Tissue Biopsy

HASPI Medical Anatomy & Physiology 04d

Lab Activity

Name(s): _____

Period: _____ Date: _____

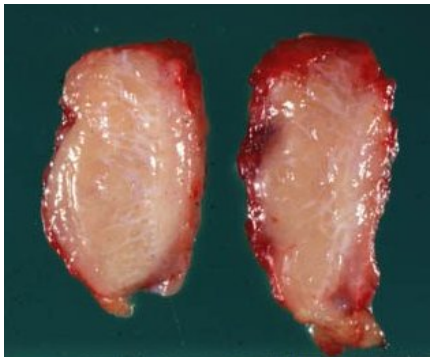
Background

What is a Biopsy?

A biopsy is a test performed to collect a tissue sample from a site in the body. These tissues are then examined microscopically to determine the health of the tissue and cells. Depending on the tissue location and suspected pathology, a biopsy may be performed in a doctor's office (skin biopsy), by a surgeon, or through interventional radiology. In addition, the biopsy procedure may differ depending on the type and amount of tissue collected.

http://stonybrookoralpath.com/images/slide.jpg

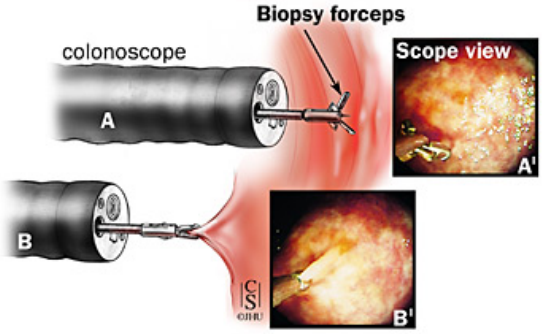
Common Types of Biopsy Procedures



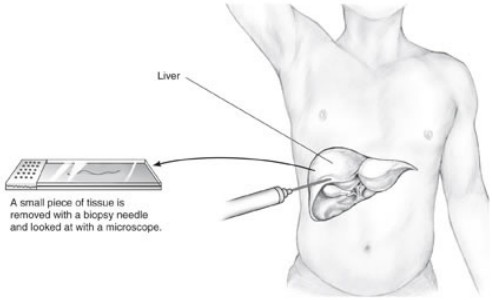
http://drbentownsend.files.wordpress.com/2010/10/biopsy.jpg

Surgical Biopsy – Surgical biopsy involves the removal of suspicious tissue through either an incision or excision. An incision biopsy will only remove a portion of the suspected tissue, while an excision biopsy involves the removal of all of the suspected tissue. For example, if ovarian cancer is suspected, a small portion of the ovary may be removed in an incision biopsy, while the entire ovary and any other surrounding tissue that looks suspicious would be removed in an excision biopsy.

Endoscopic Biopsy – An endoscope is a flexible, hollow tube that can be inserted into the body through incisions or orifices. Surgical tools can be passed through the tube to excise and collect tissues from various locations within the body. Common endoscopic biopsies that are performed include a bronchoscopy to collect tissue from the respiratory tract, colonoscopy to collect tissue from the intestinal tract, and a cytoscopy to collect tissue from the bladder.



http://www.hopkins-gi.org/Upload/200710261617_18708_000.jpg



http://digestive.niddk.nih.gov/ddiseases/pubs/liverbiopsy/images/BiopsyNeedle.jpg

Needle Biopsy – When only a small tissue sample is needed, a special needle can be used to extract cells for biopsy. This is most often used when a tumor or lump can be easily felt through the skin. Interventional radiology techniques can be used to identify the specific location of a mass, and then the needle is inserted to remove a small portion of the mass. Needle biopsy procedures may include fine-needle aspiration, core needle biopsy, vacuum-assisted biopsy, or image-guided biopsy.

Sausville, Edward A. and Longo, Dan L. "Principles of Cancer Treatment: Surgery, Chemotherapy, and Biologic Therapy", Harrison's Principles of Internal Medicine, 16th Ed. Kaspar, Dennis L. et al., eds. p.446 (2005).

