

Liver Biopsies and Special Stains: the Basics

By Amy Backer, MD and Jennifer Burchill, HTL

The adult liver is one of the largest organs of the human body, weighing between 1400 and 1600 grams (2.5% of total body weight). The liver's job is maintaining the body's metabolism. Synthesis of proteins, detoxification of both endogenous and exogenous waste products, excretion of waste products into bile, and processing dietary lipids (fats), amino acids (proteins), carbohydrates (sugars) and vitamins are among the liver's many important functions.

The job of the pathologist evaluating a liver biopsy is to assess the type and extent of hepatic injury.

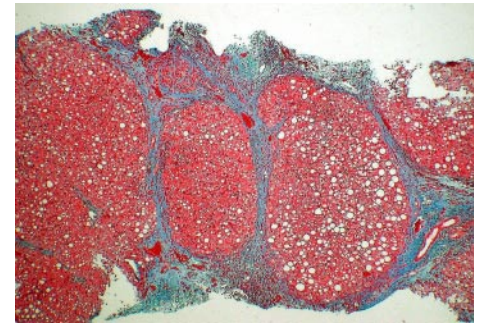
The liver receives a dual blood supply from the portal vein and hepatic artery, both of which branch and rebranch within the liver, forming a network of blood vessels that supply the entire organ. The hepatic tissue is comprised of sheets of liver cells, called hepatocytes, which are arranged into small, repeating architectural sub-units. In the field of pathology, these units have been described in two related ways, as the lobule and as the acinus. Briefly, the lobule is a two-dimensional structure that portrays the liver cells and their related vessels as a hexagon centered around a branch of the hepatic vein. From each vein, hepatocytes radiate out to portal tracts, located at the lobule's periphery. The lobule can then be divided into three zones: the "periportal" or peripheral zone, midlobular zone and "centrilobular" zone (directly surrounding the hepatic vein branch, otherwise referred to as the central vein). By contrast, the acinus is a three-dimensional structure, organized around the intrahepatic circulatory system. It is schematically represented as a semi-spherical mass of hepato-

cytes arranged around a central structure called the portal tract. There are many portal tracts within the liver, and each one contains a branch of the hepatic artery, portal vein and a bile ductule. While the acinus is usually the better descriptive term relating to clinical pathophysiology, the two-dimensional lobule is easier to visualize for the pathologist when viewing microscopic slides. Both terms are used in discussions of hepatic pathology, and provide useful insight when attempting to understand the myriad of pathologic processes that affect the liver.

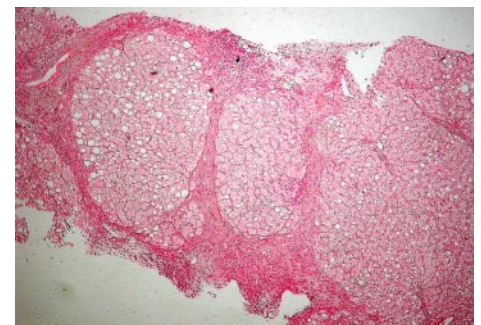
Because of its function, the liver is vulnerable to a wide variety of diseases, including infectious, metabolic, toxic, circulatory and neoplastic disorders. The disease process may be primary to the liver, as in viral hepatitis. It may be secondary, such as in cardiac disease, alcoholism or metastatic cancer. The liver responds to these injuries in several basic ways. Direct cellular injury, in the form of degeneration or necrosis, is often seen in acute liver disease. Inflammation can be seen in both acute and chronic liver disease. Regeneration can be an appropriate response to liver damage, and the liver has a great capacity to regenerate itself. Fibrosis is an irreversible consequence of severe, often chronic, liver damage. The combination of fibrosis and regeneration is seen in end-stage liver disease, and is called cirrhosis. The job of the pathologist evaluating a liver biopsy is to assess the type and extent of hepatic injury.

Needle biopsy is a safe and effective means of sampling the liver, and although wedge biopsies and resection specimens are also used as sampling methods, needle biopsies are by far the most common liver specimen that the pathologist encounters. While percutaneous biopsies are useful in assessing diffuse liver disease, radiographically directed fine needle aspirations have become commonplace in sampling smaller, focal lesions, such as primary or metastatic neoplasms.

