

HCP Antibody Stability Analysis

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16-May-2024

BEBPA HCP Conference

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2024 HCP CONFERENCE

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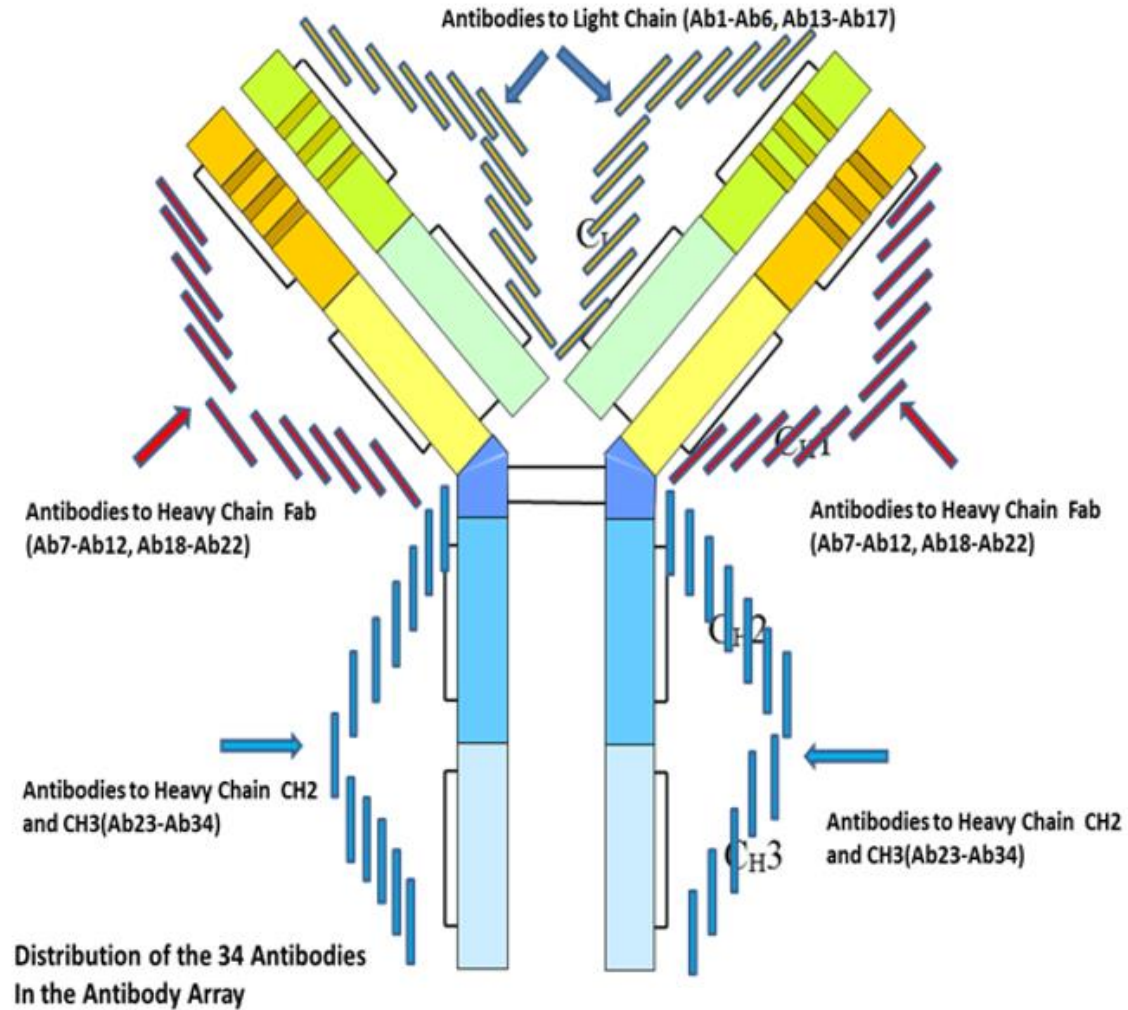


Topics Covered Today:

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

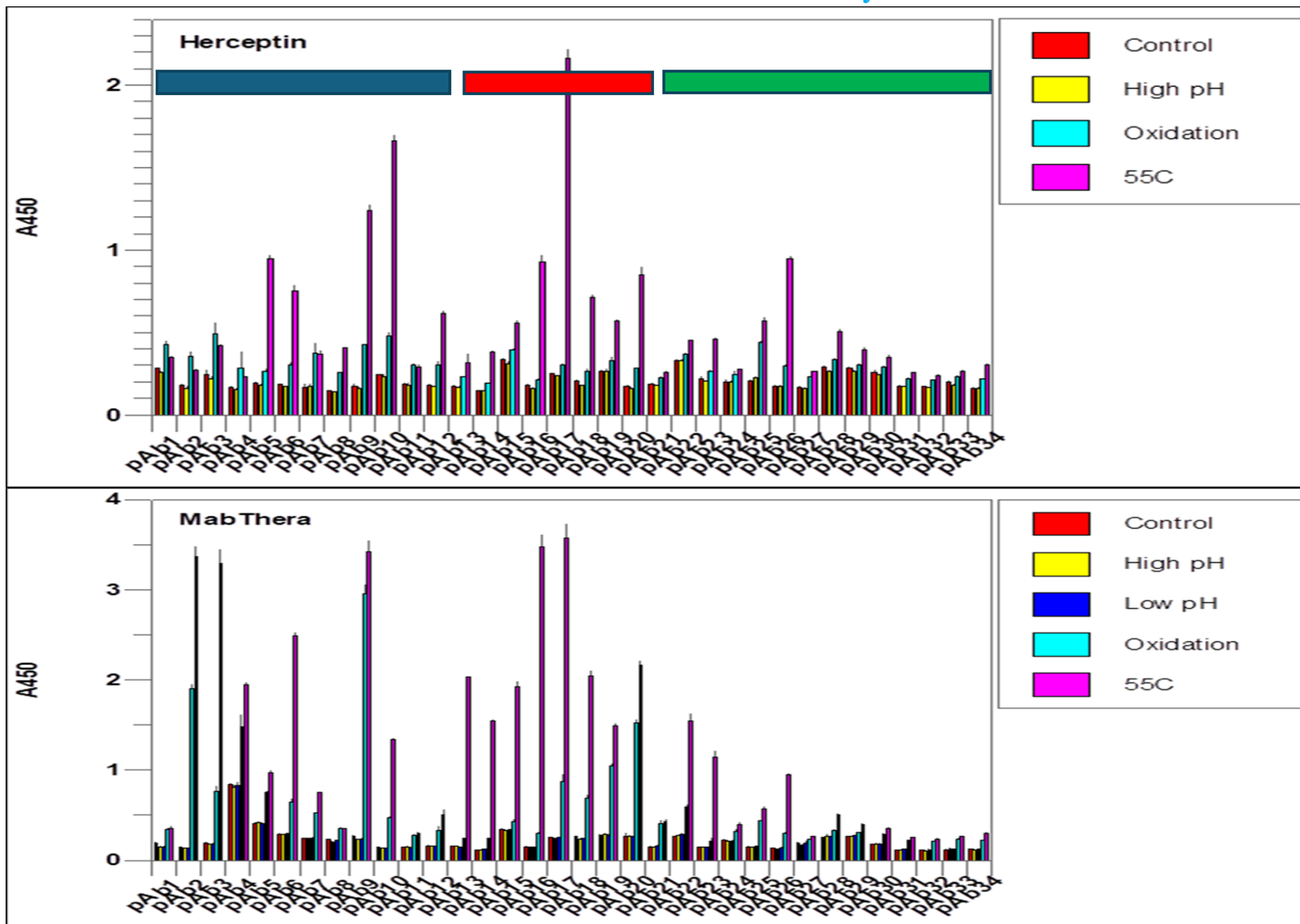


Distribution of the 34 pAb: pAb 1-12 (variable region); pAb 13-34 (constant region)

