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# Wet-Mount Sampling Techniques in Fish

# 25

Diagnostic cytology plays a major role in the diagnosis and management of diseases affecting mammals, birds, and reptiles, whereas diagnostic microscopy using wet-mount techniques plays a major role in the diagnosis and management of fish and amphibian diseases. Diseases readily diagnosed using wet-mount preparations include ectoparasite infestations, lymphocystis, fungal infections, and certain bacterial infections such as columnaris disease (*Flexibacter columnaris*).

## Sampling Techniques

Common samples used for wet-mount preparations in the evaluation of fish and amphibian patients include the mucus smear (skin scraping), fin biopsy (fin clip), gill biopsy (gill clip), and fecal. Fluid samples, such as coelomic fluid, are also collected and evaluated in the same manner as for other species and not as wet-mount preparations. Blood collection has been discussed in Chapter 12.

Fish and aquatic amphibians should be handled quickly (less than 1 minute) and safely during an examination and for sample collection. These animals tend to writhe violently when handled, which predisposes them to traumatic injury. Also, the mucus layer covering the skin makes the skin slippery and proper restraint difficult and unsafe. Safe handling may require sedation or general anesthesia to prevent excessive stress and trauma to the patient. Tricaine methanesulfonate (MS222 Sigma Chemical Co., Saint Louis, MO) in a concentration of 100–150 mg/L is commonly used for fish anesthesia. Higher concentrations are generally required for amphibians (500 mg/L to 3 g/L) (Wright, 2006). Special precautions should be given to the acidification of tricaine-treated water that is poorly buffered. The acidosis created by unbuffered tricaine can cause potentially harmful metabolic changes in the patient (Smit et al., 1979; Groff and Zinkl, 1999).

Latex surgical gloves without powder should be used when handling these animals to protect their delicate skin from the trauma. Fish and amphibian skin is sensitive to human handling, and the natural oils, soaps, lotions, alcohol, and other chemicals on the handler's skin will damage the skin of these animals. The mucus layer that covers the skin of aquatic animals is a protective surface that should be preserved during handling. Using dry paper or cloth towels, cotton gloves, or bare hands during handling will remove an excessive amount of the mucus coating and damage the skin. Latex gloves should be rinsed with water, either from the fish tank or with distilled water, before handling a fish or aquatic amphibian. It should be noted that some salamanders have tail autotomy sites that result in the loss of the tail with improper handling (Wright, 2006).

An examination includes visual evaluation of the overall body condition, skin, and external structures. The fins are spread for a thorough examination. The oral cavity should be evaluated by carefully opening the mouth. The gills of fish are examined by lifting the opercular covering. The external gills of some aquatic amphibians are examined by direct visualization.

## Mucus Smear

The mucus smear is the most commonly used and useful sampling method for the evaluation of the fish patient. Because the skin is the first line of defense against infectious agents, cutaneous lesions are common. Suspected cutaneous bacterial or fungal infections and infestations with ectoparasites are common indications for the evaluation of a mucus smear. Ectoparasites irritate or damage the skin causing epithelial hyperplasia and increased mucus production, which causes the skin to appear thickened and cloudy.

The procedure is simple; a sample of the mucus covering the surface of the skin is obtained by either pressing

