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**Novel approach to assess parvovirus disinfection efficacy by aptamer-based assay**

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**Abstract:**

**Background/Objective:** This study aimed at assessing the potential of nucleic acid aptamer in distinguishing between intact virus and physically/chemically deformed virus particles.

**Methods:** An automated single-stranded (ss)-DNA SELEX (systematic evolution of ligands by exponential enrichment) was performed on recombinant (r)-VP2 (30-553 amino acid of 584 amino acids) protein of canine parvovirus 2a (CPV-2a) for 12 cycles. Enrichment of possible ssDNA binder was evaluated by sequencing of the nucleic acid product generated through SELEX. Abundant sequences by rank were tested by incubation with coated rVP2 and CPV virus (both in intact form and after treatment with heat and peracetic acid (PAA) solution) followed by PCR amplification and gel electrophoresis. Cross-binding specificity of the candidate sequences were evaluated by using intact and heat-treated feline parvovirus (FPV) and porcine parvovirus (PPV).

**Results:** Mid-cycle (6th) and end-cycle (12th) sequencing of SELEX products revealed enrichment of individual sequences having maximum coverage of ~9% and ~1% of the total sequence in the 12th cycle and 6th cycle, respectively, compared to the <0.01% maximum coverage in the 1st cycle. However, upon replacement of rVP2 with CPV, only heat-treated (at 95 ºC) and disinfectant (~5% PAA) treated CPV but not the intact CPV showed binding interaction with the candidate sequences as observed by gel electrophoresis. This was supported by lack of CPV antibody binding sites in rVP2, heat-treated CPV and ~5% PAA treated CPV compared to intact CPV as indicated by OD450 measurement (p < 0.01). No cross-binding activity of the candidate sequences was found for either of intact and heat-treated forms of FPV and PPV.

**Conclusion:** The binding interaction of the aptamer candidates to heat and chemical denatured CPV rather than intact CPV together with no cross-binding to closely related virus indicates the capability of the aptamer candidates to distinguish between intact and deformed CPV.

**Keywords:** parvovirus, disinfection, aptamer assay