Criteria for Antibody Specificity

Antibody:

- ☐ The pattern of immunoreactivity agrees with known patterns of protein expression?
- ☐ Immunoreactivity co-distributes with specific mRNA, e.g., by *in situ* hybridization or Northern?
- ☐ Immunoreactivity co-localizes with appropriate organelles or structures, e.g., plasma membrane, nucleus, mitochondria)?
- ☐ There is agreement between changes in immunoreactivity and known changes in protein and/or gene expression, e.g., following tissue injury?
- ☐ Appropriate profiles of reaction are observed on proteome microarrays? *This is a potentially powerful technology for examining antibody specificity, but presumes that relevant proteins or sequences are represented on the array, and that the presentation is comparable to that in the tissue.*
- ☐ A single reactive species is identified on Western blotting of extracts of tissue or cells known to express the antigen, but not in extracts lacking the antigen? *If the Ab selectively binds to a protein of the predicted size, it is possible that the protein contributes to observed tissue immunostaining. Although multiple bands should raise concern, multiple bands can sometimes indicate the presence of splice variants, secretory intermediates, differences in post-translational modifications such as glycosylation, or degradation. Immunoblotting of proteins resolved on reduced versus non-reduced SDS gels can also provide information on the nature of the epitope, which can then be correlated with other information.*
- ☐ A single reactive species is immunoprecipitated from extracts of tissue or cells known to express the antigen?*This approach (or blots of native gels) may be required to confirm reactivity with conformational epitopes that may be lost following denaturation and SDS-PAGE.*
- ☐ Immunoreactivity is lost following competition or pre-adsorption with purified antigen? *This approach can be used confirm biochemical specificity of the Ab for the antigen, but does not exclude cross-reaction with other proteins in cells or tissues. The approach requires the availability of significant amounts of purified antigen and it is not always possible to achieve complete inhibition.*
- ☐ The expected amino acid sequence is obtained following mass spectroscopy of protein immuno-isolated from cell or tissue extracts? *This approach approximates the state of the art for confirming biochemical specificity and tissue expression of the protein of interest. However, it does not prove that the same protein is responsible for reactivity of the fixed and embedded tissue.*
- ☐ There is codistribution of immunoreactivity using two or more antibodies that recognize different protein epitopes? *Ideally, the epitopes should be associated with different domains of the same protein.*