SHANTEL HEBERT-MAGEE: So the difference between fine needle aspiration and fine needle biopsy is that you're attempting to get more tissue by doing fine needle biopsy. Not only do you want more tissue, but you want tissue that also has intact architecture, typically what we would define as a core. In order to do biopsies, we typically use larger needles or needles that have a different cutting mechanism.

In the past, we've used a 19 gauge needle in attempts to acquire larger tissue fragments. However, we didn't always get intact architecture. Now that we have biopsy needles that are actually cutting and yielding tissue with intact architecture, it is important that we actually cover the difference in being able to process these specimens.

In this section, we'll discuss proper cytologic slide preparation for on site and off site evaluation. In addition, I would like to introduce my fellow technician that I am so happy to work with, Mr. Konrad Krall.

OK, so when it comes to slide preparation and smearing, I think it's important to recognize the basics. First of all, you only want to put one drop of material on the slide. Putting more than one drop of material on the slide is going to lead to too much specimen. And so therefore, the excess specimen will no longer be on the slide. And it will end up on the table.

I'm really good at reading slides. I'm not that good at reading tables. So therefore, in order for me to be able to make a diagnosis, it's imperative that you only put one drop on the slide.

If you look at the slide, you can see there's one drop placed near the top. How we make the smear, we take and put the cover slide-- or actually the smearing slide-- directly on top of the slide. Pushing it down by diffusion principle, you're going to have a nice circle that is formed. And then you want to pull in one fluid motion. When you pull in one fluid motion, you see a nice oval. And that is typically what you would see when you have a good smear.

The endosonographer can evaluate the tissue grossly. Here we can see there are two slides. Utilizing the standard FNA needle, we have tissue fluid, which happens to be dissociated tissue fragments. What are those? Cells, background fluid and cell groups.

However, we have another slide which has an intact core. We can tell that this is not just blood. Because normally blood appears red.

What we have on the slide is tan, white, orange, gelatinous material. And then we have a hard firm tissue fragment. So even if there is not a pathologist in the room, the endosonographer should feel confident that if their needle is in the right location, they have obtained diagnostic material.

Now Conrad will show us how to actually perform, once again, a smear and touch imprints when we obtain material, using the standard fine needle biopsy needle. Conrad will demonstrate how to actually perform touch imprint, a slide evaluation. It's important that you pick up the biopsy specimen with non-serrated forceps.

Serrated forceps will actually crush the tissue. And it be distorted, when it comes to actual evaluation under the microscope for histologic interpretation. I think it's important that you only gently push the material on the slide, allowing only the superficial cells to adhere. You do not want to push too hard. Because then you'll cause crush artifact, distorting the cells that are on the slide and also distorting the biopsy specimen.

After pushing the biopsy along the slide, you want to take and transfer this biopsy, using the forceps to the specimen jar, typically formalin, for histologic processing. There are instances in which you do have dry aspirates. Typically that happens when you have a very fibrotic lesion.

And we know that when you do seven passes, it's diminishing returns. So in those situations, I think it's important to utilize all the different tools in your armamentarium, at which point of time, you can utilize suction or wet section. However, typically we do not use wet section.

The problem with using wet suction, in particular, is that saline may actually be expressed onto the slide. And that would also dilute the diagnostic material. So we try to avoid that. However, if you happen to have a dry aspiration, I would encourage using other methods, either using a biopsy needle or using a larger 19 gauge needle if you're not getting enough material with using the standard 25 or 222 gauge needles.

The difference between cytology and histology is as follows. Cytology is the microscopic evaluation of disruptive tissue fragments. Typically this is going to be cells and cell fragments and blood. You will receive this from aspirations, brushings, washings, fluids and scrapings.

Whereas histology happens to be the microscopic evaluation of intact tissue fragments. This will be procured from biopsy and larger surgical resection specimens. FNB specimens can be processed as routine histological specimens if the specimen is received intact.

Sometimes when you do fine needle biopsy, the specimen will come out fragmented. If you get tissue fluid, meaning cells, small cell groups and blood, you will want to process it as routine cell block. However, if you get an intact core specimen, meaning retained architecture, the tissue has minimal to no blood. It is OK to process it as routine histology.

How would you do that? You would take and place it in a tissue bag or you will wrap it in tissue paper and then placed it in a cassette. And it should be fine for processing.