Use of Biopsy Tests

Biopsies can be used to identify abnormal conditions in tissues and cells. Most commonly, biopsies are used to determine whether a lesion or mass is benign or malignant, and can also assist the pathologist in determining the type of cancer. Biopsy can also help determine the extent and type of many inflammatory conditions such as colitis or vasculitis. Other uses of biopsy include determining the extent of kidney disease, types of infectious diseases, metabolic conditions, fertility, and organ transplantation rejection.

http://www.drday.com/rumors/images/pathslides.jpg

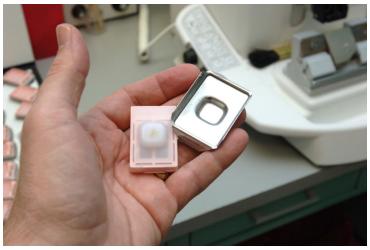
Slide Preparation of a Biopsy

Following the collection of a biopsy, the tissue is sent to the pathology laboratory. A lab or pathology technician is responsible for fixating, slicing, mounting, and staining tissue samples onto a slide so the pathologist can review the sample microscopically and make a diagnosis. The results are sent to the patient's physician to be communicated to the patient.

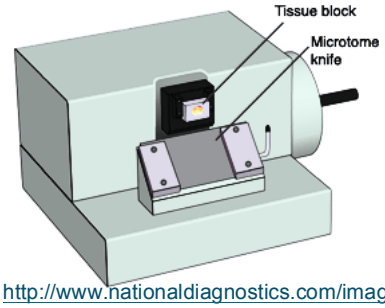
Fixation - It is important to preserve the histological structure and integrity of a tissue before examining it microscopically. A section of the sample is cut and transferred to a tissue cassette. The cassette is labeled and placed in a chemical, such as formalin, that will preserve the sample. Fixation is followed by bathing the sample in ethanol and/or xylene to dehydrate the tissue.

http://pathology.utscavma.org/wp-content/uploads/2008/03/path-club-pics-024.JPG

Embedding – Following dehydration of the sample, the tissue is placed in a mold that fits within the tissue cassette and paraffin wax is poured over the sample. The paraffin replaces any water remaining in the tissue. For this reason, it is important to completely cover the tissue sample. The wax is cooled until hardened.



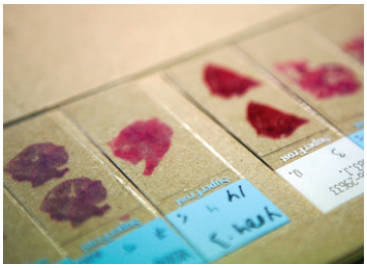
http://www.newcomersupply.com/media/products/imag0130.jpg



Sectioning - As soon as the wax has hardened, the sample is removed from the mold and placed in a microtome that will slice the sample into very thin sections. The thin sections allow for easy viewing under the compound light microscope. The paraffin wax also prevents the microtome from smashing the cells together during slicing. Good slices are then placed on glass slides, allowed to dry, and the paraffin wax is removed from the sample before staining.

http://www.nationaldiagnostics.com/images/h1_4a.gif

Staining – Once the sample has been fixated and mounted on a glass slide, stains are used to identify cellular components. There are many stains and staining procedures that can be used depending on the type of tissue and cell parts to be visualized. Once the staining procedure is complete, the tissue is permanently mounted and covered with a cover slip to protect the sample. The slide is now ready for the pathologist to review.



http://www.helsinki.fi/hub/1_2009/kuvat/cell.jpg

Materials

10 ml Formalin
Hinge-top vial
Slide/coverslip
10 ml Paraffin Wax
Tissue Cassette
Forceps
Razor
Microscope
Permount
Base Mold
Scalpel
Gloves
Goggles
Face Mask
Graduated Cylinder
Masking Tape/Marker
Paper Towel

Staining Solutions IN ORDER
Share with Class

1.7% Soap solution (90° C)
Distilled water (90° C)
Distilled Water (room temp)
Tap water
Mayer's Hematoxylin
0.5% Lithium Carbonate solution
1.0% Eosin solution

Procedure

Scenario:

You are part of the pathology laboratory team at HASPI Hospital. A patient biopsy sample has been submitted to your lab. The patient had a large portion of the liver removed surgically due to late stage liver cancer. Your responsibility is to prepare the submitted biopsy sample and determine whether the remaining liver tissue is healthy, or whether it also contains abnormalities requiring additional portions of the liver to be excised.

