

Stability Testing in Immunohistochemistry

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HSL-Advanced Diagnostics is a large tertiary referral centre. In 2023, the laboratory is expected to perform over 340,000 IHC tests and continues to experience growth rates between 15-20% per year. Due to the high workload and the esoteric nature of testing provided, a high proportion of tests are performed using concentrated primary antibodies. Since 2020, the laboratory has had a dedicated process improvement project to reduce waste while increasing the quality of service. As part of this wider project, the laboratory sought to establish stability data for diluted antibodies and tissue sections. The aim of this project was (1) anticipate workload demand; (2) establish stability data for each test using concentrated antibody; (3) tailor diluted antibody production to optimise demand versus stability; (4) develop a system of continuous monitoring and adjustment of diluted antibodies produced and (5) replicate this study for tissue stability. The aim of this poster is to provide Biomedical Scientists with a framework from which to develop a similar project for their service.

Keywords: Immunohistochemistry, Stability Testing, Primary Antibodies, Tissue Controls

Methods

Antibody & Control Stability Testing

- All concentrated primary antibodies and control slides were prepared with sufficient volume for testing at Week 1, Week 2, Week 3, Week 4, Month 2 and Month 3. This considered the possible need for repeat testing.

- In a high volume IHC laboratory, many routine antibodies and their controls slides will be in constant use at room temperature for almost the entirety of their period of use, e.g. Oestrogen Receptor, CD20, AE1/AE3, Ki67 etc.

- Testing was carried out across a range of automated IHC instruments, including Leica Bond III, Roche Ultra & Dako Autostainer 48.

- For both antibody and control stability testing, a "worst-case scenario" was employed to cover expected usage profiles.

- The laboratory facility is a temperature-controlled, air-conditioned environment. Temperature monitoring is carried out using the Kelsius CoolCheck™ system.

Concentrated Antibody Stability

- All diluted antibodies were stored at room temperature (range 22-26°C) for the duration of this study. All tissue sections used in the testing of antibody stability were cut fresh.

Tissue Control Stability

- All control slides were stored at room temperature (range 22-26°C) for the duration of this study. All antibodies used in the testing of control material stability were prepared fresh.

Stability Test Assessment

- All stained slides were independently double assessed by at least one Biomedical Scientist and one Consultant Pathologist. Result was determined using a binary grading system (Pass/Fail).

