The Total Test Approach to Standardization of Immunohistochemistry

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Histopathology continues to serve as the mainstay for diagnostic and therapeutic decisions in almost all forms of neoplasia. Yet, as previously described,1 surgical pathologists in routine practice have problems recognizing many of the more subtle criteria, and even experts are far from infallible.2 It has been written that “All pathologists would probably agree that the interpretation of histologic sections is subjective, often extending, through a spectrum of possibilities”3 and that “the final morphologic diagnosis often is hedged with uncertainty.”4 In some circumstances, the hedges loom exceedingly tall, so tall that even though “we stand on the shoulders of giants,”5 we can scarcely see over them.

It has been argued1 that rigorous scientific studies of the accuracy, reliability, and reproducibility of histopathologic diagnoses are rare but nonetheless disconcerting and that significant disagreement between pathologists exceeds 25% for difficult but clinically important diagnostic distinctions. With regard to malignant lymphomas, even the experts are seen to encounter difficulties: “It seems to me that pathologists are getting into difficulties in the area of non-Hodgkin’s lymphoma. Not only do we clinicians have trouble in understanding pathologists, but at times they seem unable to understand each other.”6 Studies of diagnostic consensus for malignant lymphomas describe a level of agreement as low as 35% for practicing pathologists and 50% for experts applying the recently derived REAL classification. Attempts to predict prognosis by using histologic criteria for the grading of tumors are fraught with even greater difficulty.1 The fact that morphologic criteria still constitute the ‘gold standard’ for diagnosis in many conditions reflects not only the success of pathologists in coming to terms with subjectivity but also in some measure the lack of an alternative and more accurate diagnostic method. A serious and less well-recognized corollary is that in most cases we lack a reliable means of validating the surgical pathology diagnosis on which therapy is predicated.

SPECIAL STAINS

It may be argued that special stains evolved to meet just this need. The first special stains were variations of the basic biological dyes. Specific histochemical stains soon evolved based on the in situ identification of active enzymes within cells by the application of colorogenic substrates.7 Many histochemical methods were, however, technically exacting, both in terms of tissue preparation and staining protocol, a situation that led to recognition of the critical importance of appropriate positive and negative controls for interpretation of the findings.

Although the methods were challenge enough, the reagents themselves were often poorly characterized and somewhat variable from manufacturer to manufacturer and lot to lot. In the United States, the Biologic Stain Commission (BSC) was founded in 1944 as a nonprofit corporation in large part to address the problem of standardization of chemical stains.8 The BSC achieved considerable success with regard to biological dyes, but with the advent of immunohistochemistry, special stain technology was about to take a new leap forward, with great opportunities and new challenges in standardization.

THE ADVENT OF IMMUNOHISTOCHEMISTRY FOR ROUTINE FORMALIN PARAFFIN SECTIONS

In 1978 immunohistochemistry was only 4 years old. Nonetheless, imbued with the enthusiasm of relative youth and fortified by a mere 6 years of hands-on experience in the field, I was able to write of “the potential value of immunohistochemical methods as an immediate aid to diagnosis, and more importantly as a tool for the reshaping and redefining of current histologic criteria.”4 At that time, the whole field of immunohistochemistry as applied to routine formalin paraffin sections was encompassed in a score or so of manuscripts from around the world. By 1986 the literature had surpassed the 1000 mark, and I was moved to add, “Widespread application of immunohistochemical methods, by virtue of their great specificity, inevitably will transform histopathology from something resembling an art into something more closely resembling a science.”7 Although the utility of immunohistochemistry is accepted today by most pathologists, my optimism as to the scientific application of the method appears to have
TABLE 1. The Total Test*