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

A

mouse IgG1	231	ICTVPEVSSV	FIFPPKPKDV	LTITLTPKVT	CVVVDISKDD	PEVQFSWFVD	DVEVHTAQTQ
mouse IgG2a		APNLLGGPSV	FIFPPKIKDV	LMISLSPIVT	CVVVDVSEDD	PDVQISWFVN	NVEVHTAQTQ
mouse IgG2b		APNLEGGPSV	FIFPPNIKDV	LMISLTPKVT	CVVVDVSEDD	PDVQISWFVN	NVEVHTAQTQ
human IgG1		APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK
rabbit IgG		PPELLGGPSV	FIFPPKPKDT	LMISRTPEVT	CVVVDVSQDD	PEVQFTNYIN	NEQVRTARPP
mouse IgG1	291	PREEQFNSTF	RSVSELPIMH	QDWLNGKEFK	CRVNSAAFPA	PIEKTISKTK	GRPKAPQVYT
mouse IgG2a		THREDYNSTL	RVVSALPIQH	QDWMSGKEFK	CKVNNKDLPA	PIERTISKPK	GSVRAPQVVY
mouse IgG2b		THREDYNSTI	RVVSTLPIQH	QDWMSGKEFK	CKVNNKDLPS	PIERTISKIK	GLVRAPQVYI
human IgG1		PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKSLPA	PIEKTISKAK	GQPREPQVYT
rabbit IgG		LREQQFNSTI	RVVSTLPIIH	QDWLRGKEFK	CKVHNKALPA	PIEKTISKAR	GAPLEPKVYT
mouse IgG1	351	IPPPKEQMAK	DKVSLTCMIT	DEFPEITVE	WQWNGQPAEN	YKNTQPIMDT	DGSYFVYSKL
mouse IgG2a		LPPPEEEMTK	KQVTLTCMVT	DFMPEDIYVE	WTNNGKTELN	YKNTPEVLDS	DGSYFMYSKL
mouse IgG2b		LPPPAEQLSR	KDVSLTCLV	GFNPGDISVE	WTSNGHTEEN	YKDTAPVLDS	DGSYFIYSKL
human IgG1		LPPSREEMTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTPEVLDS	DGSFFLYSKL
rabbit IgG		MGPPREELSS	RSVSLTCMIN	GFYPSDISVE	WEKNGKAEDN	YKTTPVALDS	DGSYFLYNKL
mouse IgG1	411	NVOKSNWEAG	NTFTCSVLHE	GLHNHHTEKS	LSHSPGK		
mouse IgG2a		RVEKKNWVER	NSYSCSVVHE	GLHNHHTTKS	FSRTPGK		
mouse IgG2b		NMKTSKWEKT	DSFSCNVRHE	GLKNYYLKKT	ISRS PGK		
human IgG1		TVDKSRWQQG	NVFSCSVMHE	ALHNHYTQKS	LSLSPGK		
rabbit IgG		SVPTSEWQRG	DVFTCSVMHE	ALHNHYTQKS	ISRS PGK		

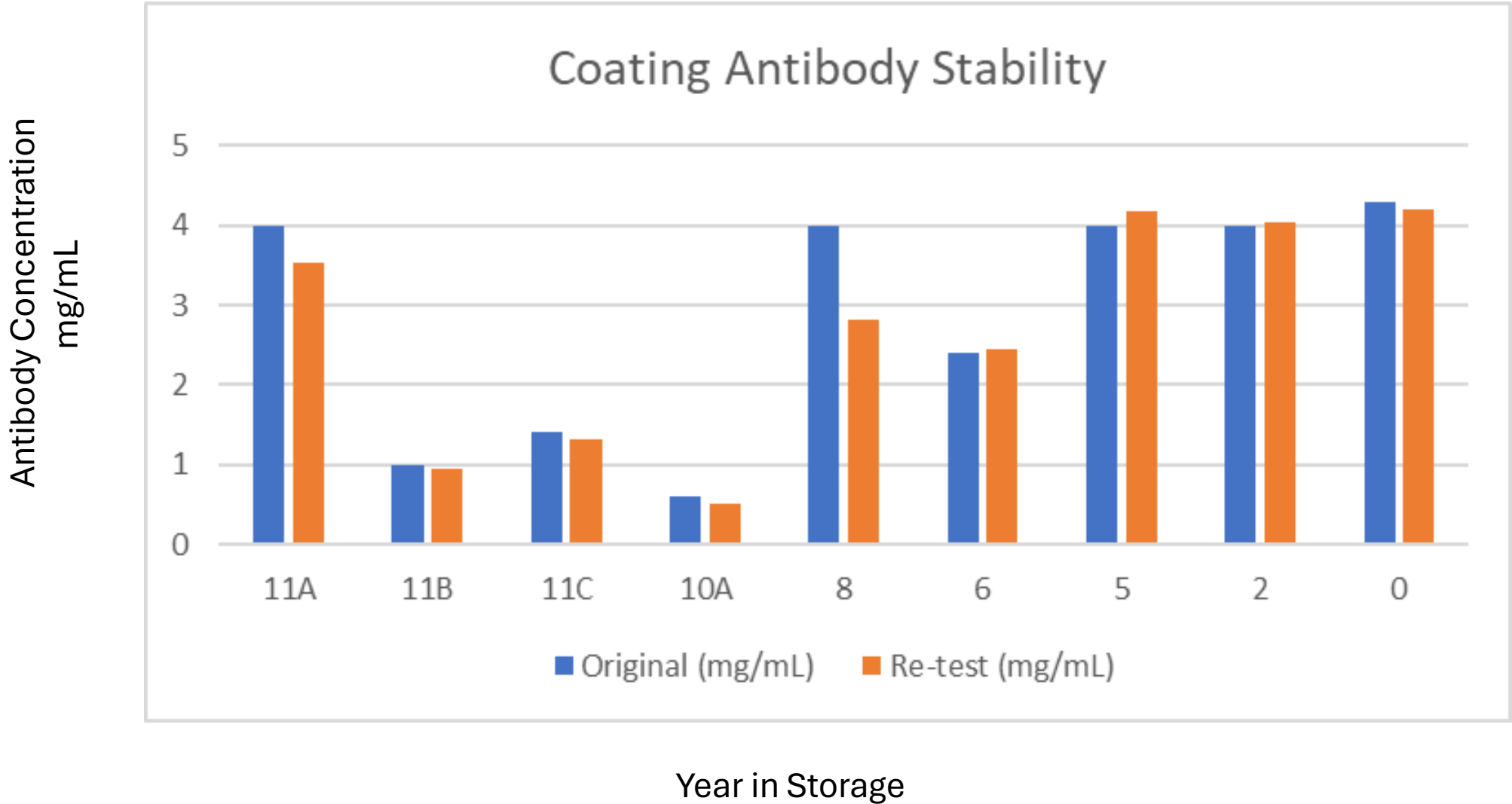
B

Wild Type:	5'-GGATGTGCTCACCATTCTCTGACTCC-3'	T254S:	5'-GGATGTGCTCACCATTCTCTGACTCC-3'
	3'-CCTACACGAGTGGTAAATGAGACTGAGG-5'		3'-CCTACACGAGTGGTAAATGAGACTGAGG-5'
	<div style="display: flex; justify-content: space-around; width: 100%;"> Thr Thr </div>		<div style="display: flex; justify-content: space-around; width: 100%;"> Ser </div>
T252M:	5'-GGATGTGCTCATGATTACTCTGACTCC-3'	T252M-T254S:	5'-GGATGTGCTCATGATTCTCTGACTCC-3'
	3'-CCTACACGAGTACTTAATGAGACTGAGG-5'		3'-CCTACACGAGTACTTAATGAGACTGAGG-5'
	<div style="display: flex; justify-content: space-around; width: 100%;"> Met </div>		<div style="display: flex; justify-content: space-around; width: 100%;"> Met Ser </div>

