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DETECTION OF INFECTIOUS PANCREATIC NECROSIS VIRUS BY  
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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A microplate double antibody, sandwich enzyme-linked immunosorbent assay (ELISA) was employed for the detection of infectious pancreatic necrosis virus (IPNV).

The minimal dose to detect IPNV-VR299 strain by the method was about 10 to 20 ng protein or  $10^4$  50% tissue culture infective dose per well. No reaction was observed against infectious hematopoietic necrosis virus or supernatant fluid from normal cell cultures. The specificity of ELISA was also confirmed by the blocking test.

The antigenic relationships among five strains of IPNV were examined by ELISA and found to be divided into three groups, namely: (i) VR299 strain, (ii) Buhl and Bonnamy strains, and (iii) Powder Mill and d'Honnincthun strains. When the viral polypeptides of five strains of IPNV were analyzed by SDS-polyacrylamide gel electrophoresis, the strains were also divided into three groups by the molecular weight of protein designated as  $\beta$  (VP<sub>2</sub>). This result was consistent with the reaction patterns obtained by ELISA described above.

A group of rainbow trout fry was experimentally infected with IPNV-VR299 strain and then examined for infection by virus isolation *in vitro* and detection of IPNV antigen by ELISA. In an early stage of the infection, the infective titers and antigen titers in the fishes corresponded well with each other. After one week post inoculation, the virus titer decreased rapidly, but IPNV antigen was consistently present in the host during the entire period of the experiment. This finding indicates that the virus antigen still remained in the body after inactivation of the infective virus.

The results obtained in the present study provide evidence that ELISA is a rapid and reliable method for diagnosis of IPN in both the laboratory and the field.