Laboratory Test Failure Profile

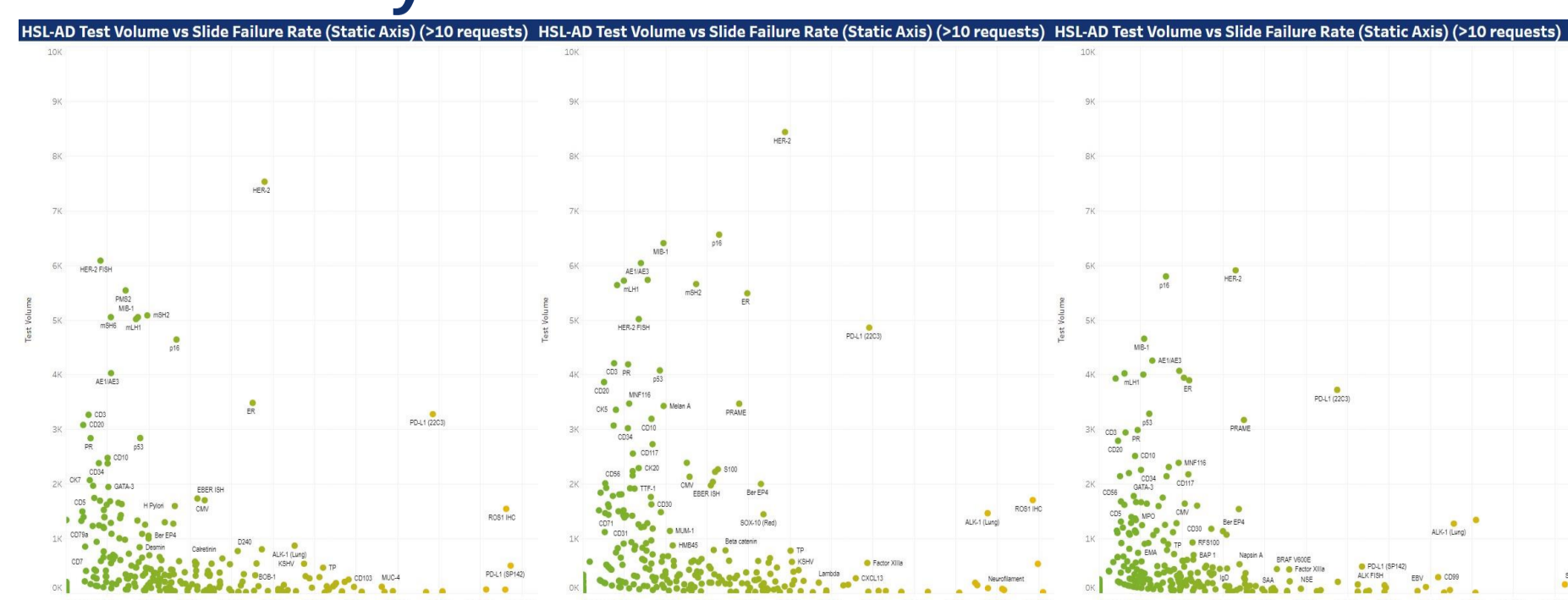


Figure 1. Test Failure 2021, 2022, 2023 (YTD)

Results

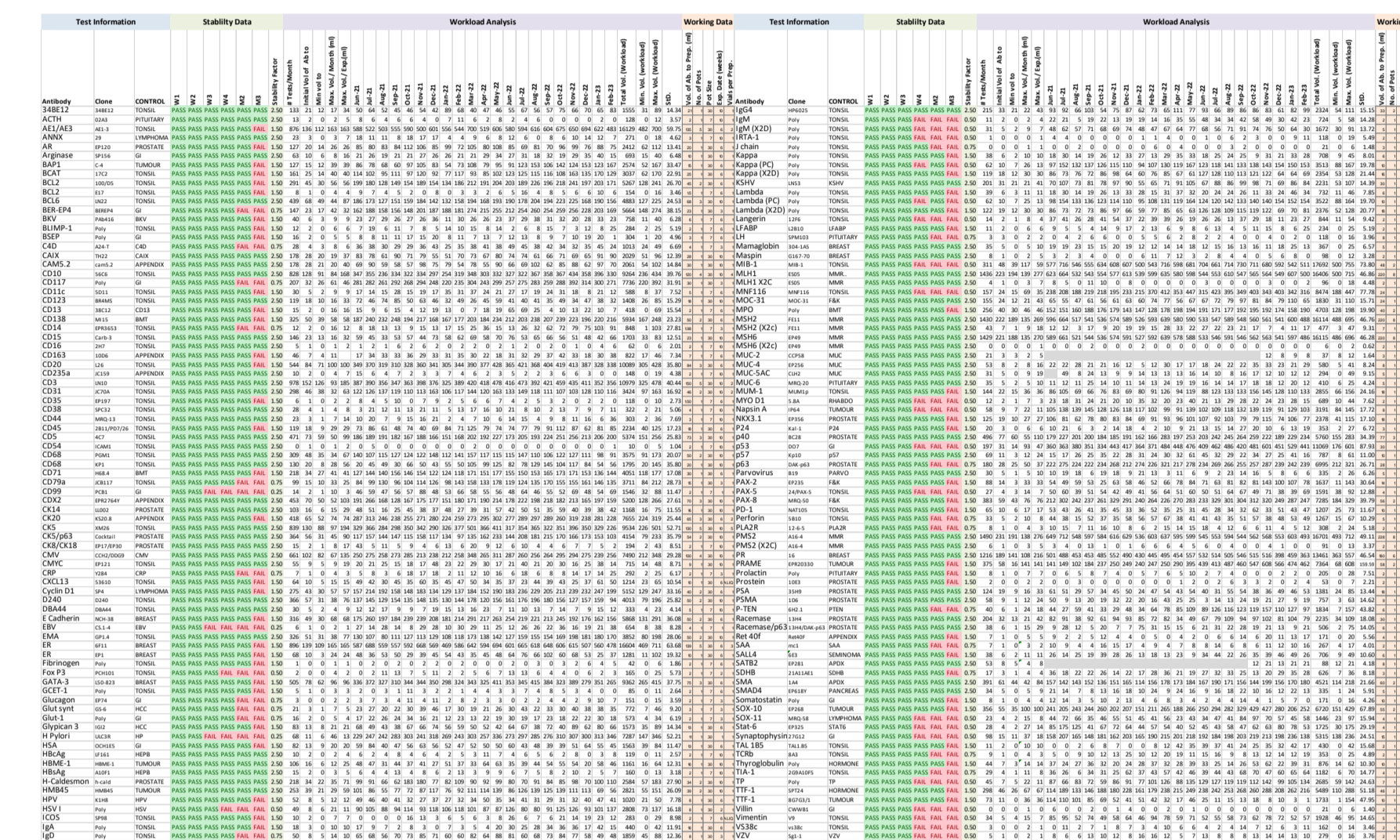


Figure 2. Antibody Stability and workload analysis

Table with columns: Test Information, Stability Data, Working Data, Test Information, Stability Data, Working Data, Test Information, Stability Data, Working Data. Rows list various antibodies and their performance metrics.

