

Investigation of Diagnostic Monoclonal Antibody Structural and Performance Stability Over Time

B-010

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BACKGROUND

Monoclonal antibodies (MAb) are common and reliable tools used in diagnostic tests. They are favored in diagnostic medicine due to their inherent lot-to-lot reproducibility and shelf-life stability. Manufacturer directed shelf-life dating of monoclonal antibodies commonly ranges from 6-18 months. This dating may be conservative; monoclonal antibodies stored at -20°C may have functional shelf lives that extend beyond 10 years.

METHODS

Retention samples of two lots of monoclonal antibody monospecific for Human CK-MB were obtained. These samples had been stored at -20°C in buffered solutions of PBS with 0.05% sodium azide at pH 7.2 with manufacture dates of May 2002 (Lot A) and April 2019 (Lot B). Both lots share a common cell line. In 2019, structural stability of Lot A was compared to recently manufactured Lot B via SDS-PAGE. Performance of Lot A was examined in 2020 by antigen down ELISA, then again in 2026 by antigen down ELISA and sandwich ELISA. The antigen down assay was developed by passively coating purified Human CK-MB antigen to a 96-well microtiter plate and detecting with the MAb of interest. A sandwich assay to detect Human CK-MB was developed with the MAb of interest passively coated to a 96-well microtiter plate as capture antibody; polyclonal goat anti-CK-MM (with reactivity to both CK-MB and CK-MM) was used for detection. Performance of Lot B was examined by both antigen down and sandwich ELISA within a year of manufacture, then again six years later. Performance assays were not developed prior to 2020, thus no performance data exists for Lot A prior to 2020.

