Service update

Addition to anatomic pathology test menu: Liver Biopsy with Staining Panel

A new Liver Biopsy with Staining Panel (HISTOLIV) has been created by IDEXX Reference Laboratories to optimize interpretation of liver biopsy submissions. This test code combines histopathologic interpretation of the hematoxylin and eosin (H&E) stained section(s) with a panel of histochemical special stains (not including immunohistochemical stains) to evaluate and grade (if applicable) any hepatic changes. Evaluating the H&E stained section(s) concurrently with the panel of special stains will streamline the biopsy interpretation and reporting process. Additional non-liver sites/lesions can be ordered using the HISTOLIV additional site/lesion codes.

Test code	Test name
HISTOLIV1	Liver Biopsy with Staining Panel
HISTOLIV2	Liver Biopsy with Staining Panel and 1 Site/Lesion
HISTOLIV3	Liver Biopsy with Staining Panel and 2 Sites/Lesions
HISTOLIV4	Liver Biopsy with Staining Panel and 3 Sites/Lesions

Hepatic specimen preparation will include the following stains: H&E, copper (rhodanine or rubeanic acid), trichrome, iron, and reticulin. Copper staining is utilized to identify occult or pathologic elevations. Trichrome highlights the extent and distribution of fibrosis and is part of the assessment of cirrhosis. Reticulin is used to assess hepatic architecture and changes, such as proliferation, collapse, or loss of hepatocytes. Iron staining helps identify previous hepatic damage or inflammation by highlighting hemosiderin.

The liver specimens will be evaluated and graded according to published guidelines by the World Small Animal Veterinary Association (WSAVA) Liver Standardization Group.[1,2] Please note that grading criteria are canine and inflammatory specific, and therefore, they may not be applicable in every liver biopsy case.

Liver biopsy can be utilized to:

+ Diagnose acute and chronic liver conditions.
+ Differentiate inflammatory from noninflammatory disease (e.g., neoplasia, toxicity).
+ Distinguish between primary chronic inflammatory conditions (e.g., chronic hepatitis or immune hepatitis) and secondary chronic inflammatory conditions (e.g., nonspecific reactive hepatitis).
+ Identify congenital abnormalities (such as ductal plate malformations and vascular anomalies).

In the absence of a specific cause, knowledge of the nature and extent of underlying liver pathology may still be helpful in optimizing management and assessing prognosis.

Submission guidelines

History

A thorough clinical history is critical for accurate histologic interpretation. This includes the following:

+ Patient signalment (breed, sex, age)
+ Lesion(s) description (appearance of the liver grossly or via diagnostic imaging)
+ Clinical signs and duration of signs
+ Clinicopathologic data (CBC, chemistry, bile acids)
+ Results of any previous diagnostics (imaging, prior biopsy, cultures)
+ Any possible drug/toxin exposure, such as azathioprine, sulfas, doxycycline, phenobarbital
+ Clinical differentials

Specimen requirements

Accurate assessment of liver disease with histopathology relies on evaluation of a representative specimen. Generally, this requires enough liver to include 12–15 portal triads for histologic evaluation. This can typically be obtained via 2–3 needle biopsies that are 2–3 cm long, 5–8 laparoscopic specimens using 5 mm forceps, 2–4 surgical biopsy specimens using 8 mm punch, or a wedge resection 1 cm deep (to avoid capsular artifact). Prior to obtaining a specimen, exclusion of a coagulopathy is advised.

Some disease processes can be irregularly distributed throughout the liver and the severity of a disease may vary between lobes, thus submission of a minimum of 3 separate liver lobes is strongly encouraged for diffuse, nonfocal, or irregular disease. If there are focal or specific lesions of interest, they should be identified in the clinical history and submitted in separate jars. Ultrasound guided percutaneous biopsies of liver have a risk of sampling error (i.e., harvesting small specimens or nondiagnostic specimens due to variation of histological changes among liver lobes).

