The editorial by Fletcher and Fletcher in the August 2002 issue of *AJCP* in response to news that the Food and Drug Administration (FDA) is making plans to recommend a “standardized” immunohistochemical test for the KIT protein (CD117) highlighted the clash of cultures that is emerging in diagnostic surgical pathology. Largely based on the interpretation of histologic images, this profession has long relied on the individual practitioner’s inherent pattern-recognition skills, experience, and clinical acumen, attributes not easily quantifiable. However, with the introduction of techniques to identify individual molecular attributes of cells in histologic sections, surgical pathology is moving more and more into the realm of quantifiable traits. Because therapies now are being directed toward individual molecular targets, it is becoming the responsibility and purview of surgical pathologists to make clinical judgments on the presence of, and the quantity of, specific molecular constituents of cancer cells. This also means that the diagnostic practices of surgical pathologists are coming under the scrutiny of regulatory agencies such as the FDA, which believe that it is their purview to regulate the manner in which these interpretations are made.

By far the major procedure in surgical pathology by which specific molecular constituents of cells are identified is immunohistochemical analysis. As a technique, immunohistochemical analysis is “quirky,” in that it is applied to a wide variety of tissues that have been treated and processed in different ways, it involves several crucial steps (each of which can affect the final result), and it is highly operator dependent. It is a technique that can have quality assurance applied to it by the appropriate use of controls, but it is not a technique that easily lends itself to standardization, even with attempts to automate it. As an adjunctive test to the standard evaluation of histologic tissue sections and cytologic preparations, immunohistochemical analysis has been very useful in establishing and refining clinical disease entities and in the appropriate classification of individual tumors for clinical purposes. However, no single immunohistochemical test defines a single entity, and in any group of well-defined lesions by histopathologic and clinical criteria, there are “outlier” cases with aberrant immunohistochemical profiles. Most practitioners of surgical pathology are aware of the limitations of the technique and of biologic variability, and it is the rare pathologist who has not “ignored” at some time an immunohistochemical result (positive or negative) in a diagnostic workup. This is in keeping with the concept of medical diagnosis being the result of an integration of many data and is not the result of a single binary test result. However, in the assessment of a specific molecular target, the result must be binary: either the target cell expresses the target molecule to the degree required for therapy or it does not.

On the face of it, one would think that immunohistochemical analysis would be an excellent quantitative technique: the amount of antibody binding to the tissue should be directly related to the amount of antigen. The problem, of course, is that nothing is standard in the evaluation of clinical tissue specimens. The degree of antigen degradation will vary from sample to sample depending on time of intraoperative anoxia, time elapsed from resection to fixation, type of fixative used, amount of fixative used, and accessibility of fixative to tissue (thickness of sectioning, piling of tissue slices on top of one another). The simple act of freezing tissue before fixation tends to greatly affect subsequent immunohistochemical analysis for reasons not well understood. During...
fixation, antigens tend to be masked, by intramolecular crosslinking, coagulation, or both. This masking can be relieved by a number of mechanisms, which in themselves are difficult to precisely standardize (proteolytic digestion or by boiling, steaming, microwaving, or autoclaving histologic sections in a variety of buffers). Antibody lots will vary in affinity and concentration (especially polyclonal antibodies) and may degrade with storage. It is not unusual for an immunohistochemical laboratory to have to retiter dilutions between antibody lots.

For each immunohistochemical test, conditions must be titrated to obtain the correct “threshold level” of detection. This is especially crucial for tests involving an antigen that has biologic significance only when expressed to a certain degree. This is best accomplished by titrating conditions to a sample with known characteristics. Another problem is that the most widely used methods of antibody binding involve the enzymatic deposition of colored compounds, a technique that has a small dynamic range, making assessment of widely variable antigen concentrations difficult. In addition, in most adjunctive diagnostic uses of immunohistochemical analysis, it is more often the presence or absence of staining, often augmented by patterns of staining of cellular substructures, that is used. Focal weak staining for an antigen in one case may be considered “supporting evidence” for a diagnosis, while in another case strong diffuse staining will lead to the same conclusion. This type of diagnostic leeway is not allowable when immunohistochemical analysis is to be used as a quantitative (or euphemistically a “semiquantitative”) assay.

The article by von Wasielewski et al in this issue describes the innovative use of tissue microarrays, which were used to share a wide range of test samples with a group of reference laboratories in the evaluation of estrogen receptor (ER) status in breast cancer by immunohistochemical analysis. In the same study, investigators also performed some limited experiments that showed, at least in the case of ER and as long as formalin was used as a fixation agent, that the immunohistochemical detection of ER was not greatly influenced by the pH of the formalin buffer or up to several days of fixation time. Their study suggests that variability in subsequent immunohistochemical practices and interpretation of results was a greater source of diagnostic error. If we can extrapolate these results (and these types of rigorous studies should be performed and published routinely for crucial immunohistochemical determinations), it would seem that, indeed, variability in the immunohistochemical technique and interpretation should be targets for standardization.

