

MAFF project FC1136: Research on the identification, diagnosis and significance of notifiable and emerging virus diseases of fish and shellfish

Application of new molecular biology methods for detection of fish pathogenic viruses

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In many studies we need to determine the tissues in which pathogens can be found. This is relatively simple in the case of large pathogens such as many bacteria, protists, metazoan parasites etc., as they can be readily visualised in histological sections of tissues under a light microscope. However, viruses cannot be visualised directly under a standard light microscope as they are extremely small. For instance, the diameter of one infectious pancreatic necrosis virus particle is 60 millionths of a millimetre. As we cannot visualise viruses directly under a light microscope, indirect methods of detecting viruses have been developed. Until recently, such methods were based on detecting the protein(s) which make up the outer coat of the virus, and are found in infected cells. The protein is detected using a specific antiserum that has been labelled in some way e.g. with a fluorescent tag, or an enzyme that produces a particular colour reaction when exposed to a specific chemical. The problem with that approach is that it is not always possible to produce antisera against all of the viruses of interest.

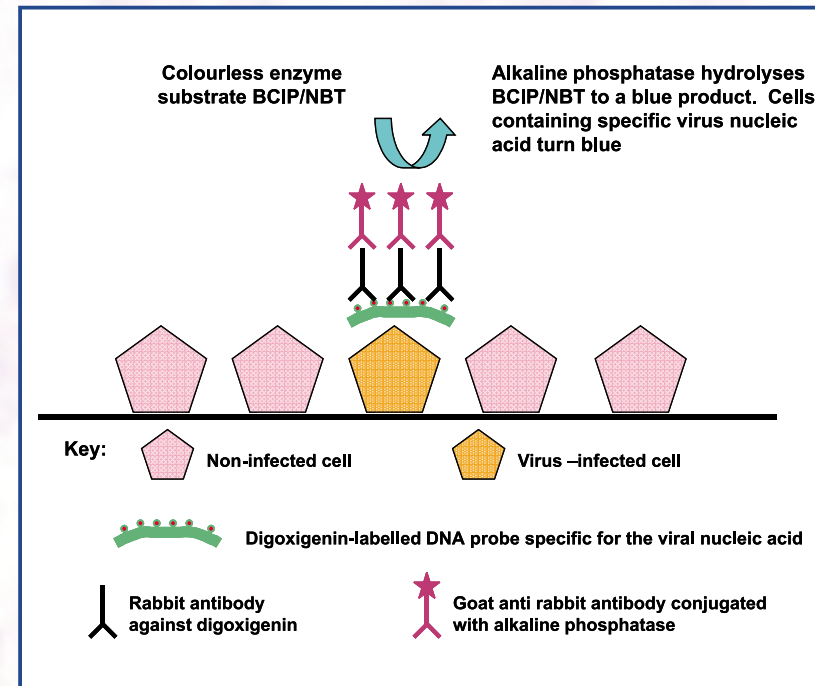


Figure 1. Principle of in situ hybridisation. Cells or tissue sections are attached to histological slides, and a previously constructed digoxigenin labelled DNA probe binds specifically to complementary sequences of the virus nucleic acid, if present in those cells or tissues. Excess probe is washed away and the probe attached to the viral nucleic acid is detected with a sandwich of antibodies (Ab). The first Ab is specific for the digoxigenin on the probe and the second Ab is specific for the first Ab. This second Ab is conjugated to an enzyme (e.g. alkaline phosphatase, AP). Finally, the slides are incubated with BCIP/NBT, a substrate of AP. The enzyme activity causes the substrate to turn blue, so cells containing the virus nucleic acid appear blue when the slides are examined under a microscope.

With the advent of molecular biology techniques, a new approach has been made possible. Instead of detecting the protein coat of the virus, the nucleic acid contained within that coat is detected. Each virus type has a unique sequence of molecules, called nucleotides, that make up its "genetic code". Once we know that sequence we can produce gene probes that will attach specifically to the nucleic acid of the virus. We can label the gene probe with a fluorescent tag, or an enzyme (as done with antibodies), and in the technique known as in situ hybridisation (ISH) (Fig 1), we can detect and localise the virus in histological sections of tissues under a light microscope. At the CEFAS Weymouth Laboratory we have developed the ISH method for viral haemorrhagic septicaemia virus (VHSV) (Fig 2), spring viraemia of carp virus (SVCV) (Fig 3) and koi herpesvirus (Fig 4). We are also using an ISH method for infectious salmon anaemia virus (ISAV) (Fig 5) developed by colleagues at the FRS Laboratory, Aberdeen. As well as providing information on the pathogenesis of the virus (e.g. VHSV was detected by ISH in the gills of fish one day post-infection, Fig 6), the ISH method can be used in virus diagnosis, particularly when the virus cannot be detected by the standard virus isolation method. The ISH method can also be used to confirm virus diagnosis when new non-approved methods such as the polymerase chain reaction are used.

