Questions about frozen tissue, preanalytic variables, tumor content

June 2018—*Boaz Kurtis, MD, laboratory and medical director of Cancer Genetics in Los Angeles, took the following questions from attendees at a webinar on NGS in routine non-small cell lung cancer biomarker testing.*

Can NGS be done with fresh frozen paraffin-embedded tissue?

It can, and we have experience with that. Any specimen type that you want to put into a clinical next-generation sequencing assay should be independently validated, and fresh frozen would be an example. There are different techniques you can use to extract DNA from those samples. If you were to anticipate a unique specimen type like fresh frozen, whether it's fresh frozen alone or fresh frozen and later paraffin embedded, it would be advisable, and this is in the guidelines for analytical validation, that you include such specimen types in your validation of the assay.

How do you work with your customers to control preanalytic variables?

This is often the bulk of what I do on any given day. There are certain things no one can control for after the sample has been delivered to us because you can't turn back time, and there are other things we can control for. The most obvious example is tumor content. One thing we can do to control for that is employ dissection techniques to enrich for it.

Another thing we can do to control for other preanalytical variables is the amount of tissue. For example, even if the tumor content as a percentage of the overall sample was very high, let's say it was 80 or 90 percent, but the absolute amount of tumor tissue is low, to control for that we can section more tissue from the block.

But the question is specifically how we work with our clients to control for those variables. In that example, we would need to talk to the client to get permission to potentially exhaust the block if we feel it would be needed to yield valid results from NGS testing. Alternatively, we would ask the client if it has another block.

Pathology reports accompany all the specimens. I and my colleagues review the details of the report, even the gross description, to see if other blocks were harvested or put together as a result of this procedure that might be equally, if not more, suitable for the testing based on what we read in the report. I have also gone so far as to scan digital images of H&E slides and share those images in a live setting. We review the histology and make sure we're on the same page in identifying what constitutes tumor for testing within the sample, because sometimes even that might not be clear. It's particularly important if you want to micro dissect or do a dissection technique on the sample. You need to make sure you know what the tumor to dissect is. On some occasions I have consulted with the referring pathologist using digital imaging to make that determination.

How do you assess tumor percentage or tumor content? Do you use a computer or image-based algorithm?

In our laboratory, the assessment of tumor percentage is very traditional: It's a trained pathologist's eyes. And I fully recognize that among pathologists there will be variability. My general rule of thumb is that variability that is within plus or minus 10 percent is acceptable such that if one pathologist says 50 percent, anyone who says between 40 and 60 percent would be considered to be concordant with the first pathologist.

How do we validate around that? You would take samples in which the tumor content has been assessed as part of your validation. And it would probably be most advisable to have the tumor content assessed by more than one

pathologist to make sure there's consensus. And then you can take that content value and put that sample into validation testing.

Once you've tested a large number of samples that have had their tumor content determined by a consensus measurement, you can establish a cutoff for tumor content required for your test. That said, as a medical director you do have discretion to allow samples into the testing process even if they don't meet your tumor content threshold. That is something we can do; it's part of practicing medicine. As licensed physicians we can make those determinations at the level of the laboratory.

I do that not uncommonly for a variety of reasons, because I feel there could be something in that sample that's worth testing. In those situations where I proceed into testing a sample that has less than the determined or predetermined tumor content threshold, I include a disclaimer. It's built into our report. We are basically disclaiming our ability to perhaps detect a mutation should there be variations in tumor content beyond our control.

Perhaps false negativity could be a possibility, and that is the reality of laboratory testing. As long as you make the reader of the report aware that is a potential factor, it's acceptable. You are within your limits of practicing pathology and lab medicine to proceed with testing on those types of samples.

Can you foresee a time soon when a lab can use only NGS in lung cancer cases?

I think that era is already here. You also have to take into account who these labs serve—different types of communities in the health care field—and that can inform the lab's decision to use or not use any other non-NGS testing platforms. But an NGS-only paradigm is already taking place today.n[hr]