positions 511 to 537). Actual hybridization was achieved by mixing 5 µl of the NASBA reaction product (prediluted by a factor of 40) with 20 µl of a solution containing 0.75 M NaCl, 75 mM sodium citrate, 0.8 mg of BSA/ml, 2 × 10⁻⁶ copies of one of the allele-specific ruthenium-labeled detector probes, and 2 × 10⁻¹² copies of the bead-immobilized capture probe. Each NASBA product was analyzed in separate hybridization reactions with one of the allele-specific probes by incubation at 60°C for 5 min followed by 30 min at 41 ± 0.5°C. Positive hybridization was measured by quantification of the ECL signal using the NASBA QR System ECL reader (Organon Teknika) from 14 separate trials were calculated for each probe. Background ECL signal from hybridization of the Mut probe to WT RNA ampli-
cation products. Establishment of ECL positive cutoff values. In order to evaluate raw ECL signals generated by the Basic Kit CCR5 NASBA assay on clinical samples, cutoff values for WT and Mut probe signals must be validated. The need to calculate independent cutoff values for WT and Mut signals stems from the fact that the two probes detect different target sequences on different amplification reaction products (see Materials and Methods). Therefore, background signals with these two distinct hybridization-based detection sys-
tems will not necessarily be equal. Consequently, we evalu-
ated two methods for determining cutoff values (see Mate-
rials and Methods) so as to determine the most appropriate means of evaluating raw data obtained from the application of this assay to clinical samples.

Results from the analysis of negative-control samples with each probe were used to determine the mean negative-control ECL values, as well as the standard deviation, for 14 samples. The mean negative-control WT probe ECL signal was 340 U, with a standard deviation of 279. By this method, the WT ECL cutoff was defined as 1,735 U. Similarly, the mean negative-control Mut probe ECL signal was 232 U, with a standard deviation of 115. Thus, the defined cutoff for the Mut probe was 807 U. In the alternative approach, the mean ECL signal from the analysis of each probe on its alternative control template material was determined, along with the associated standard deviation. The mean ECL signal obtained with the WT probe hybridized to Mut RNA amplification products was 100 U (standard deviation, 165). Therefore, the background cutoff, calculated as the mean plus 5 standard deviations in this system, was 925 U. The mean ECL signal obtained from the Mut probe hybridized to WT RNA amplification products was 1,087 U (standard
WT allele and Mut allele; Mut/Mut, homozygous Mut alleles.

TABLE 1. Summary of CCR5 Δ32 genotype results per specimen type

| Specimen type (n) and assay | No. of genotype results/total tests per genotype* | % of genotypes identified |
|----------------------------|-----------------------------------------------|--------------------------|
|                            | WT/WT | WT/Δ32 | Δ32/Δ32 |
| PBMC (21)                  |        |        |        |
| Standard NASBA             | 16/16  | 4/4    | 1/1    | 100   |
| Basic Kit                  | 16/16  | 4/4    | 1/1    | 100   |
| DNA PCR                    | 16/16  | 4/4    | 1/1    | 100   |
| Whole blood (21)           |        |        |        |
| Standard NASBA             | 13/13  | 7/7    | 1/1    | 100   |
| Basic Kit                  | 13/13  | 7/7    | 1/1    | 100   |
| DNA PCRb                   | 9/13   | 7/7    | 1/1    | 80.95 |
| Dried blood spots (21)     |        |        |        |
| Standard NASBA             | 13/13  | 7/7    | 1/1    | 100   |
| Basic Kit                  | 13/13  | 7/7    | 1/1    | 100   |
| DNA PCRb                   | 9/13   | 7/7    | 1/1    | 80.95 |
| Buccal scrapings (21)      |        |        |        |
| Standard NASBA             | 17/17  | 3/3    | 1/1    | 100   |
| Basic Kit                  | 17/17  | 3/3    | 1/1    | 100   |
| DNA PCRc                   | 8/17   | 0/3    | 1/1    | 42.8  |
| Plasma (63)                |        |        |        |
| Basic Kit                  | 56/56  | 6/6    | 1/1    | 100%  |
| DNA PCRd                   | 0/15   | 0/4    | 0/1    | 0.00  |