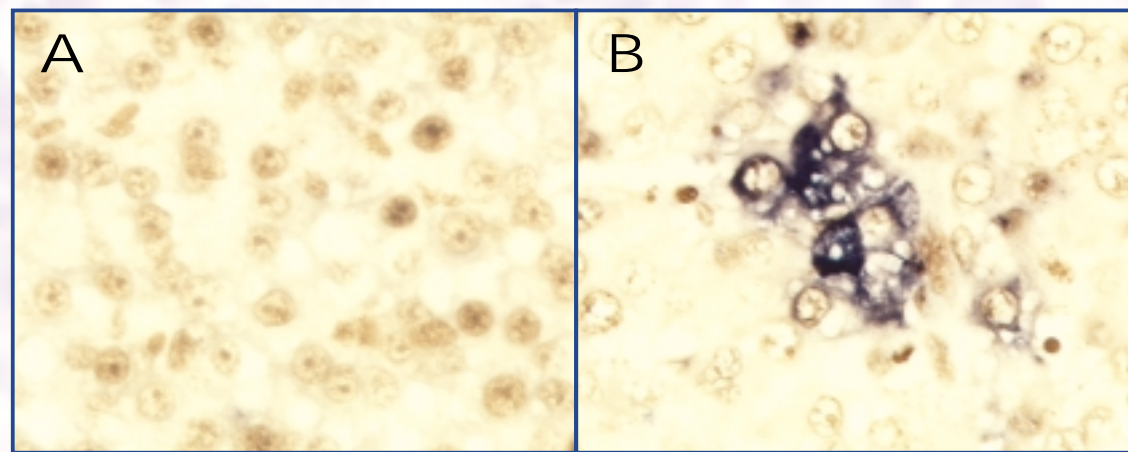


Figure 2. Detection of VHSV in rainbow trout liver. A) control, B) blue colour shows VHSV-positive cells in infected tissue.

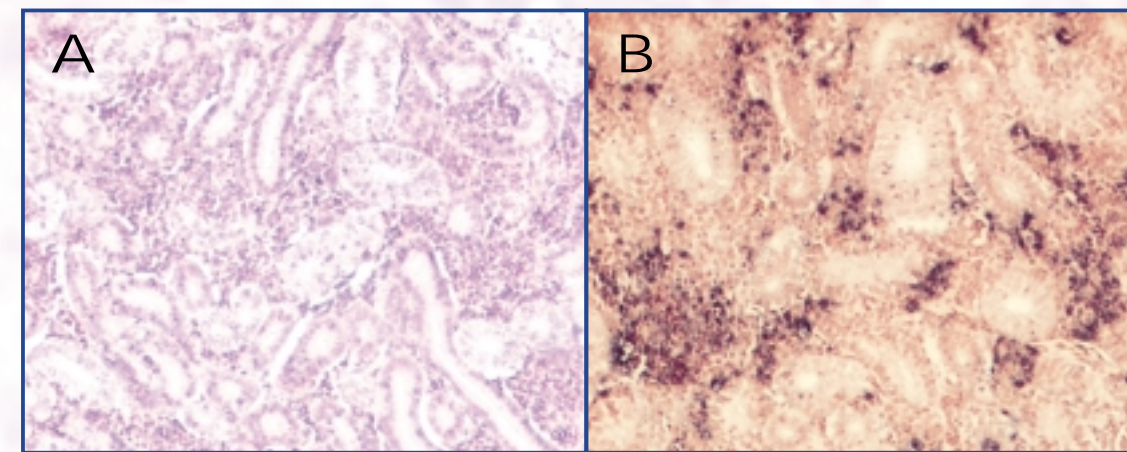


Figure 3. Detection of SVCV in carp kidney. A) control, B) blue colour shows SVCV-positive cells in infected tissue.

