DETERMINATION OF PROSTATE SPECIFIC ANTIGEN mRNA IN PERIPHERAL BLOOD
BY RT-PCR AND A SIMPLE CHEMILUMINOOMETRIC HYBRIDIZATION ASSAY
IN A HIGH-THROUGHPUT FORMAT

E. Emmanouilidou¹, P.C. Ioannou¹, T.K. Christopoulos², K. Polizois³

¹ Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Athens 15771, GREECE.
E-mail: ioannou@chem.uoa.gr
² Department of Chemistry University of Patras, Patras 26500, GREECE
E-mail: tkc@chemistry.upatras.gr
³ Department of Urology, General District Hospital "G. Gennematas", Athens, GREECE

Abstract

In recent years, the mRNA for prostate-specific antigen (PSA) is being investigated as a potential marker for molecular staging of prostate cancer. We report a simple, rapid and sensitive assay protocol for the quantification of PSA mRNA in peripheral blood by using a recombinant RNA internal standard (IS) and reverse transcriptase polymerase chain reaction (RT-PCR). The IS RNA has the same primer binding sites and size as the target. Total RNA from the sample is coextracted with a constant amount of IS RNA and the mixture is subjected to reverse transcription and amplification. Amplified sequences are labeled with biotin during PCR by using a biotinylated upstream primer. The products are heat-denatured and hybridized with oligonucleotide specific probes (for PSA and IS) that are immobilized in microtiter wells. Immobilization of oligonucleotide probes is achieved by passive adsorption of their conjugates with bovine serum albumin. The hybrids are measured using alkaline phosphatase-labeled streptavidin and a dioxetane chemiluminogenic substrate.

The ratio of the luminescence values obtained for the PSA mRNA and the RNA IS is a linear function of the initial amount of PSA mRNA present in the sample prior to RT-PCR amplification. The linear range extended from 50 to 500000 PSA mRNA copies, and the overall reproducibility of the assay, including RT-PCR and hybridization, ranged from 7.3% to 21%. Samples containing total RNA from PSA-expressing LNCaP cells give luminescence ratios that are linearly related to the number of cells in the range of 0.04 to 400 cells. The method was applied to PSA mRNA determination in peripheral blood of healthy individuals, patients with benign prostate hyperplasia, patients with prostate cancer as well as patients with other types of localized cancer.