Negative Reagent Controls in Diagnostic Immunohistochemistry: Do We Need Them? An Evidence-based Recommendation for Laboratories Throughout the World

Richard W. Cartun, MS, PhD

During the development and application of immunohistochemical techniques to diagnostic pathology, controls have played an important role in ensuring that the appropriate result has been obtained. The positive control is a tissue or collection of cells that contains the target protein (antigen) and confirms that the primary antibody and detection system have worked properly. The negative control, used primarily for primary antibody validation, is a specimen that does not contain the target protein and, as a result, should show no immunoreactivity. The negative reagent control (NRC) is an additional tissue section (frozen or paraffin) or cytology smear prepared from the patient specimen that is treated identically to that of the test slide(s) except for the omission of the primary antibody. Its primary purpose is to identify nonspecific reactivity due to factors or components other than the primary antibody.

In the early days of diagnostic immunohistochemistry, the NRC was important because of the use of crude detection systems that often showed cross-reactivity with endogenous immunoglobulins (Fig. 1A) or with other proteins present in the test specimen that could be confused with specific immunoreactivity. In addition, with the introduction of avidin-biotin–based detection, nonspecific reactivity with endogenous biotin was frequently observed, especially following heat-induced epitope retrieval complicating interpretation (Fig. 1B). The introduction of sensitive and specific polymer-based detection systems has eliminated most of these issues.1

A critical examination of the use of NRCs in diagnostic immunohistochemistry today reveals several issues in play. First and foremost, they consume precious tissue or cells that could be used for additional testing. This is especially true today, when laboratories are seeing more fine-needle aspirate biopsy specimens and diagnostic needle cores that require ancillary immunohistochemical and/or molecular testing for targeted therapies for patients with cancer. Second, depending on the type of laboratory running the immunohistochemical tests (eg, general surgical pathology vs. a specialty laboratory such as urology, GI, or GYN), NRCs can represent as much as 50% of the slides in a given run (personal observation). This overwhelming volume can be a major burden on the staff and the instruments processing these slides. Finally, laboratories incur a major expense in terms of labor, supplies, and reagents for running these additional slides, for which there is no reimbursement. As a result, the routine use of NRCs in diagnostic immunohistochemistry has been challenged.2

In 2008, I made a decision to stop running NRCs routinely except for antibodies requiring avidin-biotin detection. My decision was based on 3 factors: (1) the...
FIGURE 1. A, Section of the stomach showing nonspecific reactivity of inflammatory cells with HRP-conjugated secondary antibody; negative reagent control slide (2-step immunoperoxidase with DAB chromogen, ×400). B, Section of the kidney demonstrating endogenous biotin in tubular epithelial cells following heat-induced epitope retrieval; negative reagent control slide (LSAB immunoperoxidase with DAB chromogen, ×400). C, Section of skin showing pronounced melanin pigment; negative reagent control slide (polymer immunoperoxidase with DAB chromogen, ×400). D, Section of lymph node with metastatic malignant melanoma showing melanin pigment (brown) and immunoreactivity for HMB45 (red) (polymer immunoperoxidase with AEC chromogen, ×400). E, Section of lymph node with metastatic malignant melanoma showing diffuse immunoreactivity for HMB45 (immunoalkaline phosphatase with fast red chromogen, ×400). F, Section of lung tissue showing anthracotic pigment and macrophages containing hemosiderin; negative reagent control slide (polymer immunoperoxidase with DAB chromogen, ×400).
observation of no nonspecific staining on thousands of NRCs run over several years using polymer detection; (2) the slide capacity consumed by NRCs on our new, fully automated IHC platforms installed in the fall of 2007; and (3) the detection cost per slide as a result of a reagent rental agreement used to acquire these new automated IHC instruments. At the same time, I added “Negative Reagent Control” to our antibody dictionary in our laboratory information system so that my colleagues could order an NRC following the examination of their immunohistochemical slide(s) if they felt there was nonspecific staining present or an endogenous pigment that might complicate interpretation (Fig. 1C). During this time I also instructed them to always look for “internal” negative controls within the patient specimen to validate specific immunoreactivity. My good friend and colleague, Hadi Yaziji, MD, always points out at our Annual International Retreat on Applied Immunohistochemistry and Molecular Pathology, “when a panel of antibodies is run on a given specimen, each individual antibody serves as a negative reagent control for the other antibodies.” Why not take advantage of this when more than 1 antibody is run on a case? In addition, pathologists can use alternative chromogens such as AEC in place of DAB (Fig. 1D) or immunoperoxidase phosphatase detection with a high-contrast red chromogen for cases with endogenous pigments (Fig. 1E). This has proven especially useful for our Dermatopathology service.

