marinescotland
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Scottish Government
Riaghaltas na h-Alba
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VIRUS DIAGNOSTICS AT MARINE SCOTLAND

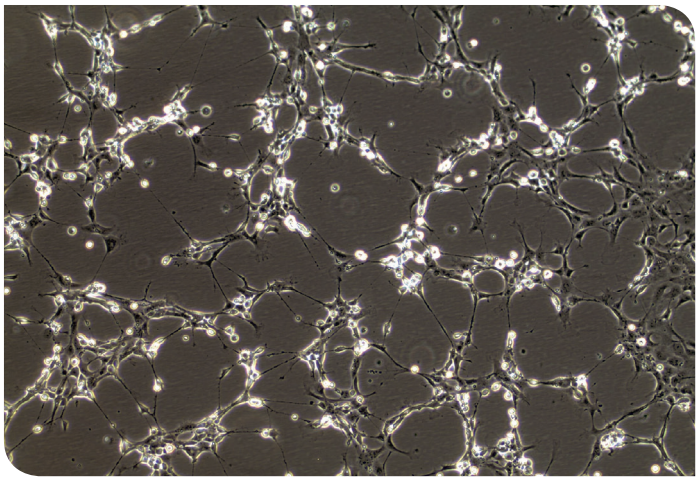

FIGURE 1
VHSV INFECTED BF-2 CELLS DEMONSTRATING CPE.

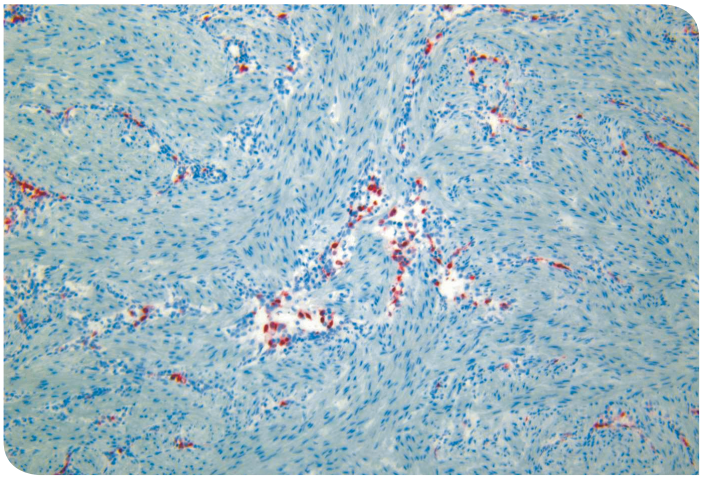

FIGURE 2
AN ISAV IHC POSITIVE HEART SECTION. ISAV POSITIVE CELLS INDICATED BY RED STAINING.

Introduction

Marine Scotland Science (MSS) staff carry out diagnostic and surveillance tests for numerous viruses including those listed in Council Directive 2006/88/EC, infectious haematopoietic necrosis virus (IHNV), infectious salmon anaemia virus (ISAV), koi herpesvirus (KHV), spring viraemia of carp virus (SVCV), white spot syndrome virus (WSSV) and viral haemorrhagic septicaemia virus (VHSV). The group also responds to requests for testing for other viral pathogens, such as, aquareoviruses, infectious pancreatic necrosis virus (IPNV), piscine nodaviruses, salmonid alphaviruses (SAV) and oyster herpesvirus (OsHV). All diagnostic investigations undertaken by MSS include a full histopathological screen of moribund animals.

Virus testing methods

Infectious heamatopoietic necrosis virus (IHNV) and Viral haemorrhagic septicaemia virus (VHSV)

MSS Fish Health Inspectorate (FHI) sample brain or heart, spleen and kidney from immature fish, and gonadal fluids from mature fish for IHNV and VHSV screening. Tests are carried out by cell culture isolation on fathead minnow (FHM) cells for IHNV and bluegill fibroblast (BF-2) cells for VHSV. Microscopic examination of cell cultures for cytopathic effect (CPE) is performed (Fig. 1). A real-time reverse transcription PCR assay is also available to screen samples for VHSV.

Identification of both viruses is made by an enzyme-linked immunosorbent assay (ELISA). The test normally takes 14 days for negative screening.

