Antibody validation by combining immunohistochemistry and protein extraction from formalin-fixed paraffin-embedded tissues.

Aims: Personalized cancer treatment strategies depend on comprehensive and detailed characterization of individual human malignancies. Clinical pathology, particularly immunohistochemical evaluation of biomarkers in tissues, is considered to be the approved standard for diagnostic and therapeutic decisions, having a direct influence on patient management and therapy. Although antibody-based approaches are established and integrated successfully into both clinical and research applications, for personalized treatment regimens new demands have been placed on the quality, reproducibility and accuracy of antibody-based assays. To ensure the accuracy of specific antigen detection in immunohistochemistry, we introduce a novel approach for antibody validation. Methods and results: In a tandem approach we used the same archival tissue of interest for antibody validation by combining extraction of immunoreactive proteins from
formalin-fixed, paraffin-embedded tissue with Western blot analysis and immunohistochemistry. This procedure allows for specification of the antigen detected and for localization of the protein in the tissue. Of the 32 antibodies tested used in research and routine diagnostics, 19 showed reliable specificity in both assays. Conclusion: This study emphasizes the advantage of combining suitable methods to ensure reproducibility and specific antigen detection. Based on our results, we propose a novel step-by-step strategy to validate antibody specificity and reduce variability of immunohistochemical results.

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