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Evaluating the Effect of Culture Supernatant of *Pseudomonas aeruginosa* on Removing the Inhibitory Effect of Heparin in Real-Time PCR Test

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Abstract

BACKGROUND: Heparin is a sulfated glycosaminoglycan. Blood is a common source for DNA detection in all kinds of samples, and anticoagulants such as heparin and ethylenediaminetetraacetic acid (EDTA) are used to prevent coagulation. Because heparin has a strong inhibitory effect on polymerase chain reaction (PCR), it is not used in samples that will be tracked by DNA. There are physical, chemical, and enzymatic methods to eliminate the inhibitory effect of heparin on PCR test.

OBJECTIVES: First, to compare the intensity of the inhibitory effect of two anticoagulants, heparin, and EDTA, on the Real-Time PCR (qPCR), and then to investigate the impact of the heparinase enzyme present in the medium culture extract of *Pseudomonas aeruginosa*, on removing the inhibitory effect of heparin during the real-time PCR.

METHODS: In the present study, two blood samples containing heparin and EDTA were subjected to a real-time PCR test to check the intensity of the inhibitory effect. Then, the medium culture extract of *Pseudomonas aeruginosa* was added to the heparinized blood sample infected with *Escherichia coli* bacteria in two groups with different conditions. In the first group, the DNA in the heparinized blood sample was extracted by the phenol-chloroform isoamyl alcohol method. Then, these samples were incubated with the extract of *Pseudomonas aeruginosa* bacteria culture medium at different hours, but in the second group, the samples were incubated at different hours before DNA extraction. Also, the DNA concentration in both groups was measured by a Nanodrop device, and finally, all samples were subjected to a real-time PCR test.

RESULTS: The results of the research samples showed that although the heparinized blood sample contains more DNA concentration than the EDTA blood sample, it completely prevents genome replication. Also, incubating heparinized blood with *Pseudomonas aeruginosa* culture medium extract before DNA extraction for more than 24 hours removes the inhibitory effect of heparin during the real-time PCR, even at a lower cycle threshold than the EDTA-containing sample.

CONCLUSIONS: The *Pseudomonas aeruginosa* culture medium extract may enable researchers to use heparinized blood samples for genome amplification and diagnosis without using expensive and limited commercial heparinase enzyme.

Keywords: EDTA, Glycosaminoglycan sulfate, Heparinase, *Pseudomonas aeruginosa*, qPCR

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Figure Legends and Table Captions

Figure 1. Comparing DNA Concentration Extracted From Blood Samples Containing Ethylenediaminetetraacetic Acid (EDTA) and Heparin in Serial Dilutions of 10^{-4} (S1) to 10^{-1} (S5).

Figure 2. *Escherichia coli* Genome Replication Curve Based on the Cycle Threshold (CT) of Three Groups of Physiological Serum, Blood Containing Ethylenediaminetetraacetic Acid (EDTA), and Heparin.

Figure 3. *Escherichia coli* Genome Replication Curve Based on the Cycle Threshold (CT) in Two Heparin A (HA) and Heparin B (HB)Groups in Different Durations (2, 4, 6, 8, and 24 h) and the Positive Control Sample (PC).

Figure 4. *Escherichia coli* Genome Replication Curve Based on Cycle Threshold (CT), HB Group in different Durations (48 and 72 h), and the Positive Control Sample (PC).

Figure 5. Standard Curve, Standard Samples Along With HB Treatment Group in Different Durations (24, 48 and 72 h) in Real-Time PCR Test.

Figure 6. Graphic Diagram of the Statistical Comparison of the Results of the Three Groups of Heparin B in Different Durations (24, 48, and 72 h) With the Sample Blood Containing Ethylenediaminetetraacetic Acid (EDTA).