HCP Antibody Stability Analysis

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BEBPA HCP Conference

Badge of Recognition

Celebrating your contribution as a speaker on May 16, 2024 to

2024 HCP CONFERENCE

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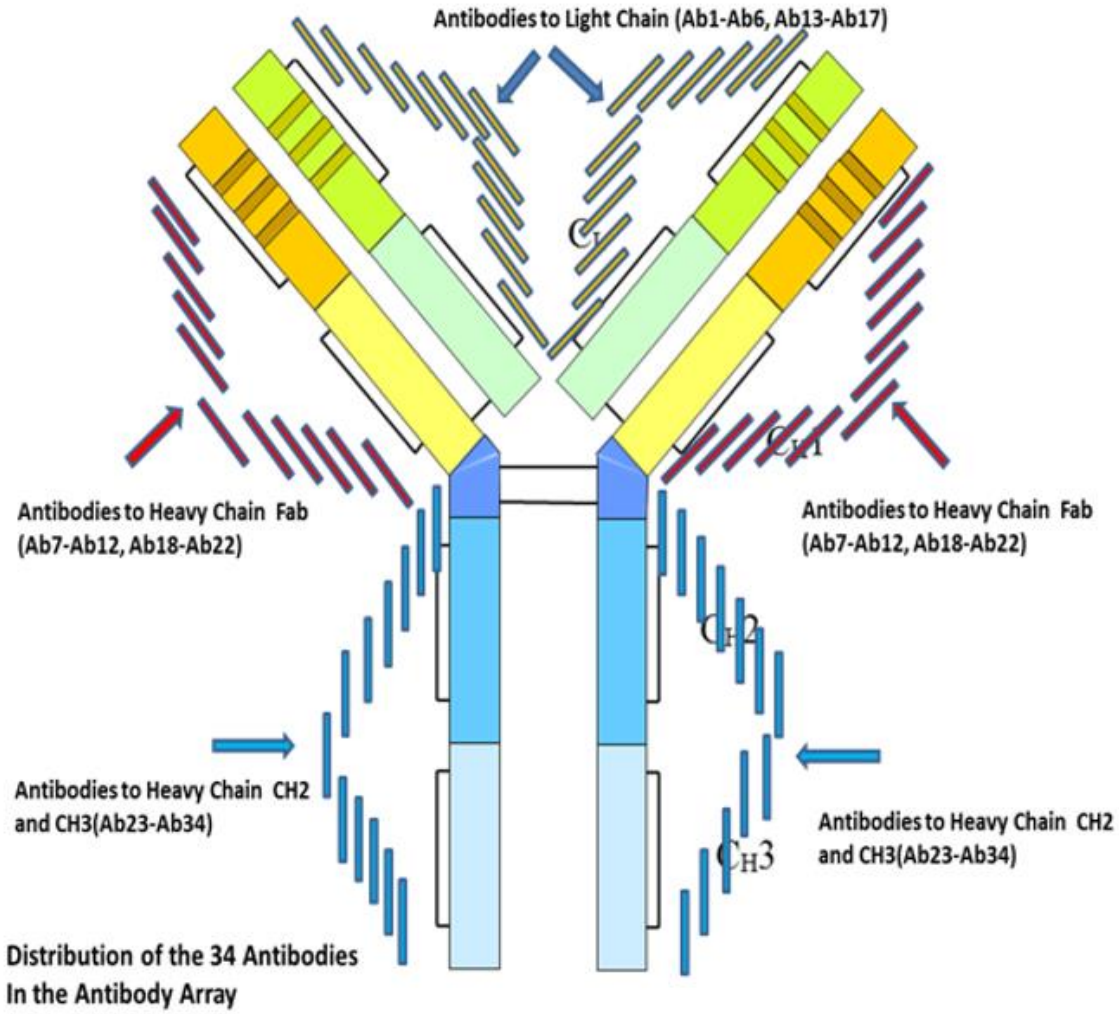
Topics Covered Today:

1. The PCA Technology.
2. Long-term HCP Antibody and Antisera Stability Studies.
3. Conclusions.

1. The Protein Conformational Array (PCA) Technology

PCA Technology Covers the Whole mAb

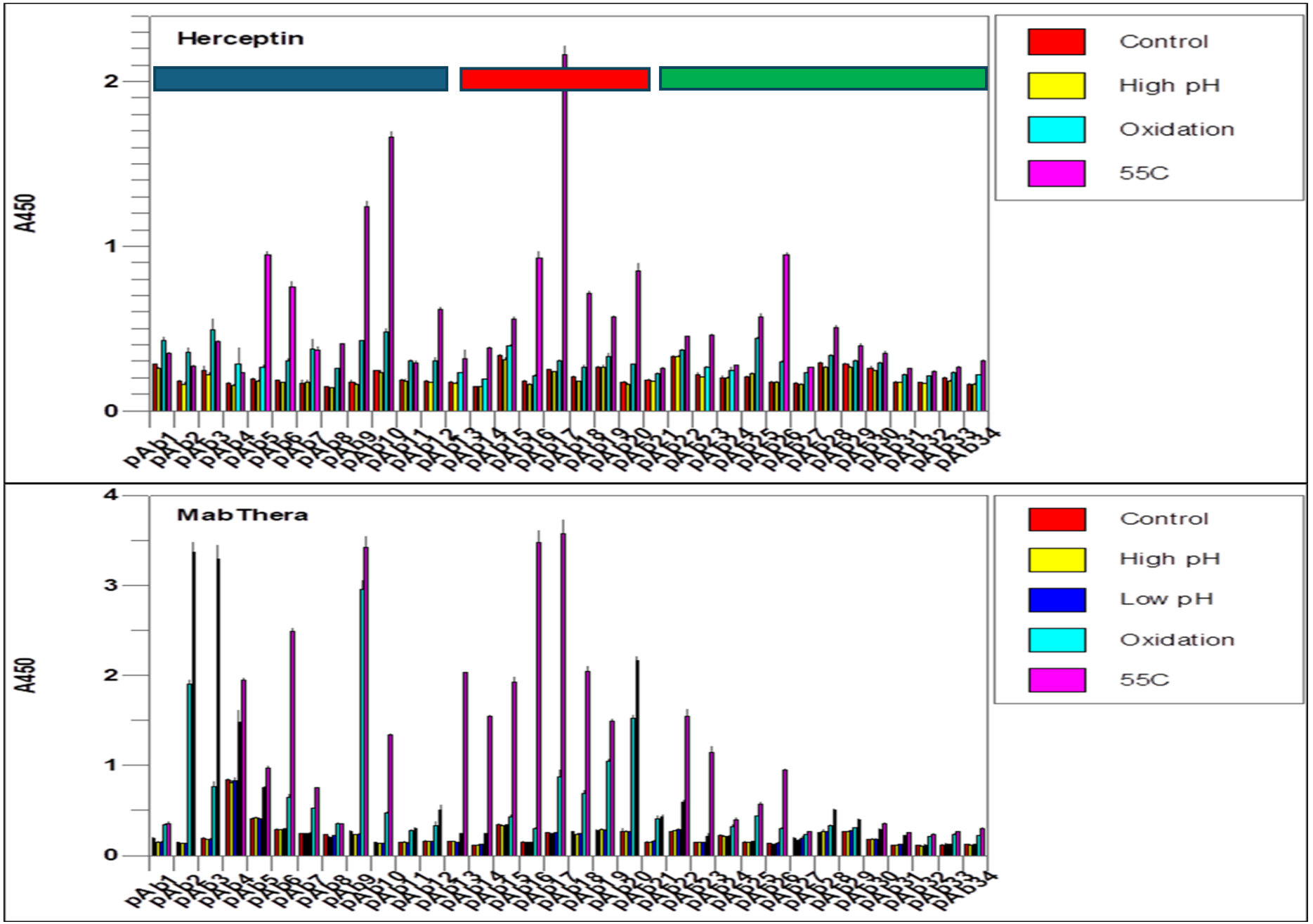


Distribution of the 34 pAb: pAb 1-12 (variable region); pAb 13-34 (constant region)