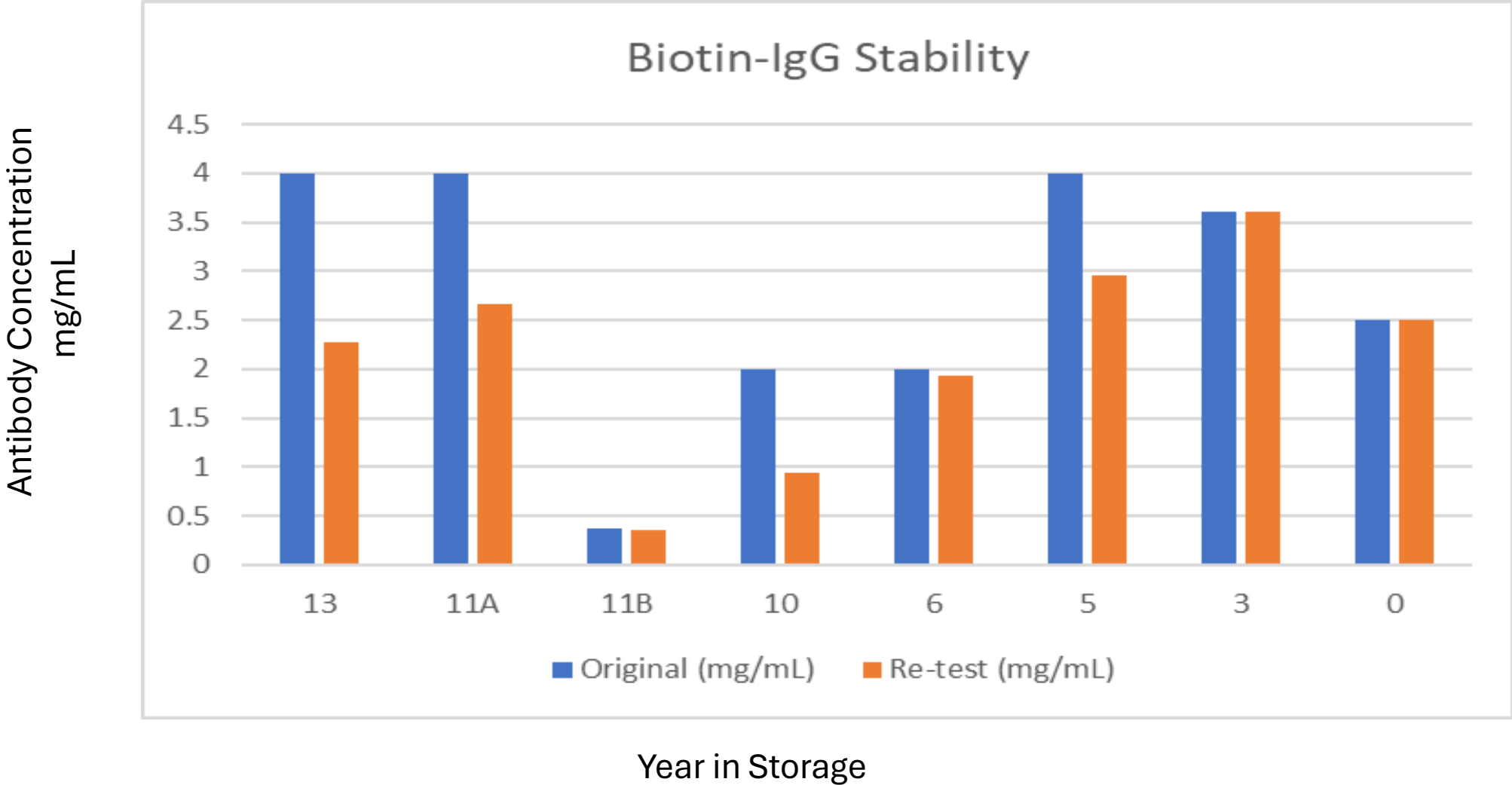
Alignment of the amino acid sequences of mouse IgG1, 2a, 2b, human IgG1 and rabbit IgG heavy chain constant region. The residues that are important for interaction with protein A are indicated by shading and the neighboring domains of the interaction core are surrounded by dotted squares. (B) DNA sequence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

2. HCP Antibody and Antiserum Stability Analysis

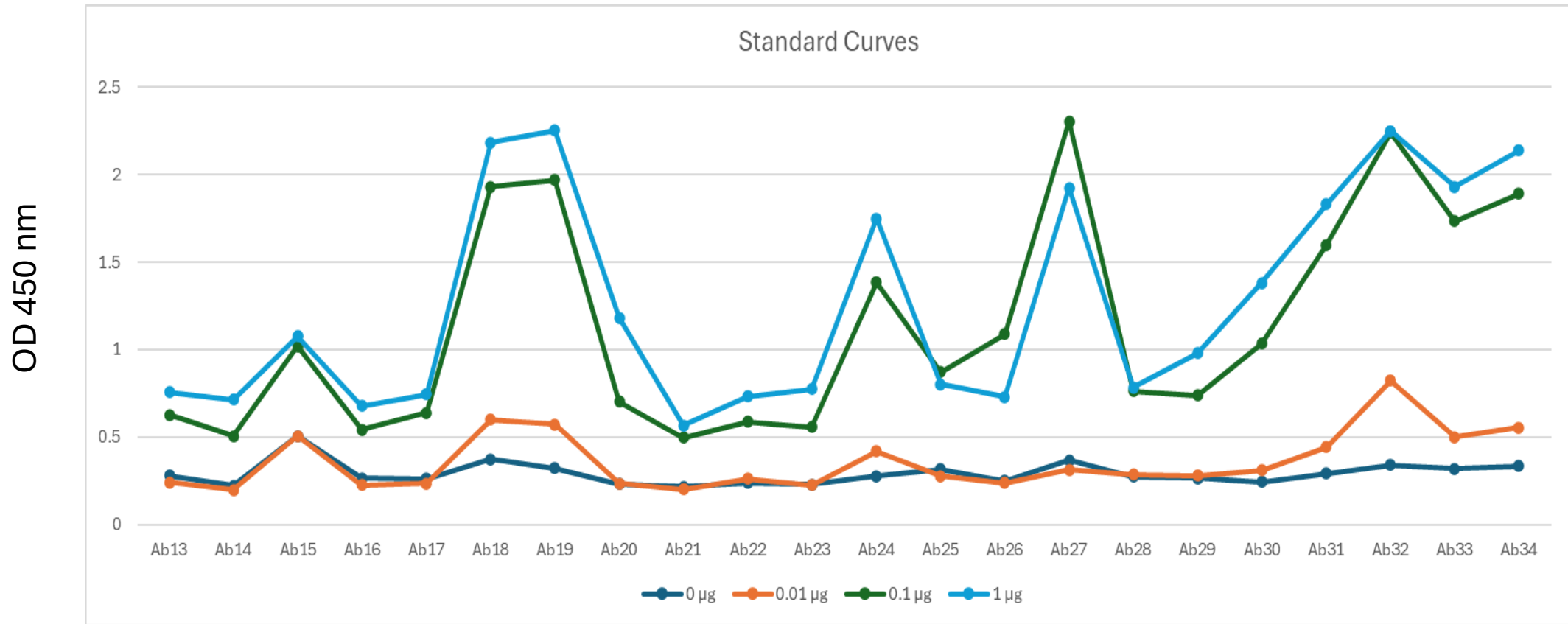
Coating Antibody Total Protein Concentration



Reporting Antibody Total Protein Concentration



PCA ELISA Standard Curve (constant regions)

