# Application of Digital PCR for Evaluating the Performance of Nucleic Acid Extraction Kits in the Detection of Avian Paramyxovirus Serotype-1

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**Abstract**

**Background:** Newcastle Disease (ND), remains one of the most devastating infectious diseases affecting the global poultry industry. The disease is caused by the Newcastle Disease Virus, taxonomically as *Avian Paramyxovirus serotype-1* (APMV-1), a negative-sense, single-stranded RNA genome. Currently, real-time reverse transcription PCR (qRT-PCR) is the standard technique for routine diagnostics. Digital PCR (dPCR) represents a novel nucleic acid detection technology that enables direct absolute quantification of target molecules without the need for a standard curve. In this workflow, nucleic acid extraction is a critical step, as the quality and integrity of the extracted genetic material directly determine the efficiency and accuracy of molecular diagnostic outcomes.

**Objective:** This study aimed to apply qRT-PCR in conjunction with dPCR to evaluate the efficiency of various commercial nucleic acid extraction kits for the detection of APMV-1.

**Methods:** Three commercial kits, two magnetic bead-based (A and B) and one spin column-based (C) were evaluated. The reference virus used in this study was APMV-1/Queensland-V4 (lentogenic strain). By utilizing primers and probes specific to the *Matrix (M)* gene. Cloacal swab samples from poultry, pre-confirmed as negative for ND via qRT-PCR, were spiked the reference virus at concentrations 1.325 to 53,000 copies/µL of sample. Following extraction with each respective kit, diagnostic performance was evaluated using both qRT-PCR and dPCR. The evaluation criteria included the detection range, limit of detection (LOD), linear regression analysis ($R^2$), repeatability and recovery rates.

**Results:** Using qRT-PCR, extraction kits A, B, and C demonstrated a detection range of 2.65–53,000 copies/µL, with 100% limits of detection of 2.65, 1.325, and 2.65 copies/µL, respectively. All kits showed excellent linearity, with $R^2 > 0.996$, and good repeatability, with overall coefficients of variation (%CV) between 1.6% and 7.9%. In parallel with qRT-PCR, all samples were also evaluated using dPCR. By dPCR, all kits exhibited a detection range of 5.3–53,000 copies/µL, with 100% LOD values of 5.3, 1.325, and 2.65 copies/µL for extraction kits A, B, and C, respectively, and All kits showed excellent linearity, with $R^2 > 0.996$. In recovery testing, extraction kit B demonstrated significantly higher recovery rates than extraction kits A and C ($p < 0.05$).

**Conclusion:** The magnetic bead-based extraction kit B demonstrated outstanding performance for APMV-1 nucleic acid extraction, particularly when combined with dPCR, providing the highest sensitivity and recovery rates. These results support its use for developing highly accurate and reliable molecular diagnostic standards for Newcastle disease.

**Keywords:** Newcastle disease virus, Nucleic acid extraction kits, Digital PCR, Real-time RT-PCR

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