False-positive result from Bayer Versant HIV-1 bDNA viral load assay, with a possible role for light leakage after inadequate maintenance of the analyzer

Multiple reports have noted the occurrence of false positive results when HIV viral load assays have been used qualitatively to diagnose HIV infection (de Mendoza 1998, Rich 1999, Daar 2001, Salimnia 2005, Gallant 2005). These false positives have been reported both for the PCR-based RNA amplification and the branched-DNA (bDNA)-based signal amplification techniques. Suggested causes for these erroneous results include cross-contamination, non-specific hybridization, and incorrect specimen processing. Here we report on the occurrence of a false-positive HIV viral load result using the Bayer Versant HIV-1 RNA 3.0 bDNA assay. Testing and maintenance of the Bayer System 340 Analyzer used in the performance of this assay led us to conclude that leakage of the chemiluminescent signal, or “crosstalk,” between sample wells in an inadequately maintained analyzer likely led to the false-positive result (Gibbs 2001).

The patient was a 28-year-old male who was referred for HIV testing after a needlestick injury from a needle contaminated by blood from a newly diagnosed HIV-positive patient. He was given post-exposure prophylaxis consisting of zidovudine, lamivudine and nelfinavir for 4 weeks. Baseline and 6-week HIV-1/2 antibody enzyme immunoassay (EIA) testing produced a negative result. An HIV viral load assay was ordered at the same time as the 6-week EIA for which the patient’s blood was collected in a Greiner K_2 EDTA tube. This tube was centrifuged and then frozen at minus 70°C within 65 minutes of collection. The following day, the
Versant HIV-1 RNA 3.0 bDNA assay was performed according to the manufacturer’s instructions, using the Bayer System 340 Analyzer, and produced a result of 955 copies/ml. No error code was generated by the analyzer during this test.

Immediately after this test was performed, a Bayer technician examined the analyzer by prior arrangement (see below). It was discovered that the read head of the instrument was not adequately compressing the tops of the sample wells, potentially resulting in light leakage between wells. Maintenance was performed to correct this problem. A repeat viral load bDNA assay on the same serum 2 days later produced a result of <75 copies/ml. Additional EIAs performed 3 days and 45 days after the initial viral load assay were repeatedly negative (7 and 12 weeks since the needlestick exposure). An additional viral load assay using new serum collected 2 days after the first viral load also produced a result of <75 copies/ml. In 4.5 months since the needlestick exposure, the patient continues to be asymptomatic, with negative HIV testing, and is presumed to be HIV-negative.

For several weeks prior to this test, the analyzer generated intermittent error reports due to abnormally high luminescence intensities for certain standard specimens. The analyzer did not indicate problems with the read head during our regularly scheduled operator-performed maintenance. We notified the manufacturer and a maintenance visit was arranged, as described above. Meanwhile, we sought to determine whether light leakage could be occurring by placing an empty strip of sample wells just below the strip of wells containing the standards. Table 1 shows the luminescence results of the standard and empty wells in rows 1 and 2, respectively. In
row 3 are the luminescence results for routine patient samples in the strip immediately below the empty strip. There was a strong correlation between the luminescence intensities of the empty wells and the adjacent wells immediately above, below and diagonal to each empty well (p<0.01). It is notable that the sample well immediately below our patient’s initial (presumed false-positive) well had a very high luminescence intensity, corresponding to a viral load of >500,000 copies/ml (data not shown).

After preventive maintenance was performed and the read head compression was corrected, we again tested the assay for possible light leakage as described above, using empty wells in the strip below the standard wells. The results are shown for standard, empty, and routine patient wells in rows 4, 5 and 6, respectively, of Table 1. There was only a marginal correlation between the empty and adjacent wells (p>0.05).

In summary, we present a case of a false-positive HIV result using the Versant HIV-1 bDNA viral load assay, apparently caused by inadequate maintenance resulting in light leakage between sample wells during luminescence reading by a Bayer System 340 Analyzer. While we present one way that a false-positive result may be generated by a viral load assay, it is important to note that other false positives previously reported with multiple assay types show that there can be diverse causes. Clinicians using HIV viral load assays for HIV diagnosis must be cognizant of the potential for false-positive results, and prepare their patients for this possibility. In addition, the phenomenon we report here could conceivably cause known HIV-positive patients' viral loads to be
erroneously reported above certain critical values, indicating treatment failure where none may be occurring, and leading to unwarranted changes in clinical management. This case underscores the need for proper maintenance of the apparatus used in a chemiluminescent assay to ensure that light leakage is not occurring between samples. Even isolated deviations of the luminescence intensity of standard specimens should prompt thorough evaluation of the assay, and preventive maintenance of the analyzer, before further clinical use of the assay. Future design modifications of these assays may need to incorporate improved detection of light leakage.

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References
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<thead>
<tr>
<th></th>
<th>relative luminescence</th>
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<tbody>
<tr>
<td><strong>before maintenance:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (standards)$^4$</td>
<td>7870 8602 651.6 60.63 7.709 1.826 2.075 1.318 0.800 0.388 8.445 2200</td>
<td>0.944$^2$</td>
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<tr>
<td>2 (empty wells)</td>
<td>6.107 6.554 1.728 0.253 0.073 0.072 0.211 0.081 0.053 0.054 0.334 1.614</td>
<td></td>
</tr>
<tr>
<td>3 (routine patient samples)$^5$</td>
<td>317.0 86.99 1.495 3.871 5.831 6.629 184.2 1.280 11.68 0.948 13.67 40.58</td>
<td></td>
</tr>
<tr>
<td><strong>after maintenance:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (standards)$^4$</td>
<td>8384 7980 518.3 57.79 6.318 3.326 3.213 1.233 1.312 0.558 7.227 2341</td>
<td>0.572$^3$</td>
</tr>
<tr>
<td>5 (empty wells)</td>
<td>0.023 0.014 0.022 0.002 0.006 0.000 0.002 0.006 0.002 0.006 0.015 0.000</td>
<td></td>
</tr>
<tr>
<td>6 (routine patient samples)$^5$</td>
<td>1617 1027 5.258 91.37 28.75 1.135 0.770 22.33 0.888 180.3 1.619 1.971</td>
<td></td>
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$^1$ Spearman rank order correlation coefficient, for correlation between empty well luminescence and the total luminescence of standard and routine patient sample wells immediately above, below and diagonal to the empty well.

$^2$ p<0.01

$^3$ p>0.05

$^4$ rows 1 and 4 comprise different sets of standard specimens

$^5$ rows 3 and 6 comprise different sets of patient specimens