PCA is Multifaceted

Testing Condition	PCA ELISA Detection	Sensitivity	Resolution
Temperature Stress	Yes	0.1% (5 ng impurity in 5 µg testing sample)	Epitope-based, 3-6 Amino Acids
Low pH	Yes	High	Epitopes
High pH	Yes	High	Epitopes
Oxidation	Yes	High	Epitopes
Glycosylation	Yes	High	Epitopes
Aggregation	Yes	High	Epitopes
Bioassay Difference	Yes	High	Epitopes
Light Stress	Yes	High	Epitopes

PCA Detection of Human mAb HOS Stability

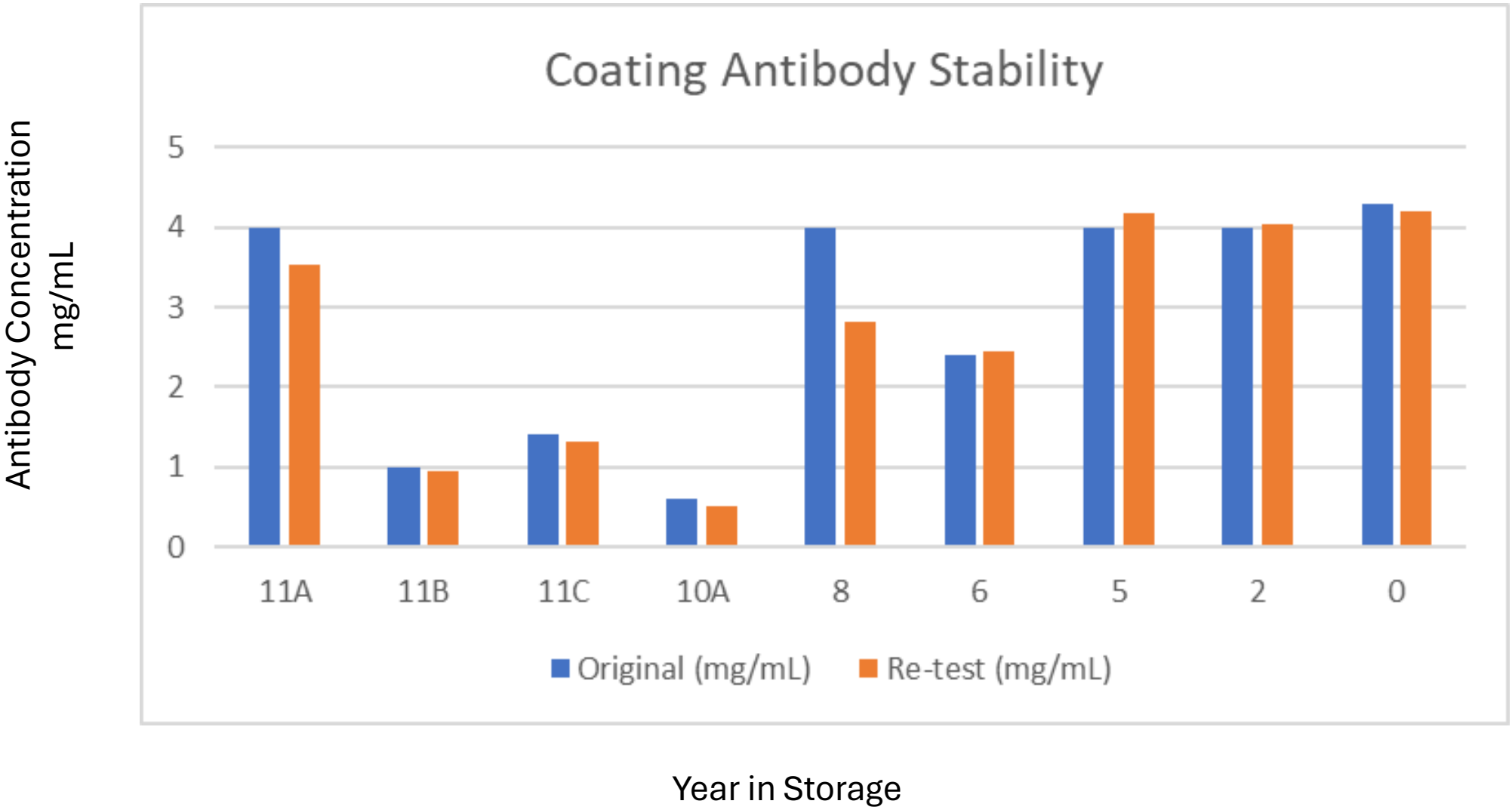


Human, Mouse and Rabbit IgG Constant Region Have High Homology

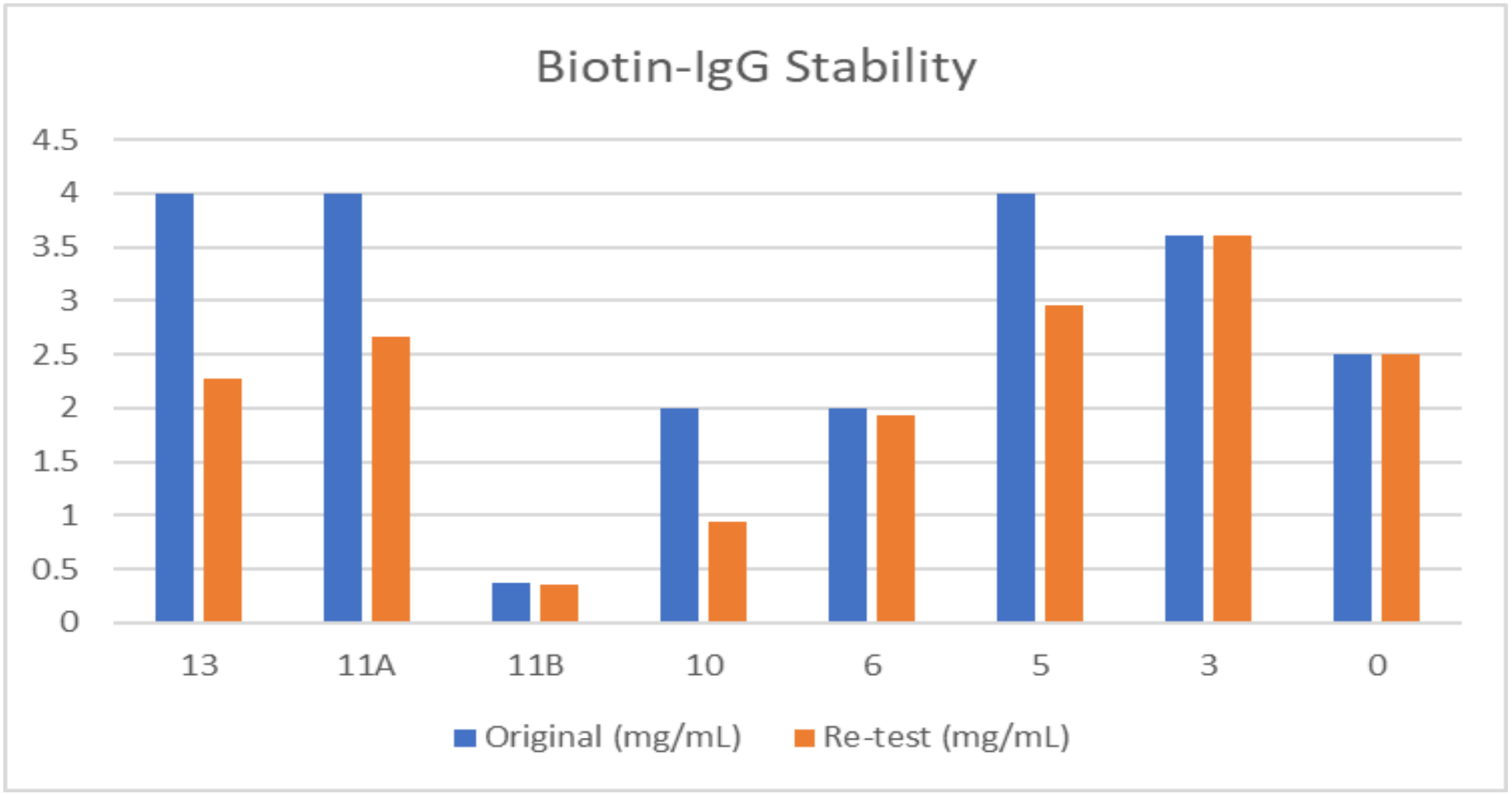
Alignment of the amino acid sequences of mouse IgG1, 2a, 2b, human IgG1 and rabbit IgG heavy chain constant region. The residues that are important for interaction with protein A are indicated by shading and the neighboring domains of the interaction core are surrounded by dotted squares. (B) DNA sequence of PCR primer pairs for mutagenesis. Replaced nucleotides are underlined.