The following information was submitted to the lab along with the biopsy sample:

Patient ID: 439015 Age: 63 yr. Gender: Male

Lab No: 50928829 Biopsy No: 543983 Collection Date: 10/5/2012

Referring Physician: Dr. Janice Sizemore

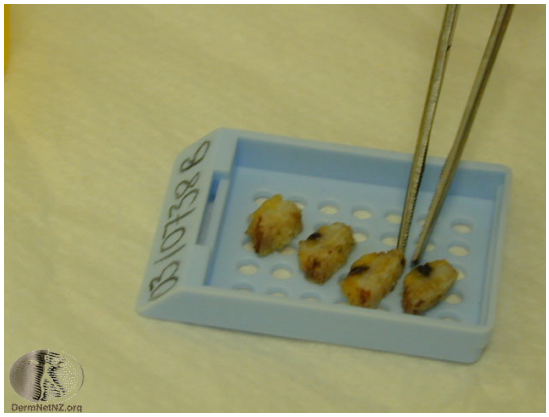
Specimen: Excision Biopsy, Liver Cancer

Notes:
All cancerous tissue removed surgically. Biopsy sample taken from visually healthy liver tissue. Check for abnormalities to determine if any further tissue removal is required.

Use the following directions to fixate, embed, section, and stain the submitted biopsy sample. Since you are working in a lab group, each individual should take responsibility for at least one part of the procedure.

PART A: Fixation

		✔when complete
Step 1	Using the scalpel, CAREFULLY cut an 2 cm x 2 cm x 2 cm section from the biopsy sample provided by your teacher.	
Step 2	Using forceps, place the sample in the center of the tissue cassette and close the cassette.	
Step 3	Using the masking tape and marker, write the name of your lab group on the hinge-top vial.	
Step 4	Using the graduated cylinder, fill the hinge-top vial with 30 mL of formalin.	
Step 5	Place the tissue cassette in the formalin and close the top on the vial.	
Step 6	Place the vial in a safe place, and allow the tissue to fix for AT LEAST 24 hours. The tissue can fixate longer if needed.	



http://dermnetnz.org/doctors/lesions/images/histol6.jpg

PART B: Embedding

Base Molds

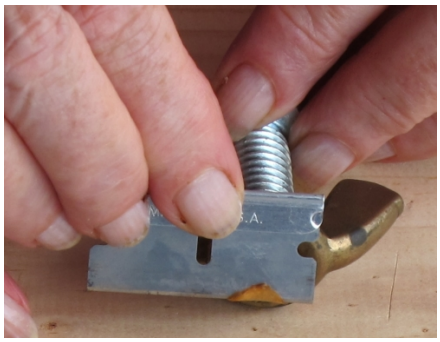
http://www.medimeas.com/product/mtem.jpg

		✔when complete
Step 1	Once the sample has been allowed to fixate for at least 24 hours, remove the tissue cassette from the formalin and place it on a paper towel.	
Step 2	Remove the tissue sample from the cassette and place it in the base mold. If the sample does not fit within the base mold, use a scalpel to trim off excess tissue.	
Step 3	Rinse the tissue cassette to remove excess formalin.	
Step 4	Place the base mold containing the tissue sample back into the cassette.	
Step 5	Your instructor will have melted paraffin wax available in a beaker. Obtain 5-10 mL of wax in a small beaker or graduated cylinder.	
Step 6	Make sure the tissue sample is towards the center of the base mold.	
Step 7	Pour the paraffin wax into the base mold and over the tissue sample. The wax should pour easily. If the wax has slightly hardened, place in the microwave for 10-20 seconds.	
Step 8	Try to make sure that the entire tissue is covered. The paraffin should solidify in about 30 minutes.	
Step 9	Once hardened, the embedded tissue sample and paraffin can be easily pushed out of the base mold.	
Step 10	If there are any cracks in the paraffin simply re-melt and set again.	
Step 11	The tissue block is ready for sectioning, and can be maintained at room temperature for years if necessary.	

