

A Guide to the Analysis of the Mammary Gland

Mammary Gland Whole Mounts

Introduction

The mammary gland, the organ that defines mammals, achieves its ultimate purpose during lactation after its development and functional differentiation have been induced through the hormonal stimuli of pregnancy. The most common methods to analyze the gland are whole mount and histology, RNA and protein assays. This CD-ROM is intended to show you how to prepare whole mount samples.

Methodology Videos Script

Screen 1 – Operating Site and Materials Needed

This is a typical set-up for the collection of mammary glands from a mouse.

The basic equipment needed for this procedure includes: a dissecting microscope, microscope slides with a frosted end, number 2 pencils, histocassettes, 50 ml centrifuge tubes, razor blades, scintillation vials and dissecting board (we just use the lid of a Styrofoam cooler)

Not shown here are glass cover slips and a Pasteur pipette with a rubber bulb.

You will also need a 60-degree incubator.

To collect the mammary gland tissue you will need one inch metal pins, bent forceps, blunt end surgical scissors, jewelers forceps, cotton swabs, and a squirt bottle of 70% EtOH. Sterilization is not an absolute necessity but the instruments should be clean and free of debris.

The solutions needed include water and EtOH in a 30%, 50%, 70%, 90%, 95% and 100% solution. These are reused or replaced as necessary. Additional solutions are a fixative, a staining solution, a clearing solution, a mounting medium, a medium for transitioning to embedding and an embedding medium. We will use Carnoy's fixative, Carmine Alum stain, Histoclear for clearing and Permount for mounting.

We will use a 1:1 mixture of xylene to paraffin for transitioning to embedding and paraffin for embedding. The xylene paraffin mix and the paraffin should be kept in the 60-degree incubator for at least an hour.

Label the slides to be used with the date, experiment number, mouse name or other pertinent information.

Screen 2 – Locating the Mammary Gland

This is a sketch of the mammary glands of a mouse. Five pairs of mammary glands extend from the base of the neck to the inguinal region.

This demonstration will show a simple method for evaluating the overall morphology of the number 4 glands which are easiest to dissect. Whole mounts can be prepared from any stage of mammary development. You are able to evaluate the extent of ductal outgrowth and alveolar development. If you are studying a mouse which is unable to lactate, wait one day after the birth of her first pups to make sure lactation is not occurring. Sacrifice on the second day before involution begins.

Collect all necessary equipment and supplies prior to sacrificing the animal. Autolysis, which can interfere with the biochemical and morphological analysis of the tissues, begins as soon as the animal has been sacrificed. Pin the mouse to a dissecting board at all 4 extremities with the ventral side up.

Here are the #1, 2 and 3 mammary glands. These are the thoracic mammary glands. Here are the #4 and 5 mammary glands. These are the inguinal glands. The glands are easier to identify when the animal's fur is dry. Reduce the amount of hair that gets into the specimen by wetting the fur with 70% EtOH.

Screen 3 – Mammary Gland Dissection

Make an incision that looks like an inverted Y. Avoid cutting through the peritoneum. Using bent dissection forceps grasp and lift the skin. Make the first incision using blunt surgical scissors. Begin just above the genitals at the midline and extend 2.5 cm towards the head of the animal. Separate the skin from the peritoneum.

The next two incisions should also begin at the mid-line point between the two #4 nipples. Cut 1.5 cm with the tip of the scissors pointed toward the hind legs and between the #4 and #5 nipples.

Using blunt ended scissors or a cotton swab, separate the skin flaps from the underlying peritoneum. The mammary glands will remain attached to the skin flaps. The free end of the skin flap is pinned to the dissecting board. The fat pad extends all the way to the back so the skin flaps have to be separated from the peritoneum all the way around to the back.

The fat pad is now exposed. Here are the #4 and 5 inguinal mammary glands. The mammary epithelium is embedded in this fat pad and these components together constitute the mammary gland.

The nipple of the #4 mammary gland lies here. It is important to include the area closest to the nipple. The mammary ducts grow starting at the nipple and extending into the fat pad. A specimen which does not include the nipple area, especially a smaller, nonlactating specimen, may be missing a majority of the mammary epithelium.

This is the lymph node of the #4 gland. It can be distinguished from the surrounding tissue by its shape and color. It is easier to see the lymph node in a virgin mouse than in a lactating mouse.

The lymph node can be located by this intersection of blood vessels which overly the lymph node. These blood vessels form an inverted Y.

Screen 4 – Removing the Mammary Gland

For a mammary gland that you wish to process for histology, grasp the fat pad near the nipple and gently separate and cut it away from the skin using blunt ended scissors.

Spread the mammary gland and lymph node in the center of a pre-labeled glass slide. It is useful to center the specimen so that there will be room to surround it with Permount later. Take care not to allow hair or air bubbles to be caught under the tissue. The margins of the gland should be extended as far as possible to promote adhesion and reveal critical structures. Inspect under the microscope and remove any air bubbles caught under the tissue, gently press from the middle of the specimen outward.

