Evaluation of the Quantiplex Human Immunodeficiency Virus Type 1 RNA 3.0 Assay in a Tertiary-Care Center

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The Quantiplex human immunodeficiency virus type 1 RNA 3.0 Assay (bDNA) (Bayer Diagnostics, Walpole, Mass.) produced linear and reproducible (intra-assay and interassay) results over its quantification range of 50 to 500,000 copies/ml of plasma with 96% specificity. A threefold or 0.5-log10 change or greater was clinically significant for serial patient samples.

Serial human immunodeficiency virus type 1 (HIV-1) RNA testing is an essential component of management for individual HIV-positive patients (1, 2). The Quantiplex HIV-1 RNA 3.0 Assay (bDNA) (Bayer Diagnostics, Walpole, Mass.) is a signal amplification nucleic acid probe assay for the direct quantification of plasma HIV-1 RNA (3). In validating this assay for use in our laboratory, we examined its (i) specificity, (ii) linearity over its reportable range of 50 to 500,000 copies/ml of plasma, (iii) intra-assay (well-to-well) variability, (iv) interassay (run-to-run) variability, (v) variability between serial patient samples and its clinical significance, and (vi) copy numbers, compared to those of the (previous) 2.0 version. We also compared a plastic tube to the standard glass tube used to collect the blood sample. Many laboratories have switched to the plastic tube to avoid breakage.

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Bayer Diagnostics (formerly Chiron) provided frozen plasma for the specificity and linearity testing. The other samples were obtained from HIV-positive patients at the Veterans Affairs Medical Centers in Washington, D.C., and Baltimore, Md. Peripheral blood was collected by venipuncture, kept at room temperature, and centrifuged within 4 h at 1,000 × g for 10 to 15 min. The plasma was frozen at −80°C. Each specimen was then tested according to the manufacturer’s directions (Quantiplex HIV-1 RNA 3.0 Assay [bDNA] package insert; Bayer Diagnostics). A run of a complete plate contained up to 80 samples; a half-plate run contained 35 samples. One person prepared and analyzed all of the plasma samples.

Seventy-four HIV antibody-negative plasmas were analyzed by using three runs and three kits from two kit lots. For 71 samples, the HIV-1 RNA level was below the detectable limit of 50 copies/ml. The three remaining samples gave values of 85, 114, and 237 copies/ml of plasma. These values were well below the range reported for acute HIV infection of 27,200 to 1,600,000 copies/ml (4). These low quantities were considered false positives, and the specificity of this assay was 96%. This is consistent with the manufacturer’s statement of intended use; this test is not to be employed for the diagnosis of HIV infection (Quantiplex package insert). However, clinicians do order HIV-1 RNA assays to screen for acute infection before seroconversion has occurred. If this particular assay is being used, caution must be taken to not interpret a low positive value as proof of a genuine HIV infection.

Seven samples from a serial dilution panel were analyzed eight times each by using two runs and two kits from the same kit lot. As illustrated in Fig. 1, the Quantiplex HIV-1 RNA 3.0 Assay (bDNA) gave linear results over its quantification range of 50 to 500,000 copies/ml. The least-squares linear regression for the median log10 copies per milliliter was as follows: y = 0.075 + 0.983x, with r2 = 0.996.

In the intra-assay study, matching plasma samples in adjacent wells were assayed by using four runs and three kits of the same kit lot. Table 1 shows little variation between wells. There also was agreement on four samples being <50 copies/ml and one sample being >50,000 copies/ml. In designing this study, it was assumed that using two varieties of Vacutainer EDTA tubes would not contribute significantly to the variability. The data supports this assumption, and K3 EDTA liquid in glass tubes and K2 EDTA sprayed in plastic tubes can be used interchangeably with this assay.

