Erroneously Low or Undetectable Plasma Human Immunodeficiency Virus Type 1 (HIV-1) Ribonucleic Acid Load, Determined by Polymerase Chain Reaction, in West African and American Patients with Non-B Subtype HIV-1 Infection

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The polymerase chain reaction (PCR) assay for plasma human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) inadequately quantitates virus load for some non-B HIV-1 subtypes because of genetic diversity in the gag region targeted by the PCR primers. Unexpectedly low or undetectable plasma HIV-1 RNA findings by PCR were a clue to non-B HIV-1 infections in patients in whom plasma HIV-1 RNA was found to be substantially higher when determined by a branched-chain deoxyribonucleic acid assay.

Genetic diversity is a quintessential attribute of HIV type 1 (HIV-1). On a global scale, HIV-1 exists in a variety of genetic strains, called "clades" or "subtypes." The genetic sequences of the subtypes differ from each other by ∼30% in the env region (which encodes the outer envelope) and by ∼14% in the gag region (which encodes an inner structural protein). The greatest genetic diversity exists in central Africa, where HIV-1 is believed to have originated. All known subtypes are present in Africa. Subtype B HIV-1, which dominates among infections acquired in Europe and the Americas, is numerically insignificant in Africa [1].

Virus quantitation by means of reverse transcriptase–PCR (RT-PCR) analysis relies on detection and amplification of a single conserved viral genetic sequence. Genetic variation in the putatively conserved sequence has a marked negative impact on the ability of the test to quantify virus. The RT-PCR test approved for clinical use by the US Food and Drug Administration (FDA; Roche Amplicor MONITOR version 1.0) amplifies a 142-bp sequence by use of a single pair of primers (SK462/SK431) and a probe (SK102). These bind to a conserved region of the HIV-1 gag gene sequence derived from subtype B.

Several laboratory-based studies [2–4] show that this RT-PCR test inadequately quantitates many viruses of subtypes A and E because of mismatches between the primers and the regions in which they anneal to the gag of subtype A [5]. Subtype E, a recombinant virus, contains the gag of subtype A. An alternative test, the branched-chain DNA (bDNA) test (bDNA Quantiplex 3.0, Bayer Diagnostics) detects HIV by hybridization to multiple sequences, derived from multiple subtypes, that span almost the entire 2700-bp length of the pol (polymerase) gene. One would predict that genetic variation would have less impact on a test that uses multiple conserved sequences.

Although both assays are linear over their stated dynamic ranges for subtype B, the RT-PCR test results in a virus load determination for subtype B that is consistently 0.45 log higher than that found by use of the bDNA test when the 2 tests are directly compared [6]. However, the bDNA test is better able to quantitate subtypes A and E than is the FDA-approved RT-PCR test. For subtypes A and E, virus load determined by the bDNA test is up to 3 logs higher than that found by the RT-PCR test [2, 3]. An RT-PCR test in development that uses additional primers (Roche Amplicor MONITOR version 1.5) has been reported to better quantify subtypes A and E [2–4]. Subtype A is the dominant subtype (>90%) in West Africa. Subtype E occurs in some parts of Asia, especially Thailand.

At a clinic in Bronx, New York, unexpectedly low or undetectable (<400 copies/mL) virus loads measured by the FDA-approved RT-PCR MONITOR test were noted in patients who were recent immigrants from West Africa. (West African immigrants make up ∼4% of a clinic population of 800.) In contrast to laboratory-based comparisons between the RT-PCR test and the bDNA test, to our knowledge, the cases detailed here are the first descriptions of patients identified in the context of clinical care who had virus loads erroneously determined to be low or undetectable by the RT-PCR test because of the presence of non-B HIV-1 infection. Either the patients had declining CD4 cell counts while on antiretroviral therapy (ART), despite repeatedly undetectable virus loads as shown by the RT-PCR test, or they had undetectable virus loads while not on ART (table 1).

