From Under the Microscope

A Biopsy Specimen Travels to Hammond

Have you ever wondered what happens between the time a lesion is removed and the time the doctor calls the patient with the results? I'd like to tell you what an important role our histology department plays in the process by explaining how tissue gets processed and how this lets us not only diagnose the patient's condition but also how to help design therapies to treat these conditions.

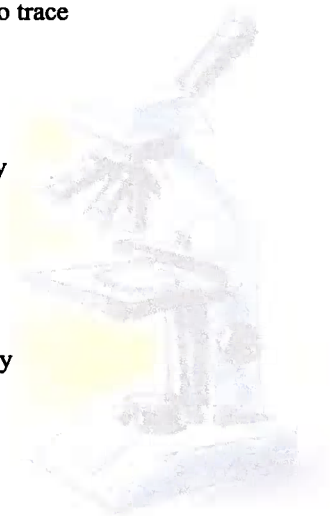
To illustrate this process, I will explain step-by-step how the tissue is handled from beginning to end using a specific example. All names and examples are fictional.

Ms. Barbara A. Smith (40 years old) notices a new black-tan spot on the back of her knee. She visits her dermatologist who recommends an excisional biopsy which means the grossly visible lesion will be completed removed. This is done because the dermatologist wants to know not only what the lesion is, but also whether the lesion has been completely removed microscopically.

The lesion and the skin tissue immediately surrounding it are removed by a physician and the tissue is placed immediately into a container filled with a solution called "formalin" which is a mixture of formaldehyde and water. This solution fixes the proteins in the tissue to make the tissue firmer to cut and to preserve the proteins from degradation. This formalin-fixation also helps preserve the DNA in the tissue in case evaluation of the genetic material in the cells of the lesion is necessary in designing a therapy to treat the lesion.

The appropriately labeled container (Ms. Barbara A. Smith, 40) is sent to Alverno for further processing. Imagine how important it is to make sure that this container is accurately labeled so that it is not confused with Ms. Barbara K. Schmidt's skin biopsy, or even the biopsy of Ms. Barbara A. Smith (60). To help prevent any mix up, the material is assigned a case number (accession number) with the container appropriately labeled with the accession number as well as the patient information.

This tissue is removed from the bottle, then inked with special pathology-paint (which permanently sticks to the tissue) on the outside of the tissue by one of our pathology or lab assistants so that the margins can be evaluated; think of the white paint surrounding a football field to mark what is "in-bounds" versus what is "out-of-bounds." The tissue is then cut into thin slices and put into cassettes that have the accession number printed on them. Imagine how important it is to make sure the right tissue goes into the right cassette because with every step in the process, it becomes more and more difficult to trace this material back to the 40 year old Barbara A. Smith.



Oil and water (actually wax and water) don't mix, at least at first. In order to cut the tissue even more thinly so that the pathologist can eventually see through the tissue, the tissue must eventually be able to be put into wax (paraffin). To do this, the tissue is "processed" in machines which remove the water (formalin is mostly water) with chemicals (mostly alcohols) and allow paraffin to take water's place in the tissue without disrupting the proteins and DNA in the cells which make up the tissue. The cassettes are designed so that the chemicals pass through the cassettes but the tissue stays inside. After having the wax replace the water, the tissue is very thinly sliced on special machines called microtomes which cut ribbons of wax with the tissue safely inside the extremely thin wax ribbon. These ribbons are carefully transferred to appropriately labeled glass slides to which the ribbons of wax and tissue are permanently attached.

Under the Microscope Cont'd

To see the tissue under the microscope, the tissue must be dyed with special dyes that distinguish cell-structures that are acidic and structures that are basic. DNA is acidic and most proteins tend to be basic as a rule. Because these dyes are more water-friendly, we have to get rid of the wax to get back to the water state. Yes, we're going backwards. Special staining machines are used to remove the wax (the tissue sticks to the glass slide) and put the water back. To make things even more complicated, the water has to be removed *again* after the tissue is dyed. Because the tissue is more stable without water and because the tissue is clearer under the microscope without water, we have to go back through the chemical process one more time to remove the water using alcohols and other chemicals as an intermediary and finally to a water-insoluble resin that preserves the tissue for long periods of time and that is clear so that the light from the microscope can pass through the tissue. All this time, the tissue remains fixed safely to the glass slide.

The glass slide (or sometimes slides) with the stained and resin-preserved tissue is given to the pathologist with the appropriate paperwork. With all of the steps in this process, it is so critical that the slides with Ms. Smith's tissue must match up with her identity. Barcoding and scanners have greatly reduced the possibility for error but not completely eliminated it. The pathologist looks at the tissue under the microscope and decides what the lesion is based on what it looks like under the microscope and whether the margins are free of the lesion microscopically. If there is any question about what the lesion is, fortunately the proteins in the tissue have been preserved and special stains called immunohistochemical stains can be used to help identify specific proteins associated with specific lesions or tumors.

The tissue <u>not</u> transferred to the glass slides is stored in the wax in the original appropriately labeled cassettes and can be kept safe for many years. Any leftover tissue which was not put into cassettes and processed for whatever reason is only stable for a few weeks or so.

In this age of molecular medicine, the DNA of the cells is getting increasingly more important in designing treatments for various tumors. The DNA can be extracted from the tissue in the wax and analyzed for the presence of certain genes which are correlated to the response of some tumors to certain chemotherapy drugs. These drugs or combinations of drugs are available to treat not only a specific type of tumor but also to Ms. Smith's tumor specifically. This is the reason why now we are getting better at creating patient-specific therapies which can be tailor-made to treat a patient's tumor.

Although much of the staining and tissue processing is done by machines at Alverno, all of the tissue slicing is still done by real people, whether it's the dermatologist, the pathology assistant, or one of our histo techs. Ms. Smith's well-being depends on them.

So as we celebrate the holidays, let's celebrate the Alverno travel agents: the pathology assistants, laboratory assistants, and all the people in the histo lab who make anatomic pathology happen!

Submitted by:

Dr. Mark vanGorder