Specificity of antibodies

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Whether polyclonal or monoclonal antibodies should be used in immunohistological staining is sometimes a matter of personal preference. Most often, however, the type of antigen and the tissue to be studied will influence this selection (Table 1). Because monoclonal antibodies are of uniform affinity, monoclonal antibodies with high affinity are preferred for immunohistology in oder to avoid loss of antigen staining due to dissociation of antibody from the epitope site.

Table 1: Polyclonal and monoclonal antibodies in immunohistology

Features	Polyclonal antibodies	Monoclonal antibodies	Pool of monoclonal antibodies
Specificity	Heterogenous antigen specificity (cross-reaction) Limited reproducibility (batch variation)	High and reproducible antigen specificity Homogeneity (no batch variation)	High and reproducible antigen specificity
Sensitivity Signal strength	High sensitivity Usually good or excellent in immuno-histology	Antibody dependent Low to moderate signal strength in immuno-histology	Usually good or excellent Pools can overcome problems of single monoclonals
Advantage	High signal strength High fixation tolerance in immunohistology	High specificity and unlimited supply	High signal strength High specificity and unlimited supply
Disadvantage	Production of polyclonal immune sera with limited reproducibi-lity Background problems due to possible cross-reactions	Lower signal strength than with polyclonals Low fixation tolerance in immunohistology	Restricted availability of suitable monoclonals

All prepared and purified antibodies must be submitted to quality and specificity testing against the antigen plus several unrelated or closely related proteins. Proteome microarrays containing proteins from the relevant organism and microarrays with unrelated proteins represent an ideal format for an assay to prove antibody specificity. This will allow the simultaneous screening of thousands of proteins for possible cross-reactivity (MICHAUD GA et al., 2003). Protein microarrays can be generated on planar supports (planar technology) quite comparable to those of DNA chip technology. For example, reverse-phase protein arrays can be constructed with multiple microspots each containing selected antigens or cell lysates. After incubation, the bound antibodies will then be detected by use of specifically labeled antibodies. Alternatively, particle based technologies may be employed. The capture molecules (antigens) are immobilized on microsperes which are coded according to

fluorescence and size. The identification of target molecules is then done by the discernibility of the respective microsperes in a flow cytometer. Finally, a very important way is the functional test of antibodies in selective immuno-stainings of known tissues and in staining of complex tissue arrays (tissue microarrays).

Tissue microarrays appear to be greatly useful as validation tools and, generally, in biomarker research (BATTIFORA H, 1986; WAN WH et al., 1987; HOWAT WJ et al., 2005). By arraying multiple normal tissues and different tumor types, one can analyze molecular targets and their histological distribution. Also, staining patterns of multiple antibodies are easily compared and evaluated for specificity. Interfering reactions due to unwanted antibodies are readily detected and, finally, the quality of the specific staining against the background is evaluated. In this context, the staining intensity of a serially diluted immune serum can be taken as criterium for the antibody titer. Tissue arrays are especially beneficial and effective tools in finding novel molecular targets.

Cross-reactions arising from different antibody molecules (reacting with different antigens) is a major problem observed with polyclonal immune sera, but unexpected cross-reactions can be also detected in some cases with monoclonal antibodies. Possible cross-reactivities may be due to

- **Antibody heterogeneity (polyclonal, monoclonal antibodies)**:
  - *polyclonal immune sera* with different antibody populations;
  - *monoclonal antibodies* with heavy and light chain variety after fusion of a myeloma cell (synthesizing one heavy and one light chain) with a lymphocyte (synthesizing one heavy and one light chain).
- **Intrinsic cross-reactivity of monoclonal antibodies:**
  - *same epitope* on different antigens;
  - *similar determinants* on related chemical structures;
  - *divergent and convergent determinants* in evolutionary related molecules;
  - *coincidental expression of determinant shape* in unrelated structures.

The inherent advantage of monoclonal antibodies due to their unique specificity will be lost when the antibody reacts with an epitope that is common to two or more antigens. While cross-reactivity of polyclonal antibodies can be eliminated by dedicated absorption schedules, this measure will not work with monoclonal antibodies due to their monospecificity.

Methods of antibody testing have to reconcile all the techniques for which the antibodies will be employed in the future. This holds especially true in immunohistology with aldehyde fixed and paraffin embedded specimens when a certain number of epitopes in a given antigen will not "survive". Consequently, corresponding monoclonal antibodies (against these epitopes) cannot detect the antigen. In this situation, the possibility of cellular antigen staining will be greatly enhanced by the use of polyclonal antibodies. In order to overcome such problems, one can try a mixture of monoclonal antibodies (with different epitope specificities) derived from different clones.

Storage of antibodies

Collected hyperimmune sera or monoclonal antibodies from culture or ascites fluid may be stored in aliquots at -20° (or -80°C) in tightly capped tubes. Alternatively, immunoglobulin fractions can be prepared by salt precipitation (ammonium sulfate precipiation), ion exchange chromatography or other prparative techniques prior to further testing and storage. In the case

of enzyme or fluorochrome labeling, optimal results can be expected f. e. by purification of specific antibody molecules using immunoaffinity chromatography.

Antibody molecules may be protected and stabilized against a variety of stress factors. For example, the addition of glycerol proved useful as storage stabilizer in freeze-thaw cycles. Then, biomolecules are an attractive alternative to chemicals as protective substances. To this aim, *Ectoin* (low molecular, zwitterionic and hygroscopic organic compound) and *Hydroxyectoin* isolated from the halophilic bacterium *Halomonas elongata* have been proposed and which have proved useful as protecting and stabilizing agents for proteins against a variety of stress factors (GALINSKI EA et al., 1985; KNAPP S et al., 1999).

Selected publications for further readings

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Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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