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Table 1. Comparison of bDNA and RT-PCR tests in patients with unexpectedly low virus loads as assessed by the RT-PCR test.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Birthplace</th>
<th>Subtype</th>
<th>Antiretroviral therapy</th>
<th>CD4 cell count, cells/mm³</th>
<th>Virus load, copies/mL</th>
<th>RT-PCR test</th>
<th>bDNA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>M</td>
<td>Ivory Coast</td>
<td>Non-B</td>
<td>ZDV+3TC</td>
<td>128</td>
<td>&lt;400</td>
<td>Not done⁶</td>
<td>Not done⁶</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>F</td>
<td>Ghana</td>
<td>Non-B</td>
<td>d4T+3TC</td>
<td>82</td>
<td>&lt;400</td>
<td>Not done⁶</td>
<td>Not done⁶</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>F</td>
<td>Nigeria</td>
<td>Non-B</td>
<td>3TC+d4T+Idv</td>
<td>167</td>
<td>1314</td>
<td>92,112</td>
<td></td>
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<tr>
<td>4</td>
<td>39</td>
<td>M</td>
<td>Liberia</td>
<td>Non-B</td>
<td>3TC+d4T</td>
<td>167</td>
<td>543</td>
<td>51,088</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>F</td>
<td>Nigeria</td>
<td>Non-B</td>
<td>None</td>
<td>533</td>
<td>1502</td>
<td>4909</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>F</td>
<td>Nigeria</td>
<td>Non-B</td>
<td>None</td>
<td>18</td>
<td>&lt;400</td>
<td>88,000</td>
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</tr>
<tr>
<td>7</td>
<td>37</td>
<td>M</td>
<td>New York</td>
<td>Non-B</td>
<td>ZDV+3TC+Idv</td>
<td>153</td>
<td>232</td>
<td>10,295</td>
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<tr>
<td>8</td>
<td>32</td>
<td>M</td>
<td>New York</td>
<td>Non-B</td>
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<td>360</td>
<td>613</td>
<td>37,985</td>
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<tr>
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<td>28</td>
<td>M</td>
<td>New York</td>
<td>Non-B</td>
<td>None</td>
<td>39</td>
<td>1900</td>
<td>555,900</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>F</td>
<td>New York</td>
<td>Not done</td>
<td>3TC</td>
<td>35</td>
<td>7187</td>
<td>Not done</td>
<td></td>
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<tr>
<td>11</td>
<td>28</td>
<td>F</td>
<td>Benin</td>
<td>Non-B</td>
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<td>398</td>
<td>&lt;50</td>
<td>3408</td>
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<tr>
<td>12</td>
<td>23</td>
<td>F</td>
<td>Ghana</td>
<td>B⁵</td>
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<td>78,168</td>
<td>7,202,040</td>
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<tr>
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<td>F</td>
<td>Mali</td>
<td>Non-B</td>
<td>ZDV+3TC+NVP</td>
<td>200</td>
<td>112</td>
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<tr>
<td>14</td>
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<td>Non-B</td>
<td>None</td>
<td>309</td>
<td>&lt;400</td>
<td>76,142</td>
<td></td>
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<tr>
<td>15</td>
<td>21</td>
<td>F</td>
<td>Ghana</td>
<td>Non-B</td>
<td>d4T+3TC+Nevirapine</td>
<td>507</td>
<td>&lt;50</td>
<td>5099</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** 3TC, lamivudine; bDNA, branched-chain DNA; d4T, stavudine; F, female; Idv, indinavir; M, male; NVP, nevirapine; RT-PCR, reverse transcriptase–PCR; ZDV, zidovudine.

* Subtype determined by serologic assay [6].

① Unexpectedly low virus load was identified in retrospect; the bDNA test was not done concurrently with findings of low virus load by the RT-PCR test. For patient 1, virus was undetectable by the RT-PCR and bDNA tests when his CD4 cell count rose to >600 cells/mm³ while he was receiving antiretroviral therapy.

② Subtype analysis not performed; patient was identified in retrospect, after her death.

③ Subtype determined to be B on serologic assay. A confirmatory genotypic assay is planned.

