CONTROLS

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■ Reagent and tissue controls are necessary for the validation of immunohistochemical staining results. Without their use, interpretation of staining would be haphazard and the results of doubtful value. More specifically, controls determine if the staining protocols were followed correctly, whether day-to-day and worker-to-worker variations have occurred, and that reagents remain in good working order. Furthermore, all procedures designed for in vitro diagnostic use must be monitored by reagent and tissue controls.

REAGENT CONTROLS

■ Because of the subjective nature of the test, the relevant reagents must be controlled within routinely conducted quality assurance programs both by the manufacturer as well as by the user. The prime objective is to ascertain whether the primary and secondary antibodies are specific for their target antigens. Various immunochemical techniques, such as double diffusion, immunoelectrophoresis and rocket electrophoresis may be useful for obtaining this information. It is however imperative to test the primary antibody in immunohistochemistry, first for optimal dilution on positive tissue and then against an expanded panel of additional tissues known to either contain or not contain the antigen. The secondary (link) antibody should be affinity-absorbed in order to render it non-reactive to the tissue proteins. Quality control programs should be documented by proper record keeping on dilutions, diluents, incubation times, and dates on which any procedural changes are introduced.

Of all the components used in an immunochemical staining system, the primary antibody is, without doubt, the most critical, although occasionally other reagents may need to be replaced.

To ascertain the specificity of a primary antibody of the polyclonal type, it is best to replace the same with an affinity-absorbed antiserum or immunoglobulin fraction. Affinity absorption of the primary antibody with highly purified antigen is the ideal means to obtain a valid negative control for differentiating specific from nonspecific staining. The problem is that purified antigen is rarely available in clinical histology laboratories, and that such preparations are very expensive. Practically, therefore, most laboratories use as a control either non-immune serum or its immunoglobulin fraction from the same species as the primary antibody or select a primary antibody of irrelevant specificity.

Because soluble aggregates present in immunoglobulin fractions may contribute to nonspecific staining, these fractions, if used as controls, should be the product of identical isolation methods, be of comparable age and contain nearly identical protein concentrations. They are therefore likely to contain similar amounts of the aggregates. Omission of the primary antibody or use of diluent in its place are ineffective controls. An example for the preparation of a negative reagent control for an IgG fraction of a polyclonal antiserum is illustrated as follows:

PROTEIN CONCENTRATION OF IGG FRACTION: 4.8 g/L, recommended dilution is 1:200, resulting in a final protein concentration of 4.8/200=0.024 g/L.

NON-IMMUNE RABBIT IMMUNOGLOBULIN FRACTION: 20 g/L; required dilution to be determined as follows: 20 g/L ÷ 0.024 g/L = 833

DILUTION OF NEGATIVE REAGENT CONTROL:1:833, or one part non-immune rabbit immunoglobulin fraction in 832 parts buffer.

For primary antibodies of the monoclonal type, use of another irrelevant antibody is probably the best negative reagent control, although non-immune mouse antibodies of the same subclass are now available also for this application. Tissue culture media used for the propagation of monoclonal antibodies have been employed occasionally but are not recommended.

The paramount objective in the selection of a good control in all cases is to imitate all facets of the primary antibody step except for the antigen specificity. Buffers used for the dilution of antibodies and controls must be identical. If these points are not observed, confusion may result from background staining in the positively stained section, but not in the negatively processed control, or vice versa.

■ **TISSUE CONTROLS** can be of either the negative, positive or the internal type.

NEGATIVE TISSUE CONTROLS Specimens serving as negative controls must be processed (fixed, embedded) identically to the unknown, but not contain the relevant tissue marker. An example would be normal liver serving as control for hepatitis B surface antigen-positive liver.

POSITIVE TISSUE CONTROLS Again, these controls must be processed identically to the specimen but contain the target protein. In some cases, it will be advantageous to have this control tissue stain only marginally positive, so

as to monitor not only for the presence of the antigen, but also for any possible *loss of sensitivity.* This loss might not be apparent if only intensely staining controls are used. Controls for loss of sensitivity would be particularly important, for example, when staining tumors. In this case, staining intensity frequently varies with the degree of tumor differentiation.

INTERNAL TISSUE CONTROLS Also known by the name "built-in" control, this control is ideal because the variables of tissue fixation between specimens and controls are eliminated. Built-in controls contain the target antigen not only in the tissue elements under scrutiny, e.g., tumors, but also in adjacent normal tissue elements. One example is the presence of S-100 protein in both melanoma and normal tissue elements, such as peripheral nerves and melanocytes. Built-in controls have the additional advantage that no separate positive control sections are required.

Battifora[1] recommended staining for vimentin as a means for an internal control. Because of its presence in blood vessels and stromal cells, vimentin is distributed ubiquitously and therefore encountered in every tissue sample. Monoclonal antibody V9 recognizes an epitope on vimentin that is partially susceptible to fixation with formaldehyde and thus functions as a "reporter" for the quality of tissue fixation. Other diagnostically useful tissue markers often show fixation-induced alterations that parallel those of vimentin. Through extrapolation to other antigens, it is possible to assess the quality of fixation and to facilitate the selection of fields for diagnostic interpretation.

STATUS QUO

■ As in the clinical laboratory, quality control in immunohistochemistry is of great importance. However, contrary to quantitative immunoassays, e.g., enzymeimmunoassays (EIA or ELISA) where numbers afford definitive quantitative information over standardization and control, the results of immunohistochemical staining, itself an art, must also undergo subjective interpretation by variably experienced pathologists.[2-4]

Quality control and quality assurance in immunohistochemistry therefore will continue to remain one of the most important issues in need of attention, both by the manufacturers as well as by the user in the histopathology laboratory. In 1993, Taylor[2] proposed the development and distribution of tissue reference standards for all pathology laboratories for quality control and quality assurance. This has yet to be realized. The continuing introduction of a multitude of different antigen retrieval procedures promoting the advantages of one retrieval buffer or heating source over another, has made efforts in the standardization and control over a qualitative procedure even more challenging.[5,6] However, as in other branches of the clinical laboratory, automation did contribute significantly to the increase in consistency in, and the control over immunohistochemical staining.

Many publications have appeared during the last ten years on this subject in general and on specific issues and applications of quality control. For more information, read the editorial by Taylor,[4] and the approved guideline for internal quality control.

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