Branched DNA (bDNA) Technology for Direct Quantification of Nucleic Acids: Research and Clinical Applications

Janice A. Kolberg, Douglas N. Ludtke, Lu-Ping Shen, Will Cao, Darrah O'Conner, Mickey S. Urdea, Linda J. Wuestehube, and Marcia E. Lewis

Introduction

Over the past decade a number of new technologies, including the polymerase chain reaction (PCR) (Mullis and Faloona, 1987), have emerged for the detection and quantification of nucleic acid molecules. Distinct among these is the branched DNA (bDNA) assay which, unlike its target amplification counterparts, uses a signal amplification scheme to enhance detection of physiologic concentrations of target nucleic acids. The basis of the bDNA assay involves the specific hybridization of bDNA and enzyme-labeled oligonucleotide probes to target nucleic acids; this is described in detail in the accompanying chapter in this book, entitled "Branched DNA Technology for Direct Quantification of Nucleic Acids: Design and Performance." The bDNA assay is inherently quantitative and nonradioactive, and has proven to be a reproducible and accurate means of quantifying nucleic acid molecules. The bDNA assay offers several advantages for research and clinical applications; it:

- provides direct quantification of nucleic acid molecules at physiological levels. Since bDNA assays do not require highly purified nucleic acid preparations, inhibitors of enzyme-dependent amplification techniques are of no concern.
- allows a wide diversity of specimen types to be used.
- can be used with reference standards to ensure accuracy for applications requiring absolute quantification, or without standards for applications requiring only assessment of relative changes in nucleic acid concentrations.
- exhibits a high level of sensitivity (moderately expressed genes can be detected using as few as 50–100 cells). The level of sensitivity can be

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modulated by altering the number of target-specific extenders to accommodate specific applications.
- is thoroughly tested for within-lot and between-lot precision to ensure reproducibility (2.2- to 3-fold changes can be discerned as statistically significant).

One of the earliest applications of the bDNA assay was the quantification of viral nucleic acids as a measure of viral load — the amount of virus in the patient. In the management of patients with viral diseases, viral nucleic acid measurement serves to complement other serological, biochemical, and histological measures of disease. For some viral diseases, viral nucleic acid levels have been correlated with disease progression and clinical outcome. Moreover, changes in viral nucleic acid levels in response to therapy may be clinically relevant. Thus, the use of the bDNA assay to measure viral nucleic acids may facilitate a more rational approach to therapy by providing clinicians with information needed to follow viral load throughout the course of disease, select and adjust treatment protocols, and evaluate the efficacy of therapeutic regimens.

The potential prognostic and therapeutic value of measuring viral load using the bDNA assay is only just beginning to be realized. For example, studies of hepatitis C virus (HCV) infected patients undergoing interferon therapy have shown that lower pretreatment serum HCV RNA levels measured with the bDNA assay are predictive of response (Davis et al., 1994; Lau et al., 1993; Yamada et al., 1995). Also, the rate at which serum HCV RNA levels decrease during interferon (IFN) therapy as measured with the bDNA assay has been shown to be informative (Orito et al., 1995; Terrault et al., 1994). A recent study found that the bDNA assay distinguished a more significant difference in serum HCV RNA concentration between complete and incomplete responders than did competitive RT-PCR (Toyoda et al., 1996), indicating that serum HCV RNA concentrations when measured with the bDNA assay are a better predictor of clinical response than when measured by competitive RT-PCR. Similar studies using the bDNA assay to measure hepatitis B virus (HBV) DNA have shown that different patterns of change in serum HBV DNA levels may be useful in distinguishing response to therapy and in modifying the therapeutic regimen of patients with hepatitis B (Hendricks et al., 1995; Hosotsubo et al., 1994; Watanabe et al., 1993). As was the case for comparative studies of HCV RNA assays, comparative studies of HBV DNA quantification assays showed that the bDNA assay exhibits greater sensitivity and precision in measuring HBV DNA in clinical specimens than do other assays (Kapke et al., in press; Zaaijer et al., 1994).