Non-B HIV-1 infection was demonstrated by an investigational assay for antibodies to subtype-specific peptides derived from env sequences [7] that was performed at the New York City Department of Health (NYC DOH). Individuals from geographic regions where the prevailing subtypes have evolved over time can harbor strains exhibiting substantial genetic differences from the representative peptides used in the serotype assay. Consequently, the serotype often does not correlate with the genotype [8] because of serologic cross-reactivity, especially between subtypes A and C and, to a lesser extent, between subtypes B and D. Infection due to recombinant strains containing antigenic material from ≥2 subtype parent strains (i.e., an AB recombinant strain) poses further problems for the serologic subtype assay. Therefore, the NYC DOH only reports subtypes as B or non-B on the basis of the serologic assay results and recommends confirmation by genotypic sequencing.

Initially, 6 West African patients were identified whose low virus loads by the RT-PCR test were incompatible with clinical status or with declining CD4 cell counts. Specimens from these 6 patients were submitted to the NYC DOH. All 6 had non-B HIV-1 according to the serologic assay. The commercially available bDNA test and RT-PCR test gave markedly discrepant results for virus load in 4 patients, with the bDNA test indicating substantially higher virus load.

The patient who initially inspired our concern was a West African man (patient 1) whose steady decline in CD4 cell count from 262 to 128 cells/mm³ over 10 months occurred despite a repeatedly undetectable virus load (RT-PCR test, <400 copies/mL) while he was taking lamivudine and zidovudine. With the addition of a protease inhibitor, his CD4 cell count rose to >600 cells/mm³. Patient 2, from Ghana, presented with wasting and a CD4 cell count of 82 cells/mm³; yet her virus load was undetectable (RT-PCR test, <400 copies/mL) after only 1 month of stavudine-lamivudine therapy. She returned to Ghana before bDNA assessment could be done. In 2 other West Africans (patients 3 and 4), CD4 cell counts steadily dropped over several years of observation in the clinic, despite repeatedly undetectable virus loads (RT-PCR test, <400 copies/mL) during nucleoside-analogue therapy. Only when virus load was measured by the bDNA test could the failure of treatment regimens to fully suppress virus be recognized. Patient 5, from Nigeria, presented with lymphadenopathy, thrombocytopenia, and CD4 cell count of 311 cells/mm³. Virus load was repeatedly undetectable (RT-PCR test, <400 copies/mL) while the patient was not on therapy. On a subsequent determination, a substantial discrepancy between RT-PCR and bDNA findings was demonstrated. Patient 6, from Nigeria, presented with newly diagnosed HIV infection, wasting, CD4 cell count of 18 cells/
mm$^3$, and virus load of <400 copies/mL by the RT-PCR test. By contrast, her virus load as assessed by the bDNA test was 88,000 copies/mL.

After it was recognized that non-B HIV-1 could be underestimated by the RT-PCR test, several US patients with unexpectedly low or undetectable virus loads were evaluated for non-B HIV-1 infection. Consequently, 3 US patients (patients 7–9) in New York City were identified who acquired non-B HIV-1 via sexual contact. Virus load measured with the bDNA test was substantially higher than the unexpectedly low or undetectable virus load found by the RT-PCR test.

Patient 7, a homosexual man from New York City, had a baseline CD4 cell count of 110 cells/mm$^3$ after only 3 years of HIV infection, despite a seemingly low virus load set point of 831 copies/mL (RT-PCR test) before ART. During ART, his virus load remained <400 copies/mL on repeated RT-PCR testing, but his CD4 cell count never rose. On further testing, he was found to have non-B HIV-1; his virus load was 232 copies/mL (RT-PCR test) compared with 10,295 copies/mL (bDNA test) while on ART. Neither he nor his sex partners had traveled abroad.

Patient 8 sought HIV testing after learning that his only sex partner of >9 years had died from long-standing AIDS. His initial CD4 cell count was 360 cells/mm$^3$, and his virus load was repeatedly undetectable (RT-PCR test, <400 copies/mL) while he was not on ART. He had non-B HIV-1; virus load as assessed by the bDNA test was 37,985 copies/mL, whereas his virus load was 613 copies/mL as assessed by the RT-PCR test. In retrospect, his partner, patient 10, who had attended the clinic regularly for years, had a surprisingly low virus load despite long-standing and severe immunosuppression. She had had a previous relationship with a West African man. She may have acquired subtype A HIV-1 infection from her West African partner and passed it on to her US-born partner in New York City. No blood was available after her death for determining the subtype of her HIV-1 infection.

