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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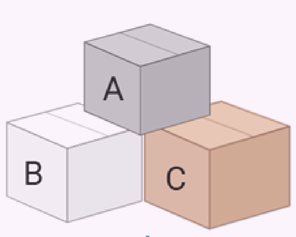
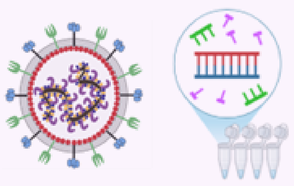
BACKGROUND

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). 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.

METHODS

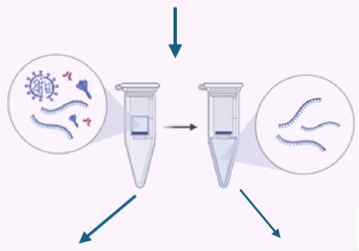


Three commercial kits, two magnetic bead-based (A and B) and one spin column-based (C) used in laboratory operations were selected for evaluation



The reference virus used in this study was APMV-1/Queensland-V4 (lentogenic strain) was prepared. By utilizing primers and probes specific to the Matrix (M) gene

The initial viral concentration was determined under conditions optimized to eliminate matrix effects



Spiked the reference virus at concentrations 1.325 to 53,000 copies/µL of cloacal swab

Viral RNA was extracted using different commercial kits: Kit A, B, and C

RNA amplification was quantified using qRT-PCR and dPCR assays 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 values are 0.9963, 0.9951, and 0.9976, respectively. , 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, R^2 values are 0.9994, 0.9989, and 0.9967 respectively. In recovery testing, extraction kit B demonstrated significantly higher recovery rates than extraction kits A and C ($p < 0.05$).

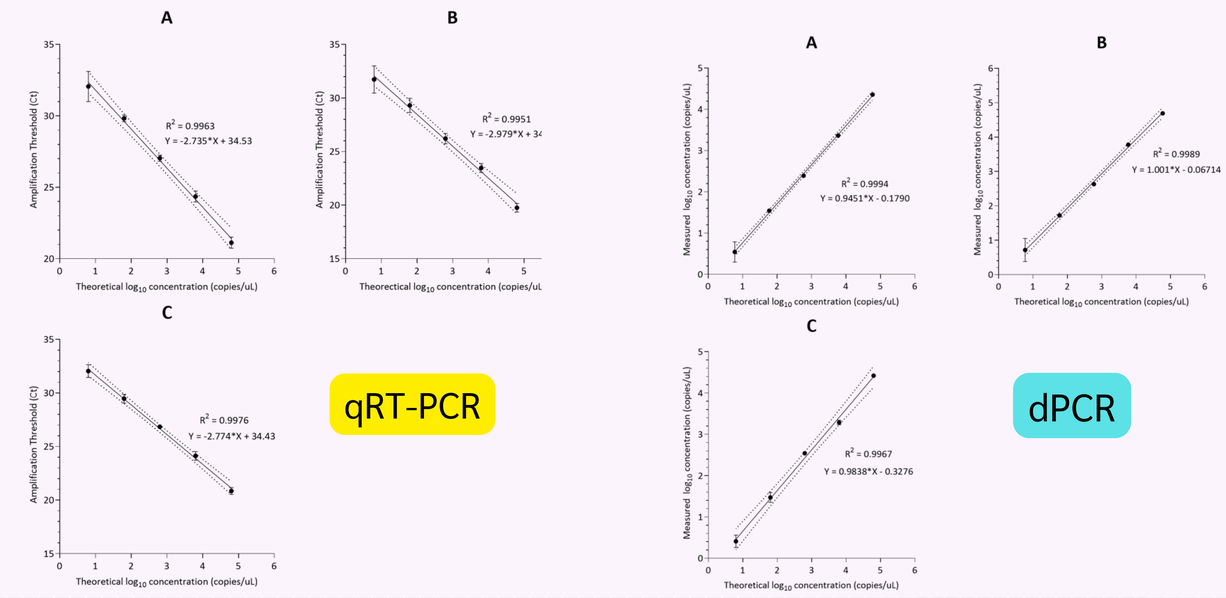
RESULTS (cont.)

qRT-PCR

Virus count	Brand A			Brand B			Brand C		
copies/ul	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
$5.3x10^4$	21.409	21.272	20.673	20.220	19.500	19.554	20.811	20.539	21.178
$5.3x10^3$	23.937	24.441	24.686	22.973	23.696	23.708	23.940	23.836	24.585
$5.3x10^2$	26.912	26.897	27.284	26.051	25.835	26.742	26.844	26.744	26.931
53	29.556	29.983	29.922	29.926	29.400	28.602	29.095	29.403	29.898
5.3	30.872	32.411	32.890	33.121	31.426	30.630	32.716	31.815	31.595
2.65	32.591	32.313	32.521	33.386	31.384	31.779	32.342	32.499	32.577
1.325	34.553	33.157	UND	33.682	32.919	33.285	32.815	UND	UND
0 (NTC)	UND	UND	UND	UND	UND	UND	UND	UND	UND

dPCR

Virus count	Brand A			Brand B			Brand C		
copies/ul	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
$5.3x10^4$	21,900.00	23,600.00	22,800.00	42,900.00	50,600.00	54,600.00	25,000.00	28,700.00	25,700.00
$5.3x10^3$	2,230.80	2,393.80	2,320.60	5,816.40	6,159.80	6,076.70	2,232.90	1,838.90	1,765.10
$5.3x10^2$	261.90	219.50	260.90	382.60	460.10	445.60	329.00	379.00	338.30
53	33.27	36.44	34.92	46.16	58.42	53.80	25.59	40.56	25.02
5.3	5.10	1.83	4.56	2.56	11.89	4.57	2.56	1.83	3.65
2.65	1.68	UND	0.93	3.36	3.64	5.55	2.52	0.91	0.92
1.325	UND	UND	0.90	2.51	4.45	1.79	UND	0.89	1.79
0 (NTC)	UND	UND	UND	UND	UND	UND	UND	UND	UND



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.

ACKNOWLEDGEMENTS

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