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# HISTOLOGY IN FISH DISEASE DIAGNOSIS

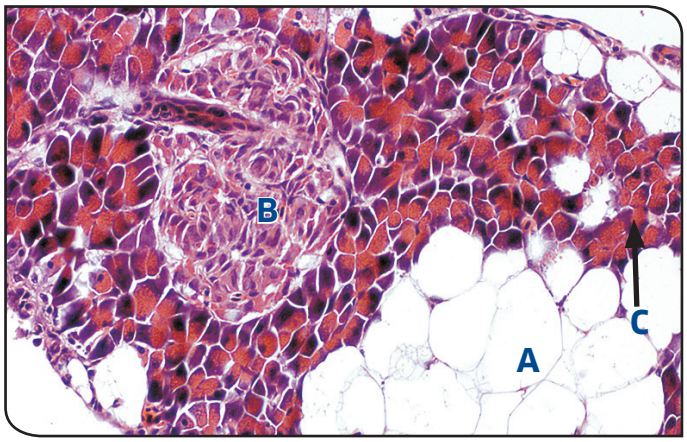


FIGURE 1.
HAEMATOXYLIN AND EOSIN (H&E) STAINING OF NORMAL SALMON. PANCREAS. A= FAT BODY, B = ENDOCRINE PANCREAS, C = EXOCRINE PANCREAS.

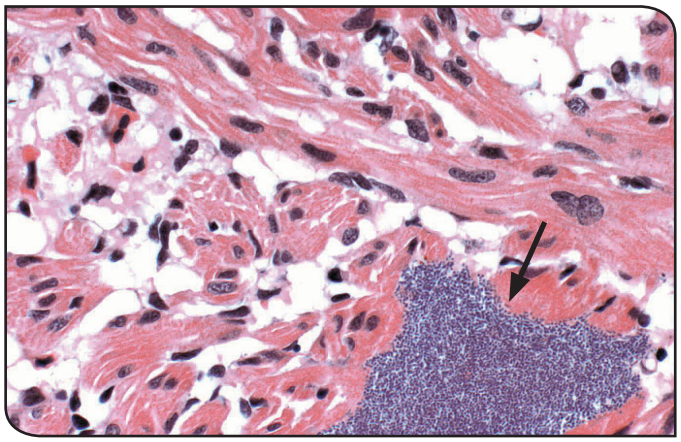

FIGURE 2.
H&E STAIN DEMONSTRATING BACTERIA (ARROW) IN RAINBOW TROUT HEART.

## What is histology?

Histology involves the microscopic examination of thin, stained tissue sections in order to study their structure and function and, in the case of histopathology, to determine changes which may be due to pathogens and disease.

Marine Scotland Science staff carry out diagnostic and research histology and have a key role in disease diagnosis. Stained fish tissue sections are prepared and examined by light microscopy and any tissue changes resulting from infectious or non-infectious disease are identified and described. Immunohistochemical methods are also used to detect specific pathogens in tissue sections.

## Preparation

Following sampling, fish tissues are placed in an aqueous fixative. This fixative preserves the morphology (structure and chemical constituents) of tissues and cells, so that they are capable of withstanding further preparatory steps without change. It is essential that tissues are fixed within a very short time after death to avoid disintegration of tissues or cells by the action of their own enzymes.

Following fixation, tissues are gradually dehydrated to remove any tissue water, using a series of graded alcohols. The tissues are then 'cleared', which involves treatment with a substance that mixes completely with both the dehydrating fluid and the embedding agent. Next the tissues are embedded in molten paraffin wax and cooled to harden the wax so that thin sections can be cut using a microtome and then mounted onto glass microscope slides. The wax is removed from the sections before staining.

## Staining

After clearing and rehydration, the tissue sections can be stained using biological stains or dyes. Haematoxylin and Eosin (H&E) is the most widely used histological stain because of its ability to reveal a wide range of different tissue components (Figs. 1 and 2).

Gram's stain is a staining method for differentiating bacteria. The technique is based on the capability of bacteria cells walls to retain the crystal violet dye in the Gram stain during solvent treatment. The cell walls for Gram positive micro-organisms retain the primary violet as they have a higher peptidoglycan (sugars) and a lower lipid content than Gram negative bacteria (Fig. 3).