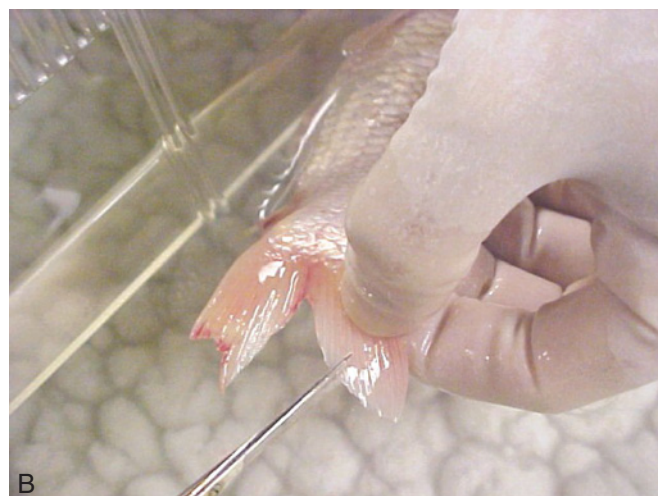


**Fig. 25.1.** Obtaining a sample for a mucus smear from a fish (*Cyprinus carpio*).

the surface of a clean microscope slide onto a skin lesion or a small sample of mucus is gently scraped from the skin surface. A scraping can be obtained by using a dull scalpel blade, the blunt edge of a scalpel blade, a spatula, or the edge of a microscope slide. The scraping motion should be performed in a cranial to caudal direction with the normal lay of the scales (Figure 25.1). A light scraping using gentle pressure should be emphasized as this technique is not analogous to a mammalian skin scraping, but rather a method of harvesting a small mucus sample. Excessive pressure applied during the process will damage the skin. After the mucus sample has been transferred to the surface of a microscope slide, a drop of the tank water is added before the addition of the coverslip. The sample is examined under the microscope as a wet-mount preparation. Wet-mount preparations should be examined immediately following sample collection because many of the pathogenic protozoal parasites die quickly under the microscope. A drop of methylcellulose solution (Carolina Biological Supply Company, Burlington, NC) can be added to the sample to slow fast-moving protozoa to aid in their identification. Wet-mount preparations are used to identify ectoparasites, fungi, and bacteria and are more useful in fish diagnosis than air-dried smears stained with Romanowsky stains. If there is a delay in the evaluation of the sample, then the slide should be stored in a humid chamber, such as a plastic container containing moist gauze or towels, to prevent drying.

### Fin Biopsy

A fin biopsy or fin clip is performed when visual lesions are observed involving the fin or a mucus smear has failed to identify the cause of a generalized skin

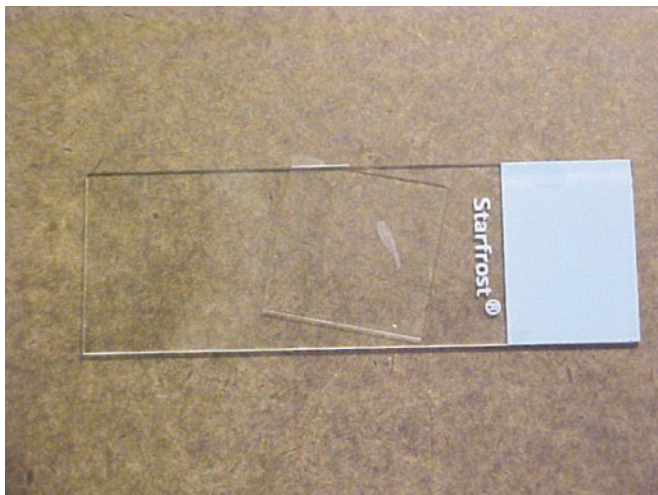


**Fig. 25.2.** (a) Obtaining a fin biopsy from a fish (*Oncorhynchus mykiss*); (b) obtaining a fin biopsy from a fish (*Cyprinus carpio*).