* CCR5 genotypes: WT/WT, homozygous WT alleles; WT/Mut, heterozygous WT allele and Mut allele; Mut/Mut, homozygous Mut alleles.

a) First-round DNA PCR gave positive results for 12 of 21 samples (including 3 that were weakly positive); second-round PCR using the template from the first-round PCR on the remaining 9 samples gave 5 more positive results, increasing the total number of positive results and percentage of genotypes identified to 17 of 21 and 80.95% respectively.

b) First-round DNA PCR gave positive results for 8 of 21 samples (all weakly positive); second-round PCR on the 13 negative samples yielded one more positive result, increasing the total number of positive results and percentage of genotypes identified to 9 of 21 and 42.8%, respectively.

c) DNA PCR results were negative for all samples after second-round PCR.

d) DNA PCR results were negative for all samples after second-round PCR.

A total of 147 different clinical samples were analyzed in this study (results of this analysis are summarized in the following sections). Results obtained with the WT probe using the two different cutoff values derived above were 100% concordant; results obtained with the Mut probe by the two methods were also 100% concordant. However, it should be noted that one of the samples had a Mut probe ECL value of 5,418 U, putting it above the cutoff value for this probe by a single ECL unit. In a clinical situation, such a sample would require retesting in order to confirm the result. For subsequent analysis of all clinical samples, we conservatively used the higher cutoff value for each probe.

Validation of NucliSens Basic Kit CCR5 Δ32 genotyping assay using PBMC. Nucleic acids isolated from 2 × 10⁸ PBMC derived from 21 patients were subjected to standard NASBA, NucliSens Basic Kit, and DNA PCR amplification as described in Materials and Methods. The results were scored positive or negative for the WT and Mut CCR5 probes based on the ECL signals and established cutoff values for both the standard NASBA assay (14) and the NucliSens Basic Kit assay as described above. Both CCR5 WT and Mut in vitro-transcribed RNA controls were included with each assay run. In addition to validating the NucliSens Basic Kit assay against the standard NASBA assay, DNA PCR was performed from another aliquot of the same nucleic acid extracts for all 21 patients. As shown in Table 1, all three methods were able to determine the CCR5 genotype and all results were 100% concordant. Representative CCR5 Δ32 genotyping results obtained by all three methods from five patients are shown in Fig. 2. Panel 1A shows results obtained by the standard NASBA assay, panel 1B depicts results with the NucliSens Basic Kit assay, and panel 1C depicts results from DNA PCR amplification. Assay results indicate that patient CC89 (lanes 2) is homozygous for the Δ32 deletion mutation (Δ32/Δ32), patients CC43 and CC95 (lanes 3 and 5, respectively) are heterozygous for the Δ32 mutation (WT/Δ32), and patients CC94 and CC114 (lanes 4 and 6, respectively) are homozygous for the WT CCR5 alleles (WT/WT).

CCR5 Δ32 genotyping using alternative specimen types. (i) Whole-blood and dried blood samples. The standard NASBA assay, NucliSens Basic Kit assay, and DNA PCR were used independently to perform CCR5 Δ32 genotyping on 50 and 100 μl of whole blood and dried blood spots derived from 15 HIV-1-infected patients and 6 uninfected healthy volunteers. For all samples tested, sufficient ECL signals were generated to accurately determine allele status by both the standard NASBA and NucliSens Basic Kit assays. PCR amplification products obtained from whole blood were visible on ethidium bromide-stained 2% agarose gels for all samples, although the bands were faintly visible in three cases (data not shown). Of the 21 dried blood spots, PCR amplification products were visible on ethidium bromide-stained gels in 9 cases, faintly visible in 3 case, and not visible in the remaining 9 cases. A second round of amplification using 1 μl of product from the first-round PCR gave visible bands in five of the nine samples that were negative after first-round PCR. Four of the samples could still not be typed after the second round of PCR (Table 1). Genotyping results from all three methods were 100% concordant for all comparable samples. Furthermore, the results from PBMC samples were also 100% concordant. Examples of CCR5 genotyping by standard NASBA (Fig. 2, A panels), the NucliSens Basic Kit (B panels), and DNA PCR (C panels) obtained from whole-blood samples (panels 2A, 2B, and 2C) and dried blood spots (panels 3A, 3B, and 3C) from five patients are shown in Fig. 2. The stability of CCR5 mRNA detection in 100-μl dried whole blood spots was assessed by testing multiple same-sample aliquots after storage for 3, 6, and 12 months on blood collection filter paper cards. As shown in Table 2, detectable signals were obtained for all five samples that were processed at the end of 3, 6, and 12 months. Although in two cases the signals decreased over the 12-month period, the ECL signals were at least double the established positive cutoff values. Genotype results correlated 100% with those obtained on corresponding samples processed within 48 h of spotting.