<table>
<thead>
<tr>
<th>Testing Process</th>
<th>Quality Assurance Issues</th>
<th>Responsibility</th>
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<tr>
<td>Specimen acquisition and management</td>
<td>Specimen collection, fixation, processing, sectioning</td>
<td>Surgical pathologist, sometimes clinician</td>
</tr>
<tr>
<td>Technology and methodology</td>
<td>Reagents, protocols, sensitivity, specificity</td>
<td>Pathologist or technologist</td>
</tr>
<tr>
<td>Analytical issue</td>
<td>Qualifications of staff intralaboratory and inter-laboratory proficiency testing of procedures</td>
<td>Pathologist or technologist</td>
</tr>
<tr>
<td>Interpretation and reporting</td>
<td>Criteria for positivity and negativity in relation to controls</td>
<td>Pathologist or technologist</td>
</tr>
<tr>
<td></td>
<td>Experience and qualifications of pathologist, proficiency testing of interpretational aspects, diagnostic and prognostic significance, appropriateness and correlation with other data</td>
<td>Surgical pathologist and/or clinician</td>
</tr>
</tbody>
</table>

* Data are from Taylor.1

Immunohistochemistry has become an established method in surgical pathology—every (well, almost every) large academic center in the world uses the method routinely, some staining 10,000 or more slides per year. Immunohistochemistry is also offered by reference laboratories and even by many small community hospitals, where it is applied to the occasional case. But therein lies the rub; enormous variability has developed in terms of the reagents available, the detection methods used, and the interpretation and reporting of immunohistochemical findings. For these reasons, among others, the members of the BSC began, in the 1980s, to turn its attention to immunohistochemistry, sponsoring a series of workshops that were attended by pathologists, manufacturers, and representatives of the Food and Drug Administration.10,11

THE COMPLETE IMMUNOHISTOCHEMICAL STAIN

Immunohistochemistry is technically complex, and no aspect of this complexity can be ignored, from the moment of collecting the specimen to issuance of the final report. In this context, anatomic pathologists should be prepared to follow the fine example of their colleagues in clinical pathology and should broaden their responsibility to cover all aspects of the immunohistochemical assay.12,13 The complete immunohistochemical stain (Table 1) begins with the identification of a need for the performance of immunohistochemical studies and progress through procurement of the specimen, identification of the appropriate stain, selection of the proper reagents and protocol, and correct performance of the protocol with the appropriate controls. It concludes with an evaluation of the results and a written report. An attendant goal of long-term follow-up (outcome analysis) remains just that, a goal.

Clinical Question and Test Selection

Selection of the appropriate stain for a particular diagnostic problem should be an integral part of the process. Such a selection cannot be made in isolation from the clinical situation (diagnostic problem) on the one hand or the capabilities and limitations of the immunohistochemical laboratory on the other, including those limitations imposed by the experience of the staff and the type of tissue (frozen or fixed) available for study. The blunderbuss approach of ordering a panel of 10 or more stains on every suspected large cell anaplastic tumor can no longer be justified economically or academically. For each stain used in a particular case, the pathologist should ask, and answer, the question, “What will a positive/negative result add to the diagnosis?” If the answer is not known in the field of pathology or to the individual pathologist, then the stain should not be performed, because it will not add to the diagnostic equation.

In the face of the diagnostic conundrum that cannot be resolved by orthodox morphologic criteria, the pathologist has 2 main options. The first and more common modus operandi is to select stains from a panel that, in the experience of the pathologist, have proven to be of value in a particular diagnostic area (eg, anaplastic tumors, malignant lymphoma). The second approach uses an algorithm with sequential panels of selected stains that introduces a certain degree of logic and thought into the process but may compromise the turnaround time due to sequential staining.15 Once the appropriate stains have been selected, the determinations of whether such stains can be performed successfully on the tissue available, whether the necessary reagents are at hand, and whether the detection methods are tried and true must be made; all these determinations will be readily apparent from review of the within-run (same day) and run-run (day-to-day) control records, which are an intrinsic part of an established quality assurance program. Although obvious, it can scarcely be overemphasized that without detailed knowledge of the performance characteristics of each primary antibody, it is not possible to interpret the final result, even in the presence of controls that perform as expected.