In the interassay study, 35 patients were phlebotomized

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once, and their plasmas were frozen, thawed, and assayed twice. Three or four samples were compared at a time by using 11 runs and five kits of the same kit lot. As shown in Table 1, the run-to-run variability was low. However, its effect was still apparent: four of five runs of four samples and two of five runs of three samples had values all higher or all lower than those of the previous run. The median decrease of 15% from the first to second run is likely the result of the extra freeze-thaw cycle. Interassay variability was also examined with an in-house control (Fig. 2). Each run had one specific well dedicated to hold this control: mean, 17,166 copies/ml; standard deviation (SD), 2,828; percent coefficient of variation, 18.5%; median and 95th percentile changes were derived from copies per milliliter as follows: percent change = (100 × (sample 2 [S2] − sample 1 [S1])/S1); fold change = S2/S1 if S1 > S2 or S2/S1 if S2 > S1; log change = (log S1 − log S2). The medians and ranges of samples per milliliter were based on the averages of S1 and S2.

The in-house control was pooled plasma frozen in 1-ml aliquots at 80°C; it was thawed, and analyzed twice by using 11 runs, five kits, one kit lot, and one operator. The plasma was analyzed December 1998 in adjacent wells by using four runs, three kits, one kit lot, and one operator. Each run had one specific well dedicated to hold this control: mean, 17,166 copies/ml; SD, 2,828; percent coefficient of variation, 18.5%; median and 95th percentile changes were derived from copies per milliliter as follows: percent change = (100 × (sample 2 [S2] − sample 1 [S1])/S1); fold change = S2/S1 if S1 > S2 or S2/S1 if S2 > S1; log change = (log S1 − log S2). The medians and ranges of samples per milliliter were based on the averages of S1 and S2.

Serial samples (31)

1.013
−0.115
0.928
0.119
−24
1.43
0.156
2.70
0.431
8,015
71–150,328

### Table 1. Summary of intra-assay, interassay, and serial clinical sample correlations for the Quantiplex HIV-1 RNA 3.0 assay (bDNA)

| Assay or sample (n) | Slope | Intercept | r² | P | Median change | 95th percentile change | Median copies/ml | Range copies/ml |
|---------------------|-------|-----------|----|---|---------------|-----------------------|-----------------|-----------------|
| Intra-assay (27)    | 1.006 | −0.032    | 0.989 | 0.605 | −3 | 1.13 | 0.052 | 1.67 | 0.222 | 5,941 | 61–472,203 |
| Interassay (35)     | 1.006 | −0.067    | 0.964 | 0.113 | −15 | 1.25 | 0.097 | 2.15 | 0.332 | 6,973 | 118–304,956 |
| Serial samples (31) | 1.013 | −0.115    | 0.928 | 0.119 | −24 | 1.43 | 0.156 | 2.70 | 0.431 | 8,015 | 71–150,328 |

Each P value was determined by least-squares linear regression of the log₁₀ copies per milliliter. The P value was determined by a two-tailed paired t test. Median and 95th percentile changes were derived from copies per milliliter as follows: percent change = 100 × (sample 2 [S2] − sample 1 [S1])/S1; fold change = S2/S1 if S1 > S2 or S2/S1 if S2 > S1; log change = (log S1 − log S2). The medians and ranges of samples per milliliter were based on the averages of S1 and S2.

Patient samples were drawn in a 7-ml liquid K₃ EDTA glass tube (Vacutainer no. 36-7665) and in a 4-ml sprayed K₂ EDTA polyethylene tube (Vacutainer no. 36-7861). The plasma was analyzed December 1998 in adjacent wells by using four runs, three kits, one kit lot, and one operator.

From one phlebotomy, each patient’s plasma was frozen in September and October 1998 at −80°C, thawed, and analyzed twice by using 11 runs, five kits, one kit lot, and one operator.

Obtained from clinically stable patients with no recent changes in antiretroviral therapy. The samples were analyzed from September to December 1998 by using one operator.
(intra-assay, interassay, and serial clinical samples) results over its quantification range of 50 to 500,000 copies/ml. It correlated well with the 2.0 assay, generally giving 1.5- to 4.5-fold-higher values. Either glass or plastic EDTA Vacutainer tubes can be used with this assay.

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