(ii) CCR5 genotyping from buccal smear samples. Amplification and detection of CCR5 mRNA from buccal scrapings were successful by both standard NASBA and NucliSens Basic Kit methods for all 21 healthy volunteers (Table 1). PCR amplification products were weakly positive on ethidium bromide-stained gels for 8 of 21 subjects analyzed. Second-round
PCR of samples from the remaining 13 subjects gave a faintly visible band in one case (BS 1) and no visible bands for the other 12 subjects (data not shown).

(iii) CCR5 genotyping from plasma samples. Plasma samples from 63 patients were tested using the NucliSens Basic Kit assay. A subset of 20 patients were tested by the DNA PCR method for confirmatory purposes. Genotype results were successfully obtained for all 63 patients using the NucliSens Basic Kit assay (Table 1). In contrast, no DNA PCR products were detected, even after second-round PCR, for any of the 20 samples tested by this method. The genotyping results obtained from 10 plasma samples were validated by comparing the results to those obtained using PBMC from the same patient and were found to be 100% concordant.

DISCUSSION

We have previously described a NASBA assay that provides a simple, rapid, and sensitive method for accurate genotyping of the CCR5 locus for the Δ32 deletion (14). The development of techniques for CCR5 Δ32 coreceptor genotyping can aid in understanding the effects of CCR5 chemokine receptor mutations in HIV-1 disease progression and transmission and will allow for patient stratification in therapeutic clinical trials. In the present study, we modified the original method to permit CCR5 Δ32 genotypic analysis using a NucliSens Basic Kit format. The NucliSens Basic Kit assay utilizes amplification oligonucleotides and a capture probe that are identical to those used in the original assay. The assay differs in that the target-
This method eliminates the need to purchase expensive target-specific labeled probes. Furthermore, the kit format permits end users to perform the assay without having to prepare reagents independently or engage in complex labeling chemistry procedures. This particular application of the NucliSens Basic Kit also demonstrates a user’s ability to customize the kit for a specific analyte of interest.

Our studies evaluated both the specificity and sensitivity of the NucliSens Basic Kit assay for genotypic analysis performed on multiple specimen types, including PBMC, whole blood, dried blood spots, buccal scrapings, and plasma. Our studies demonstrated that the CCR5 genotyping results obtained by the NucliSens Basic Kit assay correlated 100% with those obtained by the original NASBA assay and by DNA PCR, indicating a specificity of 100% for all specimen types. A distinct advantage of the ECL detection system used with the NASBA assays is the generation of a quantitative ECL signal, allowing for definitive scoring of a sample result. This was in contrast to DNA PCR results, with which interpretation was sometimes not as clearly defined and depended on the efficiency of the PCR amplification, the amount of product generated, sample type, and subjective evaluation of a gel electrophoresis result

The sensitivities of all three assays were comparable when nucleic acids were extracted from 10⁶ PBMC or 100-μl whole-blood samples. However, both the original NASBA assay and the NucliSens Basic Kit assay demonstrated sensitivity superior to that of the DNA PCR assay when dried blood spots, plasma, or buccal scrapings were used as the source of target nucleic acids. DNA PCR was not able to provide clear accurate genotyping results for 13 out of 21 buccal scrapings after a single round of PCR and for 12 out of 21 specimens after second-round PCR. None of the 20 nucleic acid extracts derived from plasma yielded detectable PCR results even after second-round PCR amplification. The increased sensitivity of NASBA over PCR relates to both the amount of nucleic acid target present in each sample type and the sensitivity of the detection method. The amplification target for the NucliSens Basic Kit assay is CCR5 mRNA, and although it may vary according to sample type, overall the mRNA is present in copy numbers significantly higher than the 1 copy per allele per cell target for DNA PCR. The use of radiolabeled probes should enhance the detection rate of the DNA PCR. However, the use of sensitive nonradioactive methods is simpler, safer, and less expensive for routine clinical testing. The abundance of RNA target material is most likely responsible for the capacity of the NASBA assay to use plasma as a test sample. Typically, plasma preparations are cell free and should not contain mRNA. However, it is possible that the plasma collection process leads to the lysis of cells, liberating intracellular CCR5 mRNA, which is present at high expression levels. Undoubtedly, some of the CCR5 mRNA is degraded in the plasma, but relatively rapid processing of the plasma with Boom nucleic acid isolation lysis buffer serves to preserve a sufficient quantity of mRNA for detection in the plasma.