2. HCP Antibody and Antiserum Stability Analysis

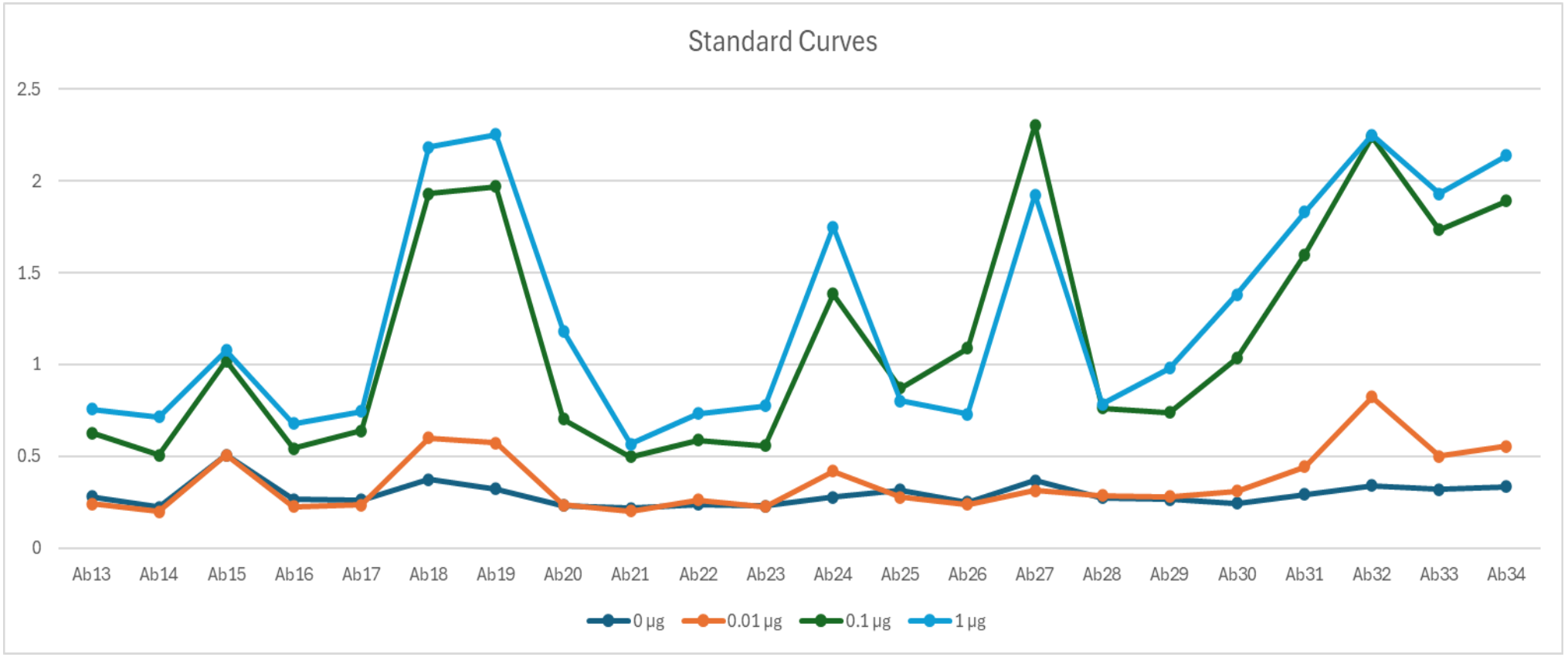
Coating Antibody Total Protein Concentration



Reporting Antibody Total Protein Concentration



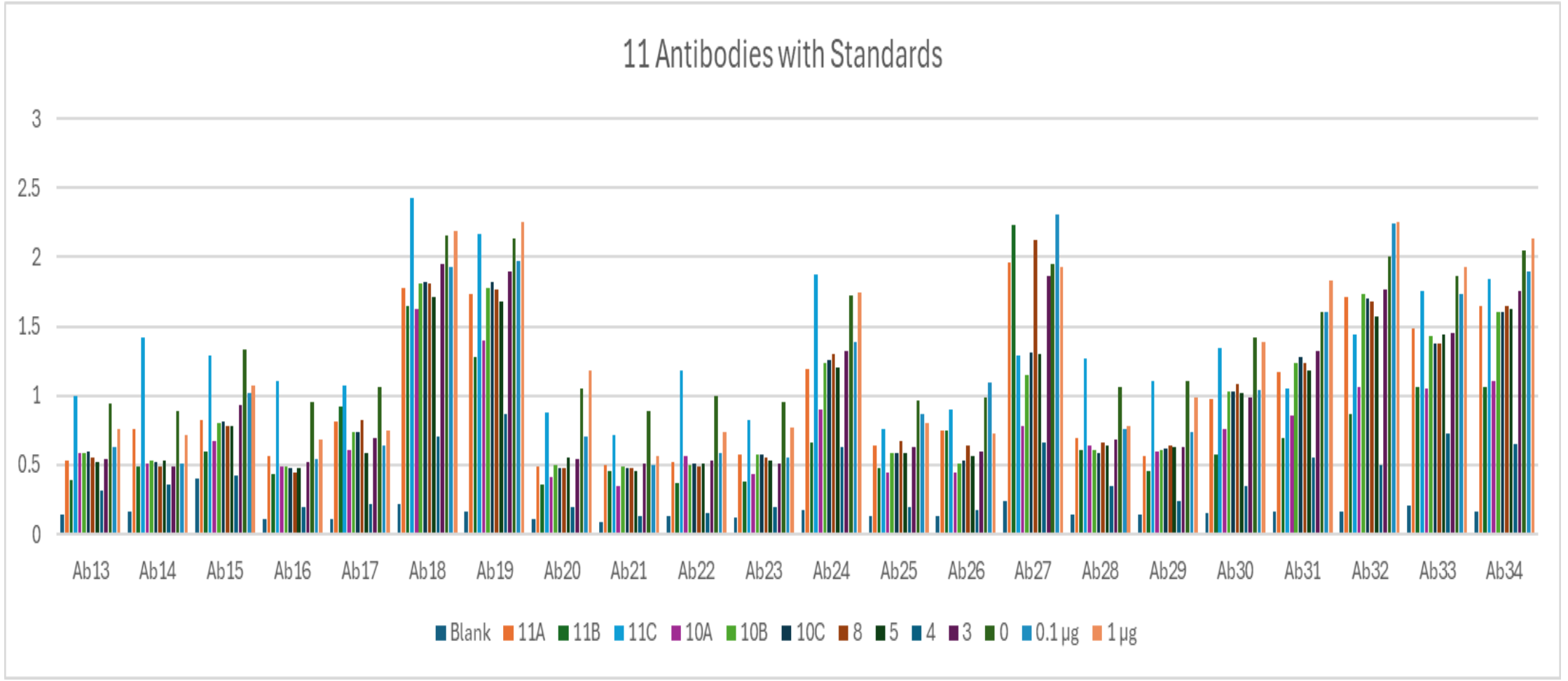
PCA ELISA Standard Curve (constant regions)