So the risk and benefits of each technique is as follows. If you were to process the specimen as routine histology, it's great, because it would go into formalin. And formalin fixation is better for tissue when it comes to staining.

However, if you happen to have disrupted tissue fragments, along with intact tissue fragments, the disrupted tissue fragments may be lost. So for example, imagine if you add a loaf of bread. And you had to hold it in your hand. If the bread is intact, it's easier to carry. And it won't be lost. But if the bread is broken down into grains, the greens will actually slip between your fingers.

So what happens is when you have disrupted tissue fragments, the smaller fragments actually leave the cassette. And they're lost during processing. So therefore, if you have intact tissue fragments, it is fine to process it as histology. However, if you have tissue fragments, plus tissue fluid, consisting of small cells and blood, it is best to process it as a cell block.

There is no difference in the technique when comparing cell block versus histology. The ultimate goal is to actually collect all of the tissue and create a semblance of architecture. Both cytopathologists and pathologists have an appreciation for architecture, right?

So if I want to evaluate a car, don't just show me the rim. I'll be able to tell you what type of car it is. But I won't be to tell you the specifications. I want to tell you if it has leather interior. I won't be able to tell you about the engine.

So therefore, the reason I'm telling you this as an analogy is that the more you give the pathologists or cytopathologists, the better and more comfortable they will be in making the diagnoses. So ultimately, cell block and histology is meant to actually collect all the tissue so that the pathologist is able to evaluate it under the microscope and give the best diagnoses.

So the difference between fixatives, preservatives and transport media. So fixatives, they actually fix the cells. So what do I mean by that? It actually cross links the tissue. And it cross links the protein.

So therefore, the specimen is not going to degrade. What is the most common fixative we use? It happens to be formalin. Now when it comes to cell preservative solutions, they also prevent the cells from degrading. But they do not cross link the proteins.

What are common preservative solutions? They happen to be ethanol and CytoLyt. Why would we use these solutions? Well, CytoLyt actually lyses the background red blood cells. So if you have a very bloody specimen, it would be advantageous to put it in CytoLyt, so you can get rid of that obscuring red blood cells.

Now the final solution we use is transport media. Transport media is going to keep the cells viable. We don't want to always kill the cells. We want the cells to sometimes be viable.

We need to be viable if we're going to do flow cytometric analysis when evaluating from lymphomas. The cells have to be alive. So we will put them in either RPMI, saline or Hank's solution. What's the difference? Well saline is the cheapest. There is no additive solution.

So if you want a marathon, right, you don't want water. You want Gatorade. You want Vitamin Water. You want something with electrolytes.

Well your cells want the same thing. They don't want to be in saline. Saline is only a homeostatic environment. But it doesn't actually have any nutrients in it.

Whereas RPMI and Hank's solution has glutamine. So if you're going to do a flow cytometric analysis and sent it to an outside lab, you want the cells to be viable as long as possible. So you have to know when to use which type of media. You don't want to actually take and place something for lymphoma into formalin. Because you won't be able to use it for flow cytometric analysis.

And likewise, you don't want to take and put a specimen in a preservative solution if you want to actually grow it in the lab for cultures. You want to take and put it in either saline or either into Hank's solution. So I think it's important to recognize how the specimen should be processed and what type of curing solution you should use.

So what is a core? A core is uninterrupted contiguous tissue fragment with retained architecture. The assumption is that if you use a core needle, you should receive a core. However, you have to recognize that a core is not dependent upon the needle. But it's dependent upon the tissue integrity.

So if you have a very fibrotic lesion. Let's say you're sampling a lymph node in the mediastinum. If it's a very hard fibrotic lymph node, you will obtain a elongated core tissue fragment.

But if the lymph node happens to be hemorrhagic or necrotic or cystic, you're going to get the worm. And the worm is going to be discontinuous tissue fragments admixed with blood or mucin or necrotic debris. So therefore, it's not dependent upon the needle. But it's dependent upon the tissue itself.

When disrupted tissue fragments are treated as a core biopsy, you stand the chance of losing diagnostic material. So let's imagine if we were stuck in the snow, and if one person is stuck by themselves, you're more likely to lose that person secondary to hypothermia. But if you're in a group and you're held together, you're less likely to lose a person to hypothermia.