Tissues in a medical lab are most often sectioned using a microtome. The tissue block is mounted on the microtome, and a razor is used to cut very thin slices to mount and stain on a slide. Unfortunately, all of the microtomes at HASPI Hospital are broken (and are extremely expensive), so we will be improvising!

PART C: Sectioning

		✔when complete
Step 1	Take the tissue block and hold it firm with the forceps on a flat surface.	
Step 2	THE RAZOR BLADE IS EXTREMELY SHARP!!! Make sure to keep the cardboard cover on the blade whenever it is not in use!!!	
Step 3	Using the razor blade, CAREFULLY begin cutting thin slices off of the tissue block. Try to cut as thin as possible, less than 1 mm in thickness. Most samples cut with a microtome are between 5-7 μm. You only need one good slice containing the tissue sample to make the slide.	
Step 4	Place the thinnest intact tissue slice flat on the glass slide.	
Step 5	The tissue must be bonded to the slide before staining so it does not come off the slide when dipped in different solutions. If an oven is available, place the slide in the oven at 65° C for 20 minutes. If an oven is not available, allow the sample to air dry on the slide for 24-48 hours.	

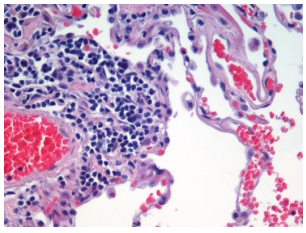


http://1.bp.blogspot.com/-vetJnE4jP9Y/Tsm8ypt3NSI/AAAAAAAAAm8/UbWHPFD93fQ/s1600/microtome4.JPG

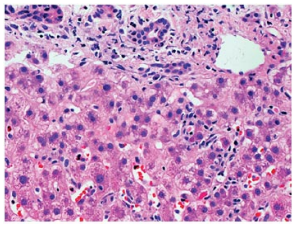
http://pathotoko.blogspot.com/2011_02_01_archive.html

Staining

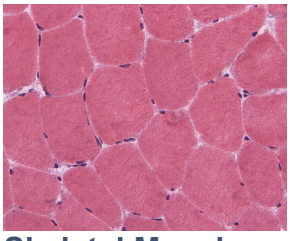
There are over a thousand staining procedures that are used in histology and pathology. One of the most commonly used staining techniques is the hematoxylin & eosin staining procedure. More than 90% of pathology slides are stained with this procedure. This is actually a two-part dye. Hematoxylin is a dye that stains the nuclei and a few other cellular components dark purple or blue. This is followed by staining the sample with Eosin Y that colors eosinophilic structures, primarily intracellular proteins, shades of pink and red. The following are examples of tissues that have been stained with the hematoxylin & eosin staining procedure:



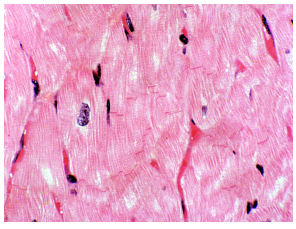
Lung



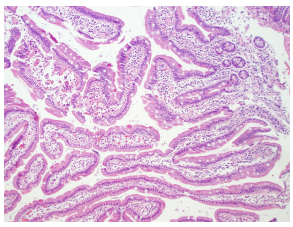
Liver



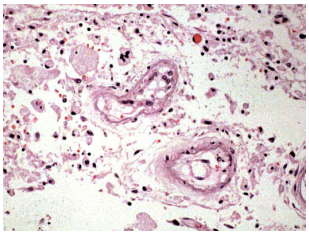
Skeletal Muscle



Cardiac Muscle



Small Intestine



Brain

PART D: Staining

		✔when complete
Step 1	Whoever is handling the slide will need to wear gloves and goggles.	
Step 2	Using forceps, check to make sure the tissue sample is bonded to the glass slide. The sample should appear dry and not move on the slide.	
Step 3	Your instructor has set up a staining station in the classroom with the staining solutions labeled and in order. Other groups will also be using the stains, and more than one slide can be placed in the solution at a time if needed.	
Step 4	Use forceps to place and remove the slide in each solution. Ensure that the entire tissue sample is emerged in each solution and use a paper towel to prevent any dripping of the solution off the slide during removal.	
Step 5	Stain your slide by following the times and order in **Table 1** below for each staining solution.	
Step 6	Let the slide dry completely, and place 1-2 drops of Permount solution over the tissue sample.	
Step 7	Carefully lay a cover slip over the tissue sample and press down lightly. Remove any extra Permount that leaks from under the cover slip with a paper towel.	
Step 8	Use a marker to write your group name on the end of your slide.	

