Controlling antisera in immunohistochemistry

Immunohistochemistry is becoming a widely used technique but how do we know what is being stained? With commercial antisera, we rely on the label, assuming that it is correct, for example, anti IgG. But what else does it stain—IgA or IgM—and at what titre? (Antiserum designed for radioimmunoassay may be unsuitable and too non-specific for immunohistochemistry as the requirements are different.) A purchased 1 ml aliquot of antiserum may be just sufficient to test for specificity against a range of tissues and a range of dilutions, and for prediluted antisera 1 ml may be nowhere near enough.

Assuming that the antiserum stains what we expect, eg, plasma cells in our test slide and also cells in a 'positive control', eg, tonsil, but not in a negative control, do we know what is staining and how do we control our antisera?

The same problem exists if we raise our own antisera, but here we have at least some idea what was injected into the animal and what contaminants may be present (eg, for absorption), and there is plenty of it for testing and for use. There are several ways of controlling our antisera and we should be aware of the meaningfulness of each.

A first approach would be to accept that we do not know what an antiserum is really staining, but it was raised against, eg, pure IgG, and some cells stain, while a negative antiserum from an animal not immunised with IgG, eg, pre-immune serum, does not stain these cells. By inference the staining shows IgG. However, one is assuming the purity of the original immunogen and forgetting that the animal may raise antibodies to a variety of substances as well as or instead of those injected, eg, autoantibodies, infectious agents, and animal house contaminants, which may give a 'specific staining'.

A second approach might be to block the binding sites on the tissue antigen with a high-avidity antibody, raised to the same antigen, but in a different, non-cross-reacting species. However, every animal will recognise a different set of epitopes or antigenic determinants on any one antigen (hence the interest in standardised monoclonal antibodies) and thus bind to different parts of an antigen, with incomplete blocking. For a small polypeptide, the first 'blocking' antibody may be large enough stereologically to impede binding by the second antibody; but on a large antigen with numerous epitopes the first antibody

may block incompletely or not at all.

A third approach probably offers the present most ideal, if inconvenient, means of determining specific staining of antisera. This is done by demonstrating abolition of putative specific staining after absorbing the antiserum with appropriate purified antigen, as described by Heyderman.¹ Preferably different material is used from that for immunisation (since the immunogen may contain traces of immunogenic contaminants, eg, lactoferrin and CEA) and the antigen is better used immobilised than simply added to the antiserum (which is wasteful) to remove the antibody physically. However, to do this, milligram amounts of highly purified antigen are required, and access to such material may present a problem, for no one is likely to have sufficient antigens to absorb all the different antisera they are likely to use.

Monoclonal antibodies, produced by the Köhler and Milstein hybridoma technique,² are unlikely to solve this problem as they may be directed against single epitopes or determinants, of only a few amino-acids, which may be present in that sequence on many different substances, and thus non-specific. A cocktail of such monoclonal antibodies may be necessary, using antibodies against defined epitopes at a high enough dilution not to recognise any epitopes singly, only collectively. The problems of controlling such a cocktail are initially the same as are presented by conventional polyclonal antisera, and our initial results using monoclonal antibodies have been disappointing in their unspecificity. One of the take-home messages of the 4th International Congress of Immunology in Paris in 1980 was that monoclonal antibodies produce at least as many problems as conventional antisera.

What can be done then? Immobilisation of antigens on to beads (Pharmacia) and absorption of antisera is not difficult, and the bonus of 'do-it-yourself' is the resulting affinity-purified antibody. Acquisition of pure antigens is more difficult. Some may be commercially available (Sigma, Miles, Calbiochem) and others may be acquired from colleagues; even in a routine pathology department one can isolate many antigens, eg, IgG (from cryoglobulins) and kappa and lambda light chains from urine. Many, however, may be unobtainable by the majority, eg, growth hormone, and in these situations perhaps the fortunate might perform absorptions of their less fortunate colleagues' antisera, or this might even be done commercially.

To encourage this we have a limited

amount of human and rabbit IgG immobilised on Sepharose 4B beads, which we would be willing to give to anyone who wishes to try this method.

We suggest that the most meaningful results in immunohistochemistry are obtained using the control system shown below:

- Patient's slide* + specific antibody
= staining
- Patient's slide* + specific antibody
(after absorption)
= no staining
- Positive control† + specific antibody
= staining
- Positive control† + specific antibody
(after absorption)
= no staining
- Negative control† + specific antibody
= no staining

*adjacent, serial sections.

†tissue fixed and processed in the same way as that of the patient.

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References

- ¹ Heyderman E. Immunoperoxidase technique in histopathology: applications, methods, and controls. *J Clin Pathol* 1979;32:971-8.
- ² Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7.

I wholeheartedly endorse the comments made by Dr Leatham, Ms Watts, and Mr Atkins and would like to suggest setting up an Immunoperoxidase Study Group to promote exchange of ideas and reagents. Interested workers are welcome to contact me at St Thomas'.

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