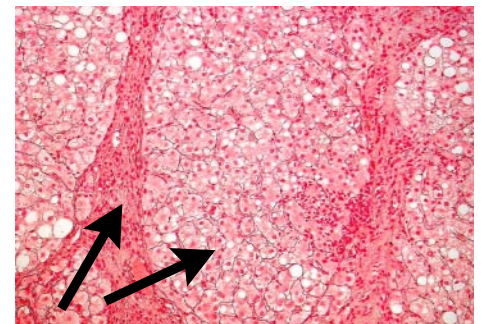
Careful handling and processing of liver biopsies is crucial for accurate histologic interpre-



Increased fibrosis in a cirrhotic liver, highlighted by trichrome stain.



Note the regenerative nodules in this cirrhotic liver (reticulin stain).



Parenchymal collapse and regeneration in a cirrhotic liver, as highlighted by reticulin stain.



PROFILE

Tom Hunton, HT

TOM HUNTON, like so many InCyte Pathology histotechnologists, was recruited into the field from a related discipline. As an Eastern Washington University student working towards a degree in microbiology, Tom received additional training before taking the registry exam in Histotechnology.

Born and raised in Spokane, Tom and his wife, Wendy, now make their home in Rosalia, Washington, where they are home-schooling their three young children. Tom enjoys carpentry, gardening, antique hunting, and spending time with his family.

Tom has worked full time for InCyte Pathology since 1990 and currently works the lone histotech shift on Saturday. He is often the departmental spokesperson, leading tours through Histology, and is well known for his practical jokes during off-production hours. Tom enjoys the creative challenge of producing a good microscope slide and appreciates the people he works with and for.

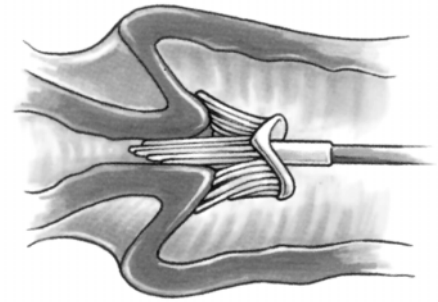


LIQUID-BASED PAP TESTING:

How to Get Maximum Cellularity With Broom-Like Pap Collection Device (Mop)

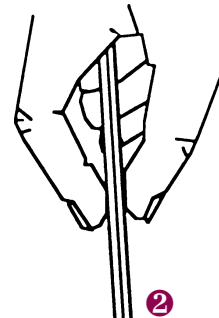
1 Collect specimen

Insert the tip of the collection device into the cervical os, jiggle instrument handle, and slip longer bristles into endocervical canal until “shoulders” of broom touch the pars vaginalis of the cervix. *Rotate clockwise*, at least five times, applying a little more pressure with each turn. The bristles of this device have been specifically cut at an angle so the epithelium will be sheared off when the device is rotated correctly and gentle pressure is maintained.



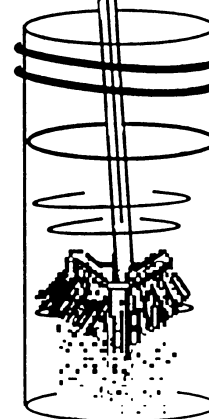
2 Rinse mop head

Rinse mop head into fixative by pushing broom head into bottom of the vial 5 times, forcing the bristles to bend apart to release the cervical material. As a final step, vigorously twirl handle between thumb and forefinger for 5 seconds.



3 Preserve the entire sample by removing mop head

After rinsing, “pop” the mop and submit the entire instrument tip. Place the collection device into the vial by placing your thumb or vial cap against the back of the brush pad. As gentle pressure is applied against the mop head, it should disconnect easily from the handle. Leave the broom head in the vial. Cells will continue to rinse from the collection device into the fixative solution while the specimen is being transported to the laboratory.



4 Cap and label vial

Tighten cap. Make sure vial is labeled with patient’s name. Send specimen and requisition form to the laboratory.

Note: The Rovers Cervex-Brush (Broom-like Device/Mop) is the recommended collection device by TriPath Imaging, Inc.



Liver Biopsies and Special Stains: the Basics, *continued*

tation. In particular, needle biopsies must be handled carefully to avoid crush artifact. Cirrhotic livers frequently yield fragmented specimens despite careful handling, and this can provide a useful clue to the underlying disease process. Following biopsy, formalin fixation and paraffin processing is needed for routine needle biopsies. At InCyte Pathology, most liver biopsies arrive by courier in late afternoon or early evening. Patient demographics, specimen sites, and other important information are double checked for accuracy, and the biopsy is assigned an accession number. The biopsies are submitted for processing the following morning. A liver biopsy must be processed for a minimum of six hours, usually completed by that afternoon. Sections are cut at various levels to ensure thorough and adequate tissue sampling, and unstained duplicate slides containing sections of tissue are also prepared for any possible subsequent studies (such as immunohistochemical stains). If the biopsy has been performed to evaluate diffuse liver disease, hematoxylin and eosin stains, as well as several special stains, are routinely performed. The slides and corresponding paperwork are ready for review by the pathologist early the following morning.

