Extraction of mRNA from coagulated horse blood and analysis of inflammation-related cytokine responses to coagulation - DTU Orbit (14/12/2018)

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Coagulated blood is a rich source of mRNA that allows the study of the regulation of expression of cytokine and other genes. However, while several methods are available for isolation of RNA from whole blood and tissues, protocols for purification of mRNA from clotted blood are not generally available. Here, a protocol for RNA extraction from highly clotted blood was optimized and the regulation of a number of cytokine genes compared to stabilized blood was studied. Whole blood samples from 10 clinically healthy horses were incubated for 24 hours at 37°C and RNA was extracted from the peripheral blood mononuclear cells present in the blood clot, homogenizing the clot by rotating knife homogenization (GentleMACS, Miltenyi Biotec) in the presence of QIAzol extraction buffer (Qiagen). The RNA extracted yielded high concentrations of total RNA (50-265 ng/μl) and quality measures (RIN=8.5-9.2), comparable with that purified by standard methods from stabilized blood. Cytokine mRNA expression was assessed by reverse transcribed quantitative real time PCR and it was found that 24-hour clotting led to a significant increase in the concentrations of mRNA of the pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-1-receptor antagonist (IL-1ra), interleukin-15 (IL-15), and interleukin-8 (IL-8). These findings that a coagulation-induced inflammation-related cytokine response takes place in whole blood upon clotting. The extraction method provides reproducible and reliable results allowing the recovery of quantifiable high-quality RNA for molecular expression analysis.

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Bovbjerg, K. K. L., Heegaard, P. M. H., EFSA Publication
Number of pages: 224
Publication date: 2010

Host publication information
Title of host publication: 2010 Annual Meeting SLB & IEIIS : Abstracts
Electronic versions:
23e3af47-7e2e-4162-a01e-59af6b28e522.pdf
URLs:
http://leukocytebiology.org/default.aspx
Source: orbit
Source-ID: 274669
Research output: Research - peer-review » Conference abstract in proceedings – Annual report year: 2011