22 Constant region antibodies

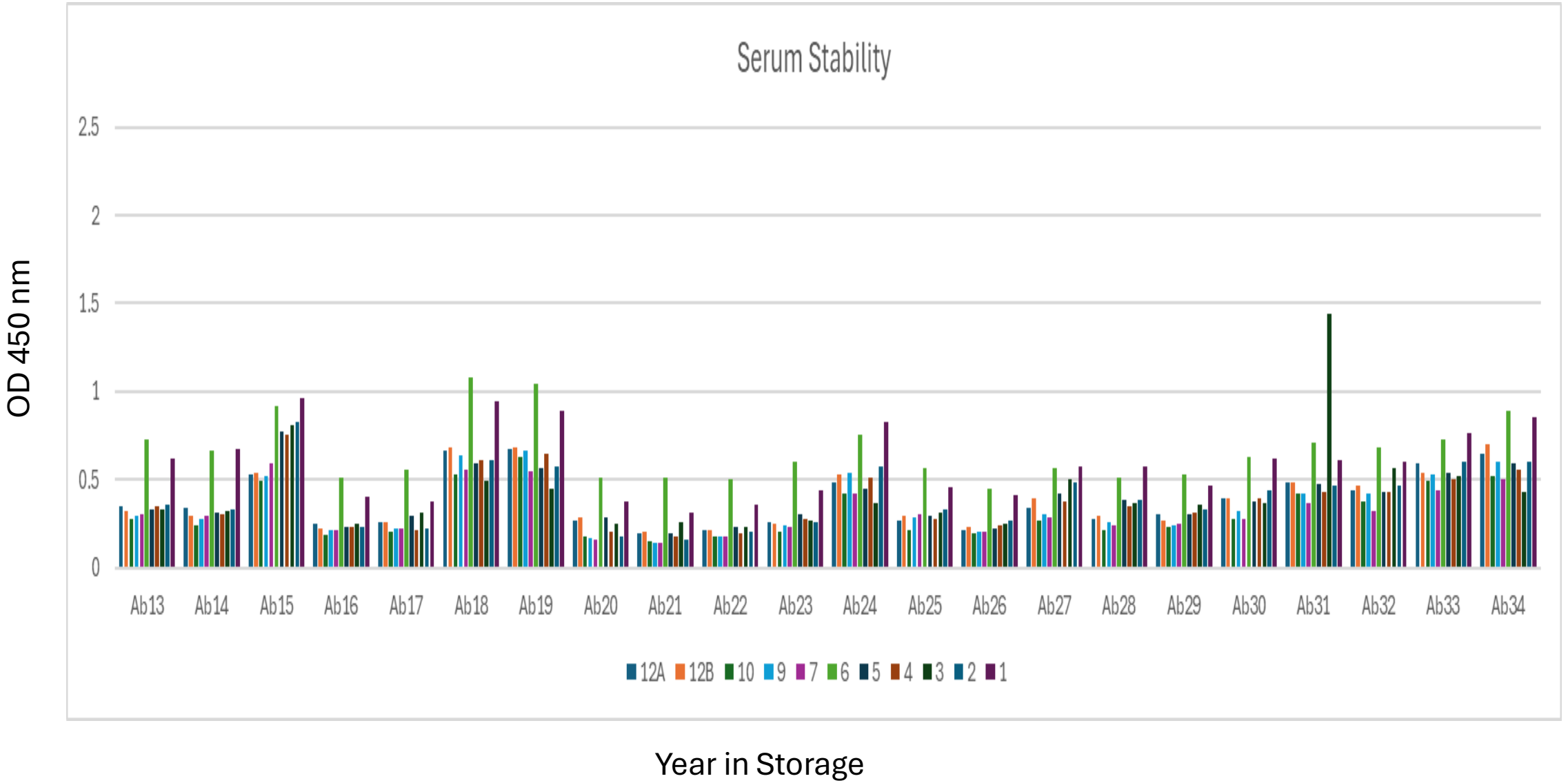
Rabbit purified IgG was treated with 8 M urea followed by coating to the Direct ELISA Plate, 22 biotin-labeled Reporting antibodies was used for the analysis.