There is also an important ongoing shift in emphasis in the use of immunohistochemical stains from a primary focus on cell and tissue markers as an aid to the recognition and classification of tumors to the demonstration of cell products, receptors, or oncogenes of possible prognostic value, and the identification of infectious agents in situ.13,14 The precision required for this latter approach places new and more exacting demands on our ability to perform immunohistochemistry or in situ hybridization in a reproducible and standardized manner.
Specimen Acquisition and Management

Aspects of tissue handling also cannot be ignored. Pathologists have sought to undo some of the adverse affects of formalin fixation either by the use of controlled enzymatic digestion or more recently by the antigen retrieval technique. The latter method (sometimes known as heat-induced epitope retrieval or unmasking) subjects sections to microwave heating in the presence of a retrieval solution that may serve to stabilize or postfix the antigens present. Although vigorous heating in a microwave oven offends basic instinct, the method has contributed to the reproducibility of immunostaining by providing a more uniform presentation of antigens in tissue sections than is otherwise present following inconsistent fixation in formalin. However, one significant caveat has been issued, namely that "as different antigen retrieval approaches are explored and propagated, there is a danger that different laboratories each will adopt a different procedure, producing varying degrees of restoration of antigenicity, that will add yet another variable to the overall process." Unfortunately this warning has come to pass. Clearly, the preference is for anatomic pathologists to adopt more uniform and rigorous procedures for the fixation and processing of tissues, extending from the moment that the specimen is removed from the body to the end of the embedding process, particularly controlling the total time in fixative and the composition of the fixative itself. The plea also was made that "if we cannot have uniform fixation, let us at least strive towards a more uniform approach to ‘unfixation,’ utilizing standardized and well described antigen retrieval procedures." The pressure for consistency in staining has grown even further because of the increasing focus on prognostic markers, where greater stringency is required.

Again, in the clinical laboratory, we collect a specimen in the right tube with the right preservative and the right anticoagulant, and we process it in the prescribed manner, all without thinking. In anatomic pathology, we largely fail to accomplish any of the analogous steps, again without thinking.

The National Committee for Clinical Laboratory Standards currently is developing detailed guidelines that address the issues of fixation, reagent selection, choice of protocol, and other aspects of the complete immunohistochemistry test. The document is in the final stages of approval, and publication is expected within the year.

Technology and Methodology

Although it is perhaps not practical to attempt to standardize a single protocol across all laboratories, because of the great variability in specimen procurement, fixation, and processing, it certainly should be possible to standardize protocols within a single laboratory. The goal is to ensure run-to-run reproducibility, evaluated against standard control sections from day to day and week to week. Achieving this goal requires careful selection of reagents, rigorous use of controls, and strict adherence to the protocol for preparation of slides, application of reagents to slides, and incubation times. Today, it is increasingly clear that, just as with complex procedures in the clinical laboratory, consistency of performance can only be achieved by automation of the score or more separate steps that constitute an immunohistochemical stain. The Model T Ford immunostainer pioneered by David Brigati more than a decade ago has given way to a wide range of automated immunostainers, the best of which will outperform even the best technologist in terms of consistency during a sustained period. Of course, such instruments should not be viewed as a replacement for a skilled technologist but rather as an adjunct technology that frees the technologist to focus on issues of quality assurance and interpretation.

The issue of selection of appropriate high-quality antibodies and reagents was addressed directly by the BSC in conjunction with the Food and Drug Administration. The result was the publication of a set of guidelines for package inserts. This document suggested not only a standard outline for manufacturers to follow in the testing and marketing of reagents but also provided a series of recommendations for positive and negative controls that could be followed by both manufacturers and performing laboratories (Table 2). By adherence to these guidelines, manufacturers will provide pathologists with more detailed information on the specificity and working conditions of antibodies than currently is available.