Figure 3. Tissue Control Stability

Antibody Stability

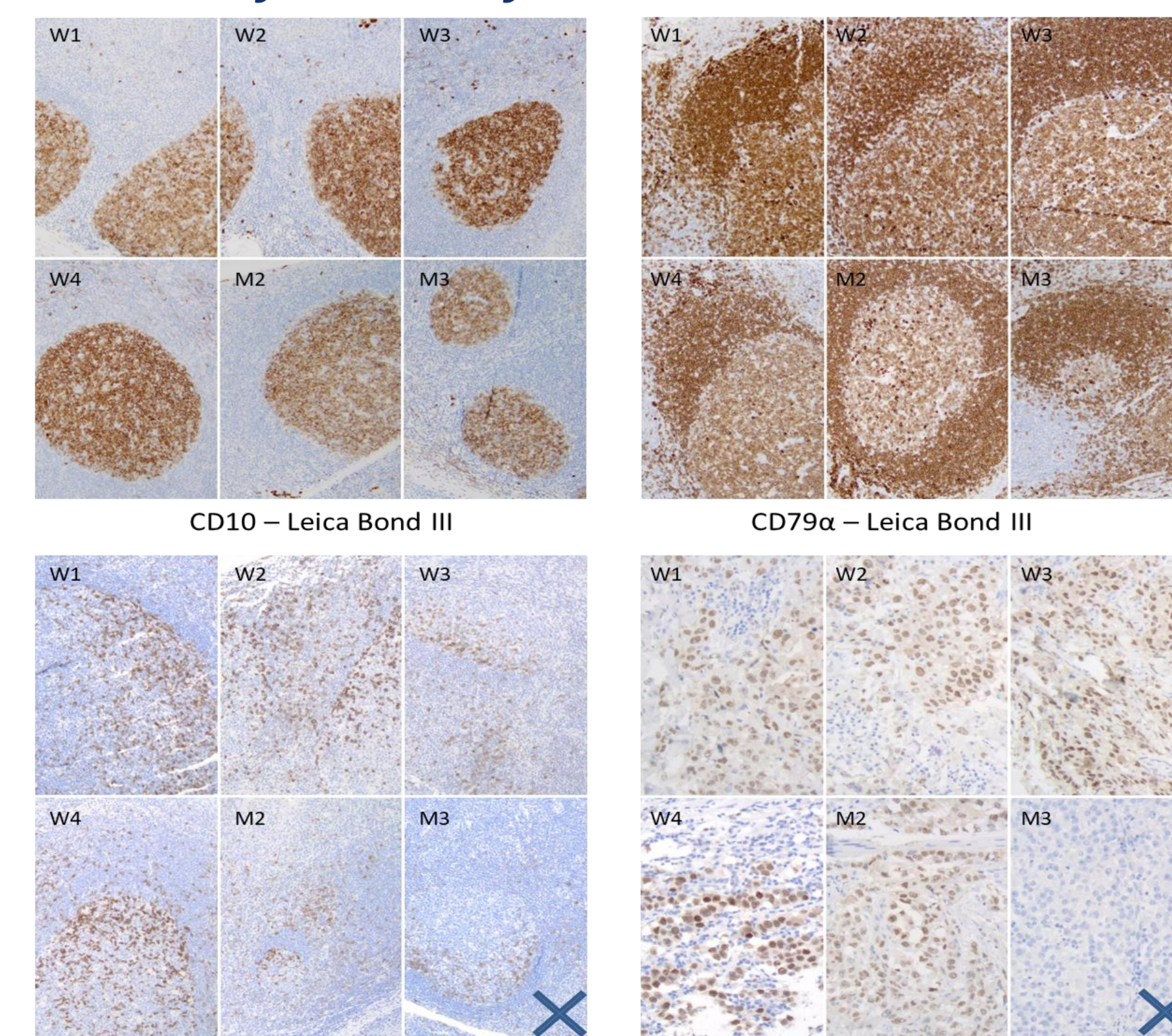


Figure 4. Antibody Stability on Stained Slides

Control Stability

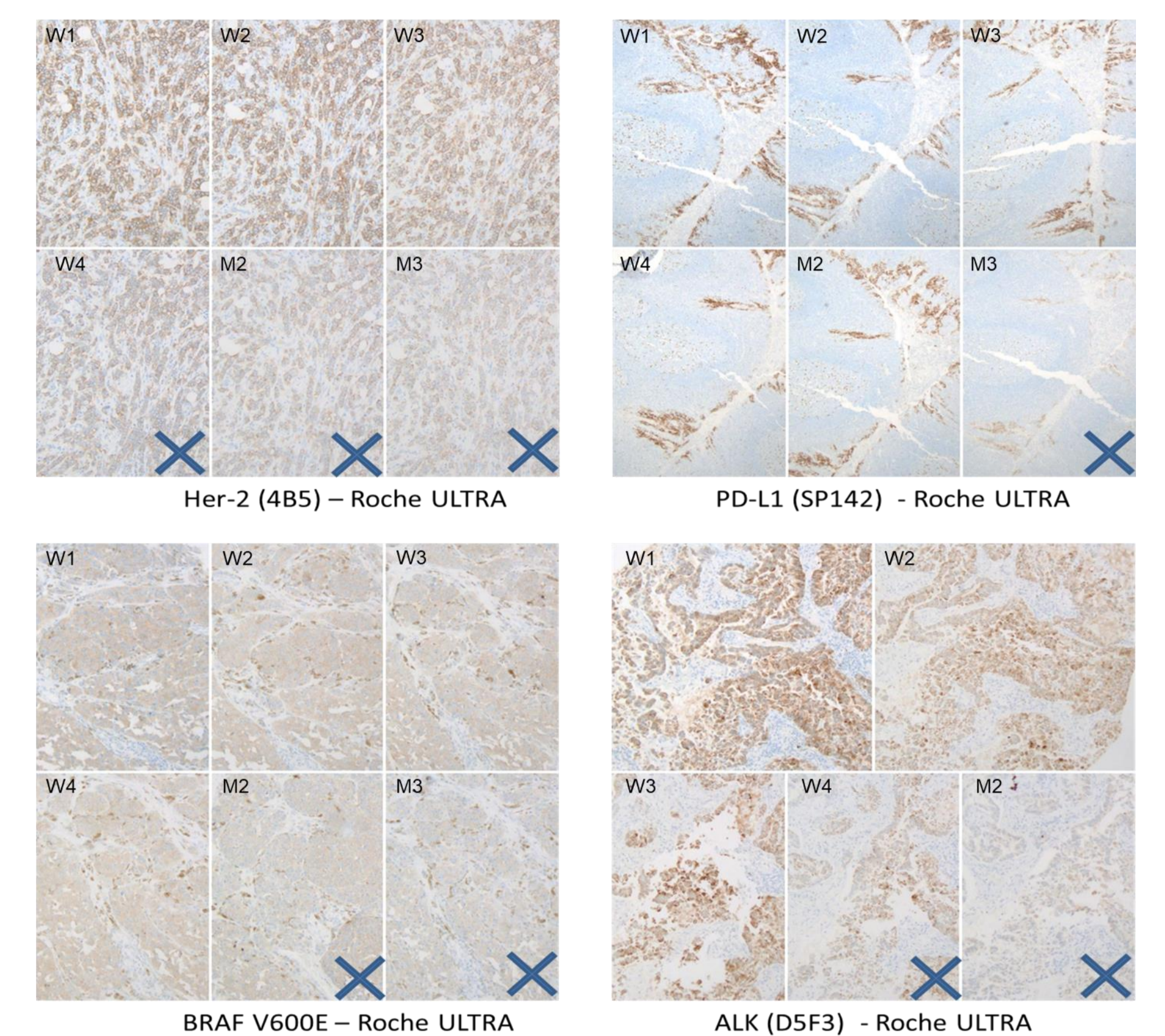


Figure 5. Control Stability on Stained Slides

Discussion & Conclusion

- Antibody and tissue stability studies provided our service with much greater insights into how our assays performed in real-time.

- The laboratory has been tracking slides failure and its root cause for a number of years (QC data collected on 1 million IHC slides), reducing failure rates while growing our testing service.

- Similar studies have been performed by a number of groups regarding the stability of tissue within FFPE blocks or as a tissue section after microtomy, but to our knowledge no group has published any findings of how it directly affected an entire clinical service^{1,2}.

- Likewise, it is standard practice for commercial IHC antibody producers to carry out stability testing of their products, resulting in established expiry dates. However, there is little information of the stability of working antibody dilutions from concentrated antibody products.

- Technically, when a laboratory prepares a concentrated antibody into a working dilution, it is manufacturing a new product. While not immediately applicable to clinical laboratories today, CE-IVDR and UKAS/ISO15189:2022 implementation will have a major impact on our services³. The results from this work allowed our service to establish expiry dates for antibody dilutions and pre-prepared control sections.

- While the results of this poster are not directly translatable into each IHC laboratory, the template used could be adopted by any laboratory to replicate this study into their clinical service, improving the reliability and reproducibility of clinical IHC testing and patient care.

References

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