Study of Potential PCR Inhibitors in Drinking Water from Riga Distribution System

B. Dalecka, L. Mezule and T. Juhna*

*Water Research Laboratory, Riga Technical University, Kipsalas 6A-263, Riga, Latvia
(E-mails: *brigita.dalecka_1@rtu.lv*; *linda.mezule@rtu.lv; talis.juhna@rtu.lv*)

INTRODUCTION

The high concentration of organic and inorganic groups of chemicals may occur in drinking water and inhibit polymerase activity and sequester DNA templates from the amplification reaction. Furthermore, to detect a low amount of pathogenic microorganisms in drinking water samples, large volumes of water are usually concentrated to very small volumes. This often results in concurrent concentration of the different inhibitors and increased interference with PCR (Green, 2012; Schrader, 2012).

To determine the inhibitory effect of potentially inhibiting substances present in the nucleic acid preparation, it has been suggested to carry out a PCR control reactions. The inhibition can be detected by changes in the threshold cycle (C_t), which indicates that lower concentrations of DNA are being amplified. Also, analysis of the PCR product is possible through a measurement of the melt characteristics of the amplicons where the change in the melt curve demonstrate modification of the PCR product (Opel, 2009).

The aim of this study was to identify if some of the chemicals abundant in drinking water from Riga have an effect on PCR assay sensitivity. In this study chlorine, humic acids and iron were examined and analysed with real-time PCR by adding different concentrations to the reaction mixture. The results of the threshold cycle (C_t) and melting curve values were compared in-between the standard curves constructed for a fecal indicator *Escherichia coli.*

MATERIALS AND METHODS

Bacterial strain and DNA extraction

Escherichia coli strain (ATCC®25922™) was grown aerobically at 37°C for 24 h in Tryptone soya broth (Oxoid, UK). The commercial kit GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) was used according to manufacturer's instructions for DNA extraction and control dilution series were prepared.

Real-time PCR analysis of inhibitors

The amplification was performed in 7300 Real Time PCR System (Applied Biosystems, USA). PCR reaction mixture of 25 µl contained 5 µl template DNA, 12.5 µl SYBR Green/ROX qPCR Master Mix (2x) (Thermo Scientific, Lithuania), 0.5 µl of primer pair EC_5 (5' AAAGCCGTGGCACAGGCAAGCGT-3') and $EC8c_2$ (5'-TCAATTTGTTATCGCTATCCAGTTGG-3') (Spierings, 1993) and required amount of PCR-nuclease free water. To enhance the effect of the chlorine, humic acids and iron, the require concentration of inhibitor was added last to reach a final reaction volume of 25 µL. Control (noninhibitors) dilution series were performed using the same protocol, with an equivalent volume of PCR water used in place of the inhibitor. In order to evaluate and compare the success and effectiveness of PCR inhibition, MS Excel 2013 was used for C_t average value and t-test statistical calculations. Each sample was tested in triplicates.

RESULTS

The average crossing threshold (C_t) values of chlorine tests demonstrated that there was no significant difference ($p>0.05$) with control dilution series. 5 mg/L and 1 mg/L of humic acids reduced the efficiency of the average crossing threshold (C_t) values while no significant change was observed for 0.3 mg/L ($p>0.05$). The obtained C_t results from the iron concentration of 4 mg/L clearly indicated PCR inhibition at the same time no significant change was observed from 0.2 and 0.1 mg/L ($p>0.05$).

Table 1. Inhibitor concentration based on the average crossing threshold (C_t) values

	C_t			
	Humic acid			
Dilution series	**Control (SD)**	**5 mg/L (SD)**	**1 mg/L (SD)**	**0.3 mg/L (SD)**
10^{-1}	12.22 (0.03)	19.66 (0.07)	14.29 (0.43)	12.36 (0.14)
10^{-3}	19.68 (0.14)	27.60 (0.38)	21.41 (0.05)	19.90 (0.19)
10^{-5}	27.24 (0.09)	36.33 (0.57)	29.41 (0.24)	27.53 (0.06)
	Iron			
		4 mg/L (SD)	**0.2 mg/L (SD)**	**0.1 mg/L (SD)**
10^{-1}	12.07 (0.07)	nd	12.09 (0.19)	11.97 (0.09)
10^{-3}	19.97 (0.14)	nd	20.10 (0.01)	20.06 (0.03)
10^{-5}	28.17 (0.12)	nd	28.30 (0.12)	28.34 (0.38)
	Chlorine			
		4 mg/L (SD)	**0.8 mg/L (SD)**	**0.08 mg/L (SD)**
10^{-1}	12.61 (0.13)	12.39 (0.03)	12.50 (0.22)	12.32 (0.20)
10^{-3}	19.57 (0.06)	19.53 (0.22)	19.46 (0.11)	19.59 (0.16)
10^{-5}	nd	26.69 (0.14)	nd	nd

nd – undetermined; SD = standard deviation

CONCLUSIONS

Three possible inhibitors were examined for the efficiency of drinking water sample analyse from Riga distribution system with real-time PCR. The results indicate that humic acids and iron inhibits the PCR reaction while no effect of chlorine possible inhibition was observed.

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