With regard to detection procedures, the BSC concluded that it was unable to recommend a standardized staining protocol from the many variations in current use. Establishing a single universal protocol would seem to be impossible, recognizing that the different laboratories have different needs, and probably is not desirable, providing that all laboratories use appropriate controls. Here again is a spin-off advantage derived from the adoption of automated methods in that, just as in the clinical laboratory, automation tends to force compliance in terms of reagent choice and protocol, placing the onus of reagent qualification on the manufacturer, with a resulting increase in quality of product from the better manufacturers. Conversely, pathologists should recognize that any departure from the manufacturer’s protocol or reagents effectively voids the responsibility of the manufacturer and places responsibility for validation totally on the performing laboratory.

Analytical Issues

The experience and training of the technologist who is performing the stain clearly are critical to this process. Again, following the model of the clinical laboratory, one major factor in ensuring reproducibility may be the growth of automation, with the extended capability for consistency and control that is inherent in the automated process. The availability of automated immunostainers has proven of overall benefit in many smaller laboratories, if not some larger ones, that do not have the luxury of highly skilled staff who are experienced in reagent titration and quality control. It is certainly our experience that the use of an automated stainer increases the reproducibility and reliability of a wide variety of immunohistochemical stains. Increasingly, other investigators report similar experience, although not all agree on the choice of system; hands-on demonstrations of the system(s) that most closely fit need and budget are necessary before initiating a purchase. With any automated system,
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Table 2. Types and Purposes of Daily Quality Control Materials for Immunohistochemistry*

<table>
<thead>
<tr>
<th>Type of Control</th>
<th>Antigen (Analyte)</th>
<th>Antibody (Reagent)</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>Positive</td>
<td>1. Nonpatient tissue or cells containing antigen to be detected and quantitated&lt;br&gt;2. Known expected result&lt;br&gt;3. (a) Fixed or processed in same way as patient sample&lt;br&gt; (b) Fixed or processed in manner shown to preserve antigen under analysis</td>
<td>Antibody reagent (of the kit) constituted in same way as intended for patient sample</td>
<td>1. Control of all steps of the analysis&lt;br&gt;2. Training of user for appearance of positive reaction. Comparison for semiquantitation of reaction&lt;br&gt;3. (a) Validates all steps of analysis, including fixation and processing&lt;br&gt; (b) Validates all steps of analysis except fixation or processing used by individual laboratory</td>
</tr>
<tr>
<td>Negative control (specific)</td>
<td>1. Tissues or cells expected to be negative by antibody (of kit)&lt;br&gt;2. Processed in same way as patient sample&lt;br&gt;3. May be portion of patient sample</td>
<td>Antibody reagent (of the kit) constituted in same way as intended for patient sample</td>
<td>Detection of unintended antibody cross-reactivity to cells or cellular components</td>
</tr>
<tr>
<td>Negative control (non-specific)</td>
<td>1. Patient tissue with components that are the same as tissue to be studied&lt;br&gt;2. Processed in same way as patient sample</td>
<td>1. Diluent (as used with antibody) without antibody OR&lt;br&gt;2. Antibody not specific for antigen or interest in same diluent as used with kit antibody</td>
<td>Detection of unintended background staining</td>
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* Data are from Taylor and Taylor and Cote.13

there is a training period during which inconsistencies and difficulties may be encountered. However, as these are resolved, the end results far exceed the quality that is achievable by even the best technologist on a day-to-day basis.

Quality Assurance.—The College of American Pathologists (CAP) defines quality assurance as a “process of assuring that all pathology services involved in the delivery of patient care have been accomplished in a manner appropriate to maintain excellence.” As such, quality assurance is more a mind set than a system, but it does incorporate the much more precise concept of quality control, which is the “aggregate of processes and techniques so derived as to detect, reduce and correct deficiencies in the analytic process.”