Why am I making that analogy? I'm making that analogy, is that, if you process FNA specimen, which consists of fragments of tissue and cells and blood and individual cells, separately, they will not survive processing. But if you actually take and processes it as a cell block, you actually take and congeal that tissue, it's more likely to survive cell processing. And you won't lose any diagnostic material. And your pathologist will be happy.

Is bigger better? The answer, inherently, you would think would be yes. But the answer can be no. We don't always need more. And more is not always best for us. An example is if you go to a Quickie Mart, you don't really need to have two huge bags of Lay's potato chips, right? One should be sufficed. And you don't need the extra calories.

Well what happens is that when you go in with a larger needle or you go and with the needle and you use suction, you're going to get more specimens. But you may not actually get more diagnostic material. So you get all the extra stuff you don't want, such as blood, such as mucin.

And what you actually want is diagnostic material. So always using a larger needle is not best. If you have a very vascular lesion, you wouldn't want to use a large 19 gauge needle in that situation. Because what happens is you're going to get more blood.

If you have a vascular lesion, you wouldn't want to use slow-pull technique or wet suction or suction at all. Because once again, you'll get more blood. So bigger isn't always better. What's better is having more diagnostic material.

So sample collection is not always reflected on what we get when processing of the tissue takes place. So what I'm saying, simply, is that what you put in is not always what you get out.

So imagine if you were actually baking a cake, right? And you take and you put all these ingredients in. Then you put it in this hot oven. Sometimes the cake gets burnt. And when it gets burnt, you lose some of the good ooey-gooeyness of the cake.

Well the same thing happens when you're processing tissue. You may do two or three dedicated passes for a cell block or histology processing. But it goes in the processor. And when it goes in the processor, it's high heat--intense heat. And what happens is that the tissue is actually cremated. And it shrinks. And it's made smaller.

So imagine if you're doing fine needle aspiration, and you have these loosely discohesive fragments of tissue, even once you take and make the nice pellet, you will still lose a certain amount of tissue.

So sometimes the endosonographer has a certain level of frustration. They feel that they gave lots of material. So the question is, was that material predominantly blood, or was it actually diagnostic material? How many dedicated passes? Could you microscopically see material floating in the jar?

If the answer to most of those questions happened to be, no, you don't see diagnostic material. You don't see things that are floating in the jar. Even though you may be giving some material, it may be insufficient for ancillary study and cell block evaluation.

So how do you process a high level cell block? I know this is the moment that you guys have all been waiting for. So I'm going to go through each step slowly and intensely to make sure that you get, how do you process the cell block?

So the first thing you want to do is actually collect material in a specimen container. So whether you use formalin, CytoLyt, or you use saline solution, you want to make sure you have something that you're transporting the specimen in. You want to take that to the lab and then transfer it from the specimen container to a centrifuge tube container. Then you want to take in centrifuge, based upon a certain revolutions per minute for a period of time, to get this nice condensed pellet.

So you want all those single cells. You want tissue fragments that actually fall to the bottom and form this nice pallet. Then you're going to take and pour off all the solution and most of the red blood cells. So you're going to decant and remove the supernatant and only be left with the nice round pellet.

To that pallet, you need to add some type of glue. So what is the glue? It's going to be plasma thrombin, HistoGel, agar, albumin. Because you want that pellet to stay together.

Once you have a nice, hard pellet, you're going to transfer that to either a tissue bag or either paper, wrap it up, put it in the cassette. Once it's in the cassette, you're going to put it on the specimen processor. The specimen processor actually dries out the tissue. It removes all the fluid and replaces it with wax. So it can take and maintain this nice, mummified type of appearance.

Then you're going to take it off of the processor and embed it in wax. Once it's embedded in wax, you're going to take multiple sections. Once you get multiple sections, you're going to find a complete section that shows all the tissue. And you're going to take and put that on the slide. Then you're going to stain it. You're going to cover slip it and send it to the pathologist.

Now that I'm giving you this detailed explanation of how to make a cell block, where can things go wrong? In every individual step. So when you're transferring from that tube to the centrifuge tube, guess what? You're going to lose a certain number of cells.

When you take and put it on the centrifuge and you try to create that pellet, guess what? You're going to lose a certain number of cells. When you take and create that nice pellet, and you transfer it over to the cassette, guess what? You're going to lose a certain number of cells.