Infectious salmon anaemia virus (ISAV)

A suite of laboratory assays is used to confirm the presence of ISAV from tissue material. The primary screening test used to detect ISAV is a real-time reverse transcription PCR targeting ISAV segment 8. If a positive sample is identified by PCR it is then confirmed as

ISAV by gene sequencing. Other diagnostic tests applied by MSS to confirm ISAV include, an immunohistochemistry assay on tissue sections that demonstrates pathological changes consistent with ISA (Fig. 2), and virus isolation using an established Atlantic salmon leucocyte cell line (TO), with CPE confirmed as ISAV by an immunofluorescent antibody test (IFAT) using a specific antibody.

Spring viraemia of carp virus (SVCV)

Brain, spleen and kidney tissues are sampled from fish species susceptible to SVCV infection. SVC virus is detected by cell culture isolation using EPC or FHM cells. Cell cultures are observed microscopically for CPE and virus identification is made by ELISA. The test normally takes 14 days for negative screening.

White spot syndrome virus (WSSV)

White spot disease (WSD) is an infection of decapod crustaceans caused by the virus white spot syndrome virus (WSSV). Samples of gill material are screened by real-time PCR and positive samples are confirmed as WSSV by gene sequence analysis.

Piscine nodaviruses v

The causal agents of viral encephalopathy and retinopathy (VER), also termed viral nervous necrosis (VNN) are classified as piscine nodaviruses. Piscine nodaviruses can infect many fish species including Atlantic salmon and several marine species such as cod, haddock, halibut and turbot. Piscine nodaviruses can be detected by cell culture isolation on the E-11 cell line. Virus identification is carried out by IFAT using a specific antiserum (Fig. 3). A reverse transcriptase PCR is also used for the rapid detection of piscine nadaviruses from tissue samples.

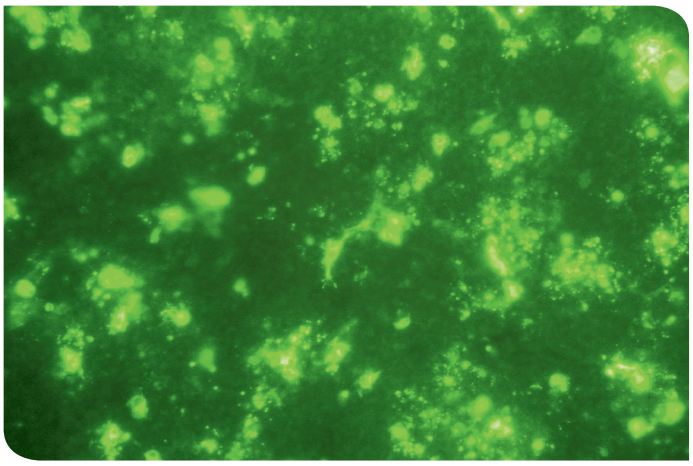

FIGURE 3
PISCINE NODAVIRUS IFAT. POSITIVE E-11 CELLS INDICATED BY GREEN FLUORESCENCE.

Sleeping disease virus (SDV) and salmon pancreas disease virus (SPDV)
The two salmonid alphaviruses SDV and SPDV are genetically very closely related and regarded as types of the same genus. Virus identification is carried out using real-time reverse transcription PCR (Fig. 4).

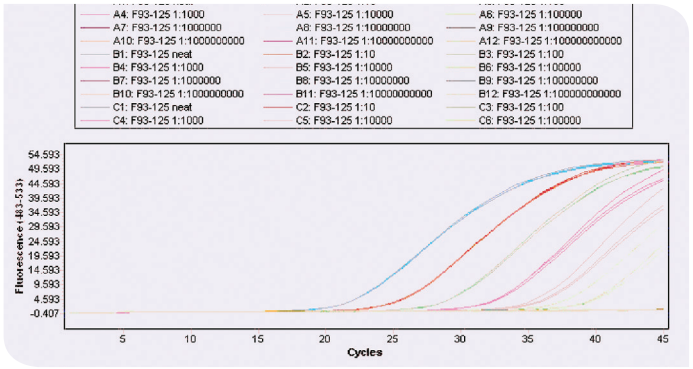

FIGURE 4
SAV REAL-TIME PCR AMPLIFICATION PLOT (SCREEN SHOT FOR ILLUSTRATIVE PURPOSES ONLY).

Oyster herpesvirus (OsHV)

Oysterherpes virus type 1 (OsHV-1) infection has caused considerable mortality levels in larvae and juveniles of several bivalve species. Gill and mantle tissues are screened using a conventional PCR and positive samples are confirmed as OsHV-1 by sequence analysis.