Comparative Study of Cell-Based and Competitive Ligand-Binding Assays for the Detection of Neutralizing Antibodies Against a Therapeutic Peptide Hormone

P. Brennecke, L. Champion, P. Doytcheva, P. Struwe
Celerion Switzerland AG, 8320 Fehraltorf, Switzerland

celerion
Translating Science to Medicine

Introduction

Neutralizing antibodies (nAbs) may affect drug efficacy/safety and/or cross-react with non-redundant endogenous proteins, potentially resulting in loss of function or serious off target effects. However, designing the proper assay for nAb testing remains challenging and requires a solid understanding of the underlying drug mechanism of action (MoA) (U.S. FDA, 2019).

In the present study we compared three different CBA and three different CLB setups for the detection of nAbs against a therapeutic peptide hormone and the endogenous counterpart. While CBA approaches measured intracellular cAMP level as a functional readout for receptor activation, the CLB methods monitored the binding of the hormone to the receptor extracellular domain as a proxy for ligand-mediated receptor stimulation.

Analytical Methods

Drug MoA and assay principle

The drug under investigation, a truncated version of an endogenous peptide hormone, binds to a G-protein coupled receptor (GPCR) on target cells, activating adenylyl cyclase and leading to increased cAMP production and downstream gene activation (Fig. 1).

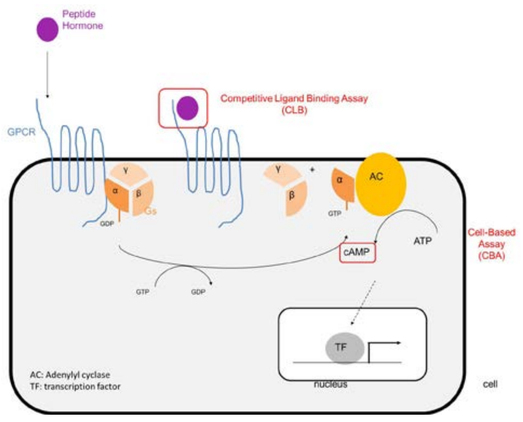


Figure 1. Drug MoA. GPCR binding and activation leads to increased cAMP.

CLB setup

Three different enzyme-linked immunosorbent assay (ELISA) formats tested the ability of nAbs to disrupt the binding of the therapeutic hormone to the extracellular domain (ECD) of the GPCR. Either the drug or the GPCR ECD was immobilized, while the other component was detected by an HRP conjugate. Samples containing nAbs were pre-incubated with the therapeutic hormone prior to incubation with the GPCR ECD, leading to a signal decrease proportional to the nAb content (Fig. 2).

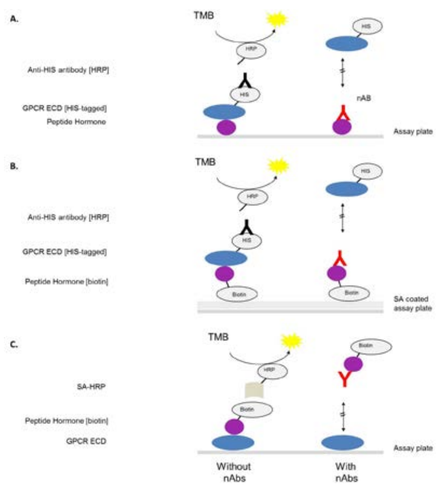
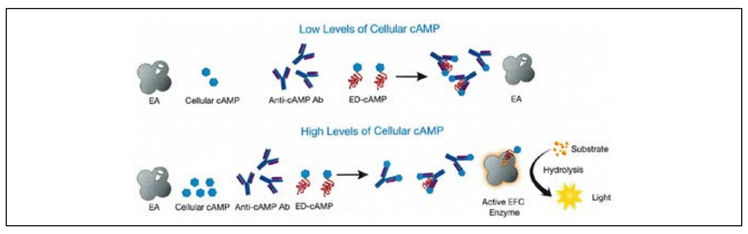


Figure 2. CLB setup (A) Peptide hormone immobilized on assay plates and HIS-tagged GPCR ECD detected by an anti-HIS HRP conjugate. **(B)** Biotin-labeled peptide hormone immobilized on streptavidin (SA) assay plates and HIS-tagged GPCR ECD detected by an anti-HIS HRP conjugate. **(C)** GPCR ECD immobilized on assay plates and biotin-labeled peptide hormone detected by an SA HRP conjugate.