When you put it on that processor, it's going to dry out the tissue and remove all the liquid and put it in wax, guess what? You're going to lose a certain number of cells. When you take it out of the cassette and the processor and embed the whole tissue into wax, when you transfer it using your forceps, guess what? You're going to lose a certain number of cells.

When you take and actually start cutting it, you're going to cut it on a microtome, like slices of bologna. And when you do this, you're going to lose the first few sections, right? You have to efface the block, so you can get this nice, smooth section. When that happens, guess what? You're going to lose a certain number of cells.

So with each step in the process, you're going to lose a certain number of cells. And we can't prevent this. So therefore, it is incumbent upon the endosonographer and the cytotechnician in the room to try to get as much tissue as possible.

So does blood in the sample matter? Typically, if you're using CytoLyt, the background red blood cells will be lysed. So therefore, you don't have to worry about having obscuring artifact from blood.

However, if you're using a different type of cell block method for preparation, you may have a lot of blood. Sometimes blood can be obscure. It can make it more difficult to make the diagnosis. However, the benefit of having a cell block is that you're capable of cutting multiple levels. And in cutting the levels, you're able to adequately evaluate the tissue without having to worry about the blood.

You typically you do not have to worry about blood. Because blood would not be a significant factor if you have a contiguous tissue fragment. So there are opportunities to lose tissue that's procured using a fine needle biopsy needle. The manner in which you would lose tissue typically takes place after the specimen comes off the processor.

So if the histotech cuts too deep within the block, diagnostic material can be lost if you're dealing with a very small biopsy specimen. This is unlikely if you have a very skilled technician. But it is possible.

So how to process a core biopsy through surgical pathology. Unlike a cell block, in which you have to take disjointed fragmented tissue pieces and create a pellet, when you have a biopsy specimen, it's contiguous tissue fragment. So it can be processed as routine histologic specimens.

I would suggest, even though it is a routine histologic specimen, typically the specimen is minute. So I would put it in a nylon tissue bag or wrap it in tissue paper and then place it into a cassette for routine histologic processing. You typically do not have to worry about blood. Because blood would not be a significant factor, if you have a contiguous tissue fragment.

So where things can go wrong. I think any time there is human intervention, by nature, things can go wrong. So with specimen acquisition, where can things go wrong? Well, it depends upon the endosonographer's technique.

Is he in the right location? Is he procuring tissue? What technique is he using to procure tissue? Is he using suction? Is he using slow pool? What type of needle is he using? If you have a very fibrotic lesion and you're using a 25 gauge needle, you may not procure sufficient material for diagnostic yield and analysis. If you're using a very large needle and it's a very vascular lesion, you may not procure sufficient material.

Also specimen processing and handling. How is the cytotech making the smears? If the smears are being made incorrectly, the pathologist will not be able to interpret the slides.

If the specimen is placed in the wrong type of solution, if you're putting it in a fixative or preservative solution, instead of putting it in a transport media, once again, something can go wrong. Obviously, labeling issues in the laboratory, things can go wrong. And even with transferring the specimens from the tube to the cassette, to the processor, to wax embedding, to cutting the section, to putting it on the slide, to staining and finally interpretation, things can go wrong.

How do we try to limit these things? Well, obviously, it takes skill, time and effort. Being conscious of knowing when things can go wrong can help prevent us from making those errors. But don't be afraid. Errors do occur. They are corrective. But the best way to prevent them is being knowledgeable.

So what's important to the pathologist, when it comes to fine needle aspiration and fine needle biopsy, I think, simply put, its tissue. We want diagnostic tissue. Fine needle aspiration, we typically utilize it when we do not need to do ancillary studies.

So for example, if you have an 89-year-old patient who has a large pancreatic mass, obstructive jaundice, you see invasion of the SMV, and the patient does not appear to be a surgical candidate, and there is no previous history of other malignancies, and you're thinking it's going to be pancreatic adenocarcinoma, typically, fine needle aspiration will suffice.

However, if you think you need to be able to do ancillary studies, a patient has a history of breast cancer, a patient has a history of lung or colorectal cancer, it's important that you have enough material. And typically, using the smaller standard fine needle aspiration needles, you may not actually procure enough material.

Now, you can do multiple passes to try to overcome this limitation. But there are times in which a pathologist actually need to have architecture. Typically, fine needle aspiration will suffice.

