The article by Bass et al titled, “A review of pre-analytical factors affecting molecular, protein, and morphological analysis of formalin-fixed, paraffin-embedded (FFPE) tissue: how well do you know your FFPE specimen?”, found in this issue, is a very ambitious review of preanalytic factors and their potential effects on tests performed to detect proteins, RNA, and DNA from formalin-fixed, paraffin-embedded (FFPE) tissue. It’s easy to be frustrated by this article, because it shows how little we know of preanalytic factors that could affect further testing. Preanalytic factors are important; this is highlighted every time someone processes tissue that is not sufficiently fixed. The next day, a proper tissue section for glass mounting cannot be cut adequately, and if cut, those sections are poor and possibly uninterpretable using traditional histochemical stains, not to mention immunohistochemical methodologies. Should we not be worrying about the condition and availability of RNA and DNA integrity in FFPE specimens?

FFPE was primarily developed and used for its ability to preserve tissue indefinitely for morphologic examination. At its inception, many special (chemical) stains were developed that have served us well for decades. It is worthwhile to pause for a moment to appreciate that the value of these histochemical stains was rooted in their ability to identify and tinctorially discriminate various subcellular components (eg, mucin, iron, glycogen, collagen, keratin, amyloid, reticulin, basement membrane, myelin, etc), and as such, represent the surgical pathologist’s initial foray into the molecular biology of the cell. This was followed by direct examination of cellular ultrastructure using electron microscopy and, beginning in the 1980s, by immunohistochemical methods. This expanded our ability to identify the expression and location of various protein antigens. Typically, these antigens are first studied using frozen sections, acknowledging that very few antigens survive formalin fixation unscathed. With time, however, we invented more sensitive detection schemes and learned how to “retrieve” these masked antigens so that they can be reliably detected. In the 1990s in situ methods were adopted to detect specific RNA and DNA targets in FFPE tissues. For all of these new technologies, we have adapted them to perform well with established tissue fixation protocols that ensured consistent histology.

With the introduction of the American Society of Clinical Oncology/College of American Pathologists guidelines for HER2 and estrogen receptor (ER)/progesterone receptor (PR) testing, we have now come to understand that preanalytic factors are important for reliable testing of these antigens.1,2 We have learned that time of fixation affects the availability of antigens detected. Not surprisingly, we have also learned that cold ischemia time is important to the viability of some antigens. There is more to be preserved in tissue than consistent histology.

We are now on the cusp of an explosion in molecular testing on FFPE tissues. The good news for pathologists is that not only are we involved in the stewardship of FFPE tissues, but we have before us an opportunity to extend our understanding of pathology that goes beyond differentiation (histochemistry), gene expression (immunohistochemistry), and ultrastructure (electron microscopy and immunohistochemistry). It is a natural next step for pathologists to explore, evaluate, and use our understanding of the genetic and regulatory elements at play in the tissues we examine. We are already fully vested and involved in the management of these tissues. We now have some responsibility to ensure that these specimens are suitable to these newer molecular studies.

Today, there are minimal general preanalytic requirements for most tissues. There are more stringent requirements for ER, PR, and HER2 testing. Although we should not rush to impose new rules on the preanalytic process, the time is right to think about how the preanalytic processes could be optimized for potential future testing. In an ideal world, all of these factors would be investigated, with deliberate determination of the best methods that should be used. But it is unlikely that this will happen. We are more likely to see tests introduced into clinical use with subsequent research demonstrating limitations because of preanalytic factors. HER2 is a good example. Now that we know cold ischemia time and fixation time are important for detection of HER2 in breast cancers, could it be important...
for detecting HER2 in other tissue, such as gastric cancer? Should we—can we—extrapolate this experience to other tumors (colon, lung, prostate, etc)?

First and foremost, the process of formalin fixation and paraffin embedding of tissues was intended for morphologic assessment of tissue. We should make sure that morphology is never compromised. Having said that, there is no way to ignore this explosion of testing that is occurring on FFPE tissues, and we should endeavor to determine how best to process, fix, and store specimens so that patients are not disadvantaged in the performance of molecular testing, either now or in the future. This review is important because it outlines all of the potential pitfalls in testing. But this review also points out how little we know about many preanalytic factors and their potential impact on testing. We need to support continued investigations into assessing many of these preanalytic factors, but should we panic if we know little about a factor? No. Testing will go on because there are other methods of assessing the integrity of specimens for protein antigens, RNA, and DNA without fully investigating every aspect of these processes. It seems appropriate to address individual specimen adequacy for a specific test with some downstream measures. For example, the Invivoscribe kits for B- and T-cell gene rearrangement (Invivoscribe Technologies Inc, San Diego, California) include an “internal control” assay that assesses the suitability of the recovered DNA for polymerase chain reaction amplification and provides an evaluation of the DNA fragment size distribution. For many assays, simply monitoring the test failure rates or positive (eg, mutation detected) frequencies can be informative of upstream deficiencies.

The article by Bass et al is a compilation of all of the potential factors that may affect testing for proteins, RNA, or DNA in tissues. Overall, this is a good framework to understand the implications of each factor. Table 1 in the article attests to the large number of factors that compound the problem. Molecular testing requires more stringent conditions than morphology. As our knowledge and experience are broadened to understand the nuances of testing, better recommendations for tissue processing, fixation, and storage will emerge. We need to be open to these possibilities and accepting of these changes when they make sense.

References