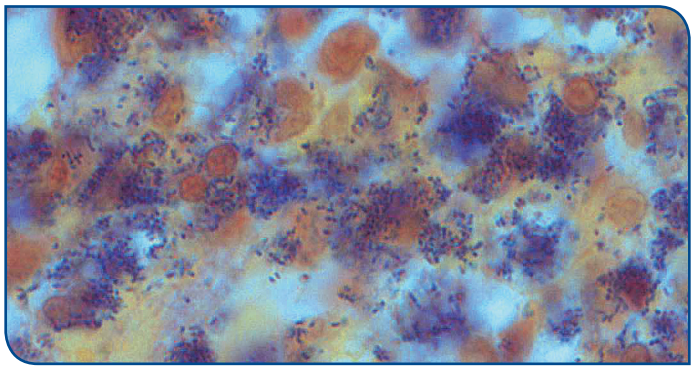

FIGURE 3.
GRAM STAINING DEMONSTRATING GRAM POSITIVE BACTERIA IN TROUT PANCREAS.

Grocott's silver reaction is used to demonstrate certain carbohydrates that are present in the fungal wall, and provide identification of infecting fungus in fish tissues. The positive material stains brown to black (Fig. 4).

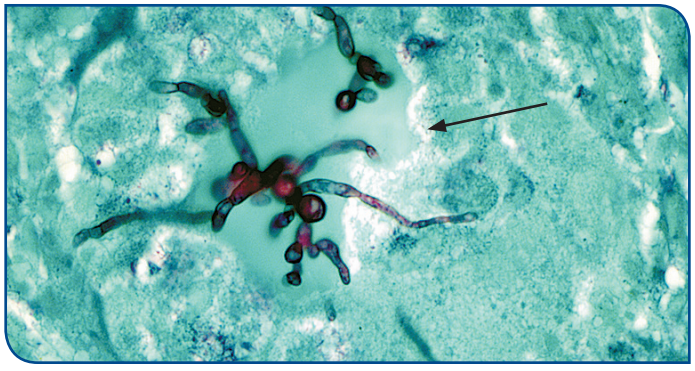

FIGURE 4.
GROCOTT'S SILVER STAINING TO SHOW FUNGAL (OOMYCETE) HYPHAE (ARROW) WITHIN RAINBOW TROUT KIDNEY.

## Immunohistochemistry

Immunohistochemical staining methods have been developed for the detection of viruses such as Infectious Pancreatic Necrosis virus (IPNV), Infectious Salmon Anaemia virus (ISAV) and nodavirus in paraffin-embedded tissue sections. Viral antigen is localised by an antibody raised against the virus and subsequent detection steps result in a coloured product that can be visualised by light microscopy (Fig. 5).

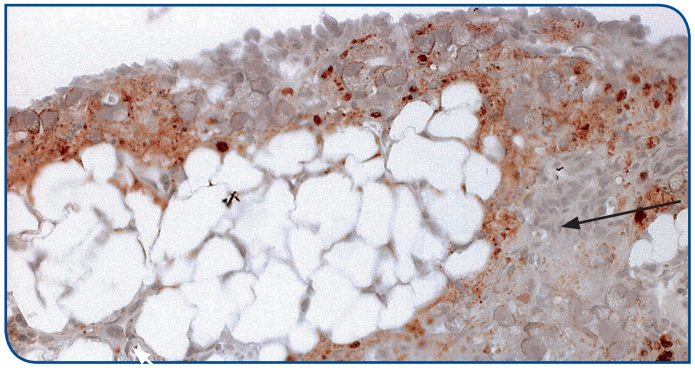

FIGURE 5.
IMMUNOHISTOCHEMICAL STAINING FOR THE DETECTION OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) IN SALMON PANCREAS (ARROW).

## Summary

Histological techniques enable the description of tissue pathology and highlight the sequence of cellular changes and their progression caused by infectious and non-infectious diseases. By examining stained sections, bacteria, fungi and parasites can be identified, and using immunohistochemical techniques, certain infectious agents can be detected in tissue sections. The increased use of digital scanning image analysis tools by MSS allows qualitative data to be generated to enhance disease diagnosis.


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