However, if you think you need to be able to do ancillary studies, a patient has a history of breast cancer, a patient has a history of lung or colorectal cancer, it's important that you have enough material. And typically, using the smaller standard fine needle aspiration needles, you may not actually procure enough material. Now, you can do multiple passes to try to overcome this limitation. But there are times in which a pathologist actually needs to have architecture.

FNB can be used for multiple different applications. Even though we can use it for standard aspirations, I think because of the increased costs, we should be judicious in when we choose to use fine needle biopsy needles. I think we should use them when we need to have architecture, particularly when looking at gastric lesions. Linitis plastica can be inconspicuous. And I think it's important to recognize that we need to have intact architecture when trying to evaluate for the presence of signet ring cells.

In addition to that, lymphoma. We need to be able to grade lymphomas. And in those cases, we need to have retained architecture. Also, for liver parenchymal diseases, hepatitities, we cannot procure enough material using the standard FNA needles in order to be able to properly evaluate, deliver. So there are indications for using a biopsy needle.

Now, we recognize that every endosonographer does not have the same level of training. Some have recently become graduates, and they're starting practices. And so their diagnostic, or more so their technical skills, may not be as sophisticated or advanced as someone who is a seasoned endosonographer. In that case, a biopsy needle may be easier for them to procure material and get a diagnoses in fewer passes.

Moreover, we also recognize that every pathologist does not have the same diagnostic skills. So therefore, it may also be advantageous to have a pathologist who feels comfortable with looking at more material. And we recognize that the biopsy needle can yield more material.

So in short, biopsy needles should be used when you need to have architecture-- gastric lesions, liver lesions and lymphomas-- and when you need to make sure you have enough material for diagnoses and ancillary studies. And you cannot procure that amount of material with the fine needle aspiration needles.

What makes a pathologist happy? Well ideally, what makes a pathologist happy is the winning lotto ticket. But we know we can't always get that.

But what we can get is diagnostic material. So having the winning specimen is most important. And what's a winning specimen? A winning specimen is a specimen that's going to be diagnostic, that's going to yield a sufficient material for ancillary studies. And we recognize that oncologists want to be able to treat patients based upon their particular molecular characterization of the tumor. So therefore, if we can get specimens that are going to yield sufficient material for ancillary studies and personalized health care, I think that every pathologist would be happy.

So what is next generation sequencing? Next generation sequencing is also known as mass parallel sequencing. It allows for tumors to be characterized based upon their molecular DNA profile.

This is particularly important when we look at lung cancers, colon cancers, and breast cancers. We no longer have to get tissue that is sufficient for on site evaluation. It's more important to get tissue for off site molecular characterization. It's important for us to be able to evaluate tissue and tumors, based upon their prognostic and predictive profile, meaning how will the tumor respond to therapy? And is there targeted therapy?

Next generation sequencing allows for you to be able to have the complete profile of a given tumor. So you can look for KRAS. You can look for ALK1. You can look for eGFR and PD-L1. And if you look at these different molecular markers, it actually can give you insight on how the tumor should be treated and what's best for the patient. And so it has huge implications, when it comes to the patient's care and overall survival.

So how much tissue is necessary for next generation sequencing? Actually, it's not that much tissue. It's actually 500 nanograms of DNA, which is probably the top of a pin cap.

The reality is that sometimes we don't get diagnostic material, meaning that we may actually procure specimen. But it's blood, or it's normal cells, and we're not actually getting the abnormal malignant cells. But if you're getting abnormal malignant cells, you're not getting necrotic debris, you're not getting blood. You only need 500 nanograms of intact viable DNA, in order to be able to do next generation sequencing. So the key is more passes, or either using biopsy needles, if you want to be able to do next generation sequencing.

So the pathologist's interaction with the oncologist can come at various different stages. Sometimes a patient has a known history of tumor or malignancy, and they're coming in for staging. So the oncologist may have certain requested tests that they want performed. Sometimes it's a new diagnoses. So we don't hear from the oncologist until we've already obtained a cell block and signed out the case.

That's why it's so important to make sure you procure enough material in order to be able to do any of the testing that the oncologist may request. And so you have to be preemptive, and make sure that you get sufficient material. And I think it's important that this is in the back of the mind of the endosonographer, the technician in the room, and if there is an on site pathologist, to be aware that tumors now can be characterized based upon their molecular profile. And oncologists want to know more than that it's just a certain type of cancer, but how the cancer will respond to a given agent.