Table 1: Staining Solutions and Time

Solution	Time	Purpose
1.7% soap (90° C)	**1 minute**	Removal of the paraffin wax
Allow to dry		
1.7% soap (90° C)	**1 minute**	
Distilled water (90° C)	**30 seconds**	Tissue rehydration
Allow to dry		
Distilled water (90° C)	**30 seconds**	
Allow to Dry		
Rinse with distilled water (room temp)	**Rinse 1 minute**	Removal of 1.7% soap solution
Mayer's Hematoxylin (room temp)	**7 minutes**	Stains basophilic structures, such as chromatin and ribosomes, purple or blue
Running Tap Water	**Rinse 4 minutes**	Removal of Mayer's hematoxylin
0.5% Lithium carbonate solution	**1 minute**	Bluing solution
Running Tap Water	**Rinse 5 minutes**	Removal of 0.5% lithium carbonate solution
1% Eosin solution	**1 minute**	Stains the cytoplasm
Running Tap Water	**Rinse 1-2 minutes**	Removal of 1% eosin
Drying	**10-20 minutes in an oven at 60° C, or allow to dry out over night in a warm space**	Dehydrates the tissues and affixes the stains

Microscope Observation

Now that the biopsy slide is complete, the slide must be observed under the microscope for any abnormalities. The pathology assistant or technician is responsible for creating the slide, while the pathologist is responsible for observing the slide and making a diagnosis. At this point, you have not spent years of your life trying to become a pathologist, so of course we do not expect you to be able to recognize abnormalities. Observing and being able to identify cellular components of your slide is an important skill to learn to be able to recognize different tissue types, and eventually to differentiate normal vs. abnormal tissue.

PART E: Microscope

		✔when complete
Step 1	Place your biopsy slide under the microscope and observe under the highest power.	
Step 2	When drawing samples, take your time and be as accurate as possible with size and color.	
Step 3	Once you have the sample focused, use colored pencils to draw what you see in the analysis section under View A.	
Step 4	Move the slide on the microscope stage to get a different view of the sample. Draw what you see under View B.	
Step 5	Use the **Table 2: Expected Results** to label and identify the following cellular components in your View A and View B drawings.	

Table 2: Expected Results

Tissue	Stain
Collagen	Pale pink
Muscle	Deep pink
Acidophilic cytoplasm	Red
Basophilic cytoplasm	Purple
Nuclei	Blue
Erythrocytes	Cherry red

Analysis

Use colored pencils and focus on details for View A and B microscope drawings.

View A

View B

Analysis Questions - *on a separate sheet of paper complete the following*

1. Briefly describe the steps and explain the importance of fixating, embedding, and sectioning a biopsy sample before staining.
2. What is the importance of exposing the slide to xylene during the staining procedure? What about ethanol?
3. What cellular components does hematoxylin stain?
4. What cellular components does eosin Y stain?
5. What is the importance of exposing the slide to acid alcohol during the staining procedure? What about ammonia water?
6. How did your slide turn out? Was it easily viewable under the microscope?
7. If applicable, what could your group have done differently to create a better tissue slide?
8. CONCLUSION: In 1-2 paragraphs summarize the procedure and results of this lab.

Review Questions - *on a separate sheet of paper complete the following*

1. What is the purpose of a biopsy?
2. Where are biopsies performed?
3. Give a short description of the three most common types of biopsy procedures.
4. Give three examples of conditions that biopsy can help diagnose.
5. Why must a biopsy sample be fixated? How is fixation done?
6. Why is a biopsy sample embedded in wax?
7. Why is it important to obtain thin samples when sectioning a biopsy sample?
8. What is the purpose of staining a biopsy sample?
9. The following image is a sample of tissue treated with hematoxylin and eosin. Label the muscle, nuclei, and erythrocytes. What type of tissue do you think this represents?