As described elsewhere,1 “anatomic pathology changed little in the 100 years preceding 1970. Sequestered in a technologic limbo, it remained relatively untouched by the new methodologies and automated systems that revolutionized the clinical laboratory. The histology laboratory performing only a few simple stains escaped the rigors of quality assurance in general and quality control in particular. To dip a slide in hematoxylin for a few minutes, then briefly differentiate it in alcohol, until it looks ‘about right’ to the technologist and ‘makes the pathologist happy’ may suffice an H&E [hematoxylin-eosin] stain, but applied to immunohistochemistry it is a recipe for disaster.”

In seeking to improve the reliability and reproducibility of immunohistochemistry, we would be well advised to take a 20-year-old page from our colleagues in the clinical immunology laboratory.1 Immunoassays for antinuclear antibodies do not differ from immunohistochemistry in any substantial way in terms of immunologic principles or performance characteristics. Even the end point is somewhat similar, expressed as the degree of staining of certain tissue components, and here also interlaboratory inconsistencies posed similar problems.1 However, there are differences in stringency of definition of the end point in that strict criteria exist for interpretation of immunofluorescence assays in relation to reference standards (controls) that have been available commercially for many years. Also, defined protocols for quality control, storage, and utilization of the various reagents are part of routine day-to-day practice in the clinical laboratory, encouraged to a large degree by clinical laboratory certification programs that are intrinsic to hospital certification but have intruded meaningfully on anatomic pathology only recently (modified guidelines of the Laboratory Accreditation Program of CAP). If we are to elevate immunohistochemistry to a level of reproducibility that matches comparable techniques in the clinical laboratory, then we must subject immunohistochemistry to a similar degree of scrutiny, including all its component parts and each stage of the procedure.1,12,13

CAP Certification and Proficiency Testing Programs.—The initiative of the BSC prompted revision and expansion of the Check List for Immunohistochemistry used by inspectors during the CAP laboratory certification process. The criteria for certification of an immunohistochemistry laboratory were expanded from 5 to 15 items, producing a much more comprehensive set of requirements for the successful performance of immunohistochemical stains. Many aspects of this expanded checklist have been incorporated in the forthcoming National Committee for Clinical Laboratory Standards guidelines, which should prove invaluable to all laboratories performing immunohistochemistry in a diagnostic setting. In addition, CAP conducts an Immunohistochemistry and Proficiency Testing Program, in which participating laboratories receive unstained paraffin sections of representative cases with instructions to perform a panel of immunohistochemical stains and controls. Following performance of
the stains, the laboratories report their findings to the CAP Cell Markers Program, where results are collated and compared. A detailed critique is provided to each subscriber. The program, therefore, serves as an external proficiency test of the performance and interpretation of immunohistochemical stains on external tissues; it does not purport to measure the efficacy of internal specimen handling and fixation procedures, a function that can only be met by internal quality control programs.

Results

Validation and Reporting.—A critical problem for immunohistochemistry has been the lack of universal controls or reference standards. Interestingly, a similar problem was encountered early in the evolution of quality assurance procedures in the clinical laboratory.16 For serum assays, it was solved by establishing large standardized serum pools and making these available to both manufacturers and laboratories. In addition, CAP “check sample” programs established other pools, samples of which were provided to the growing number of laboratories participating in proficiency testing programs. The test results from these different laboratories served to provide extensive validation of these pools as additional reference standards. An analogous CAP “check sample” program exists for immunohistochemistry, but the pool of reference materials (paraffin blocks of characterized tumors) is of limited supply, insufficient to serve as a generally available standard.