11 Coating Antibody HOS Stability Analysis

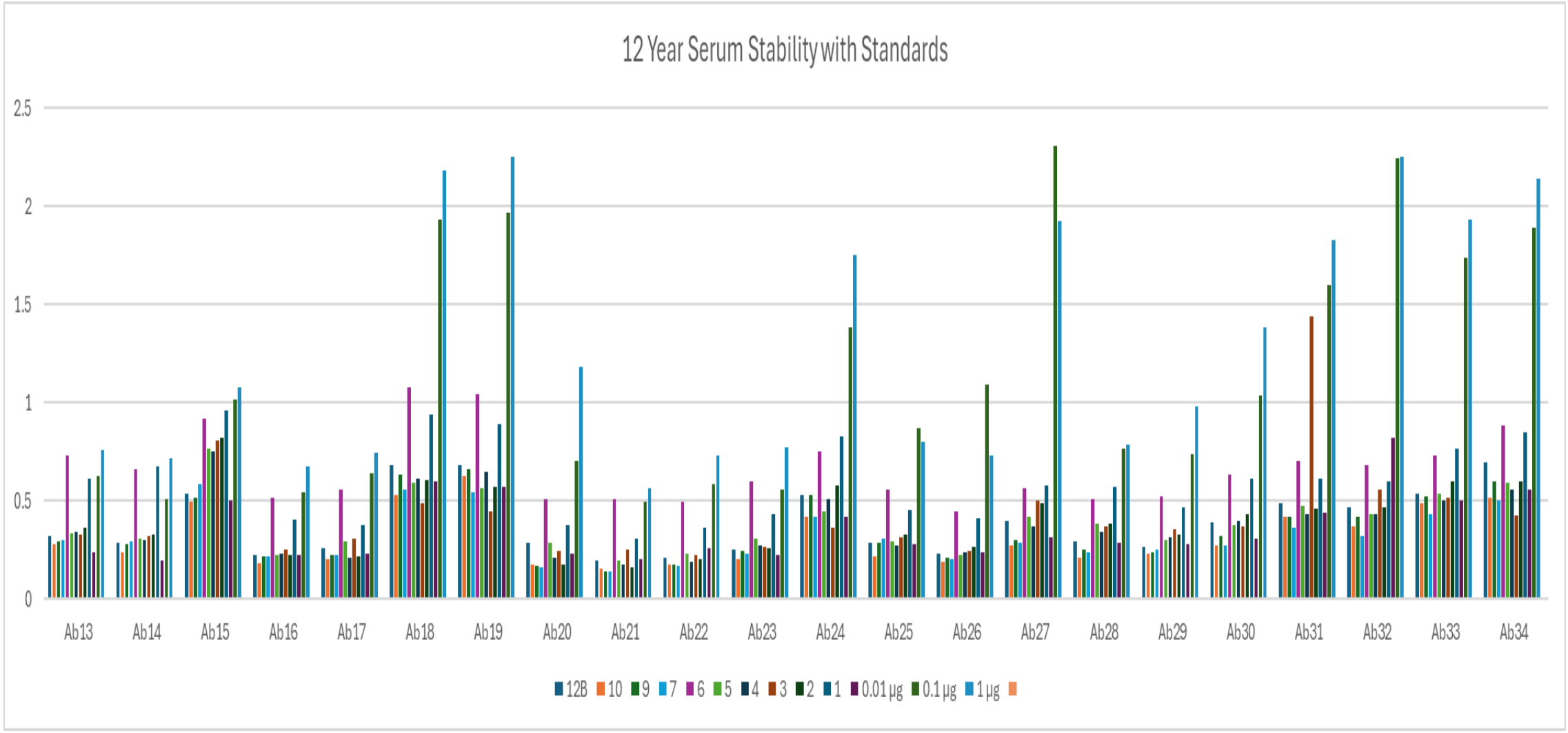


Year in Storage

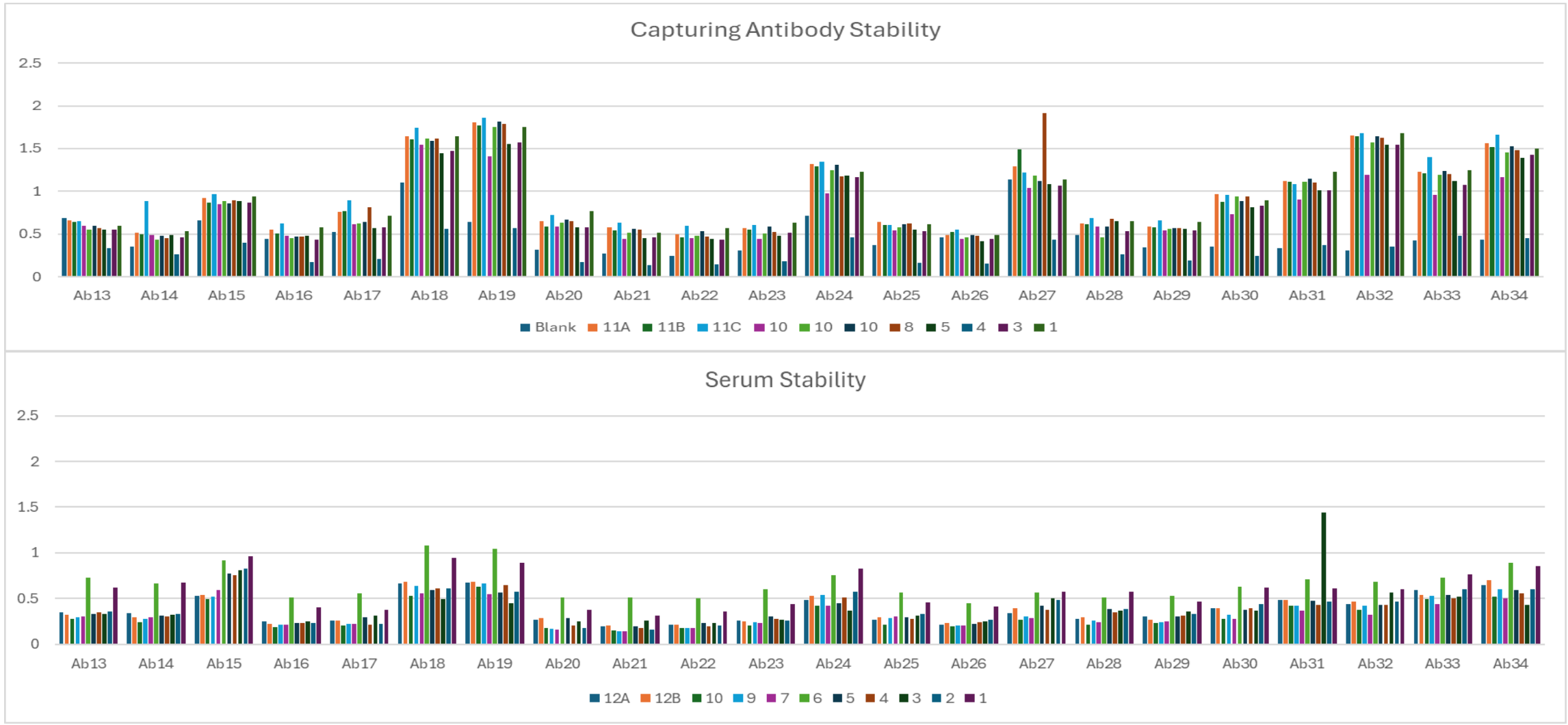
11 Anti-CHO HCP Serum HOS Stability Analysis by PCA



11 Anti-CHO HCP Serum HOS Stability Analysis by PCA



PCA ELISA Comparison of IgG Stability in Serum and Purified IgG in PBS



OD 450 nm

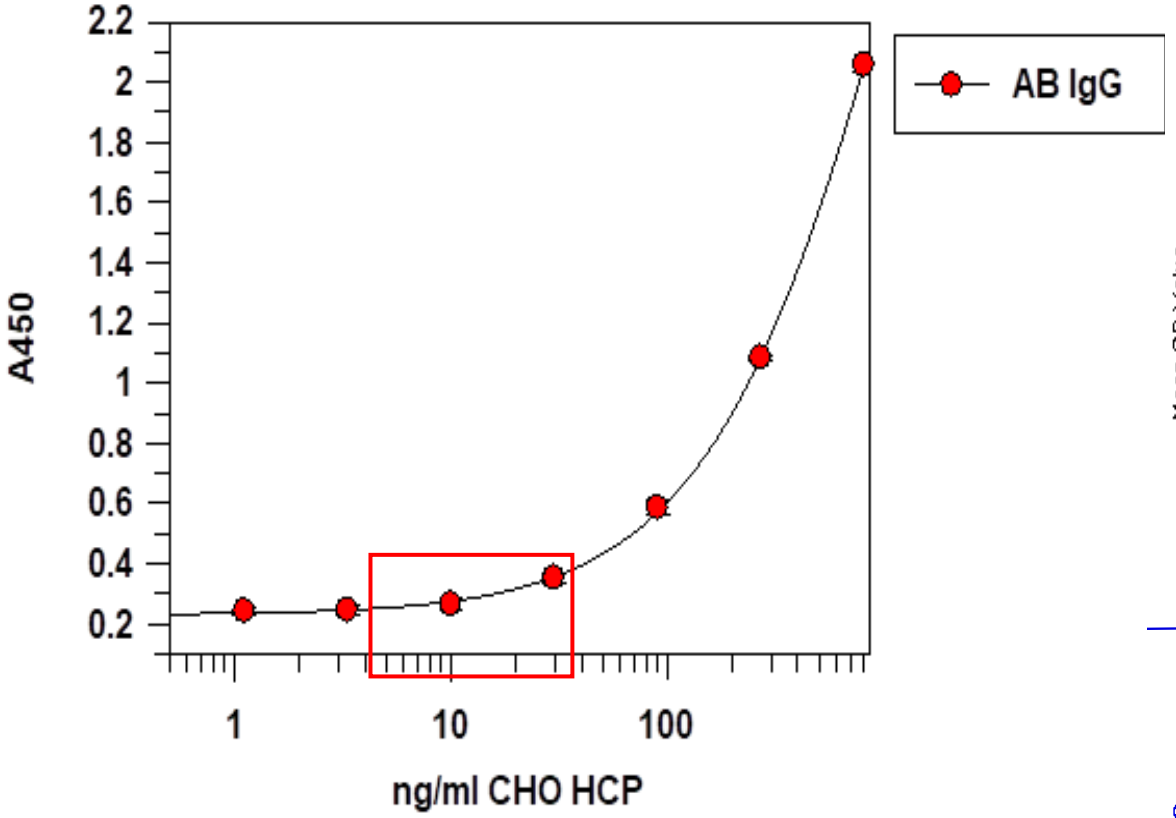
22 Constant region antibodies

Coating antibody coated at 0.1 µg/well, serum coated after 1:100 dilution, assuming maximum binding of 1 µg/well Total protein, the IgG is close to 0.1 µg/well.

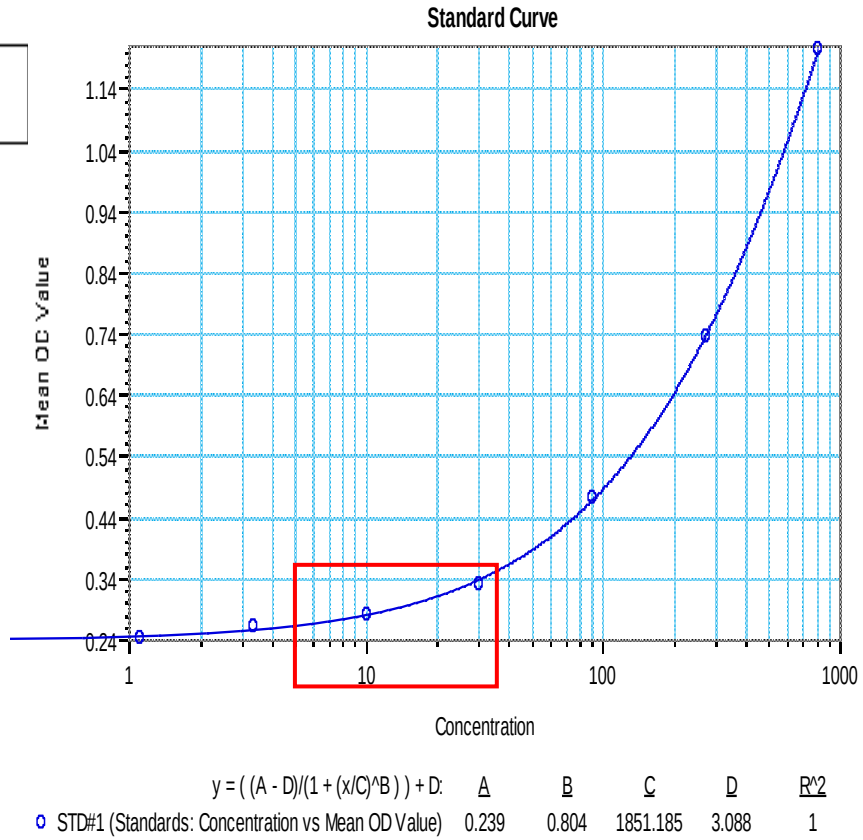
PCA ELISA Analysis of Biotin-labeled IgG Stability in PBS