RESULTS

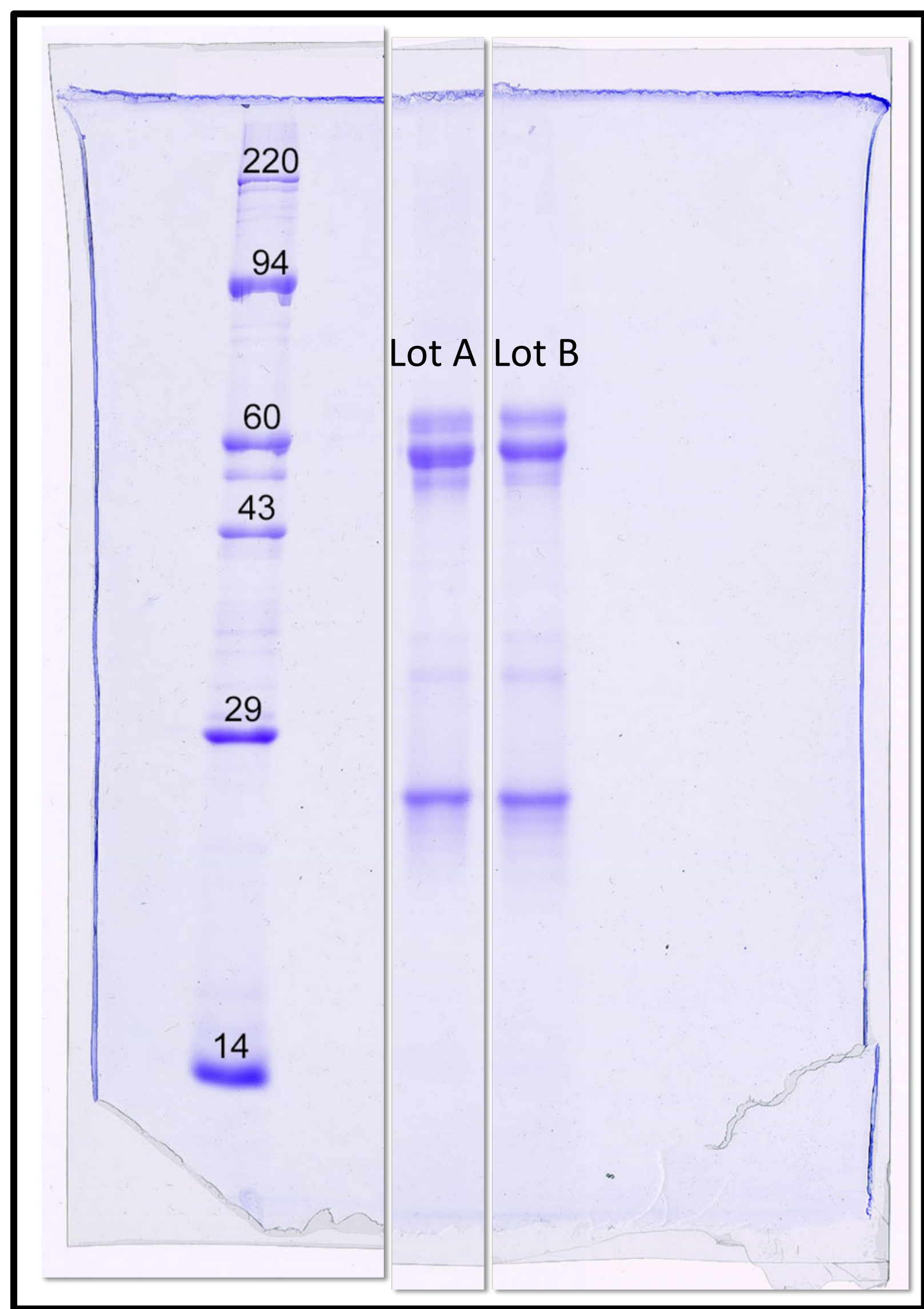


Figure 1: Anti-CK-MB MAb lot comparison of structural stability by SDS-PAGE (August 2019).

SDS-PAGE showed distinct bands corresponding to the molecular weight of complete heavy and light chains. There were no low molecular weight bands indicative of degradation species. No differences were observed between Lot A and Lot B in SDS-PAGE. Lot A and Lot B exhibited the same range of optimal working dilutions, 0.04 to 40 ng/well, in the antigen down assay. Performance was stable in the antigen down assay between 2020 and 2026. The sandwich ELISA developed in 2020

was designed to detect Human CK-MB within a linear range of 0.03-8 ng/well ($R^2 > 0.99$). Performance of Lot B in the sandwich ELISA was the same in 2020 and in 2026 when evaluated in this range. Performance of Lot A was comparable to Lot B in sandwich ELISA; in 2026 the average % difference in absolute absorbance values of the two lots was 3.4%.