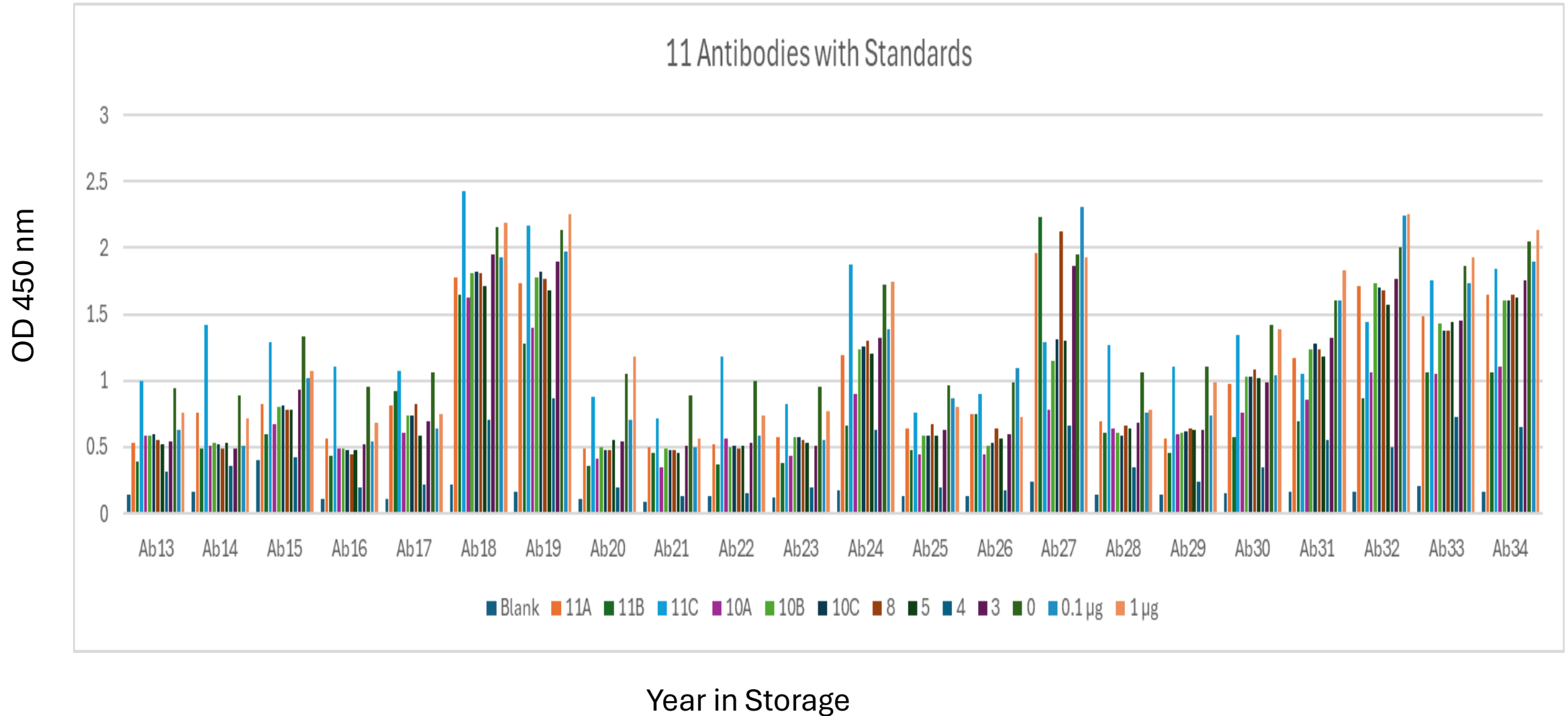


22 Constant region antibodies

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.

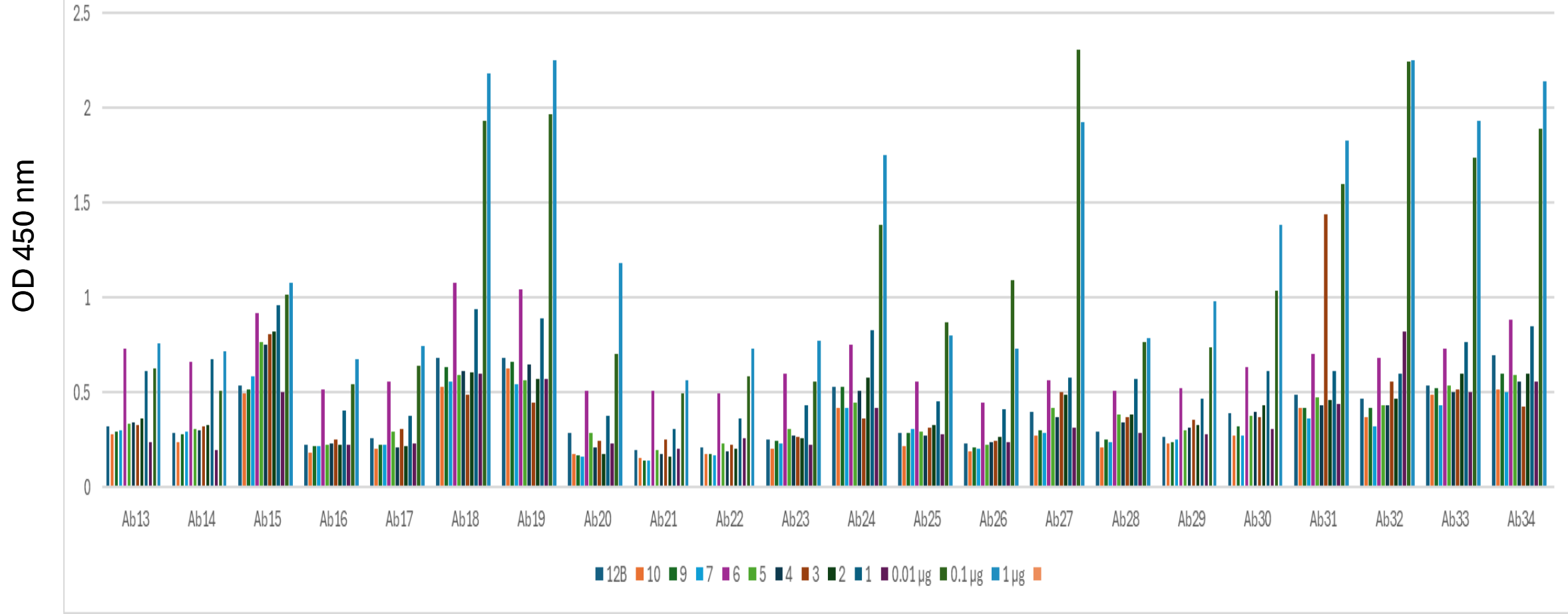
11 Coating Antibody HOS Stability Analysis

11 Antibodies with Standards



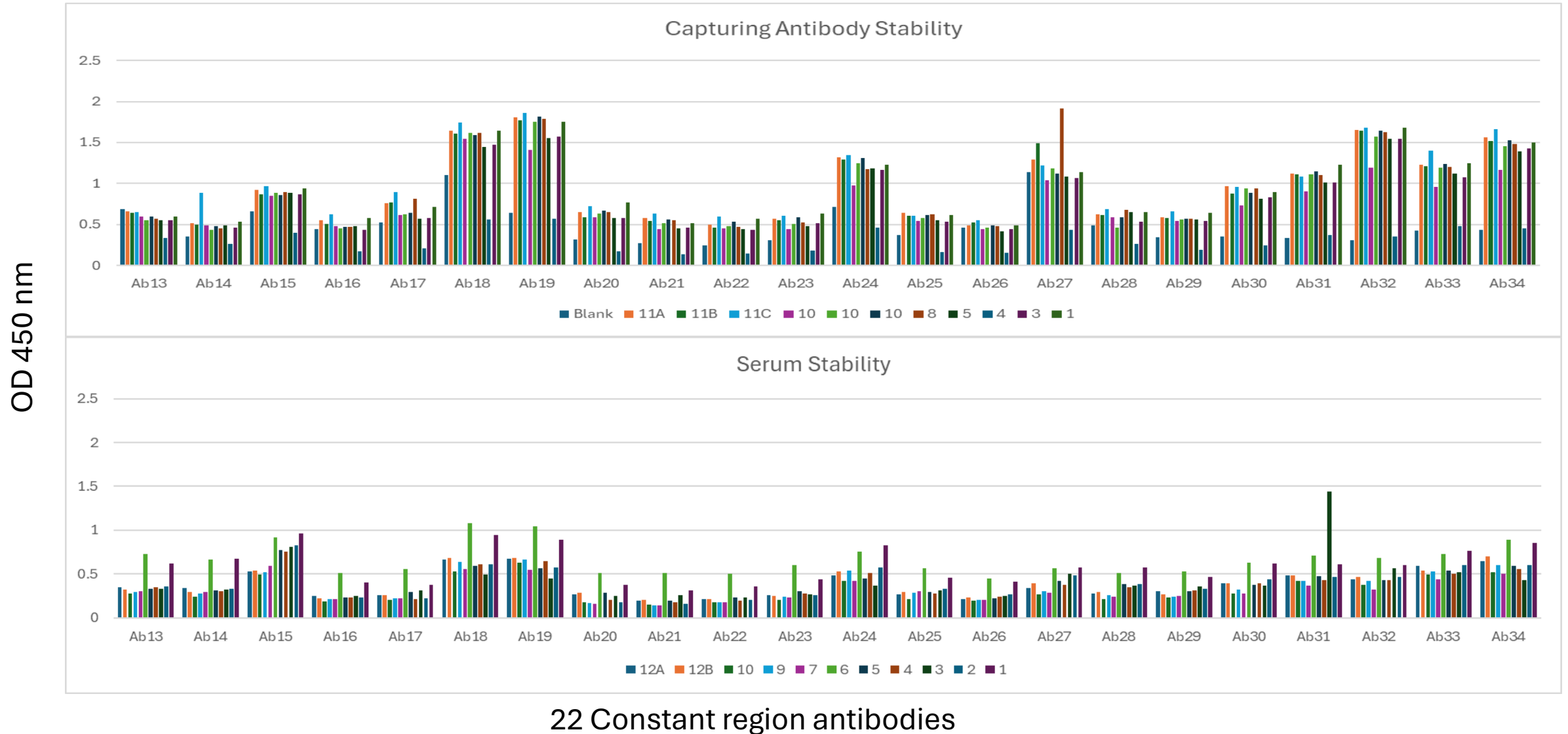
11 Anti-CHO HCP Serum HOS Stability Analysis by PCA

