

b-DNA (branched DNA) -

Proven method for the Quantification of HBV, HCV and HIV

by Dr. Peter Gohl

Because of the development of new therapeutic concepts for the treatment of Hepatitis B and C as well as HIV there is a necessity to judge the success of the therapy. To do this it is possible and advisable to determine the "Viral Load" of the patient.

Through the introduction of molecular-biological methods, one can do without the time-consuming process of cultivating pathogens.

Following the introduction of the PCR method a lot of new methods have since been developed for detecting pathogens directly.

For quantification, the b-DNA showed excellent results because the "Viral Load" is determined in its physiological concentration. It is not a DNA amplification method like PCR, but it is just a signal intensification method. Because of this, the b-DNA is not as susceptible to contamination as PCR.

A significant therapy success is obtained if it shows at least three to five-fold reduction of the basal value; this is because the viral load is subject to daily fluctuation.

Initially, the quantitative bDNA assays aimed at measurement of viral load in a clinically relevant range, but the sensitivity did not reach the level of qualitative PCR (polymerase chain reaction) methods.

Meanwhile, due to steady improvement of bDNA chemistry, the HIV assay allows for the detection of as little as 50 copies/ml, which is equivalent to PCR.

With respect to HCV (current lower limit of detection is 200 000 copies/ml), a new test generation (3.0) is available in January 2001, which is characterized by a 100-fold improvement of sensitivity (2500 copies/ml), coming very close to the sensitivity of PCR.

When compared with PCR methods, the reproducibility of bDNA results is higher, thereby increasing reliability.

Another advantage of the b-DNA is that there is no need for modifying enzymes (e.g. Taq Polymerase, Reverse Transcriptase). Therefore, so far, no false-negative results due to potential inhibitors have been noticed.

Quantification of HBV

The quantification should be performed before starting therapy with Interferon-alpha/lamivudin and corticosteroids. It is recommended that a therapy control be carried out after four weeks to check whether the medication should be modified. The b-DNA detects all known subtypes of the Hepatitis B Virus.

The virus quantification is reported in Megaequivalents (Meq/ml=10⁶ Virus equivalent/ml)

The detection limits are between 0,7 Meq/ml and 1,300 Meq/ml.

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Quantification Of HCV

New investigations show that success and duration of anti-viral therapy (interferon a or the combination of interferon with ribavirin) depends not only on the virus genotype, but also on the viral load.

The quantification should be performed before starting therapy and then controlled regularly (1-3 months) in order to be able to change and modify therapy if necessary.

The virus quantification is reported in Megaequivalents (Meq/ml=10⁶ Virus equivalent/ml).

The detection limits are between 0,2 Meq/ml and 120 Meq/ml.

Important note: The new bDNA test generation (3.0) is available in January 2001 with markedly improved (100-fold) sensitivity: Lower limit of detection: 2500 copies/ml.

Quantification of HIV

Because of advances in the therapy of HIV-infected patients there is a need to measure the effectiveness of the chosen medication. This is especially necessary in view of the development of therapy resistance and the high costs of therapy.

The b-DNA detects all known HIV-I subtypes and therefore is an invaluable aid in deciding whether therapy should be continued or changed.

The virus quantification is reported in viral copies/ml.

The detection limits are between 50 and 500.000 copies/ml.

Specimen requirement for each of the above analyses are:

5 ml EDTA blood (10 ml for HIV). Special monovettes or vacutainers must be used, not normal EDTA tubes. Always wear gloves when drawing the specimen. Do not centrifuge and do not take aliquots for other analyses. Please send only unopened tubes.

EDTA blood not Serum

At Bioscientia the sampling requirements for these analyses is EDTA blood compared to some other laboratories which accept serum.

The reason is one of quality. We wish to ensure that you receive an optimal result.

By sending us EDTA blood in a monovette, possible contamination can be ruled out and an accurate result guaranteed because we process under sterile conditions, using laminar flow hoods and strict Q.C. procedures.

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