SPEAKER 1: Let's talk about adequacy for next-gen sequencing, as well as just evaluations for all the molecular analysis that our friends have been asking us for. Right away, you want to know because you've all had the same experience, and until we had ongoing discussions with cytopathology. I would take my biopsies. They would say it's a tumor. I've got enough here to be able to tell you will run adeno versus squam, blah, blah, blah, blah, blah.

And then I'd say, well, do I have enough to run all the EGFRs and ALKs and PDLs and yada yada yada. And they would go, ah, probably. And so I take another pass, because "probably" is a crappy answer. And then you get this report that says, definitely I had no carcinoma, definitely diagnosed staged-- by the way, not nearly enough material to run any form of a molecular analysis.

So then you go to a tumor board, and nobody blames the pathologist. They blame the bronchoscopist. And so now I've got to re-bronch you, because I didn't do my job right the first time. And that's annoying. So one of the immediate advantages of ROSE, and now a better quantification of what we need to run all these tests, is the ability for the pathologist to say, you may stop now, because I have plenty of cells on this slide. And so where this has evolved-- this is very important. Because this is a very nice evolution. It's not just what's in the jar. You can use the material that's on your slides. That used to be wasted material, and we were all guilty of the 1,000 cells on the slide that someone ultimately threw away. Nine more passes into a lymph node, because they said three was enough, and you didn't believe them. So you kept going, and all you get is garbage. You never got as good a sample is that first one. So Jeff--

SPEAKER 2: Yeah, and that's-- it is changing. I think the more and more papers come out showing that the cytology smears are actually superior to cell blocks and core biopsies for doing molecular studies. And I'll go over the advantage of doing that in a second. So this varies from lab to lab, and it depends on what their panel is. Some are just doing individual like EGFR or KRAS. Now, most of them with next-gen sequencing-- they're doing different platforms. But this generally applies to most.

So each cell has roughly-- it is about four picograms of DNA. We need about 10 nanograms, so that translates to about 5,000 cells. We actually use 2,000 as our minimum number. And sometimes, especially if it's a progression of disease, and the lymph nodes are really fibrotic, and it's really tough to get tumor out, we've worked with a lot less than that, and we've been successful. But for the 50g panel, around 5,000 cells I think is what most labs would have enough.

It doesn't have to be off of a smear. It can be off of a cell block. It can be out of a core, but when using a core or a cell block, you can't really do on-site adequacy for those. You can kind of get a feel for it, but it's usually not very accurate. And another requirement is you need at least 20% of the nucleated cells to be tumor. So this is just showing that when you collect the material, you can use the smears for the DNA extraction for the molecular, or you can use the cell block. But again, you still have to wait until usually the next day or next couple of days before you'll get the results of the cell block.

And we started off using cell blocks, and it was frustrating, because so many of them-- we thought we had a really good material for a cell block, and then the cell block just doesn't have enough cells. So the smears are definitely a better way to go.

SPEAKER 1: Gives you that immediate visual representation that you had plenty of tissue.

SPEAKER 2: And this I think shows the workflow in most institutions, where they would do passes until they have diagnostic material. And then they would start putting the rest of the material into a fixative to make a cell block, or they would even do a core, but this is changing. I think we kind of mentioned these, but I'll go over them. The advantages of a cell block is it could be very cellular, but the disadvantages of using a cell block for molecular studies is you can't do on-site adequacy. You do have some degradation of the DNA, and there's an additional turnaround time, whereas using a smear, you can do the adequacy on site. Excellent DNA quality for doing next-gen sequencing.

The disadvantage, of course, is you sacrifice that slide that you're using to scrape the cells off of to do the--

SPEAKER 1: But the beauty, of course, is that communication, and there's definitely differences amongst Jeff's colleagues, because different sets of pathologists come to the lab. One will say there's adequate, and someone will say, well yeah, just give me one more slide. So they'll have been plenty, but some of them, because the slide ultimately gets used, what that sort of insurance policy of just one more slide. Well, what's it to you, right? It's another needle pass. Whoop dee doo. It's another 30 seconds of passing, sample that note again straight onto the slide and move on. And then in the end, there is always still the cell block. So if somehow there wasn't enough, even after adequacy being called at the bedside, we've got still plan b.

SPEAKER 2: Yeah, and we do work together on this. So as soon as we're in the room, and as soon as I say, OK, you have a tumor. It looks like a adenocarcinoma. Then our next goal is going to be if it's a higher stage to start getting material for molecular studies and he'd do additional pass-- usually one or two. Once you're in the lesion, it usually doesn't take much. We get one really good pass from molecular. We leave that uncover slipped, and that goes right to the molecular lab. And all of our diagnostic slides that are still part of the case. So we don't have to worry about sacrificing those. We also have our [INAUDIBLE] slides that we haven't even seen yet.

He's just talking about DNA is better preserved on the smears, as opposed to the cell block or a core. And this is just showing there are different studies. There are more and more coming out of how people are able to run these molecular studies, next-gen sequencing, off of cytology smears. And I think this is catching on more and more. Some of the bigger labs are just starting to accept smears.

SPEAKER 1: This was that evolution, right? Again, I was used to getting the entire organ, then an entire lobe, then big chunks. Now I'm getting an oh, we can't run that off of cytology specimens. Why? Why can't you? Because. OK, that's a terrific reason. So actually, as the data comes out, you very much can run off of these smears and get them extracted DNA from these smears. And why waste it? You've just seen on that microscope thousands of tumor cells. The answers that you're looking for are right there, literally.

SPEAKER 2: But by the time we finish a case that we're going to be doing next-gen sequencing, I feel confident that we have the material to do everything that we need to do to get a diagnosis and all their molecular diagnoses. And this is just saying that the studies show that using the smears for molecular is about the same as core biopsies. Ours is actually much higher, because we're doing this adequacy on site. So we rarely will have a case that we say this is adequate-- that we'll not have enough to do to do it later.

And PDL1-- that's a different story. For PDL1, we still focus on the cell block, but this hasn't been an issue. So usually one or two passes, or just after putting a drop of material on each slide-- putting the rest of the material in for the cell block. Usually, the cell block has plenty of tumor, and all we need is a 100 tumor cells to run the PDL1 test. And now they're requiring this on all of our cases.

SPEAKER 1: This is important. You'll hear a pathologist say, well, I'm not going to run that unless I have a core. Do you know how a core is defined by a pathologist? Can you pick it up with tweezers? Not kidding. So any needle can give you a quote core, but also, once you have a cell block, guess what? That's a core, because I can pick it up with tweezers. It's ridiculous. Don't be-- core to me implied that I need to-- it needs to be the size of my hand, a big wad of tumor sitting there, and not in the slightest. So the terminology is bunk. Core is anything that can be visibly seen-- therefore, picked up by tweezers. So it's all in how you label it. We can't run this unless it's a core. Here, enjoy this core sample, right?

SPEAKER 2: And all these things, they're evolving. And I think more and more pathologists are getting on board. This is just an example of a PDL1 test. The picture on the left is negative. The picture on the right is positive. I know it only looks like a small percent of the cells are staining, but those of the tumor cells. The rest of them are benign lymphocytes in the background. But that's what it looks like under the microscope, though. This just going over the panels that we have. Some of them are heme panels. The rest of them are solid tumor patterns. And this is the one [INAUDIBLE] targets small biopsies like cell blocks, even core biopsies and smears.