12 Year Serum Stability with Standards



Year in Storage

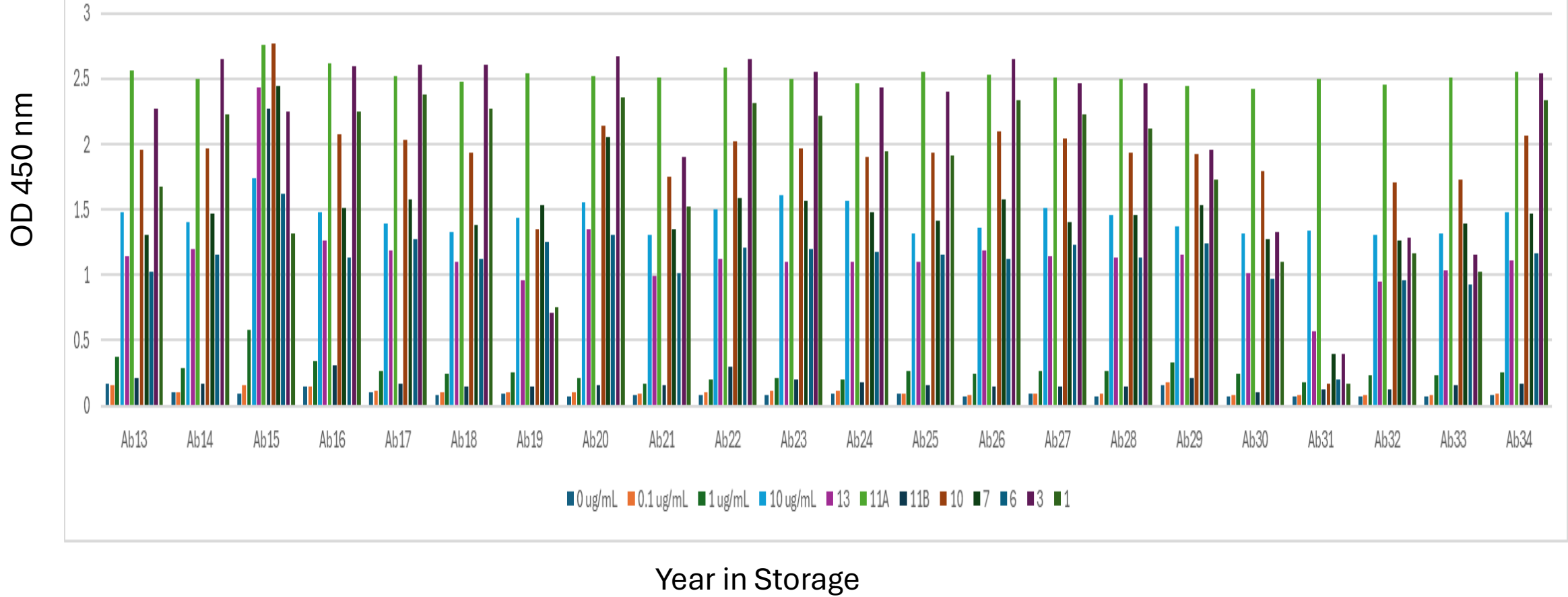
PCA ELISA Comparison of IgG Stability in Serum and Purified IgG in PBS



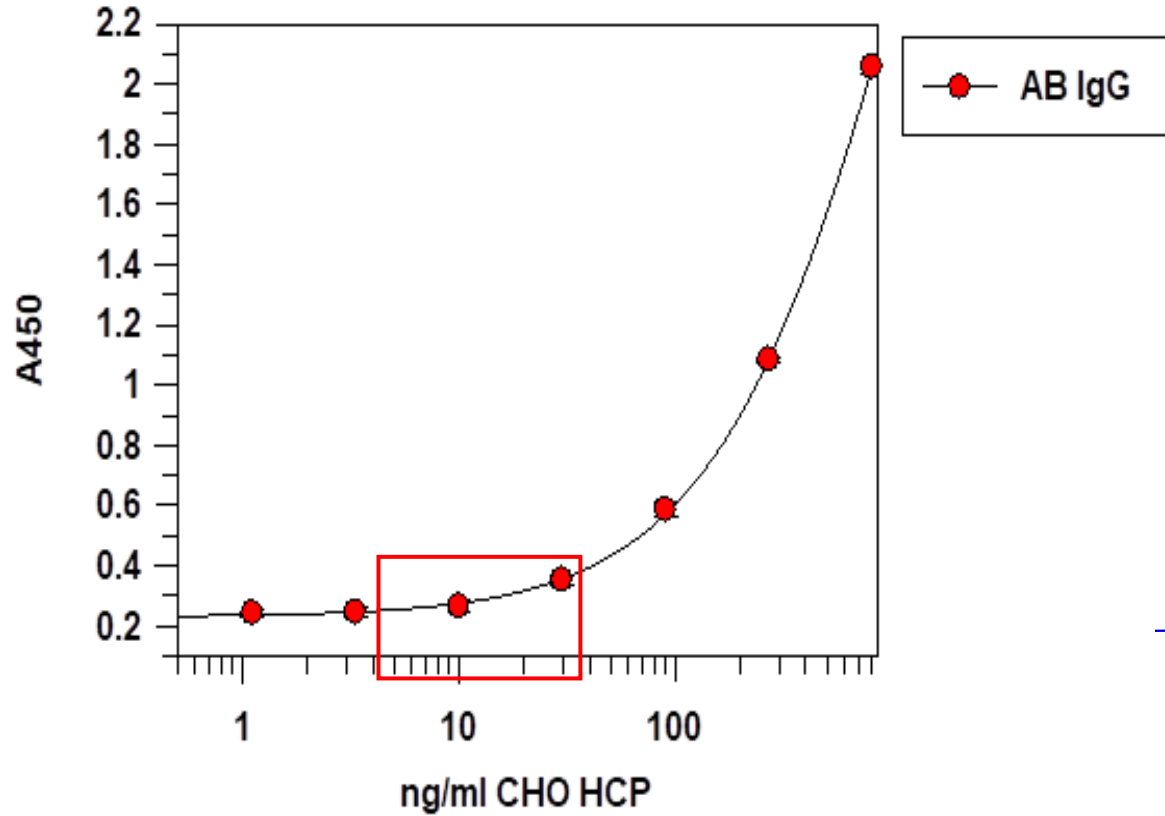
Coating antibody coated at 0.1 $\mu\text{g}/\text{well}$, serum coated after 1:100 dilution, assuming maximum binding of 1 $\mu\text{g}/\text{well}$ Total protein, the IgG is close to 0.1 $\mu\text{g}/\text{well}$.