Patient 9, a homosexual man from New York City, presented with esophageal candidiasis. Before ART, his CD4 cell count was 39 cells/mm$^3$, and his virus load was 3900 copies/mL (RT-PCR test). On later evaluation, his virus load was found to be 1900 copies/mL by the RT-PCR test and 555,900 copies/mL by the bDNA test before ART. He too had non-B HIV-1 infection. His sexual contacts occurred in New York City with men who had not traveled abroad.

We now routinely request that virus load be measured by both the RT-PCR test and the bDNA test before treatment is begun for patients likely to be infected with non-B HIV-1 subtypes, notably immigrants from Africa and patients whose low virus load seems inconsistent with their clinical status. Four newly diagnosed patients from West Africa demonstrated substantial discrepancies in virus load when tested by the RT-PCR and the bDNA tests at entry or soon after entry into the clinic. Patient 11, from Benin, was an asymptomatic pregnant woman. Patient 12, from Ghana, presented with advanced HIV infection with mental changes, salmonella bacteremia, and wasting. Although her virus load as assessed by the RT-PCR test was substantially, it was almost 2 logs higher when assessed by the bDNA test. The extremely high virus load was compatible with her advanced symptoms. Surprisingly, the subtype serologic assay revealed subtype B. Further characterization by a heteroduplex mobility assay also classified the virus as subtype B on the basis of env and gag sequences (M. Gorny, personal communication). No confirmatory genotyping has been done to date.

Patient 13, from Mali, had newly diagnosed HIV infection during pregnancy. Her virus load responded favorably to ART, with a drop from 10,000 to <400 copies/mL (RT-PCR test), but it was still 2046 copies/mL when assessed by the bDNA test. Patient 14, from the Ivory Coast, was asymptomatic when he presented for routine HIV testing. Additionally, patient 15, from Ghana, was monitored in the clinic with bDNA and RT-PCR tests because both showed an initially undetectable virus load while the patient was receiving ART. For this patient, virologic breakthrough was demonstrable repeatedly only with bDNA testing, because the RT-PCR test results remained spuriously undetectable.

In 9 other African patients (not included in the study) whose virus loads were detectable by RT-PCR testing, the RT-PCR results were comparable to the bDNA results, with the RT-PCR results consistently somewhat higher (mean difference, 0.29 log; range, 0.01–0.72 log). Thus, underquantitation of virus load by the RT-PCR test is not invariable among patients infected with non-B HIV-1. For all 6 patients with detectable HIV loads for whom repeat comparisons were done, when a substantial discrepancy between RT-PCR and bDNA results was found, the discrepancy was demonstrable repeatedly. It seems unlikely that systematic laboratory error would substantially underestimate virus load by RT-PCR in some cases but give a slightly higher value in others.

Much of the management of HIV-infected patients is guided by the virus load. Any circumstance that jeopardizes the reliability of virus load measurement has a substantial negative impact on the care of HIV-infected patients. The FDA-approved RT-PCR test poorly serves the needs of patients with HIV-1 subtype A or E infection. Although those infections most commonly afflict people outside the borders of the United States, such infections are encountered in the United States, even in people who acquired their HIV infection locally. As populations intermingle, the spread of non-B HIV-1 infection within the United States can be anticipated, especially in cities with large immigrant populations. When a patient with HIV-1 has an unexpectedly low virus load as assessed by the RT-PCR test, it would be prudent to consider the possibility of infection with
a non-B subtype. The bDNA test, which has features better suited to accommodate viral diversity in the pol gene, or the Roche Amplicor MONITOR test version 1.5, when it is available, could be used in that situation.

Acknowledgment

Bayer Diagnostics performed the bDNA tests in patients identified with non-B HIV-1.

References