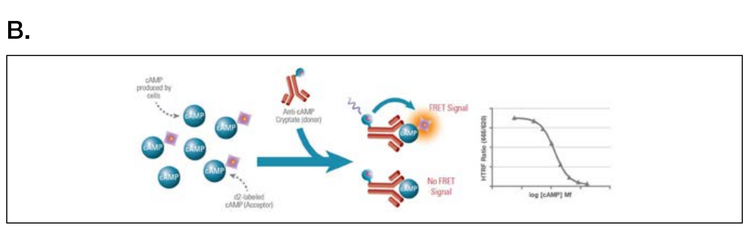
CBA setup

Three different cell-based commercial kits were used to detect the increased cAMP production downstream of GPCR activation in response to the therapeutic hormone: the DiscoverX cAMP Hunter™ Gs Cell Line combined with HitHunter cAMP Assay kit, based on competitive Enzyme Fragment Complementation (EFC) technology (Fig. 3A); a rat sarcoma cell line combined with Cisbio Bioassays` cAMP HiRange Assay Kit, based on competitive Homogeneous Time Resolved Fluorescence (HTRF) (Fig. 3B); and a rat sarcoma cell line combined with GE Healthcare`s Amersham cAMP Biotrak Enzyme Immunoassay (EIA) System, based on competitive ELISA (Fig. 3C).

A.



B.



C.

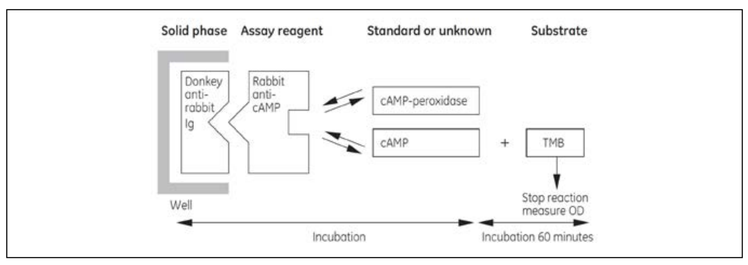


Figure 3. CBA setup (A) DiscoverX competitive EFC – signal is proportional to cellular cAMP, nAbs will decrease the signal **(B)** Cisbio Bioassays competitive HTRF – signal is inversely proportional to cellular cAMP, nAbs will increase the signal **(C)** GE Healthcare competitive ELISA – signal is inversely proportional to cellular cAMP, nAbs will increase the signal.

Assay Comparison

CLB setup

Formats A and B, where the peptide hormone was coated and the GPCR ECD was detected (Fig. 2A-B), had no or very low assay signal in the absence of nAbs (data not shown). Format C, where the GPCR ECD was coated and the peptide hormone was detected (Fig. 2C), had a good assay response proportional to both GPCR ECD and therapeutic hormone concentrations, but no signal decrease in response to two different nAb positive controls was observed (Fig. 4). Thus, neither of the CLB formats tested was sensitive to the presence of up to 2 µg/mL of nAbs.

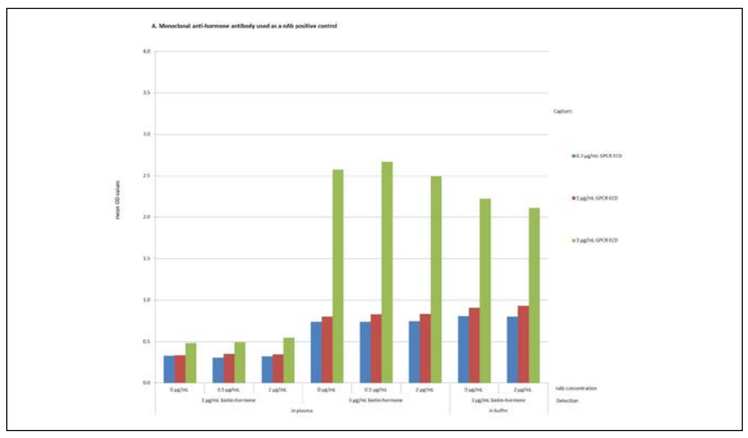


Figure 4. CLB signal in response to (A) a monoclonal anti-hormone antibody used as a nAb positive control, and to **(B)** a polyclonal anti-hormone antibody used as a nAb positive control.

CBA setup

All three commercial kits successfully detected increased cAMP production in response to increasing therapeutic hormone concentrations, although with a different EC50 (Table 1). Respectively, the normalized responses and the estimated sensitivities to two different nAb positive controls varied between the assays (Fig. 5 and Table 1).

