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Development and Optimization of an Ultrasensitive Immunoassay Method Using Amplatto™ Technology for the Measurement of Protein X to Support Pharmacokinetic Studies

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Purpose

Immunoassay is the fundamental analytical technology relied upon for the large molecule bioanalysis. There have been several recent advances in the field of low-level analyte detection. Most advances have limited effect in terms of sensitivity (<10 fold increase) and very minimal impact on increasing dynamic range.

Immuno-PCR method combines the specificity of antibody– protein binding with powerful polymerase-mediated nucleic acid amplification methods. Even though the first immuno- PCR method was reported back in 1992, it failed to become mainstream due to several challenges such as: the lack of an efficient method for the conjugation of antibody to DNA, low DNA copy number per antibody, amplification of non-specific DNA, long run time for combined ELISA and PCR amplification, and the effect of polymerase inhibitors present in biological specimens.

Here we present an immunoassay method based on Amplatto™ technology that is capable of measuring protein drug X with high sensitivity and wide dynamic range.

Figure1: Principle of Amplatto™

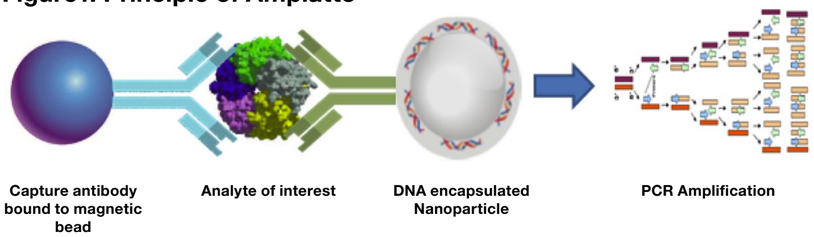
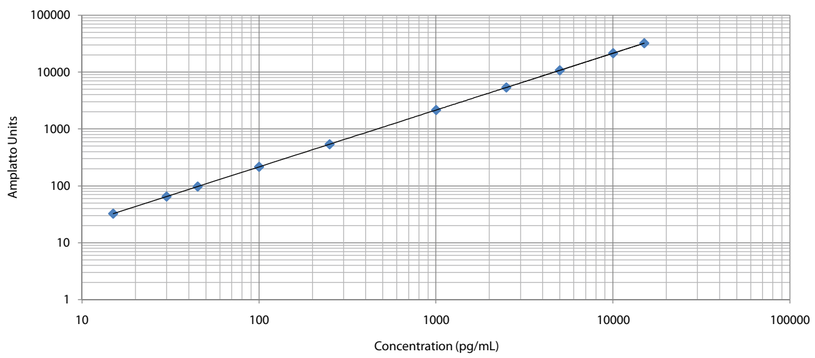


Figure2: Representative Standard Curve



Methods

Amplatto™ technology is based on the principle of immuno-PCR method. Amplatto™ relies on a proprietary nanoparticle technology that allows us to overcome the challenges associated with immuno-PCR method mentioned previously.

Briefly, the coating antibody is conjugated to magnetic nanoparticles and used to capture the therapeutic protein X from biological samples. Bound analyte is detected using a nanoparticle conjugated to antibody. The nanoparticle encapsulates reporter DNA molecules. Non-specific DNA is removed via DNase I treatment. The reporter DNA is released from the nanoparticle and the PCR is performed.

Amplatto™ technology does not require the conjugation of DNA to antibody. The ability to encapsulate multiple DNA reporters (~100 copies) per nanoparticle improves signal amplification significantly and helps overcome the effect of polymerase inhibitors present in biological specimens. Encapsulation of the reporter inside the nanoparticles also allows for removing nonspecific DNA in the assay medium to be degraded with DNase I prior to PCR amplification. Other non-specific interactions are further reduced because micro-wells with high binding capacity are no longer required since the large surface-to-volume ratio conferred by nanoparticles allows faster and more complete interactions between the antibodies anchored to the nanoparticles and the antigens in solution.

An additional advantage of Amplatto™ is the simplification of the PCR step. The PCR reagents are provided as a ready-to-use cocktail and the amplification step takes less than 15 minutes to execute.

The optimized assay was found to be 800-fold more sensitive when compared to a conventional ELISA method with six logs of dynamic range. The assay was also found to be precise (less than 20% CV for intra-batch and inter-batch precision across entire analytical range), accurate (80%-120% recovery), and linear up to a 1,000-fold dilution. The minimum required dilution (MRD) was set at 1:20. The selectivity data shows that the accuracy of the assay can be further improved (18 out of 20 lots with acceptable recovery within 15% of the nominal concentration) by increasing the MRD to 1:100. Since the assay demonstrated more than the required sensitivity, the MRD can be increased to improve accuracy.

Table 1: Recovery of Protein X in 20 individual serum

Lot #	Spiked Concentration (pg/mL)	Observed Concentration (pg/mL)	% Bias	Lot #	Spiked Concentration (pg/mL)	Observed Concentration (pg/mL)	% Bias
1	30	28	-7	11	30	27	-10
2	30	32	7	12	30	29	-3
3	30	35	17	13	30	28	-7
4	30	31	3	14	30	26	-13
5	30	27	-10	15	30	27	-10
6	30	29	-3	16	30	33	10
7	30	28	-7	17	30	38	27
8	30	26	-13	18	30	31	3
9	30	28	-7	19	30	31	3
10	30	27	-10	20	30	26	-13

Table 2: Inter-assay accuracy and precision

Run #	Concentration of Protein X (pg /mL)				
	15,000	11250	5000	45	15
Run 1	15948	11638	4694	42	13
Run 2	15685	12194	4554	39	17
Run 3	15661	11981	4676	43	13
Run 4	14601	12878	4622	46	*
Run 5	14101	13539	4467	47	17
Run 6	13476	12341	4493	40	13
Mean	14912	12429	4584	43	15
SD	917	622	86	3	2
%CV	6	5	2	7	13
%Bias	-1	10	-8	-5	-3
n	6	6	6	6	5

* Removed from calculation (%CV > 20%)

Conclusion

An immunoassay method using Amplatto™ was successfully optimized for the measurement of protein X in biological matrices to support pharmacokinetic studies. The assay was found to be highly sensitive, and specific with a large dynamic range.

Future Work

The encapsulation of the reporter DNA, off-the shelf ready- to-use reagents, and a user-friendly software tool makes this method amenable to personnel without extensive experience in PCR techniques or access to PCR-compliant laboratory facilities. Additional work will be conducted to evaluate the application of this technology to measure analytes for the purposes for biomarker measurement and anti-drug antibody measurement.