Special stains for connective tissue are among the most important and clinically relevant, and include reticulin and trichrome stains.

A wide variety of special stains can be used to evaluate liver specimens. Special stains for connective tissue are among the most important and clinically relevant, and include reticulin and trichrome stains. Reticulin stains use silver impregnation to highlight type III collagen, the sinusoidal collagen framework that supports the hepatocytes. Alterations in this framework, such as tissue collapse secondary to necrosis or cirrhosis, are readily demonstrable by reticulin staining. Type I collagen is usually present in portal tracts and within vessel walls, and is highlighted in blue by the trichrome stain. This stain accen-

tuates increases in portal fibrosis. This is an important part of the diagnosis of chronic hepatitis, in which the degree of fibrosis is an important prognostic indicator.

While small amounts of stainable iron are common in normal hepatocytes, iron overload is a pathologic process. It can be secondary to diseases such as chronic anemia and alcoholism or

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primary as in hemochromatosis. Hereditary hemochromatosis is an inherited disorder, characterized by the continuous absorption and accumulation of iron in the liver and other organs. Over time, severe liver disease can result. Special stains for iron include the Prussian blue stain, which stains intracytoplasmic iron blue and provides a light counterstain to further accentuate the iron. Often the discovery of iron accumulation by special stains is the first step in diagnosing hemochromatosis. The paraffin block can subsequently be used to determine the quantity of iron in the liver tissue, which is the most reliable tissue method of diagnosis.

The periodic acid-Schiff (PAS) stain, with or without diastase predigestion, has many uses in a variety of hepatic disease states. It highlights the basement membrane of intrahepatic bile ductules, which in biliary disorders may show damage due to inflammation. The stain also highlights lipofuscin pigment in Kupffer cells (specialized hepatic macrophages), which can pinpoint areas of active hepatocellular injury. Various carbohydrates can also be highlighted by this stain, including the cytoplasmic globules classically seen in alpha-1-antitrypsin deficiency. This is a hereditary condition that leads to accumulation of genetically abnormal (and often nonfunctional) alpha-1-antitrypsin in the liver and lung. Due to hereditary variations in biologic severity, hepatic manifestations may not become clinically evident until late in the disease, when the liver is severely

fibrotic or cirrhotic. The PAS/D stain is a relatively easy method of detecting the disease in a routine liver biopsy, and can be a useful tool in its diagnosis.

Additional special histochemical stains can be used if clinically indicated. Examples include: rhodamine stain for copper accumulation in chronic cholestasis or Wilson's disease, congo red stain for amyloidosis, and microbial stains to detect mycobacteria or fungi. At InCyte Pathology, the routine stains used to evaluate non-neoplastic liver disease include but are not limited to: reticulin, trichrome, iron and periodic acid-Schiff (with and without diastase predigestion). In addition, our extensive battery of immunohistochemical stains is particularly useful in the diagnosis and work-up of neoplastic liver disease.

Finally, the importance of good clinical history should never be underestimated, particularly when attempting to diagnose diffuse liver disease. Because the liver is vulnerable to so many different disorders, a routine biopsy will often show evidence of more than one disease process. An example would be chronic hepatitis C and alcoholic liver disease. Many different diseases produce similar histologic changes. Fibrosis can be seen in almost any chronic liver disorder. A brief history such as "chronic hepatitis C" or "rule out hemochromatosis" on the requisition slip is a requisite for timely and accurate diagnosis. Good clinicopathologic correlation leads to accurate, timely diagnosis of the disease process (or processes), and is essential for quality patient care.

Providing correct patient demographics, clinical history, and billing information saves office staff time and helps ensure accurate, reliable, and timely results.

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PathWays has items of interest for office personnel and assistants as well as for physicians, nurse practitioners, nurses and physician assistants. We recommend that, upon completion of circulation, your copy of **PathWays** be filed in the InCyte Pathology *Anatomic Pathology Services Manual* for future reference.

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