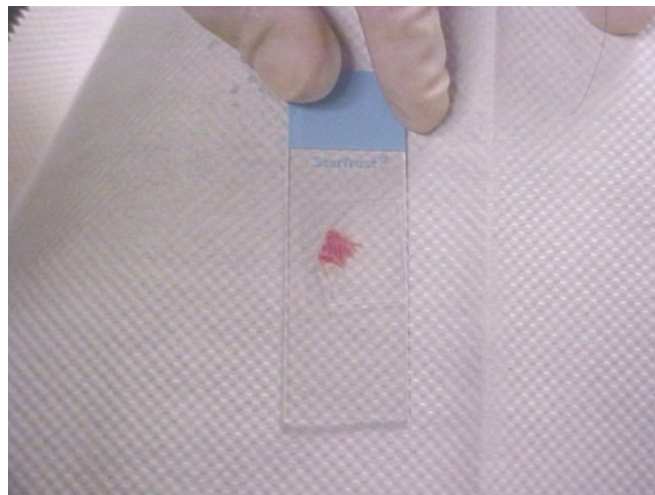
disorder involving a fish or amphibian patient. The fin biopsy is a simple procedure. While the fin is spread, a fine-tipped scissor is used to cut a small triangular wedge from the fin tissue (Figures 25.2a and 25.2b). The small biopsy wound will heal, as traumatic injury to the fins of fish is a common natural occurrence. The fin biopsy sample is placed onto the surface of a microscope slide, a drop of tank water is added, and a coverslip is applied to prepare the sample for microscopic examination (Figure 25.3). If part of a fin ray is sampled, it may be difficult to properly seat the coverslip on top of the biopsy, making the evaluation more difficult. Because fin biopsy samples are examined as wet-mount preparations, they should be examined immediately.

### Gill Biopsy

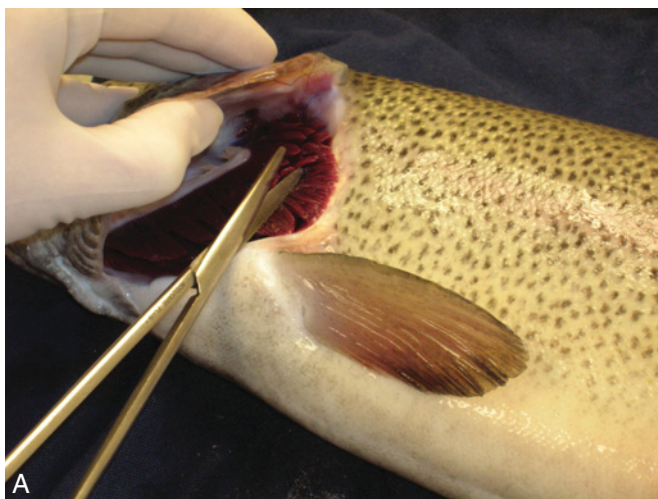
The gill biopsy is a useful diagnostic tool in the assessment of a fish patient, especially one exhibiting



**Fig. 25.3.** A fin biopsy sample as a wet-mount preparation ready for examination under the microscope.



**Fig. 25.5.** A gill biopsy sample as a wet-mount preparation ready for examination under the microscope.



**Fig. 25.4.** (a) Obtaining a gill biopsy from a fish (*Oncorhynchus mykiss*); (b) obtaining a gill biopsy from a fish (*Cyprinus carpio*).

clinical signs of respiratory disease such as piping (gulping air at the water air interface) and coughing (flaring of the operculum followed by rapid closure in an attempt to dislodge an irritant from the gills) behavior.

Before obtaining a biopsy, the gills should be examined grossly for signs of gill disease. Normal healthy gills are uniformly bright red. Pale gills are suggestive of anemia and slightly tan gills suggest methemoglobinemia (Noga, 2000). Gills with areas of discoloration, ulcerations, and missing lamellae should be noted.

A gill biopsy is performed by inserting the tip of a pair of fine-tipped scissors into the gill chamber under the operculum. The tips of a few primary lamellae are cut and removed using the tips of the scissors (Figures 25.4a and 25.4b). Bleeding is expected to be minimal if just the outer tips of the primary gill lamellae are sampled. The small gill biopsy is transferred to the surface of a microscope slide. A drop of water from the fish's environment is placed on the sample before covering it with a coverslip (Figure 25.5). The sample is examined as a wet-mount preparation under the microscope.

### Fecal Sample

A fecal sample is obtained by inserting a swab or small loop through the vent and into the intestine or by collecting feces as it is passed from the fish. This is also examined as a wet-mount.

### References

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