Table 1: Liver sampling techniques: advantages and disadvantages

Specimen type	Specimen size	Benefit	Limitations of interpretation
Fine needle aspirate	Very small—few cells	Minimally invasive	Good for diffuse processes or where diagnoses can be made on few cells (lipidosis, steroid hepatopathy, some neoplasia); lack of architecture can prevent accurate assessment of hepatitis, vascular anomalies, benign versus malignant hepatic nodules, toxic hepatopathies
Needle (ultrasound) 14 G medium and large dogs; 16 G small dogs and cats	Small (approximately 1/60,000 of liver)	Minimally invasive; larger specimen than needle	Potential fragmentation may impede grading and evaluation for fibrosis and copper; lack of adjacent normal architecture can prevent accurate assessment of benign versus malignant hepatic nodules; not indicated for congenital vascular disorders
Laparoscopic	Intermediate	Able to visualize the liver, biliary system, and pancreas during procedure; able to obtain specimens of multiple lobes/regions	Potential for small specimen that may impede grading
Laparotomy	Larger (wedge)	Able to visualize the liver, biliary system, pancreas, and other organs; can obtain specimens of multiple lobes of liver and other organs	High diagnostic specimen yield due to larger size of specimen, less potential for artifact, and opportunity to apply standardized grading

Other testing may be performed concurrently or at a later date. Specimens to be submitted for culture (use test code 401 in U.S. or AA in Canada) may include liver, gall bladder sediment, and gall bladder wall scraping if available. The copper staining performed as part of the standard liver code assessment will provide a qualitative assessment of copper accumulation in hepatocytes. For quantitative evaluation of copper accumulation (ppm), options include submission of a separate 10 gram fresh/frozen specimen (use test code 843 in U.S. or COPT in Canada) or copper quantification on the paraffin-embedded tissue block (the submitted liver biopsy specimen). Please note that the quantitative copper testing on the paraffin-embedded tissue block will destroy the tissue block. If there is a desire to hold additional testing pending the results of histopathology, specimens should be retained in-clinic and submitted to the laboratory when necessary.

What will my report look like?

Depending on the specifics of each histologic section, grading schemes (see tables 2–3) are used to characterize and provide a subjective qualitative assessment of the extent of changes. Grading criteria are chronic hepatitis specific and are not applicable to cats, all cases of hepatitis, or hepatopathies. Grading criteria are reported for distribution and amount of copper accumulation in hepatocytes and fibrosis. Qualitative assessment of apoptosis/necrosis, distribution and amount of intrahepatic iron and fine architectural changes are also assessed. Finally, a summary describing the overall hepatic changes and any further diagnostic testing recommendations is included in each report.

Chronic hepatitis grading criteria (canine only)

Table 2: Qualitative histologic hepatic copper grading

Grade	Description	Evaluation Criteria
0	No copper detected	No copper in any lobules
1	Little copper detected: solitary hepatocytes in the centrilobular area containing some copper-positive granules	1–2 hepatocytes/3.8 mm^2 (10x field), less than 10 % centrilobular hepatocytes
2	Small group of hepatocytes in the centrilobular area containing small to moderate numbers of copper-positive granules	Less than or equal to 4 clusters/3.8 mm^2 (10x field) 11%–25% CL hepatocytes
3	Centrilobular hepatocytes and some macrophages containing moderate numbers of copper-positive granules (one-third of each lobule)	26%–50% CL hepatocytes
4	Centrilobular and midzonal hepatocytes and macrophages with marked to moderate numbers of copper-positive granules (approximately two-thirds of the hepatocytes in all lobules)	51%–75% hepatocytes
5	Panlobular or diffuse presence of hepatocytes and macrophages with marked to moderate numbers of copper-positive granules	75%–100% hepatocytes

Table 3: Qualitative hepatic fibrosis grading

Grade	Degree of fibrosis	Fibrosis	Bridging fibrosis	Bridging fibrosis with nodule formation
0	Absent	Absent	Absent	Absent
1	Mild	Mild fibrous expansion (periportal and/or central)	Absent	Absent
2	Moderate	Moderate fibrous expansion	Some bridging fibrosis	Absent
3	Marked	Marked fibrous expansion	Marked bridging fibrosis	Absent
4	Very marked	Marked fibrous expansion	Marked bridging fibrosis	Present

Next steps

Pathologic interpretation primarily helps determine an individual patient's prognosis and medical (or surgical) management. The goals of treatment may include slowing the progression of fibrosis, eliminating infectious agents, chelating heavy metals for excretion, supporting remaining functional liver tissue, and addressing extrahepatic diseases contributing to a reactive hepatopathy.

References

1. WSAVA Liver Standards. Society of Comparative Hepatology. Accessed February 27, 2023. www.comparativehepatology.org/wsava-liver-standards
2. Webster CRL, Center SA, Cullen JM, et al. ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs. *J Vet Intern Med.* 2019;33(3):1173–1200. doi:10.1111/jvim.15467

For additional guidance for submitting biopsies, visit go.idexx.com/submitbiopsy and idexx.com/submitpathology.

Published June 2023.