Year in Storage

ELISA Comparison- IgG in10 Years Storage



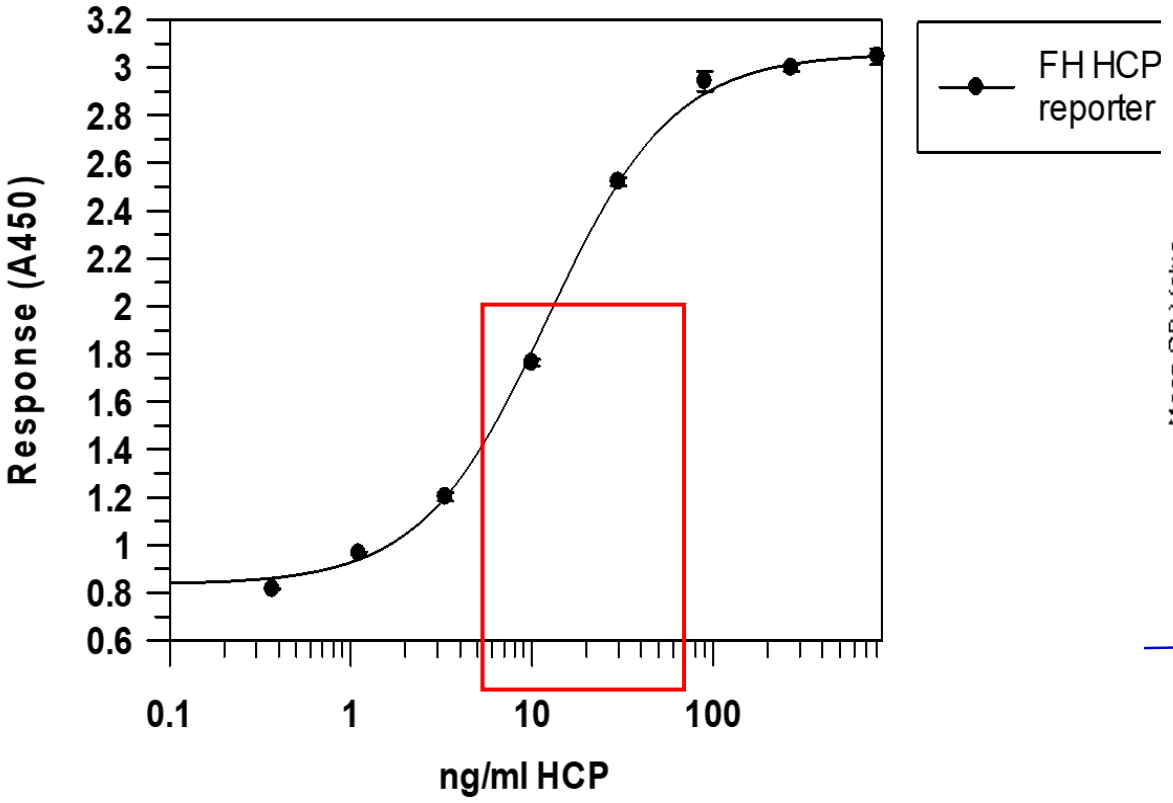
Then



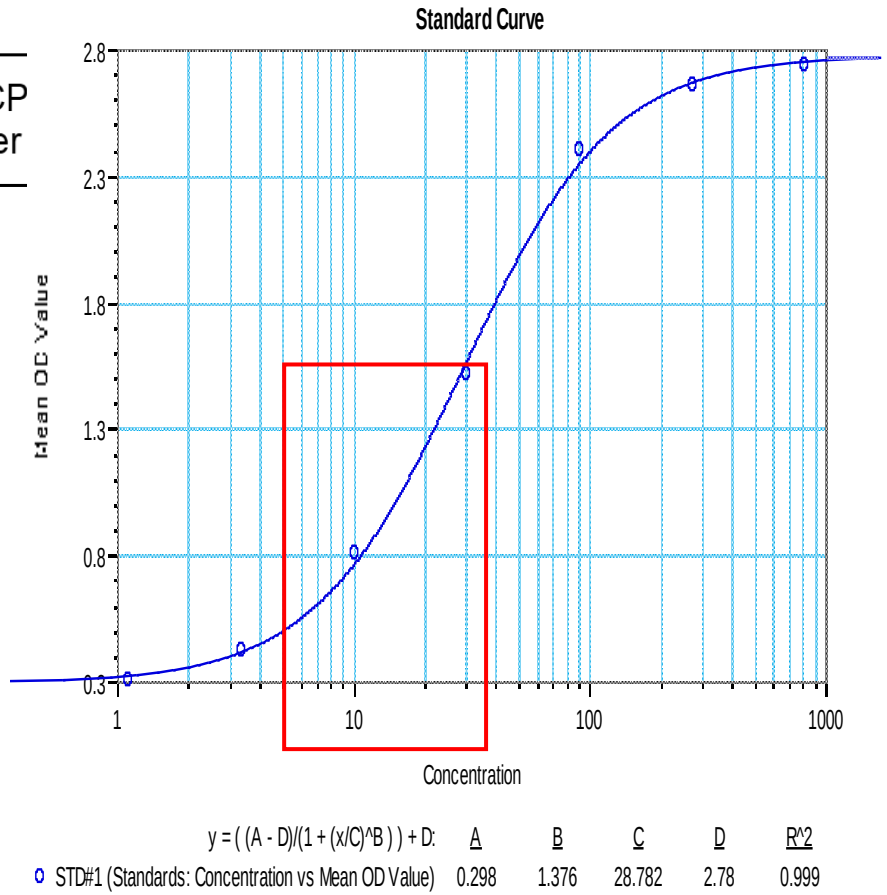
Now

Maximum OD value changed because of streptavidin-HRP change, assay sensitivity does not change significantly

