# Guidelines for Collecting Liver Samples to Characterize Liver Disease

TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory

## Introduction

Evaluation of increased liver enzyme activity or poor liver function tests can be a daunting task due to the variety of etiologies that can cause liver disease in small animals. Collection of fine needle aspirates for cytologic evaluation or larger tissue samples for histologic examination are often indicated for diagnosis. Several important guidelines should be followed to allow the best clinical information to be obtained from cytologic and histologic examination.

This document aims to improve the characterization of liver specimens submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), with an emphasis on non-neoplastic hepatopathies. These guidelines will enhance the diagnostic yield of cytologic, histologic, and toxopathologic examinations and integrated interpretations. The results obtained from any diagnostic laboratory can be improved with proper sample submission and providing pertinent and specific clinical history (see Table 1).

## Hemostasis Considerations

There are several factors to consider before collecting liver samples. Since the liver is responsible for the production of the vast majority of clotting factors, liver disease can be associated with bleeding tendencies, which raises concern for post-biopsy hemorrhage. Before collecting biopsy specimens, prothrombin (PT) and activated partial thromboplastin time (aPTT) may be indicated, preferably within 24 hours of the scheduled biopsy. PT/aPTT are especially indicated in any patients with evidence of poor liver function (i.e. elevated bilirubin, bile acids, ammonia; and/or decreased BUN, glucose, albumin, cholesterol). Laboratory findings that may indicate a high risk of bleeding include decreased PCV/HCT, platelet count <40,000/µL, prolonged PT/aPTT or buccal mucosal bleeding time (BMBT), decreased fibrinogen, and decreased von Willebrand's factor (vWF). A recent review article on canine chronic hepatitis provides more details on bleeding risk and clinical support for those animals identified to be at risk (see references).

## Cytologic Examination

Cytologic examination focuses on the evaluation of cell populations (vs. tissue architecture), as samples are typically small and collected with a needle only. While cytologic examination can provide valuable information when there are atypical cells, inflammatory cells, infectious agents, and/or other abnormal findings, there are many limitations to cytology. Cytologic examination of the liver can be diagnostic for certain neoplasms (large cell lymphoma, mast cell neoplasia, histiocytic sarcoma, etc.) and infectious agents (*Histoplasma* sp., bacterial infections, etc.). However, cytologic evaluation is often poorly sensitive for hepatocellular neoplasms like hepatocellular carcinoma and mild inflammation. Therefore, a negative cytologic examination does not rule out neoplasia or inflammation.

Cytologic specimens can be collected with ultrasound-guided fine needle aspiration of the liver. Multiple aspirates should be collected, when possible. When preparing slides, it is important to gently smear the material to a thin layer. Staining cytology slides in-clinic allows for rapid information and to ensure a diagnostic sample was obtained. However, some slides should be left unstained for the pathologist. Following these guidelines allows thorough evaluation of cell populations and cellular detail. Although cytologic examination may not always provide specific information, the cytologic report can provide guidance on differential diagnoses and future diagnostic tests when sufficient clinical history is provided. Please see the clinical history guidelines provided below (Table 1). If cytologic evaluation is attempted, animal owners should be warned that a definitive diagnosis is not guaranteed. When a diagnosis is not identified on cytologic evaluation, biopsy with histologic examination is indicated.

**Histological Examination**
Histopathology is pivotal in the diagnosis and evaluation of liver disease. Histopathology allows for examination of hepatic tissue architecture, enabling recognition of specific patterns of disease which can help lead to a diagnosis. Biopsy specimens can be collected via laparotomy, laparoscopy, or ultrasound-guided percutaneous needle biopsy. Laparoscopy is the recommended method since it is minimally invasive yet allows for larger specimen sizes needed for diagnosis. Ultrasound-guided biopsy is the least invasive method, but diagnostic accuracy is compromised due to the small sample size of the acquired specimens. Additionally, multifocal lesions may not be well represented with small samples.

When a decision is made to submit liver biopsies for histologic examination, it is important that certain guidelines put forth by the American College of Veterinary Internal Medicine, listed in Table 2, are followed. Although these guidelines were designed to aid in diagnosis of chronic hepatitis, they can be applied to other hepatopathies. Importantly, samples should be of sufficient size and quantity to increase the chance of collecting a representative diseased liver fragment and receiving a more accurate diagnosis. Proper histologic evaluation requires approximately 12-15 portal triads. To achieve this amount, a minimum of three laparoscopic or surgical biopsy specimens should be obtained. If using ultrasound-guided methods, sampling would require at least four specimens using a larger gauge needle (14 or 16 gauge) to reach the recommended amount, increasing the risk for post-biopsy hemorrhage. Since there can be significant variation of disease among liver lobes, collection from at least two lobes is recommended. Samples should be representative and taken from both central and peripheral areas. During the procedure, it is important to minimize crush/squeezing artifacts that compromise the microscopic characteristics of the biopsy. Most samples should be promptly placed in 10% neutral buffered formalin. However, fresh, non-fixed (no formalin) specimens should also be collected during hepatic biopsy procedures. Collection of at least one non-fixed sample for potential bacterial culture and/or copper quantification is recommended. At TVMDL, the same sample can be used for culture and copper quantification.