Table 1. Sensitivity comparison between CBA kits.

CBA kit	DiscoverX	Cisbio Bioassays	GE Healthcare
EC50 of therapeutic hormone	0.025 nM	0.01 nM	0.8 nM
Sensitivity to monoclonal nAb positive control at ~EC50	~20 ng/mL	~500 ng/mL	~100 ng/mL
Sensitivity to polyclonal nAb positive control at ~EC50	~250 ng/mL	~750 ng/mL	~1000 ng/mL

All three kits had a better estimated sensitivity to the monoclonal anti-hormone antibody used as a positive nAb control (Fig. 5 and Table 1). However, the Cisbio Bioassays kit showed a very small dynamic response to the monoclonal nAb control, and the GE Healthcare kit had an undesirable Hook effect (Fig. 5A). Overall, the DiscoverX system seemed to perform best, with the lowest hormone EC50 and the best estimated nAb sensitivity (Fig. 5 and Table 1). Based on this comparison, the DiscoverX CBA setup was chosen for further development and validation of a nAb assay against the truncated therapeutic hormone, as well as against the full-length endogenous hormone.

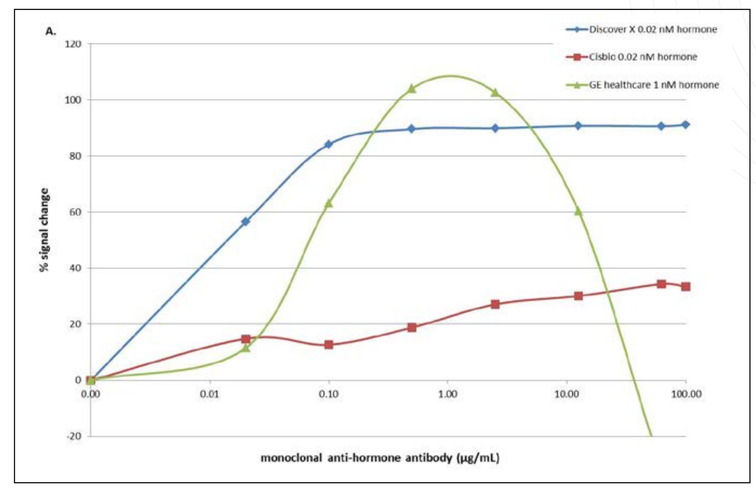


Figure 5. CBA signal in response to (A) a monoclonal anti-hormone antibody used as a nAb positive control, and to **(B)** a polyclonal anti-hormone antibody used as a nAb positive control.

Results

DiscoverX nAb assays against the truncated therapeutic hormone and the full-length endogenous hormone were fully validated with good and comparable sensitivity, assay precision, selectivity, and free drug tolerance using the polyclonal nAb positive control (Table 2).

Table 2. Validation results of CBA nAb assays.

nAb target	Therapeutic hormone			Endogenous hormone		
Sensitivity: Screening (50% Consistency)	0.19 µg/mL			0.20 µg/mL		
Screening (99% Consistency)	0.31 µg/mL			0.32 µg/mL		
Assay Precision (%CV):	**NC**	**LPC** (0.31 µg/mL)	**LPC** (1.00 µg/mL)	**NC**	**LPC** (0.33 µg/mL)	**LPC** (1.00 µg/mL)
Intra-assay	5.2	10.2	10.4	7.6	8.0	11.7
Inter-assay (12 runs)	4.3	17.5	12.0	4.6	15	16.3
Selectivity: LPC	Acceptable: 10/10 individuals met criteria			Acceptable: 10/10 individuals met criteria		
HPC	Acceptable: 10/10 individuals met criteria			Acceptable: 10/10 individuals met criteria		
Free Drug Tolerance LPC	0.50 ng/mL of free drug			2.00 ng/mL of endogenous		
HPC	4.00 ng/mL of free drug			8.00 ng/mL of endogenous		
Cross-reactivity LPC	2.00 ng/mL of endogenous tolerated			N/AP		

Discussion and Conclusions

Considering the drug MoA and the assay limitations encountered with CLB, the best performing CBA was selected for further development and validation. In conclusion, our study highlights the importance of extensive assay format comparison in order to select the method of choice when investigating nAbs. The here provided highly sensitive nAb assay lays the ground for therapeutic decisions/dosing of the peptide drug in order to have an optimal patient tailored therapy.

Reference

U.S. FDA (2019). Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection.