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Accelerated Antigen Instability Testing Reveals Quantitative Mass Spectrometry Analysis Overcomes Specimen Limitations Associated with Diagnostic PD-L1 Testing

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Introduction: Immunohistochemistry (IHC) in formalin-fixed, paraffin embedded tissue (FFPET) is widely used in clinical and research settings, but has limitations relating to epitope masking, post-translational modification and immunoreactivity loss that occurs in stored tissue by poorly characterized mechanisms. PD-L1 IHC is particularly susceptible to epitope degradation and is an ideal model for understanding signal loss in stored FFPET.

Methods: We assessed 1,124 tissue sections to understand environmental factors contributing to immunoreactivity loss in stored tissues. PD-L1 IHC using 4 clones (22C3, 28-8, E1L3N, SP142) was assessed in stored FFPET of lung and gastric carcinoma. Accelerated aging of FFPET was achieved using increased humidity, oxygen and temperature. Quantitative mass spectrometry (MS) was used alongside IHC for quantifying PD-L1. Global proteome MS analyses were used to assess proteome-wide oxidation.

Results: MS quantification of PD-L1 correlated strongly with IHC expression on unaged sections ($R2=0.745$ $P<0.001$), with MS demonstrating no loss of PD-L1 protein, even in sections with significant staining loss by IHC. 22C3 and 28-8 were most susceptible to signal loss, with E1L3N the most robust (56%, 58% and 33% reduction $p<0.05$). Increased humidity and temperature resulted in significant acceleration of immunoreactivity loss, which is largely mitigated by the use of desiccants. MS demonstrated a significant but only modest oxidation of proteins by global analyses, including PD-L1.

Discussion: Immunoreactivity loss appears to be largely driven by the presence of humidity and temperature, which may result in structural distortion of epitopes rendering them unsuitable for antibody binding following epitope retrieval. Limitations of IHC for biomarker analysis in stored tissue sections can be complemented through use of MS. In some situations, MS may be preferred for retrospective analyses of archival FFPET collections.