The BSC has debated the development of a reference standard or a series of standards that could be made available for general use.1 However, as noted herein, the solution is not so simple with regard to characterized paraffin blocks that cannot be pooled and are available in limited amounts: no 2 tumors are antigenically identical. Improved multitissue blocks19 provide an interim solution. Multitissue reference standard blocks could even be prepared from the residual material of cases used in the CAP survey program, thereby providing material that already has been evaluated and validated by several hundred different laboratories. But such multitissue blocks, even though they contain minute slivers of tissue, are not inexhaustible. The BSC has proposed the possibility of developing infinite amounts of standard reference materials in the form of multitissue blocks composed of artificial tissues, including human tumor cell lines or human tumor heterotransplants in mice with severe combined immunodeficiency disorders.1

The Immunohistochemistry Report.—In response to a request from the BSC, the Association of Directors of Anatomic and Surgical Pathology published a proposal for the organization and content of an immunohistochemistry report.20 The essential elements of this proposal are incorporated into Table 3. Adherence to this proposal would do much to introduce consistency and thoughtfulness into the field. The interpretation and significance of the findings should be presented in the context of the overall differential diagnosis. Interpretation of the presence of specific positive staining, or lack thereof, is, of course, a complex issue. It is a function of the performance and examination of the proper controls and the experience of the laboratory performing the stains, especially the pathologist responsible for evaluating the stained slides. A number of difficulties remain into this area, including some of the same problems that intrude in surgical pathology in general. Just how brown (or red) is positive? If we accept “weakly positive” as a valid finding, then how do we define “weakly negative”? What percentage of positive cells constitutes a positive tumor? How many swallows make a summer?21

Interpretation and Significance

A second aspect to interpretation relates to the way we arrive at an opinion regarding the significance of a particular set of staining results in relation to the diagnosis or prognosis of the patient. Here one can do no better than to refer once more to the principles established in the process of test selection.

These are principles that are more readily expounded than accomplished. The literature has expanded far beyond the thousand papers written by 1986 to a number exceeding that in a single year. Most surgical pathology publications now incorporate immunohistochemical findings to some degree. To keep pace with the relevant literature is a challenge that it is impossible to meet in broad perspective. Even within some specialty fields, such as hematopathology, the relevant literature on CD marker counts is still climbing, with another conference due in 2000. The journal literature can scarcely stay abreast with the productivity of the immunologists and molecular engineers, and even if it could, the reader cannot. “As a rule disease can scarcely keep pace with the itch to scribble about it.”22 Specialist journals, such

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<table>
<thead>
<tr>
<th>Table 3. The Immunohistochemistry Report*</th>
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<tbody>
<tr>
<td>1. Patient demographics and specimen identification data</td>
</tr>
<tr>
<td>2. Reference to the diagnostic problem (i.e., differential diagnosis)</td>
</tr>
<tr>
<td>3. Nature of specimen analyzed (frozen, fine needle aspiration, paraffin section, and fixative)†</td>
</tr>
<tr>
<td>4. Statement of all stains used with details of all primary antibodies (designate specificity and clone where appropriate)‡</td>
</tr>
<tr>
<td>5. Findings both positive and negative for all stains; sufficient details of patterns and controls to justify the interpretation§</td>
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<tr>
<td>6. The immunohistochemistry report should not stand alone but should be integrated into the final surgical pathology report</td>
</tr>
</tbody>
</table>

* Data are from Taylor.1
† Details of fixation and detection systems should be kept on record in the laboratory where they were performed but should be incorporated in the report, where they are an essential part of the interpretative process (eg, whether enzyme digestion or antigen retrieval was used or B5 versus formalin fixation for certain leukocyte antigens).
‡ For example anti-T cell is not acceptable; report should read T-cell antibody UCHL-1 (CD45RO). Anti-pan keratin should read anti-keratin cocktail (AE-1 + Cam 5.2 + 34βE12 + 35βH11).
§ Detailed control records should be retained in the laboratory where they were performed. Findings may also be stated in the report, where they are contributory. For example, in a breast tumor that is negative for estrogen receptor, the presence of residual normal breast epithelium that shows positivity is significant. Similarly, in an anaplastic large cell tumor that shows absence of staining for leukocyte common antigen (CD45), the presence of intermingled leukocyte common antigen–positive small lymphocytes should be described.
Appropriateness and Cost-effectiveness