On the surface, such results might argue for even tighter standardization of immunohistochemical reagents and protocols. There is an unquestioned need for accurate evaluation of molecular targets. Specific therapies for such targets are expensive, and payers for health services (patients, insurance companies, health maintenance organizations, state and federal governments) have a justified interest in not paying for therapy that does not have an appropriate indication. And although by their very nature targeted therapies tend to be of low toxicity, all therapies carry with them some risk of side effects. It therefore is an ethical obligation to obtain the most accurate assessment possible.

The question is, “What do we standardize?” The FDA has regulated various medical tests (reagents and platforms) in the practice of clinical pathology for years. It is interesting to note, however, that the closest analogy to immunohistochemical analysis in the realm of clinical pathology is the use of antibody markers in flow cytometry. While the instruments used in this procedure are subject to FDA approval, the actual antibodies used in the procedure, for the most part, do not undergo FDA approval processes, perhaps owing in part to the recognition that the use of antibodies in these techniques requires a level of individual titration and monitoring that is not easy to standardize. It is quite ironic that the antibodies in the less quantitative technique of tissue immunohistochemical analysis are being subjected to official standardization, while antibodies used in the otherwise more tightly regulated realm of clinical pathology are not.

I believe that more effort should go into the development of appropriately standardized and validated “universal” control reagents (eg, cell culture, tumor xenografts) and standardized criteria for the interpretation of immunohistochemical results. It is hard to imagine what molecularly targeted drug will be developed that has not been tested thoroughly in experimental cell culture systems, which easily can be prepared into histologic preparations that mimic tissue. DAKO (Carpinteria, CA), makers of the HercepTest for c-erb B2/HER2/neu immunohistochemical detection, provides such histologic preparations of cell line controls (negative, weakly positive, and strongly positive) for laboratories to validate their procedures, and this approach has been independently verified. In a recent AJCP article, Rhodes and colleagues used a panel of validated cell lines as a standard reference for HER-2/neu overexpression to test interlaboratory results of immunohistochemical analysis from 94 laboratories in 21 countries. Their study showed that the use of such a standard reference can help determine optimal assay conditions by comparing techniques used at laboratories that obtained appropriate results. Their study also showed that the use of such controls can help laboratories not initially obtaining appropriate results to improve quickly by providing appropriate controls in quality assurance programs. This suggests that if the controls for a specific test are validated and mandated, the reagents and platforms do not need to be mandated, as long as they produce the desired results.
As has been pointed out by Fletcher and Fletcher, officially sanctioned tests can easily be carried out poorly; this is no mechanism for ensuring reliable assay data. One might also worry about anatomic pathology laboratories being required to obtain multiple automatic immunostaining platforms to support different FDA-approved tests that in the future may require a specific instrumentation platform. We have in place effective professional bodies that monitor pathology laboratories and their practices. As suggested, rather than mandating the use of specific reagents and platforms, the FDA should work more closely with accreditation bodies (such as the College of American Pathologists) to ensure that monitoring and proficiency testing of specific tests of concern are carried out.

A final question arises: “Are histologic techniques the best way to make quantitative assessments of molecular entities?” Solution or membrane-based immunoassays (eg, radioimmunoassays, Western blots) of tissue homogenates are far more quantitative. However, we are left with a tissue equivalent of the Heisenberg uncertainty principle, since by homogenizing the tissue we cannot determine exactly the cell composition. In situ methods, on the other hand, permit us to precisely identify in what cells the molecular constituents reside, but results are more difficult to precisely quantify. The marriage of tissue microdissection and solution or membrane-based assays theoretically offers a way out of this conundrum, but the application of such technology to routine clinical practice is probably far in the future. Microdissection is a low-throughput technique that requires skilled histopathology expertise and currently is available only as a research tool. In addition, many postdissection assays are difficult to implement on the vanishingly small amount of material provided by tissue microdissection. Thus, it would seem that we are (fortunately?) “stuck” with immunohistochemical analysis and in situ hybridization techniques to make crucial clinical decisions for the near future. Pathologists will need to adapt both to new technologies and to increased regulatory scrutiny as the basis for modern therapeutics shifts in the future from purely morphologic interpretation of tissue histology to morphologic interpretation combined with various molecular diagnostic modalities.

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References