# Signal Amplification to Enable Ultrasensitive Detection and Quantification of Protein Biomarkers in Ligand-Binding Immunoassays Using *Amplatto*™ Technology

## Summary

A biomarker is a quantifiable characteristic that can indicate an individual's specific biological state. Biomarkers are currently revolutionizing the way diseases are being diagnosed and treated as they can reveal underlying biological processes, inform therapeutic deployment, and pave the way for true personalized precision medicine.[1] The investigation of signaling molecules at the protein level still faces many challenges. According to UniProt[2], there is no experimental evidence at the protein level for about 38% out of the 20,000 protein-coding human genes and only limited data is available for the endogenous protein levels for most biomarkers. The total number of proteins in human cells is estimated to be between 250,000 to one million where their concentrations vary from mM to sub aM levels.[3,4] Therefore, ultrasensitive proteomic technologies are crucial to define endogenous baseline biomarker concentrations and identify new potential biomarkers.

## Background

### Challenge 1: How to overcome limited dynamic range and low sensitivity issues in ligand binding assays?

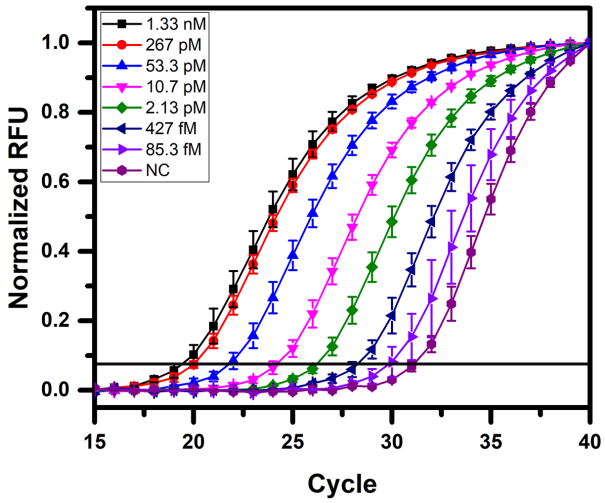


**Fig. 1** PCR curves as function of human IgG concentration obtained from immuno-PCR assay using Amplatto™ technology.

The Amplatto™ technology is based on an immuno-PCR approach and was developed to significantly improve the sensitivity of ligand-binding assays such as ELISA. It combines the high sensitivity of the polymerase chain reaction (PCR) technique with the versatility of ligand-binding assays and has shown to significantly improve the quantification range and the limit of detection compared with that of ELISAs. The signal-generating antibody-enzyme conjugate used in standard ELISAs is replaced with Amplatto™ technology which consist of an oligonucleotide carrier, where the oligonucleotide functions as a marker to be amplified and quantified by RT-qPCR. A relationship between the analyte concentration and recovered oligonucleotide marker is then established as indicated in **Fig. 1**.

