



**CPAL**

Central Pennsylvania Alliance  
Laboratory

# Technical Bulletin Number 21

## **Quantitation of Human Immunodeficiency Virus type-1 (HIV-1) RNA in Plasma Using the Branched Chain DNA Assay.**

The laboratory is now offering the Quantiplex 3.0 branched chain DNA (bDNA; Chiron/Bayer) assay for HIV-1 RNA viral load determination as performed at the Central Pennsylvania Alliance Laboratory (CPAL). Advantages of this assay include:

1. bDNA technology has a higher degree of precision and reproducibility, compared to PCR(4,5,7). The within run and between run precision of the bDNA assay from both published studies (4,5,7) and at CPAL has been excellent.
2. All known subtypes (A-H; O) are detected and quantitated with similar efficiencies. The PCR assay may either fail to detect or may underquantitate subtypes including A, E, F, G, and O (3,7).
3. The third generation bDNA assay detects HIV-1 RNA over the range of from 50 to 500,000 copies per ml of plasma in a single test run. In contrast, PCR (Amplicor; Roche Molecular) will detect 400 to 750,000 copies per ml during the first assay of a specimen. If smaller concentrations of virus are to be detected, a second, ultraquantitative PCR assay must be run, adding to the costs as well as reporting delays. In two months of bDNA testing at CPAL, 39% of the plasma samples had HIV-1 levels below 400 copies/ml.

A very recent study reported good agreement between the Quantiplex 3.0 bDNA assay and the Amplicor Monitor PCR assay and the results of the two amplification methods were highly correlated (3a). A copy of this paper is enclosed for your review.

The bDNA assay, like the Amplicor PCR test, is not to be used for an initial diagnosis of HIV infection. It is of value after the diagnosis has been established to assist physicians in managing infected patients, in predicting which patients are most likely to progress to AIDS if untreated, and to monitor patient response to antiretroviral therapy (1,2,4,6,7). Patients' bDNA results will be reported both in copies per ml and in the log (10) value of the copies per ml result. Subsequent test results will list the previous CPAL bDNA results by date for each patient and will also include the change in log (10) result from one date to the next. Decreases in HIV-1 RNA values of  $\leq 0.5$  log may indicate biological or laboratory variation while decreases of  $> 0.5$  log may indicate a response to antiretroviral therapy (4). Return of viral RNA to pretreatment levels indicates drug resistance.

The following guidelines have been established for the use of HIV-1 RNA levels in infected patients (1,4,8):

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For question about this, and other, information, call Central Pennsylvania Laboratory at 1-888-480-1422

## HIV Viral Load by bDNA *cont.*:

1. Therapy is recommended for all patients with plasma HIV-1 RNA levels above 5,000 - 10,000 copies per ml (regardless of CD4 counts) and should be considered for any patient with any detectable level of HIV-1 RNA.
2. The target level of HIV-1 RNA after therapy is < 50 to < 5,000 copies per ml plasma.
3. The minimal reduction in HIV-1 RNA indicative of a favorable response to therapy is a 0.5 log decrease in the copies/ml result.
4. A change in HIV-1 RNA that suggests treatment failure is a return to within 0.3 to 0.5 log of the pretreatment copies/ml result.

Additional guidelines have been published for determining when to monitor HIV-1 RNA levels in the plasma of infected patients (1,4):

1. When a patient is first tested, two baseline measurements, 2 to 4 weeks apart, should be made prior to initiation of therapy. The two baseline samples should also be tested using bDNA when a patient is being switched from PCR to the bDNA assay. It is unnecessary to test each of the two samples per patient by both PCR and bDNA when converting from the former to the latter assay.
2. For monitoring therapy, test 3 to 4 weeks after starting or changing therapy, every 4 weeks when making critical clinical decisions, and then every 3 to 4 months.
3. Viral load testing should be avoided during immune activation and following vaccination, interleukin 2 therapy, or concurrent microbial infections.

CPAL has provided specific instructions for collection and processing of specimens in a manner most likely to maintain HIV-1 RNA levels in plasma and prevent falsely low bDNA results:

1. Ten ml of blood should be collected in K3-EDTA tubes. Whenever possible, the patient should be instructed to come to the hospital's outpatient specimen collection facility where the specimen can be collected and rapidly processed. For maximal protection of viral RNA levels, the blood should be stored for no longer than 30 minutes at room temperature, centrifuged at 1,000 x g for 15 minutes, and the plasma immediately frozen at -60C to -80C prior to shipment to CPAL. If these processing conditions cannot be met, the blood, once collected, should not be stored for longer than 4 hours at room temperature and the separated plasma immediately stored at -20C or lower in a non frost-free freezer. Rapid freezing is essential to preserve viral RNA.
2. The plasma will be transported to the CPAL facility on dry ice. At CPAL, the specimens are stored at -80C prior to testing.

Inconsistent HIV-1 viral load levels are likely from improperly collected or processed specimens (4). Strict adherence to the recommended blood collection and processing guidelines is absolutely essential. Whole blood collected into heparin or acid-citrate-dextrose (ACD) tubes is unacceptable for viral load testing because of the greater instability of viral RNA in those anticoagulants.

Physicians with any questions concerning bDNA testing for HIV-1 RNA are encouraged to phone the Microbiology Laboratory's director or supervisor, or individuals at CPAL who are responsible for bDNA testing. At CPAL, the following people may be contacted:

## HIV Viral Load by bDNA *cont.*:

James A. Kellogg, Ph.D., Immunology Director. (717) 851-2393

Chris Woods, Operations Manager. (717) 851-4320

### References:

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