Now, for the evidence. Early this year, I queried our laboratory information system for the number of NRCs ordered by pathology staff during the period from 02/01/2008 to 02/01/2013. This search revealed a total of only “28” NRCs ordered retrospectively during this 5-year period. Keep in mind that these “28” NRCs were ordered for a surgical pathology laboratory with 55-60,000 accessions per year! Of the “28” NRCs ordered, I had ordered “22” (79%), primarily for resident/fellow education or for our “Technical Only” IHC service that we offer to other hospitals in Connecticut and elsewhere. A review of cases in which an NRC was ordered showed that the majority of tissue samples tested contained some form of endogenous pigment (eg, melanin, hemosiderin, etc.) that could be confused with immunoreactivity when DAB was used as the chromogen (Fig. 1F).

Our Immunopathology Laboratory is now running slides from an average of 850 paraffin blocks per month. If one assumes a detection cost of $10 per slide (and this number can be higher for other laboratories), this translates into a savings of over $100,000 a year for our laboratory. Keep in mind that this amount of saving is for detection cost only; a laboratory would also realize savings in terms of labor and other supplies and reagent costs. If you multiply these savings by the number of pathology laboratories performing diagnostic immunohistochemical testing in the United States, the savings will be in the millions. As everyone knows, laboratories everywhere are under intense pressure to reduce expenses and maximize efficiencies, especially now with major reductions in technical and professional reimbursements. Many laboratories have already evaluated their operations along with their clinical colleagues and have developed best practices to not only improve patient care but also eliminate unnecessary testing using evidence-based approaches. We now have the evidence that supports making NRCs optional in diagnostic immunohistochemistry when using non–avidin-biotin detection.

Therefore, I recommend that every laboratory performing diagnostic immunohistochemical testing, along with its Medical Director, evaluate their own experience with NRCs and make an “evidence-based” determination on their usefulness. If you feel that they have a useful role, then continue to run them. If not, discontinue them and preserve precious tissue for additional testing, and reduce the number of unnecessary slides that you are running, which will translate into enormous savings for your laboratory and our health care system. In 2012, the College of American Pathologists (CAP) changed their Anatomic Pathology Checklist such that laboratories not running avidin-biotin detection would no longer be required to run NRCs. I applaud them for making this change. I also encourage all laboratory regulatory agencies to reexamine the evidence and follow in the footsteps of the CAP. The biotech companies that supply the pathology community with primary antibodies and detection reagents also need to reexamine the evidence and rewrite their product data sheets regarding the use of NRCs. The decision on the need to run NRCs in the diagnostic immunohistochemistry laboratory should be left to the individual testing laboratory and its medical director based on their experience.

ACKNOWLEDGMENT
I would like to thank my colleagues in our Immunopathology Laboratory, Pamela H. Newcomb, BS, BA, HT(ASCP), Deborah Z. Stevens, BS, HTL(ASCP), and Jason Burghardt, HT(ASCP) for the wonderful work they do every day.

REFERENCES
4. CAP Today. Lab teams up to curb unnecessary testing. December 2012; 26(12).