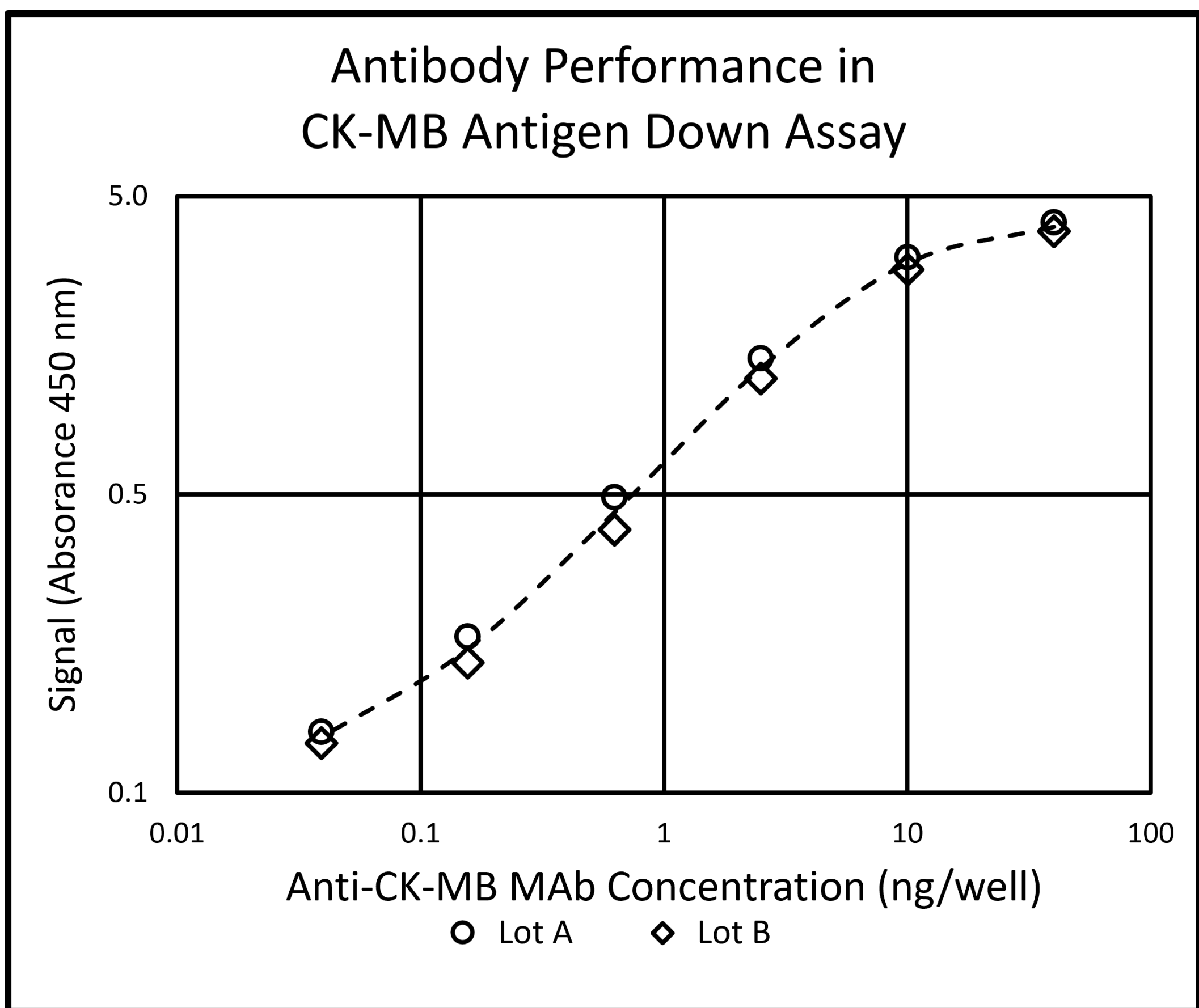


Figure 2: Lot A and Lot B performance comparison in CK-MB antigen down assay. Dashed line is the average of the two lots.