ELISA Comparison-IgG in 8 Years Storage



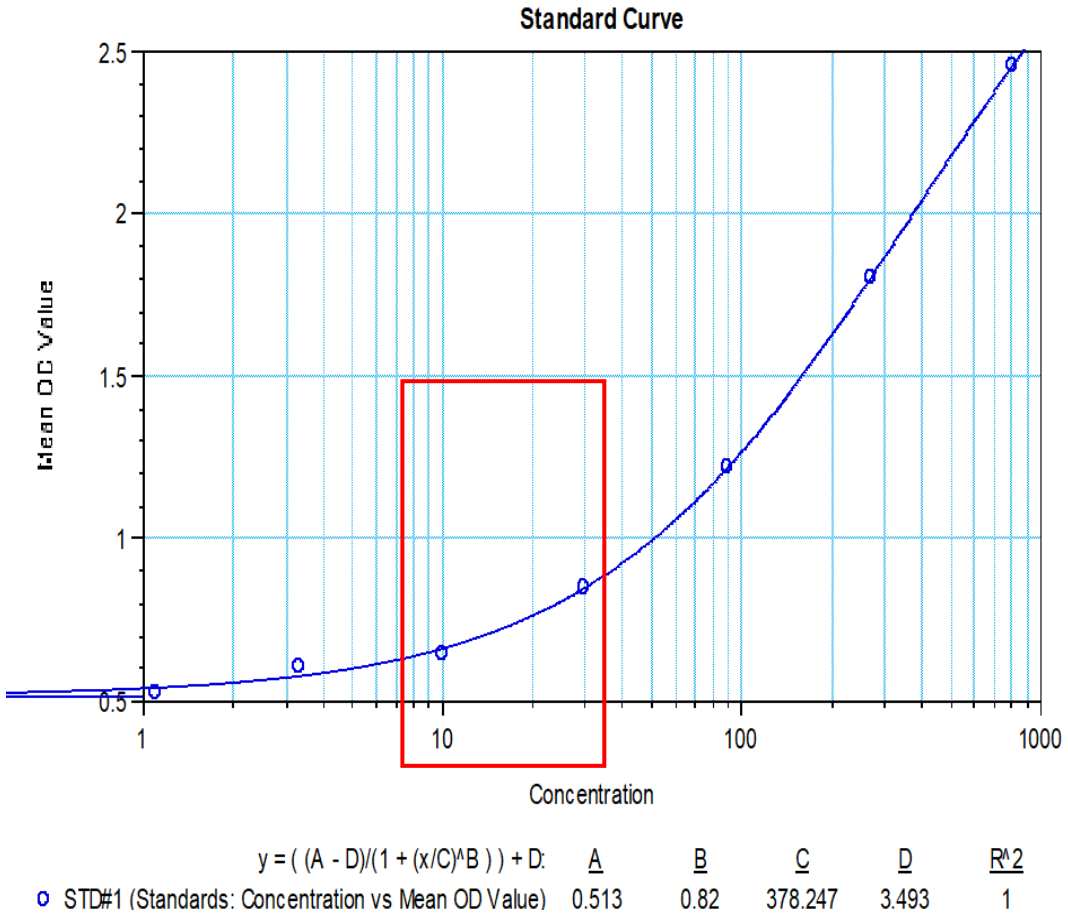
Then



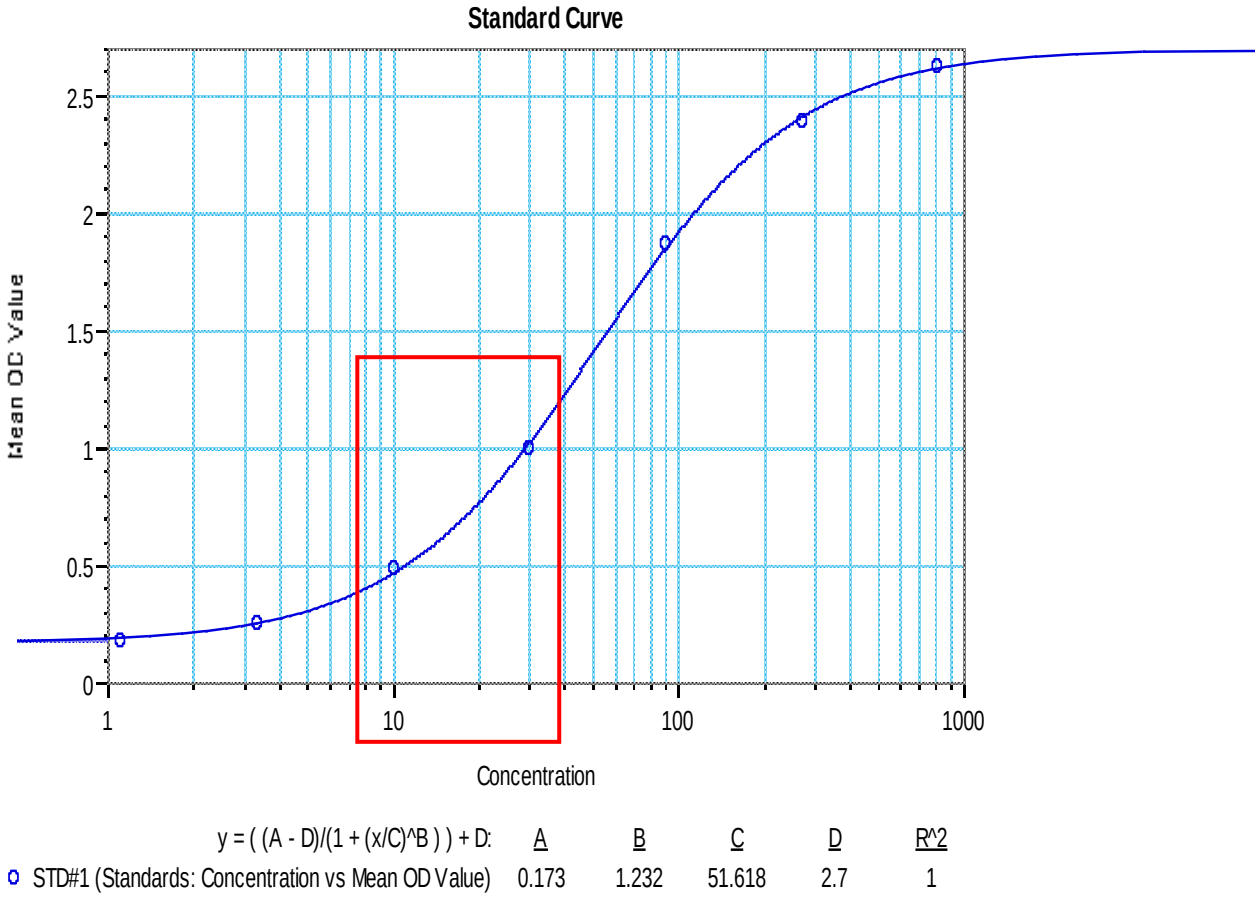
Now

Assay sensitivity does not change significantly

ELISA Comparison-IgG in 5 Years Storage



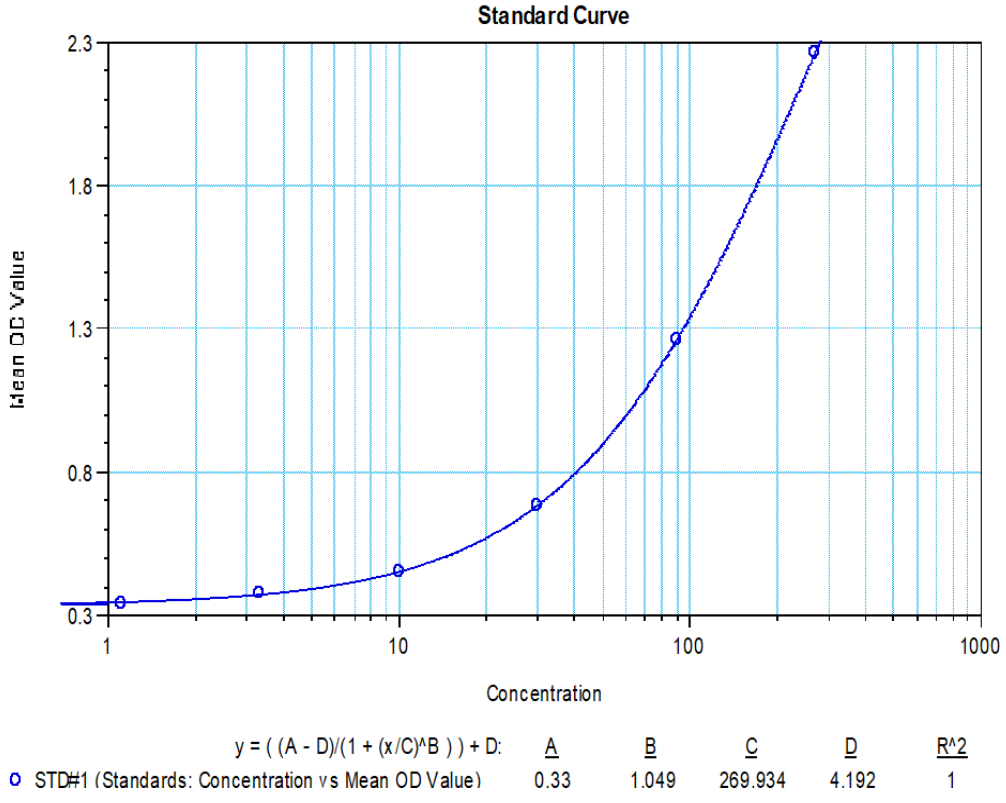
Then



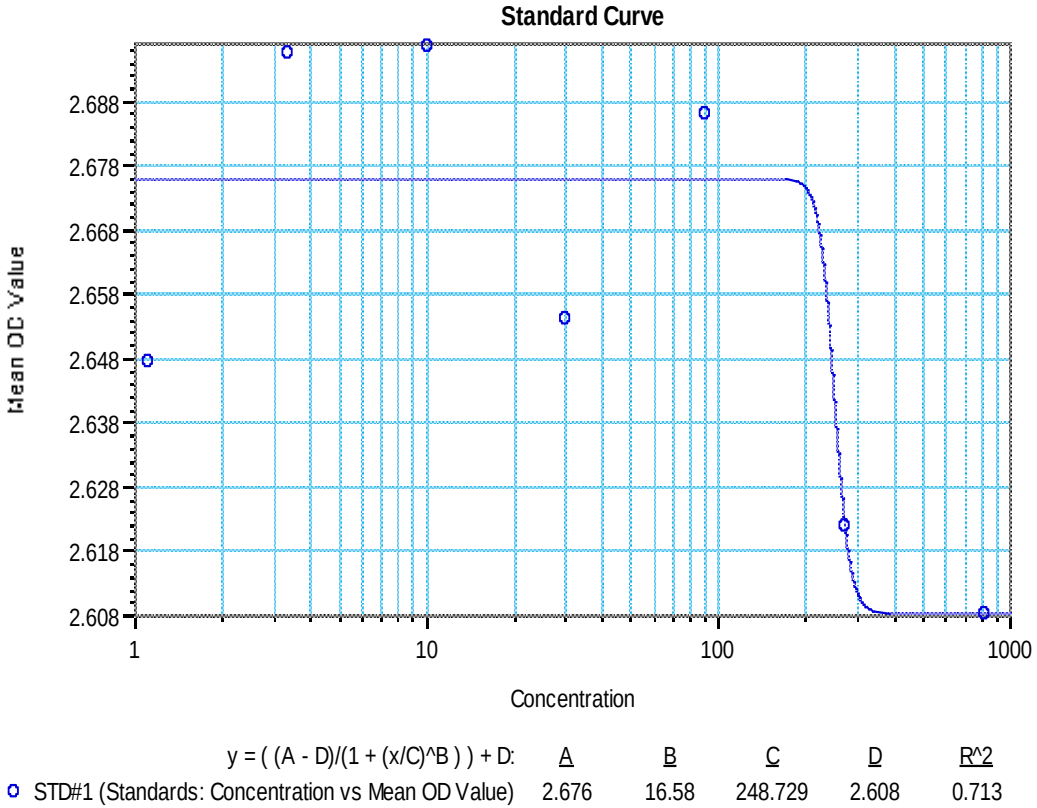
Now

Maximum OD value changed because of streptavidin-HRP change, assay sensitivity does not change significantly

ELISA Comparison-IgG in 4 Years Storage



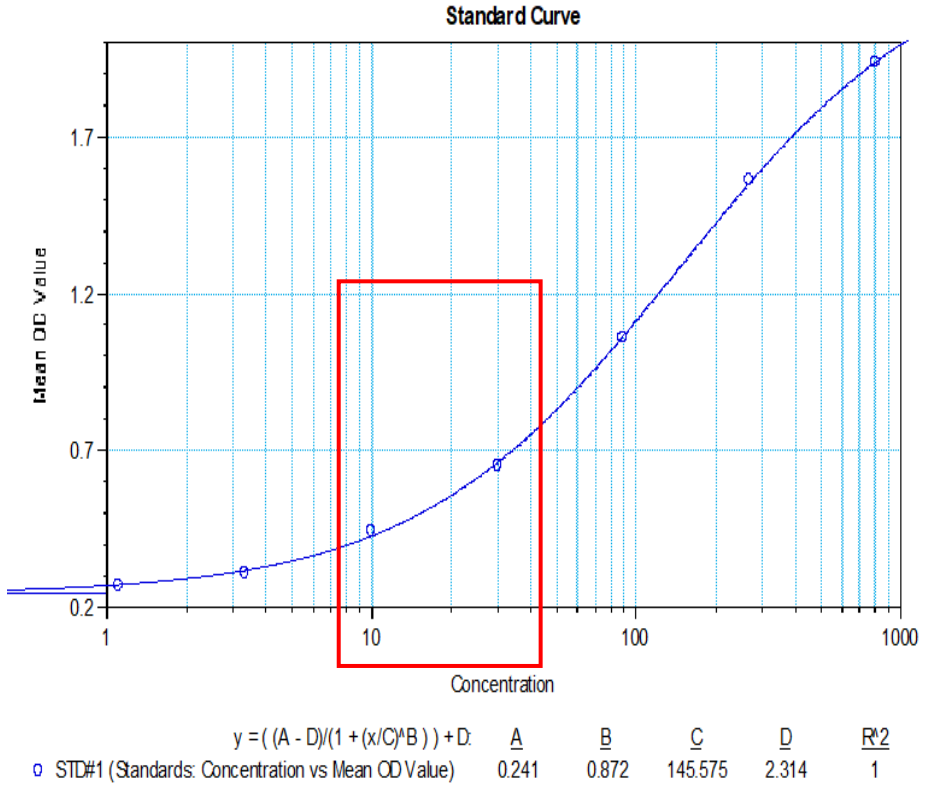
Then

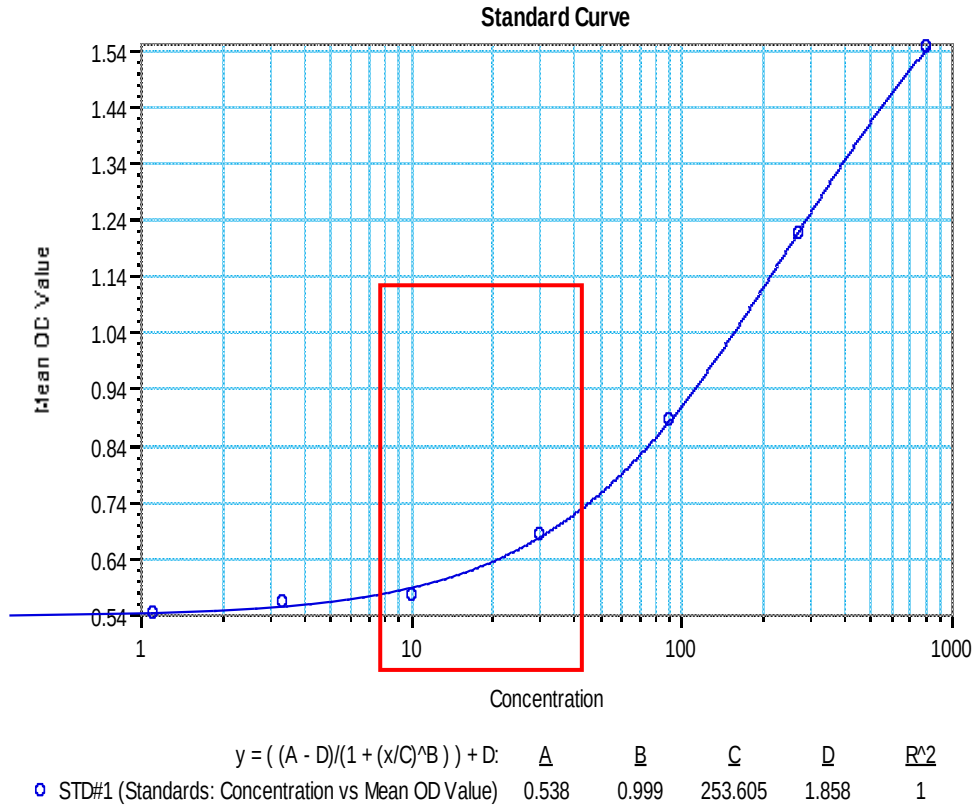


Now

Major nonspecific binding was produced during storage, biotin-IgG needs to be regenerated

ELISA Comparison-IgG in 3 Years Storage





Assay sensitivity does not change significantly

Conclusions

- Critical Reagents such as HCP antibodies and antiserum need to be managed properly.
- PCA technology provides good sensitivity in the detection of conformational changes from rabbit-derived HCP antibodies and antiserum.
- For long term storage, serum is more stable than purified IgG.
- The majority of purified anti-HCP IgG are relatively stable in PBS at -80°C, but a stability testing plan needs to be in place to monitor possible changes in reagent quality.
- Purified IgG and biotin-labeled IgG should be adjusted to 1-2 mg/mL for long-term storage to minimize aggregation formation and retain IgG stability.