Detection of gene expression by PCR amplification of RNA derived from frozen heparinized whole blood

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The development of RNA-based polymerase chain reaction (PCR) (1) has facilitated studies of abnormal gene expression in various diseases. In a clinical setting, whole blood or bone marrow specimens from patients are frequently collected in heparin and frozen without further purification for future DNA analysis (2). Although such samples are generally not considered as appropriate sources for RNA-based PCR they are commonly the only material available for molecular studies. We have designed a method for amplification of RNA from frozen heparinized whole blood or bone marrow samples.

RNA was extracted by the acid-phenol-guanidinium method (3) with a modification in the first extraction step: 2 ml of frozen whole blood were boiled for 25 s and immediately mixed by vortexing with 3 ml of 1.5×'Solution D' (6 M quanidinium thiocyanate, 37.5 mM Na-citrate pH 7, 0.75% sarcosyl, 0.15 M 2-mercaptoethanol), 5 ml of 65°C hot phenol, 0.5 ml of 2 M Na-acetate pH 4 and 1 ml of chloroform−isoamylalcohol (49:1). The extraction was carried out as originally described (3). 1−2 μg of RNA were submitted to a reverse transcription reaction using random hexamer primers. When the cDNA product was subjected to PCR no bands were seen on agarose gels after electrophoresis (Figure 1A, lane 2), but amplification was successful when the RNA was extracted from control samples of frozen non-heparinized whole blood (Figure 1A, lane 1). Moreover, a 1:5 mixture between heparinized and non-heparinized samples resulted in no-amplification (Figure 1A, lane 3). We concluded that the failure of PCR was due to the presence of heparin in the clinical samples. However, when the cDNA was treated with heparinase (4), PCR amplification was poor or negative (not shown).

We therefore investigated the effect of heparin on the activity of MLV reverse transcriptase. 0.1 unit of heparin per μg of control RNA (0.005 U/μl reaction) resulted in almost 100% reduction in the incorporation of ^{32}P-dCTP during reverse transcription. The same amount of heparin added to control cDNA also completely inhibited *Taq* polymerase. The inhibitory effects were readily reversed after treating the RNA with 3 units of heparinase I prior to reverse transcription.

We conclude that heparin inhibits both MLV reverse transcriptase and *Taq* polymerase. Incubation of heparin-contaminated RNA samples with 1−3 units of heparinase I (Sigma) per μg RNA (in 5 mM Tris pH 7.5, 1 mM $CaCl_2$, 40 units of RNasin (Promega) for 2 hours at 25°C) yielded successful PCR amplification which correlated with the concentration of heparinase in a dose dependent manner (Figure 1A, lanes 4−6). The heparinase treatment of RNA derived from frozen blood samples containing heparin before reverse transcription was therefore a necessary prerequisite for PCR amplification. Heparinase I was as effective as the more expensive heparinase II (not shown).

We have applied this approach to PCR analysis of a variety of RNA species extracted from heparinized bone marrow samples of patients with leukemia which were stored at −70°C as long as 10 years (Figure 1B). The employment of heparinase proved successful in detecting the abnormal expression of genes such as the multidrug resistance gene *mdr*1 (Figure 1B, lane 6) or the chimeric *bcr/abl* and *e2a/pbx*1 genes (Figure 1B, lanes 2 and 4) in patients with leukemia carrying the t(9;22) or the t(1;19), respectively. The method described therefore facilitates the utilization of frozen archive whole blood for the analysis of gene expression.

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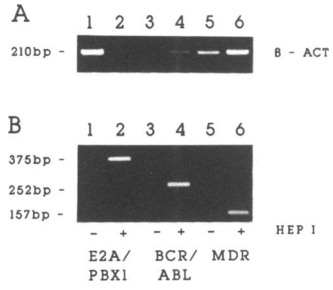

(A) The detection of β-actin (β-ACT) RNA derived from frozen whole blood (WB) by PCR: Lane 1 = controls non-heparinized WB; Lane 2 = heparinized patient WB; Lane 3 = Mixture of RNAs extracted from non-heparinized and heparinized WB in a ratio of 5:1; Lanes 4−6 = patient RNA from heparinized WB treated with 1, 2 or 3 units of heparinase I per μg RNA, respectively. (B) PCR analysis of various RNA species extracted from frozen heparinized whole bone marrow samples of patients with leukemia: Comparison of amplification with (+) or without (−) treating the RNA with heparinase I (HEP I).