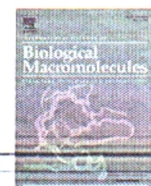
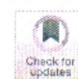
International Journal of Biological Macromolecules 209 (2022) 1298–1306

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac





Comparison of three methods including temperature, H_2O_2/ascorbic acid/sonication, and nitrous acid treatments for overcoming the inhibitory effect of heparin on DNA amplification in realtime-PCR

Pouya Alinezhad [a], Hamid Staji [a,*], Reza Narenji Sani [b]

[a] Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran
[b] Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran

ARTICLE INFO

Keywords:
Elimination of heparin inhibition
Realtime-PCR
Temperature
H_2O_2/ascorbic acid
Sonication
Nitrous acid

ABSTRACT

Heparin molecules have an inhibitory effect on DNA amplification by binding to the majority of DNA-interacting proteins. Different physical, chemical, and enzymatic methods have been used to degrade and depolymerize heparins in biomedical investigations. In this study, we aimed to evaluate some heparin degradation methods to eliminate the inhibitory effect of heparin on DNA amplification. Here, we report highly efficient, simple, and convenient methods to eliminate the heparin inhibitory effect on DNA amplification by treatments including temperature, nitrous acid, and H_2O_2/ascorbic acid/sonication. Further, treatment conditions including temperature degree and duration of treatments, the concentration of ascorbic acid, and intensity of sonication were reviewed. Target DNAs were extracted using the phenol-chloroform method. DNA concentrations and purity were analyzed before and after each treatment by Nanodrop spectrophotometry. DNA amplifications were attempted using a commercially available realtime-PCR mastermix. We found that the inhibitory behavior of heparin was well eliminated after the 85 °C/2 h, 65 °C/2 h, nitrous acid (pH = 3), and H_2O_2/ascorbic acid/sonication treatments, respectively. The further analyses indicated that the application of nitrous acid in pH = 1.5 and H_2O_2/ascorbic acid/sonication in higher ascorbic acid concentrations and sonication intensities lead to failure in DNA amplification due to the degradation of target sequences. From our experience, simple heat treatments or at the next level using nitrous acid and H_2O_2/ascorbic acid/sonication have enabled the detection and quantification of virus infection in heparin blood samples. These approaches may enable researchers to utilize blood taken in heparin tubes for genome amplification and diagnostic purposes.

1. Introduction

Molecular biology techniques are usually applied to detect a defined sequence of the genome, predominantly a gene, from an organism or tissue of interest and, thus, to identify the structure and function of this genetic element [1]. PCR (Polymerase chain reaction) as one of these techniques, is the most important new scientific automation to come along in the last hundred years. PCR, the quick, easy technique for amplifying limitless copies of any sequence of DNA, is one of those scientific advancements that warrants time-worn superlatives like revolutionary and breakthrough [2]. The PCR is a very rapid, sensitive, and enzymatic reaction and therefore sensitive to inhibitors. The existence of the PCR inhibitors, which include all materials that have a negative effect on the PCR, is a considerable obstacle to the PCR. Inhibitors can originate from the sample or may be introduced during sample processing or nucleic acid extraction. The major consequence of partly or totally PCR inhibition is a declined sensitivity or false-negative results, respectively [2,3].

PCR inhibitors are a heterogeneous group of chemicals, mostly organic compounds, for instance, bile salts, polysaccharides, urea, melanin as well as different proteins. Further, clinical specimens such as blood samples contain inhibitors including heparin, haemoglobin, hormones, IgG, lactoferrin, and myoglobin [3–5]. Heparin, a sulfated glycosaminoglycan, is one of the most widely used anticoagulants worldwide, and works by primarily inhibiting thrombin and/or Xa factor in blood coagulation cascade [6]. Due to the inhibitory effects of heparin on PCR reaction, EDTA (Ethylenediaminetetraacetic acid) containing tubes are preferred for blood sample collection in molecular

* Corresponding author at: Postal Code: 35131-19111, Iran.
E-mail address: hstaji@semnan.ac.ir (H. Staji).

https://doi.org/10.1016/j.ijbiomac.2022.04.113
Received 10 February 2022; Received in revised form 13 April 2022; Accepted 15 April 2022
Available online 21 April 2022
0141-8130/© 2022 Elsevier B.V. All rights reserved.