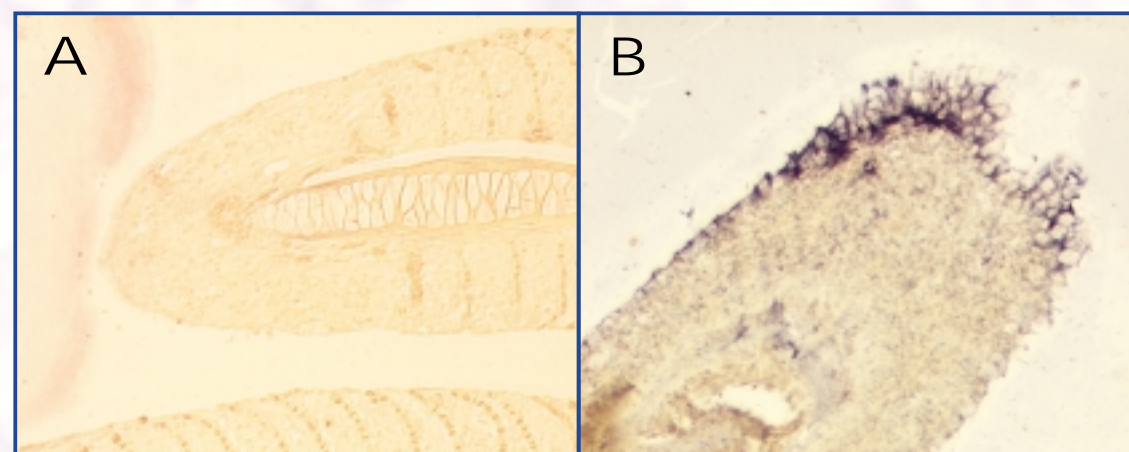


Figure 4. Detection of koi herpesvirus in koi carp gill. A) control, B) blue colour shows koi herpesvirus-positive cells in infected tissue.

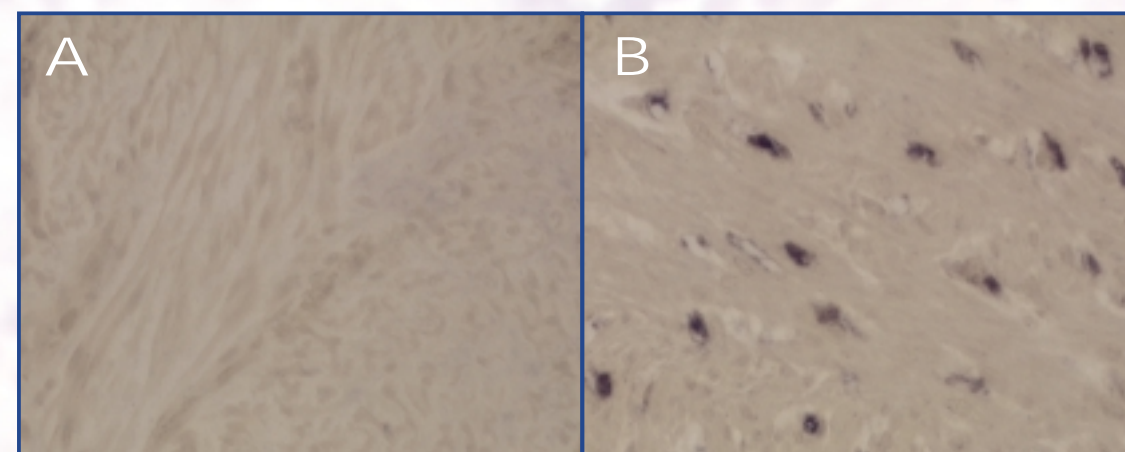


Figure 5. Detection of ISAV in Atlantic salmon heart. A) control, B) blue colour shows ISAV-positive cells in infected tissue.

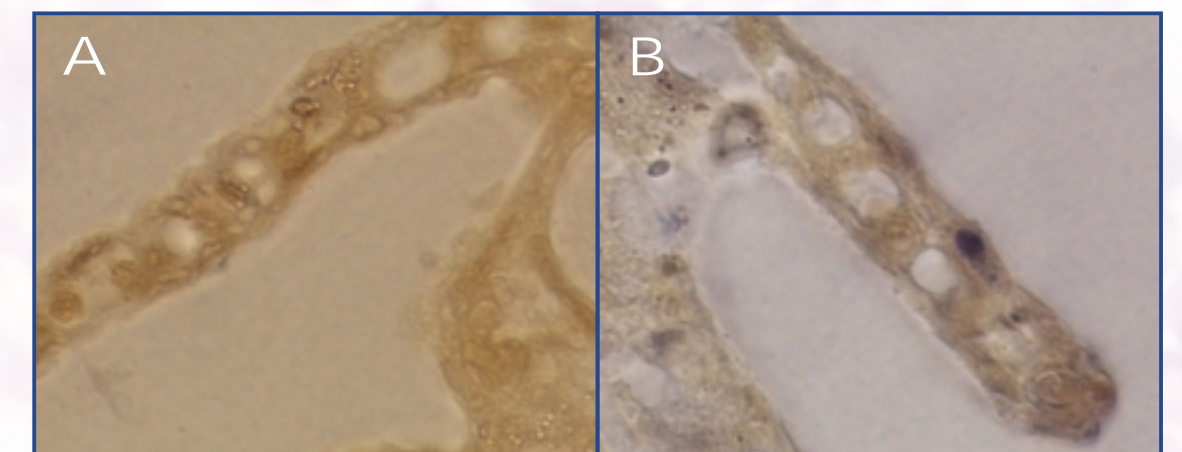


Figure 6. Detection of VHSV in turbot gill one day post infection. A) control, B) blue colour shows VHSV-positive cells in infected tissue.

ACKNOWLEDGEMENTS

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