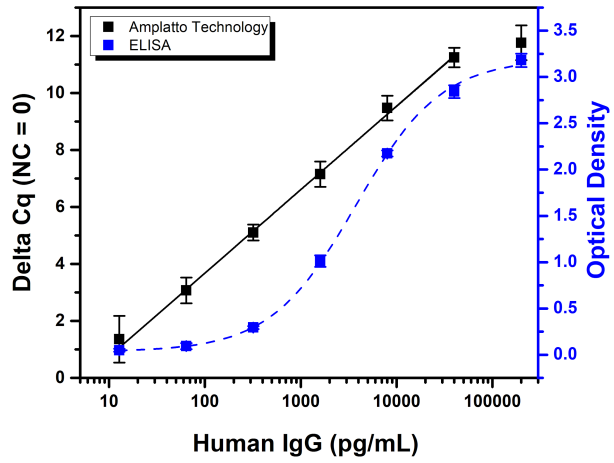


**Fig. 2** A wider dynamic range and pg/mL levels of human IgG in assay buffer compared to standard ELISA.

## Results 1

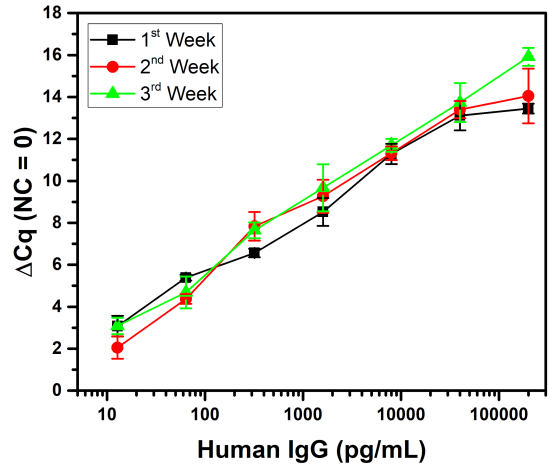


**Fig. 3** Small variability between assays as indicated by inter-assay CV.

Amplatto™ technology quantified human IgG down to fM concentration range and showed a significant broader dynamic range compared with standard ELISA as showing in **Fig.2**. Amplatto™ technology enables the accurate quantification of protein biomarkers even at the very low concentration range of

the calibration curve as the concentration-response relationship is easily described using linear regression models, which is problematic in standard ELISAs as the calibration curves are inherently nonlinear. In addition, Amplatto™ technology demonstrated a superior signal-to-noise ratio that was 4-fold higher compared to that of standard ELISA. This ultrasensitive technology enables the detection of endogenous biomarker concentrations at the protein level, their surveillance through several phases of clinical studies, and most importantly, supports the discovery of potential biomarkers that have not been yet studied due to their relatively low endogenous concentrations and limited sensitivity of the current immunoassays. Further, stability studies showed significant low inter-assay variability between immuno-PCR assays performed on different days (**Fig. 3**), demonstrating the reliability of the Amplatto™ technology.

## Challenge 2: How to study pharmacodynamic profiles for biomarkers?

The complement component 5a (C5a), a protein fragment originated from cleavage of complement C5, is a powerful inflammatory mediator and chemoattractant. The uncontrolled production of C5a appears to be a key factor in the development of pathology of many inflammatory diseases involving the complement system such as sepsis, rheumatoid arthritis and inflammatory bowel disease.[5] Clinical studies have been focused on the development of therapeutics to block these pro-inflammatory effects and to study the association between C5a levels and inflammatory diseases. Therefore, there is a considerable interest in the development of ultrasensitive detection tools to monitor C5a levels.

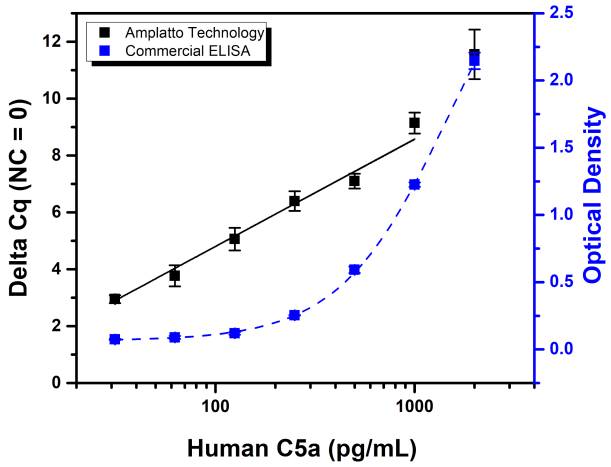


**Fig. 4** A wider dynamic range, pg/mL levels of human C5a, and enhanced signal-to-noise ratio obtained using Amplatto™ technology compared to commercially available ELISA.

## Results 2

Using AmplattoTM technology, human complement C5a could be detected in fM concentration levels with a superior dynamic range for C5a quantification compared with a commercially available ELISA kit. In this case study, Amplatto™ technology further demonstrated a superior signal-to-noise ratio that was 6-fold higher compared to ELISA.

**Conclusion:** Amplatto™ technology enables ultrasensitive biomarker detection and quantification down to fM concentration range with a superior dynamic range and signal-to-noise ratio.

## References


1. **Liu et al** *American Heart Association* **2016**, 134, 286-289.
2. www.uniprot.org
3. **Anderson and Anderson** *Molecular & Cellular Proteomics* **2002**, 1, 845-867
4. **Ellis et al** *Nucleic Acids Research* **2004**, 32, 1414-1421
5. **Monk et al** *The international Journal of Biochemistry & Cell Biology* **2009**, 41, 2114-2117