Further analysis of the liver can be aided by the use of special histochemical stains. Many of these stains are routinely performed with liver biopsies at TVMDL. Additional stains may be ordered at the discretion of the pathologist, with no additional cost to the submitting veterinarian. Commonly used hepatic stains include rhodanine to assess copper storage, Prussian blue for hemosiderin, reticulin to assess the reticular fiber network, Hall's for bile, congo red for amyloid, and Masson's trichrome for collagen. If an infectious etiology is suspected, Gram, Gomori methenamine silver (GMS), periodic acid Schiff (PAS), and acid-fast stains are often performed since they can highlight potential inconspicuous organisms. In some neoplastic diseases, immunohistochemistry (IHC) can aid in obtaining a specific diagnosis.

**Toxicopathologic Examination**
Copper-associated hepatopathy is a common cause of canine chronic hepatitis. Although once thought to be limited to Bedlington Terriers, Skye Terriers, Dalmatians, and Labradors, copper-associated hepatopathy can occur in any breed. Therefore, copper detection and quantification may be an integral portion of the evaluation of chronic liver disease.

When collecting samples for copper quantification, samples should be taken from the grossly least-affected areas of liver. Fibrotic and nodular areas should be avoided because copper concentrations can be falsely decreased in these areas. Copper quantification requires a minimum of 50 mg of liver, which equates to an entire 5 mm laparoscopic biopsy specimen, or two full, 2 cm long, 14-gauge needle biopsy specimens. Of note, a full-length 18-gauge needle specimen is insufficient and may result in erroneously low copper measurements. Once collected, the specimen destined for copper quantification should be placed in a sealed, leak-proof plastic bag or container with no formalin, saline, or other additives. To ensure the sample could also be utilized for potential culture, a sterile non-additive test tube may be ideal.

Though less ideal, copper can also be quantified in formalin-fixed tissue; however, the concentration may be falsely decreased in some cases. When histologic examination is performed at TVMDL, anatomic pathologists and diagnostic toxicologists can work together to evaluate copper quantification on formalin fixed tissues.

**Summary**

In summary, full characterization of liver disease often requires collection and submission of liver samples to a diagnostic laboratory. To enhance the quality of your submission and increase the accuracy of returned results, specific guidelines for biopsy acquisition and submission should be followed. Inclusion of a detailed clinical history is essential and will aid in the pathologist's interpretation of lesions (see Table 1 for examples). At least four laparoscopic or surgical biopsy specimens should be obtained from at least two liver lobes, including three in formalin for histologic examination, and at least one fresh specimen for aerobic/anaerobic culture and quantitative copper analysis. The provided checklist below should be utilized when collecting liver biopsy specimens. For more information on hepatic disease in dogs, please see the references below or contact TVMDL.

**Table 1: Clinical History**

| | Ideal Example | Suboptimal Example |
|---|---|---|
| **Signalment** | 10 -year-old MC Scottish Terrier | Adult dog |
| **Brief Clinical History** | Decreased appetite, lethargy for 2 months | ADR |
| **Pertinent Laboratory Findings** | Attach bloodwork or ALT – 300 U/L, ALKP – 600 U/L, Albumin WNL | Increased liver enzymes |
| **Radiographic Findings** | Attach radiology/ultrasound report or brief description of abnormalities | Sending radiographs only |
| **Current/Previous Treatment** | Currently on immunosuppressive doses of corticosteroids. Undergoing chemotherapy. Liver enzymes have not improved with liver supplements, broad-spectrum antibiotics, etc. | Being treated. Liver enzymes have not improved with treatment. |

**Table 2: Liver Biopsy Submission Checklist***

- ☐ At least 3 samples in 10% neutral buffered formalin
- ☐ Samples from at least 2 liver lobes
- ☐ Samples from both central and peripheral liver
- ☐ 1 sample in sterile, non-additive tube for culture
- ☐ 1 sample in plain, no additive tube without saline or formalin for copper
- ☐ Provide thorough clinical history (see Table 1)

*This checklist was created using ACVIM recommendations for diagnosing chronic hepatitis in dogs

**References**

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