Last but not least in considering the complete immunohistochemical stain, there are the issues of appropriateness, outcome analysis, cost, and cost-effectiveness. In these areas, there is little hard information. The accuracy of surgical pathology alone has already been found wanting in certain critical areas (eg, grading, borderline lesions of breast, dysplasias, lymphomas). A number of studies have shown that immunohistochemistry is helpful. In one early study of more than 100 anaplastic tumors, the H&E diagnosis of carcinoma or lymphoma was revised in approximately 50% of cases following basic immunohistochemical studies, providing dramatic evidence of a contribution to patient care (of 43 carcinomas, 27 were in reality lymphomas). In a separate study of 200 consecutive cases, it was concluded that immunohistochemistry contributed to the diagnosis in almost 50% and was confirmatory in the remainder. In a subsequent analysis of 557 poorly differentiated round cell or spindle cell tumors that could not be diagnosed on the basis of H&E morphologic structure, immunohistochemistry “provided a definitive diagnosis in 70% of the former and 92% of the latter.” Other analyses of difficult cases seen in consultation practices around the country, including our experience at the University of Southern California of approximately 20,000 cases during 25 years, have been strongly supportive of the need, even the necessity, for appropriate immunohistochemical studies.

Cost-effectiveness must be viewed against this background. Reagents for an individual immunohistochemical stain are not inexpensive for some proprietary antibodies; furthermore, the salaries of laboratory personnel and professional fees for interpretation by a trained pathologist must also be taken into account. A panel of 2 or 3 immunohistochemical stains that distinguishes a lymphoma from a carcinoma may save thousands of dollars that may have been incurred by additional investigations, extended hospital stays, or inappropriate therapy. As an emerging example, the detection of micrometastases by immunohistochemical methods in bone marrow or possibly lymph nodes of patients with breast cancer offers potential benefits to a large patient population with significant cost savings. Although appropriateness criteria, as proposed by RAND (Santa Monica, Calif), for such medical procedures as coronary angiography and coronary artery bypass surgery have yet to be developed for immunohistochemistry, the currently available data strongly support immunohistochemistry as a rapid and cost-effective technique that has become an essential part of surgical pathology practice. Concerted action with regard to standardization and quality assurance, coupled with progressive automation of the procedure and perhaps eventually even the reading of basic results by image analysis, will certainly enhance the value still further.

ONGOING ACTION

Much of this material, although written specifically for immunohistochemistry, applies equally to in situ hybridization methods and reagents used therein. On the technical side, all laboratories that perform immunohistochemical staining for diagnostic support are encouraged to participate in the CAP Laboratory Certification and Immunohistochemical Proficiency Testing Programs. A proposed format for reporting immunohistochemical results exists, and we would be wise to follow it. Finally, the National Committee for Clinical Laboratory Standards is expected to release quality assurance guidelines for immunohistochemistry in the near future, adding further to the momentum for overall quality improvement and consistency.

Collectively, these processes almost certainly will establish standards, but to a degree they are peripheral to the problem. The performance and practice of immunohistochemistry are aspects of the practice of medicine. It is the province of the pathologist, and rightly it is our responsibility. If, as pathologists, we do not accept this responsibility, either by conscious choice or benign neglect, then we must expect others to establish and monitor standards of performance. If this occurs in immunohistochemistry, now that we have been alerted to the problem, we know who to blame: “We have met the enemy, and he is us.” (W. Kelly, Pogo cartoon, 1970).

My thanks are owed to Myrna Cisneros for preparation of the manuscript.

References
5. Newton I. If I have seen further than other men, it is by standing on the shoulders of giants. Letter to R. Hooke. February 5, 1675.
19. Miller RT. Multitumor “sandwich” blocks in immunohistochemistry: simplified method of prep-
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