Histology in Fish Disease Diagnosis

Introduction
Staff in Fisheries Research Services’ (FRS) Bacteriology and Pathology Group carry out diagnostic and research histology and have a central role in disease diagnosis. Stained fish tissue sections are prepared and examined by light microscopy. 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.

What is Histology?
Histology is a branch of biology that 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.

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 (Fig. 1).

Gram's stain is a staining method for differentiating microorganisms. The technique is based on the capability of bacteria cell 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. 2).

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**Figure 1.** Haematoxylin and eosin (H&E) staining of normal salmon pancreas. A = fat body, B = endocrine pancreas, C = exocrine pancreas.

**Figure 2.** Gram stain demonstrating Gram negative bacteria (arrow) in rainbow trout heart.
The Periodic Acid-Schiff (PAS) reaction is used to demonstrate certain carbohydrates that are present in some tissues, and provide identification of infecting fungus in fish tissues. The PAS positive sites stain pink/red (Fig. 3).

**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. 4).

**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, viruses, bacteria, fungi and parasites can be identified, and using immunohistochemical techniques, certain infectious agents can be detected in tissue sections. The increased use of image analysis tools by FRS allows qualitative data to be generated to enhance disease diagnosis.

Figure 3. Periodic Acid-Schiff (PAS) staining to show fungal hyphae (arrow) within rainbow trout kidney.

Figure 4. Immunohistochemical staining for the detection of infectious pancreatic necrosis virus (IPNV) in salmon pancreas (arrow).