For orientation, this is the nipple area and this is the lymph node area.

If the bubbles are persistent, lift the edge of the tissue slightly and press it back down on the slide taking care not to lift the entire tissue for once it is removed it will not stick to the glass again.

Allow the specimen to air dry for about 30 seconds to increase its adherence to the glass slide and then put it into fixative to avoid further autolysis. Leave the slide in fixative for 2-4 hours. A 2-3 mm thick specimen will require at least 4 hours. The slide can be left in fixative overnight if it is convenient.

If you have a gland that is secreting a lot of milk, it may have difficulty sticking to the slide. After you place it on the slide gently blot away the milk before fixing.

Occasionally a mammary gland will float off of the slide. This usually happens with a lactating gland. If this happens further processing will have to be done by using forceps to move the specimen from one solution to the next.

Screen 5 – Staining

The next step for the mounted specimen is staining the tissue. Begin the staining process by removing the slide from fixative and washing it in 70% EtOH for 15 minutes, then 50% EtOH for 15 minutes 30% for 15 minutes and finally change to distilled water for 5 minutes.

Stain in carmine alum at least overnight. The specimen can be left in this solution longer and the stain can be reused. If the specimen is insufficiently stained, restain with fresh solution.

The next morning, dip the specimen in water to remove excess dye and then dehydrate by soaking in 70% EtOH for 15 minutes, 90% EtOH for 15 min. then 95% EtOH for 15 minutes and 100% EtOH for 15 minutes. Then place the specimen in fresh 100% EtOH for 15 minutes. This last 100% EtOH soak should be truly water free. Any water in the last EtOH soak can lead to a brownish background after clearing.

Prior to clearing, there is an opaque appearance to the tissue that disappears after clearing. The ideal length of time for clearing depends upon the thickness of the specimen. A thinner specimen, as might be found in a mouse unable to lactate, might need only a few hours in HistoClear. A 2-3 mm thick specimen might take overnight to clear.

Inspect the specimen under the microscope. If it has a brown appearance it needs to be cleared longer. If it doesn't clear despite a long time in clearing solution, the last ethanol soak may have been contaminated with water. If you suspect this case, place the specimen in fresh 100% EtOH for 15 minutes before re-clearing.

Screen 6 – Adding the Coverslip

Before mounting make sure there is no hair on the specimen. If hairs are present, remove them with forceps or a cotton tipped swab.

Using a Pasteur pipette with a rubber bulb, add a synthetic resin such as Permount on and around the specimen to create an even surface for the cover slip to lie on. This will reduce the amount of reflection you get as you look at the specimen under the microscope. For a 2-3 mm thick specimen you will need a ml or more of Permount. Place the slide on top of a few paper towels on an even surface.

Gently place the edge of the cover slip on the glass slide and slowly let it down on top of the specimen. This helps avoid trapping air bubbles. Press down gently to get an even distribution of adhesive. Expect the Permount to start oozing out as you press down on the specimen.

Avoid getting Permout underneath the slide. If this happens, you can try blotting or wiping the bottom of the slide with HistoClear. If you see streaking under the microscope, a residue of Permout may exist under the slide or on top of the cover slip. The easiest way to eliminate the streaks is by remounting the specimen.

As the Permout dries and shrinks it tends to open air gaps around the perimeter of the cover slip. Add more Permout to the edge of the cover slip to fill this gap.

Examine and document with a histology microscope. If your specimen is cloudy, it was probably not dry enough. Place it in clearing solution to remove all mounting medium as described under embedding and return it to 100% ethanol, clear and remount.

If the specimen is too weakly stained, place it for 10 minutes each in HistoClear, 100% EtOH, 95% EtOH, 90% EtOH, 70% EtOH and finally water. Then restain overnight with fresh carmine alum solution.

Screen 7 – Remove Mounting for Embedding in Paraffin

Once you have documented the specimen, you can process it further for histological analysis by embedding in paraffin and preparing thin sections. These can be stained to reveal cytological details. Remove the cover slip by placing the slide in HistoClear. The cover slip should float off and the specimen should remain attached to the slide.

Occasionally a cover slip that has been stored for a long time takes longer to float off. It should come off with continued soaking in HistoClear. One can also use forceps to gently remove the cover slip from the slide.

Scrape the tissue into a scintillation vial containing about 10 mls. of HistoClear. Decant and replace with fresh HistoClear 3 times for 30 minutes at a time.

After the last HistoClear soak, decant the HistoClear and add about 10 mls. of a 1:1 solution of xylene and paraffin incubated at 60 degrees. After one hour, decant the xylene/paraffin solution and replace with prewarmed paraffin. Replace the paraffin 2 to 3 more times and incubate at least an hour each time. The specimen can even be left overnight if it is convenient. It should then be placed on a histocassette to prepare it for sectioning.