PCA ELISA Analysis of Biotin-labeled IgG Stability in PBS

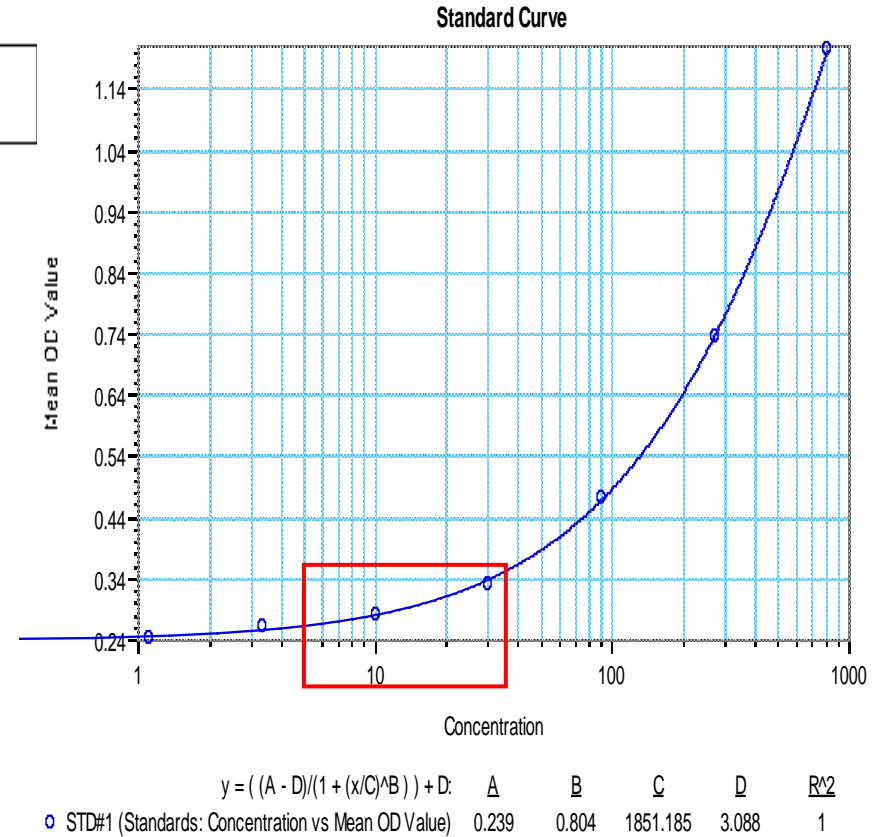
Reporting Antibody (Biotin-labeled) Stability



ELISA Comparison- IgG in 10 Years Storage



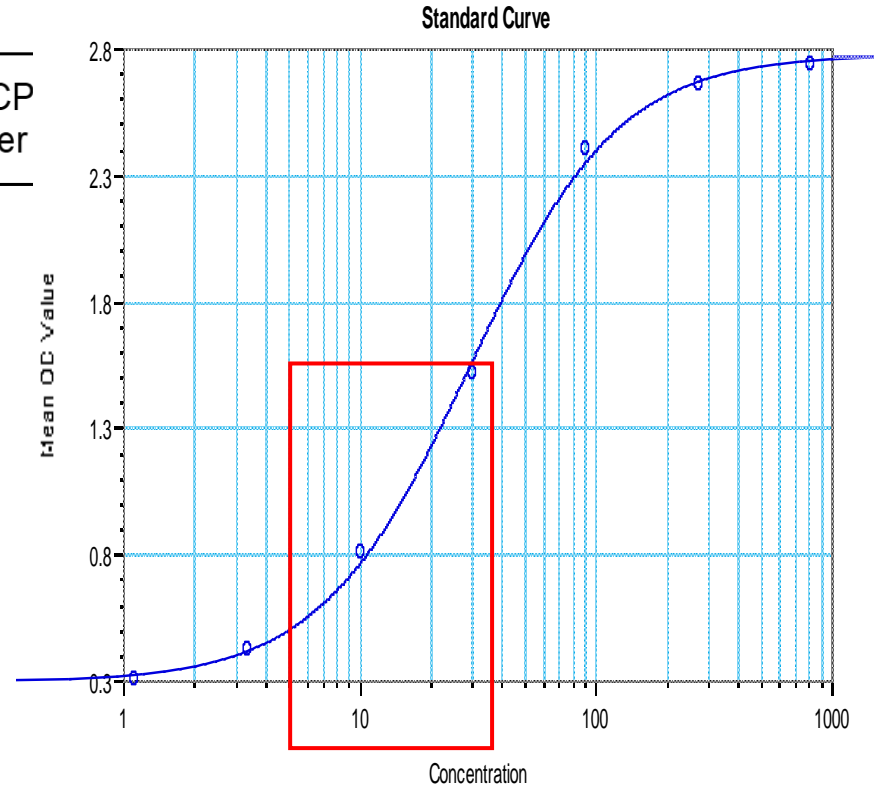
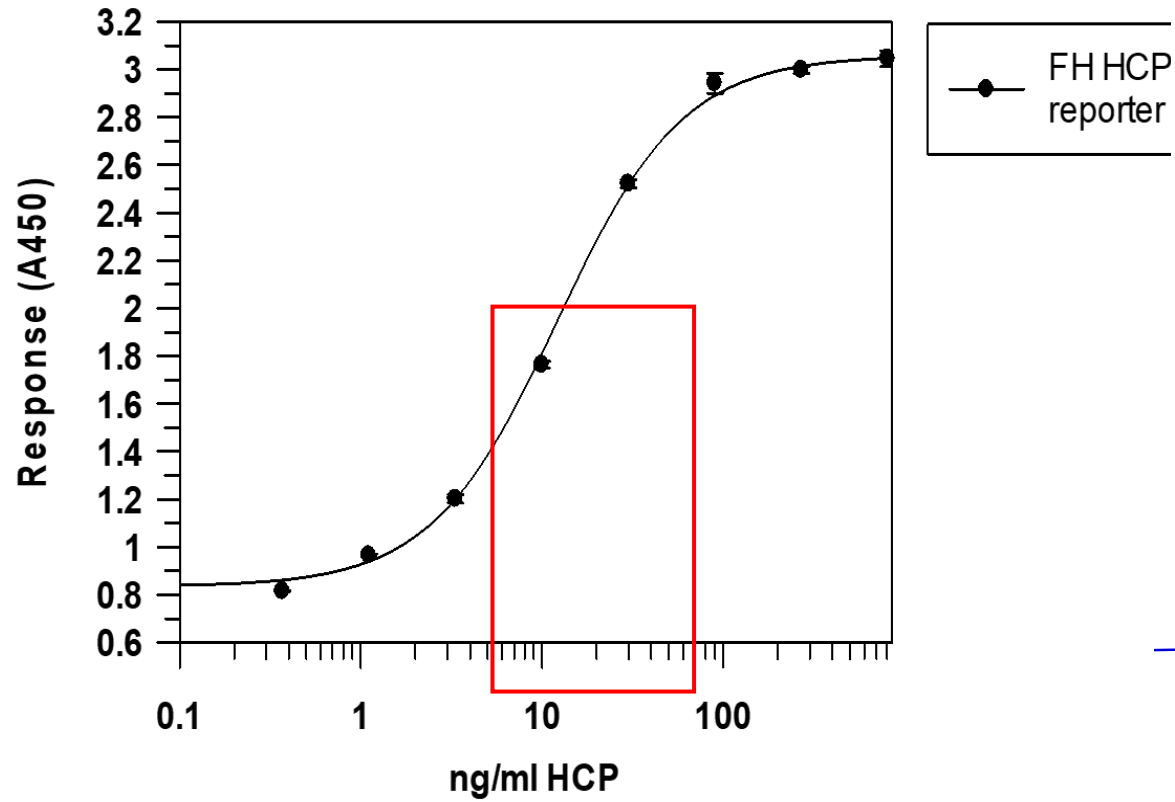
Then



