

OPTIMISATION AND COMPARISON OF THE STANDARD PCR AND THE REAL-TIME SYBR-GREEN HRM PCR FOR DETECTION OF CAV-2

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Abstract. The aim of this study was to optimise and compare the standard PCR method and the Real-Time Sybr-Green HRM PCR for detection of CAV-2. Studies were conducted on 20 CAV-2 vaccine isolates. DNA for molecular studies was isolated using a commercial isolation kit and a manual method with the use of phenol and chloroform. Due to the selection of suitable thermal conditions for individual reaction cycles and suitable reagent concentrations, both the PCR and the Real-Time PCR proved to be sensitive and specific techniques for detection of CAV-2 genetic material. The Real-Time PCR proved to be less time-consuming than the standard PCR, which is important from the clinical point of view. The performance of both reactions depended to a high extent on the DNA isolation method (the manual method showed a three times higher performance than the commercial kit). Presented results of the studies show that both the PCR and the Real-Time PCR are on target to become standard diagnostic methods for CAV-2 infections.

Keywords: CAV-2, dog, PCR, Real-Time PCR, molecular diagnosis.