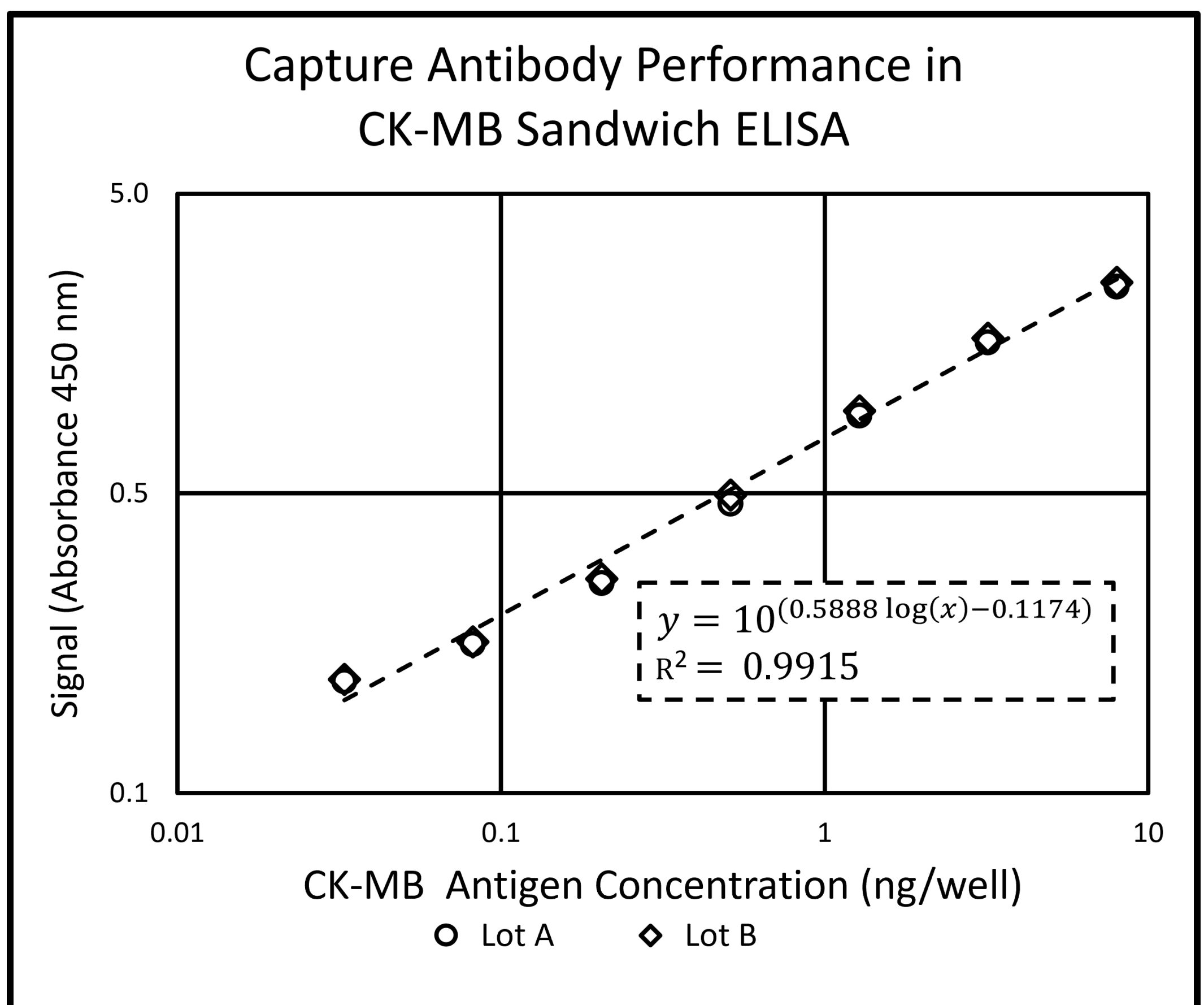


Figure 3: Lot A and Lot B performance comparison in CK-MB sandwich ELISA. Dashed line is CK-MB Standard Curve with log-log transform linear fit from Lot B data.

Results of CK-MB Antigen Down Assay Test Date January 2026		
Anti-CKMB MAb Dilutions (ng/well)	Average Absorbance (450 nm) n=6	
	Lot A	Lot B
40	4.075	3.816
10	3.116	2.839
2.5	1.427	1.224
0.625	0.489	0.381
0.15625	0.167	0.137
0.0390625	0.080	0.073
0 MAb	0.053	0.051
0 Antigen	0.052	0.050

Results of CK-MB Sandwich ELISA Test Date January 2026		
CKMB Dilutions ng/well	Average Absorbance (450 nm) n=6	
	Lot A	Lot B
8	2.449	2.529
3.2	1.593	1.644
1.28	0.907	0.942
0.512	0.461	0.493
0.2048	0.251	0.259
0.08192	0.157	0.160
0.032768	0.118	0.120
0 Antigen	0.097	0.094

CONCLUSIONS

Results of SDS-PAGE show good structural stability of Lot A for 17 years when stored at -20°C. Results of both antigen down and sandwich assay testing show good performance stability for this MAb when stored at -20°C for 6, 17, or even 23 years. While these results cannot be wholly extended to all antibodies, buffers, or storage conditions, it is reasonable to forecast extended structural and performance stability for diagnostic antibodies especially when stored at -20°C. This work joins a body of real-world evidence that supports the reputation of monoclonal antibodies as stable and reliable reagents.