The use of alternative specimen types in lieu of PBMC has specific advantages including ease of specimen collection, less specimen preparation, and enhanced specimen stability. Whole blood, dried blood spots, buccal scrapings, and plasma specimens require minimal preanalytical preparation, limited technical time, and reduced amounts of reagents compared to separation of PBMC. Dried blood spots can be collected by finger puncture, eliminating the need for venipuncture. We have demonstrated that, once dry, RNA in the blood spots is stable for a minimum of 1 year when stored at room temperature. Our results are consistent with those of other studies which have demonstrated that dried blood collected on filter paper can be successfully used for the diagnosis of HIV-1 DNA by PCR in adults (3) and children (1, 12), for quantitating plasma HIV-1 RNA (10), and for serologic subtyping of HIV-1 as well (5). Dried blood spots would be an excellent specimen source for large epidemiology or clinical outcome studies, since many samples can be easily collected and transported at room temperature to centralized laboratory facilities. This is in contrast to methods utilizing PBMC, which require larger amounts of blood and more labor-intensive cell separation procedures and may be cell count dependent. Moreover, temperature is an important variable in the shipment of nondried sample types, which need to be transported frozen on dry ice. In addition, the integrity of the PBMC and the efficiency of cell separation depend on the time elapsed between blood collection and cell separation. During the course of our studies we often found that whole-blood samples stored longer than 24 h

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**TABLE 2. Stability of CCR5 RNA transcripts in dried blood spots**

| Specimen ID* (CCR5 genotypeb) | Time zero | 3 mo | 6 mo | 12 mo |
|------------------------------|-----------|------|------|-------|
| CC103 (WT/WT)                | 80,381    | 44   | 110,944 | 70   | 77,973 | 36 | 20,998 | 1 |
| CC106 (WT/WT)                | 223,213   | 201  | 222,385 | 170  | 62,256 | 35 | 60,082 | 3 |
| CC107 (WT/WT)                | 45,378    | 54   | 26,986  | 1    | 6,563  | 22 | 3,907  | 1 |
| CC149 (WT/WT)                | 60,061    | 55   | 120,561 | 91   | 85,719 | 34 | 3,017  | 1 |
| CC113 (WT/Δ32)               | 34,747    | 121,905 | 31,596 | 127,589 | 3,216 | 144,626 | 10,973 | 56,286 |

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* ID: identification.

b WT/WT, homozygous WT CCR5 genotype; WT/Δ32, heterozygous CCR5 genotype.

c Dried blood spots were analyzed within 48 h of inoculation of blood collection cards (time zero) or 3, 6, or 12 months after inoculation of blood collection cards.

d WT, WT CCR5 allele probe; Mut, Mut CCR5 allele probe.
prior to PBMC isolation demonstrated significant reductions in the viability of the PBMC population and the total number of cells available for analysis, leading to negative or inconclusive results for DNA PCR testing (data not shown). However, the same samples, when tested by the NASBA or NucliSens Basic Kit assay, were not affected by the length of storage time, and genotyping was successfully performed from a significantly lower number of PBMC (10^5 versus 10^6 [data not shown]). Finally, the ability to genotype from buccal scrapings has the unique advantage of being entirely noninvasive.

In summary, the NucliSens Basic Kit assay is a very sensitive and specific method for determining CCR5 Δ32 genotype. Our data indicates that plasma, whole blood, dried blood spots, and buccal scrapings are acceptable alternative specimens that can be used without compromising the sensitivity or specificity of the assay.

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