Presentation number: F28

TAQ POLYMERASE REVERSES INHIBITION OF QRT-PCR BY HUMIC ACID

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Aim: To investigate the influence of humic acid (HA) and extra addition of Taq polymerase on Quantitation Real Time PCR (QRT-PCR) and test method on DNA extracted from ancient bones. Methods: Weak amplification of human target and no amplification of Internal PCR Control (IPC) may indicate a partial PCR inhibition in the sample. When the template is extracted directly from bone or other samples, humic acid can inhibit Taq polymerase. Inhibition is an especially significant problem when DNA has to be extracted from old and ancient material. In this study, we described the dose-response effect of humic acid on QRT-PCR inhibition and the effect of extra addition of Taq polymerase in overcoming the humic acid inhibition. By performing that method we also evaluated ten ancient DNA extracts on the quantitation by QRT-PCR. Results: The addition 10 - 75 ng of synthetic humic acid (Fluka) can inhibit QRT- PCR while the addition of 100 ng of synthetic humic acid completely inhibits QRT- PCR. The addition of 1,25 Unit (U) of Taq polymerase per assay (25ml) appeared to be the optimum amount in overcoming the humic acid inhibition. The best results were obtained when crude DNA extracts containing humic substances were quantified by QRT-PCR, with extra addition of 1,25 Unit (U) of Taq polymerase per assay. Conclusion: This modified procedure (with extra addition of Taq polymerase) should allow more effective QRT-PCR analysis in humic acid-containing samples.