Now

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

ELISA Comparison-IgG in 8 Years Storage



$$y = \frac{(A - D)}{1 + (x/C)^B} + D$$

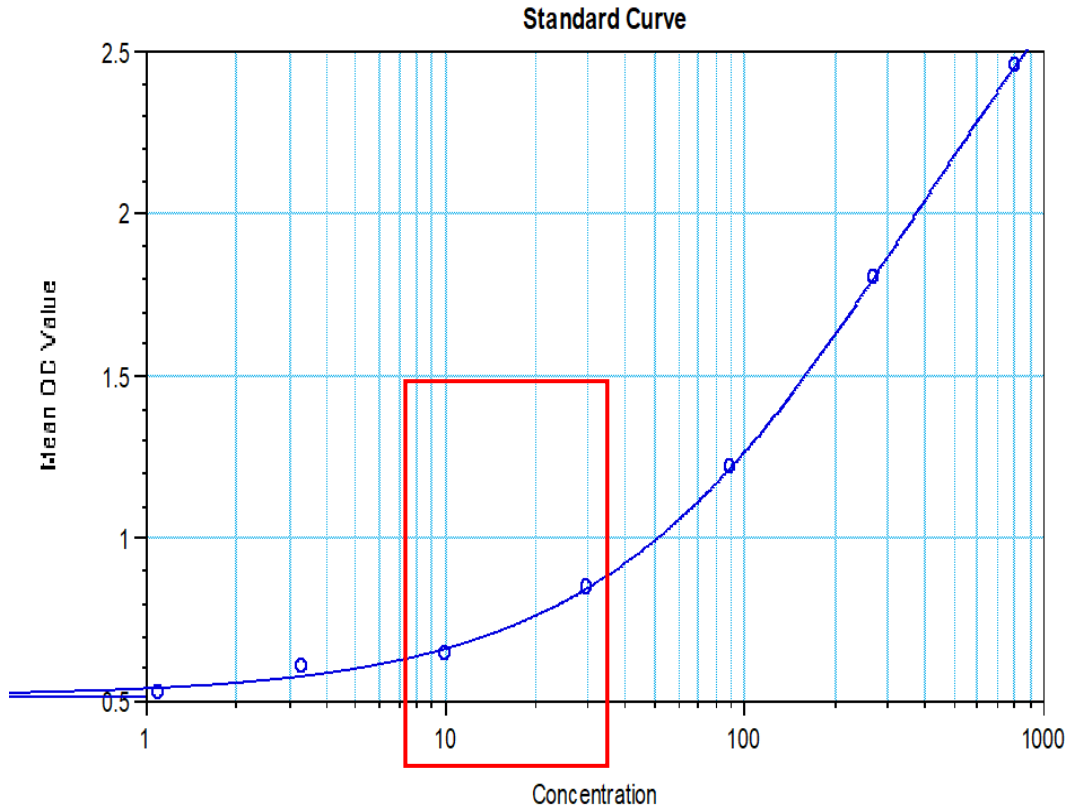
	A	B	C	D	R ²
○ STD#1 (Standards: Concentration vs Mean OD Value)	0.298	1.376	28.782	2.78	0.999

Then

Now

Assay sensitivity does not change significantly

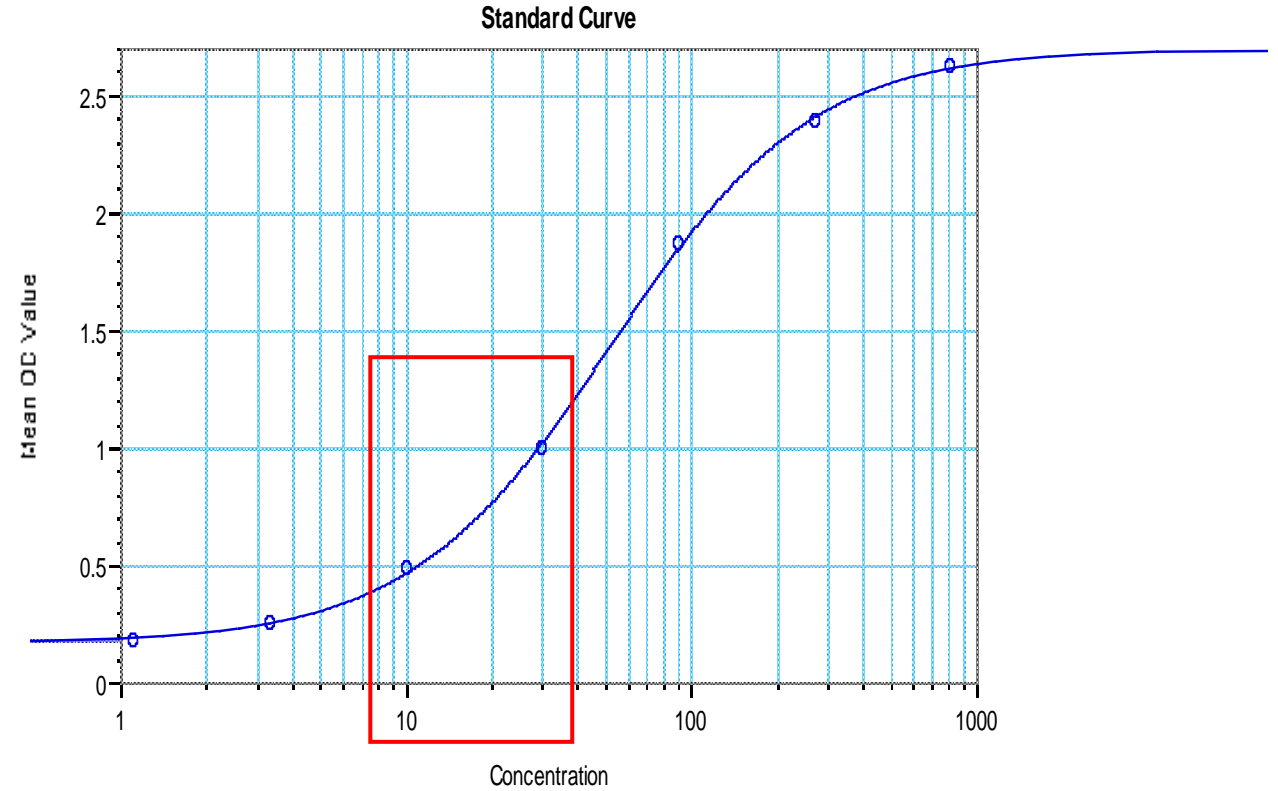
ELISA Comparison-IgG in 5 Years Storage



$y = ((A - D)/(1 + (x/C)^B)) + D$

	A	B	C	D	R ²
STD#1 (Standards: Concentration vs Mean OD Value)	0.513	0.82	378.247	3.493	1

Then



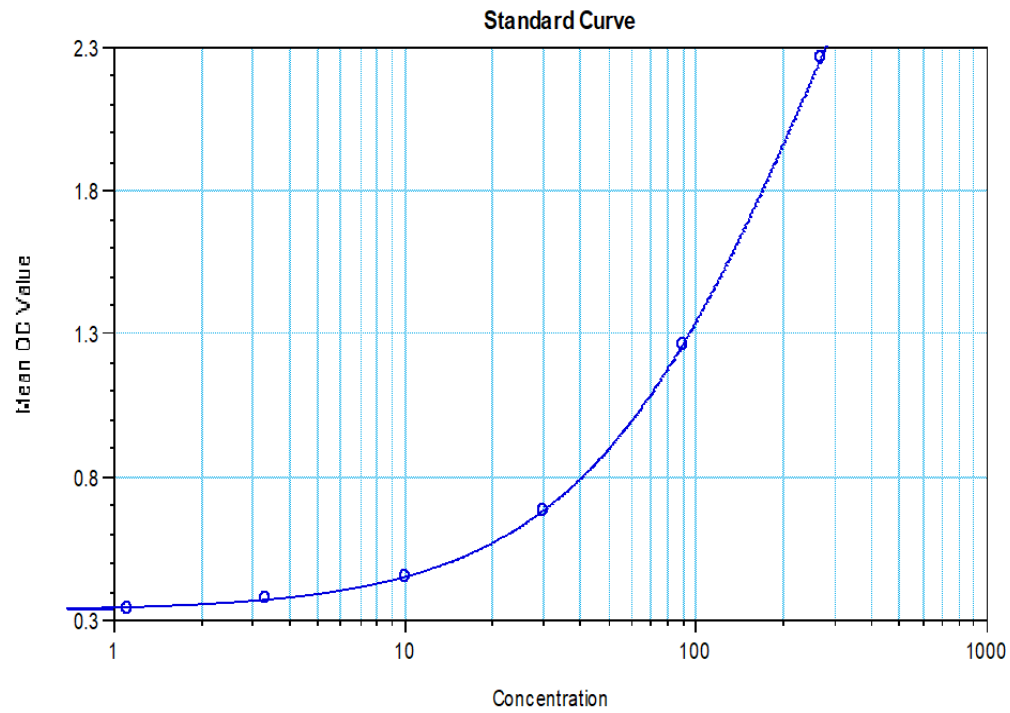
$y = ((A - D)/(1 + (x/C)^B)) + D$

	A	B	C	D	R ²
STD#1 (Standards: Concentration vs Mean OD Value)	0.173	1.232	51.618	2.7	1

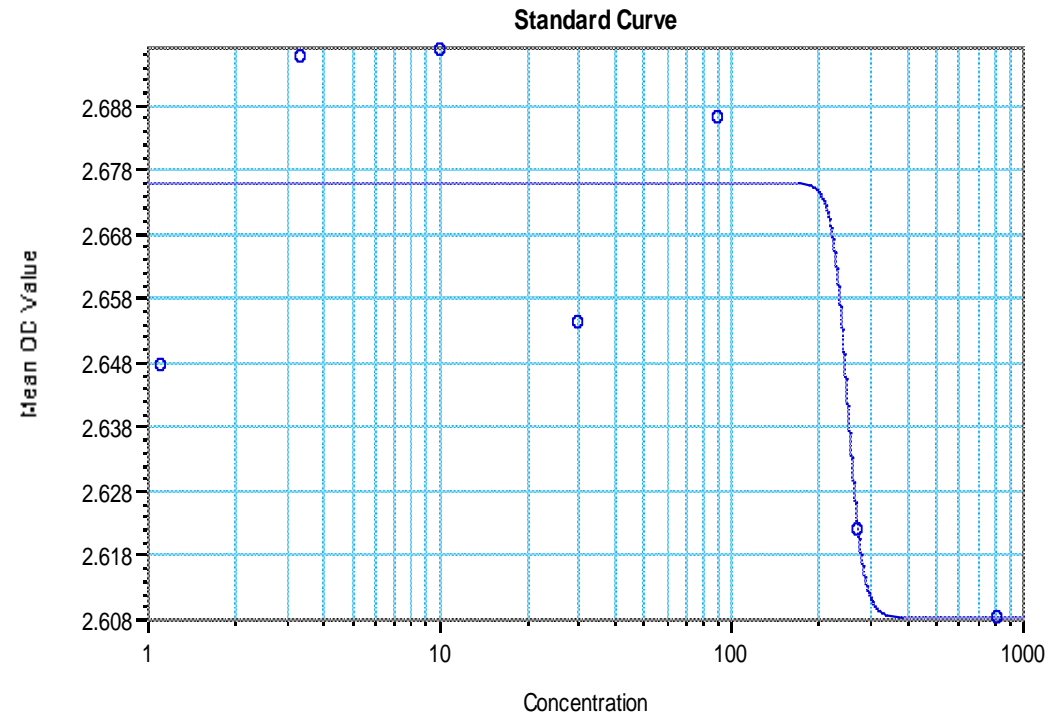
Now

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

ELISA Comparison-IgG in 4 Years Storage



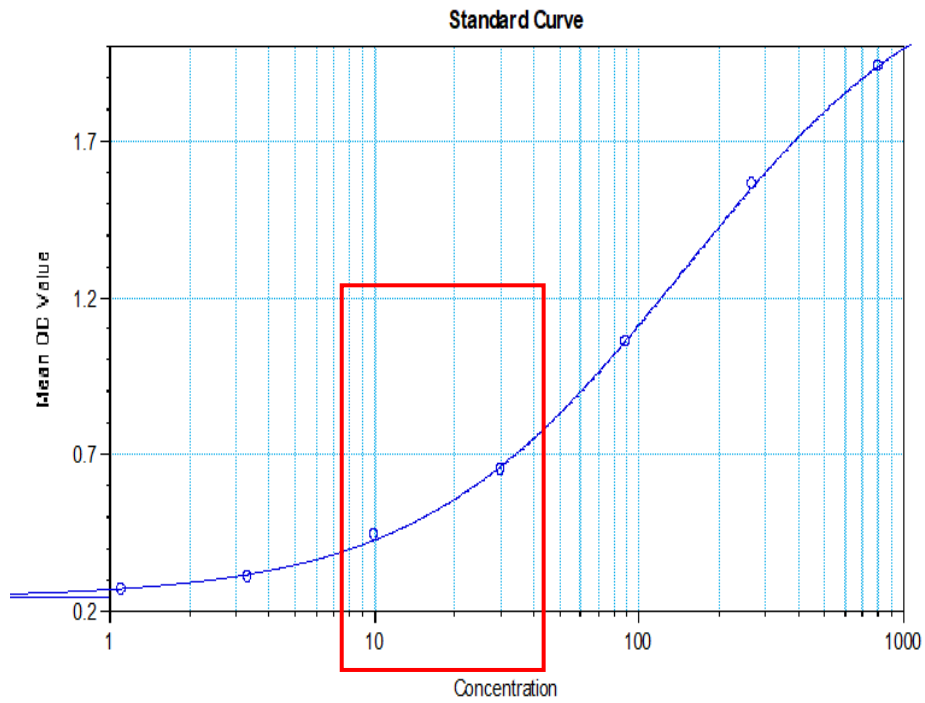
Then



Now

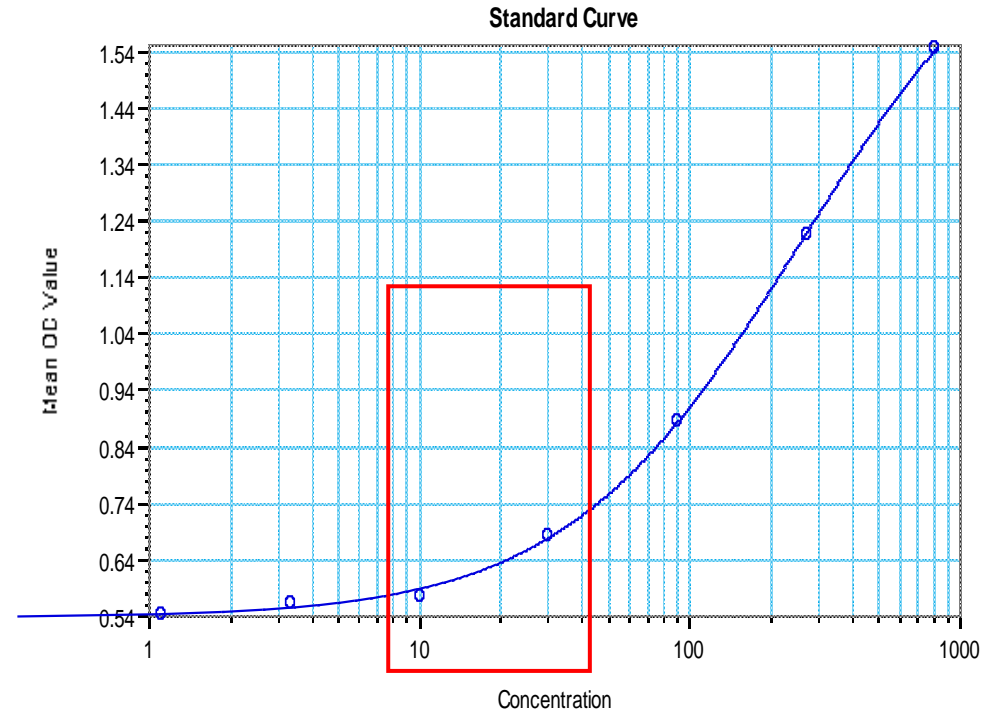
Major nonspecific binding was produced during storage, biotin-IgG needs to be regenerated

ELISA Comparison-IgG in 3 Years Storage



$y = (A - D) / (1 + (x/C)^B) + D$
 ○ STD#1 (Standards: Concentration vs Mean OD Value)

A	B	C	D	R ²
0.241	0.872	145.575	2.314	1



$y = (A - D) / (1 + (x/C)^B) + D$
 ○ STD#1 (Standards: Concentration vs Mean OD Value)

A	B	C	D	R ²
0.538	0.999	253.605	1.858	1

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.