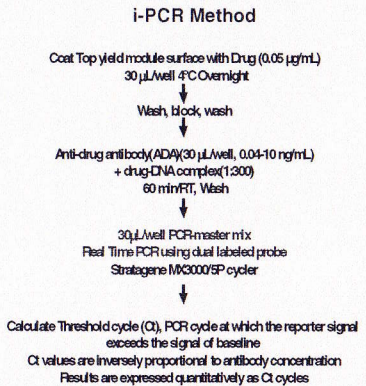


From ELISA to a Novel Immuno-PCR (i-PCR) Based Immunogenicity Assay in Quest to Improve the Drug Tolerance

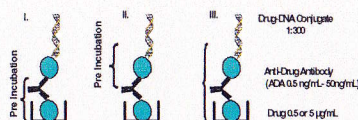
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Abstract

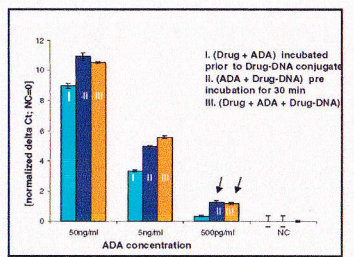
Purpose: For an immunomodulatory biologic "X" (drug), a sandwich ELISA assay was used to support first in human (FIH) studies. Electrochemiluminescence (ECL) assay with improved drug tolerance was used for single dose - dose ranging studies in disease population. This study investigated a novel immuno-PCR (i-PCR) based immunogenicity assay in attempt to further enhance the drug tolerance to support more frequent and longer term dosing with drug in addition to addressing whether the lower immunogenicity incidence observed at higher administered doses is due to drug interference. **Method:** For the bridging i-PCR assay the drug-DNA conjugate was synthesized by chemical cross-linking. Monoclonal anti-drug antibody (mAb) and drug-DNA conjugate were added to the drug coated TopView[®] modules. After washing, PCR master mix was added and real-time PCR was performed using Stratagene MX3000/5P cycler. The resulting Ct values which represent the PCR cycle at which the fluorescence signal exceeds the threshold are inversely proportional to the amount of antibody in the sample. **Results:** The ELISA assay used in FIH studies provided adequate sensitivity (70ng/mL mAb equivalent) but drug interference in ELISA assay was observed at 1:1 (mAb: drug) molar ratio. In contrast, ECL methodology improved both parameters; drug interference was 1:40 (mAb:drug ratio) and assay sensitivity was 20ng/mL. Despite the improvements offered by the ECL assay, an inverse correlation was observed in the immunogenicity incidence with increasing doses of the biologic, suggesting that upon prolonged treatment at higher doses, circulating drug still interfered with ADA detection, or inhibited the generation of an ADA response. For this particular biologic, in disease matrix, addition of an acid treatment step to the existing ELISA or ECL assays increased the background signal. Immuno PCR (i-PCR) based bridging antibody assay proved to be highly sensitive in initial studies, assay sensitivity is 40pg/mL mAb equivalent. Using mAb levels, ranging from 0.1ng/mL to 10ng/mL, detection of mAb could be achieved in the presence of 100-10,000 ng/mL of free drug. **Conclusions:** Immuno PCR assay provided a broad linear dynamic range (0.04-10ng/mL) with excellent recovery. Detection of ADA was accessible in the presence of an over 1000 fold excess of the free drug without any sample pre-treatment. Limited amount of drug for coating and Drug-DNA conjugate is needed to obtain a very robust signal due to the PCR amplification. Further optimization, assay cut point determination, and refinement of confirmation is recommended as this highly sensitive assay may detect very low amounts of antibodies compared ELISA and MSD technologies.



Optimization of Incubation Conditions

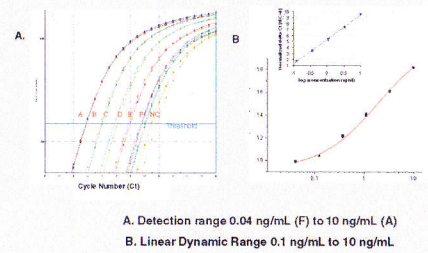


- I. Pre incubation of (Drug + ADA) followed by Drug-DNA conjugate
- II. Drug coated on the surface followed by addition of (ADA + Drug-DNA conjugate)
- III. Combined incubation of (Drug + ADA + Drug-DNA conjugate)

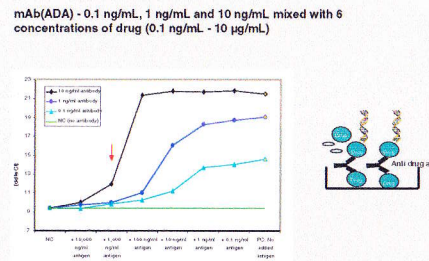


Acceptable normalized signal to noise ratio for ADA(500pg/mL) for format II and III
 Ease of use due to the pre-incubation step
 Format II was selected for further use

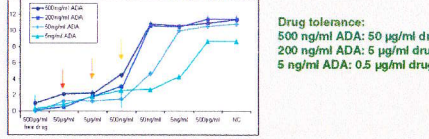
Real Time PCR Data for Assay Range



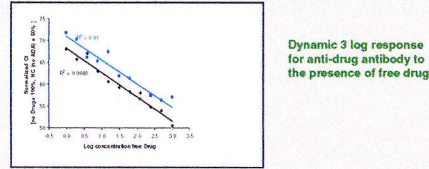
Drug Tolerance Experiment in Synthetic Serum



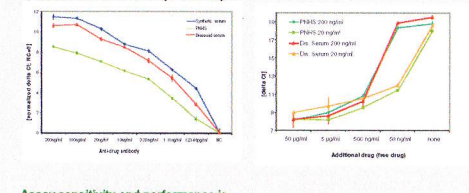
Expanded Drug Tolerance in Synthetic Serum



Linear Regression of Spiked Drug concentrations at Two Anti Drug Antibody Concentrations



Comparison of Synthetic Serum, Pooled Normal Human Serum (PNHS) Drug Tolerance in PNHS/Disease Serum



Assay sensitivity and performance is similar in all 3 matrices
 Dynamic detection range 0.1 - 200 ng/ml ADA.
 Drug tolerance: ADA to Drug ratio in disease serum 1:250

Comparison of Antibody Detection Using Two Methods

Patient #	Day after treatment	Drug PK Level in ng/mL	ADA	i-PCR
1	0	<25	Neg	Neg
	22	4000	Neg	Positive
	43	2000	Neg	Positive
	50	NA	Neg	Neg

Detection of ADA in the presence of high amount of circulating drug

Technology Comparison Clinical Study Samples (n=37)

ADA Negative Pretreatment Day 0 Samples	n=11	
	ELISA	i-PCR
	10	11
ADA Detection in Drug > 1 µg/mL Containing Samples	n=6	
	ELISA	i-PCR
	4	6
ADA Incidence	n=20	
	ELISA	i-PCR
	20	18

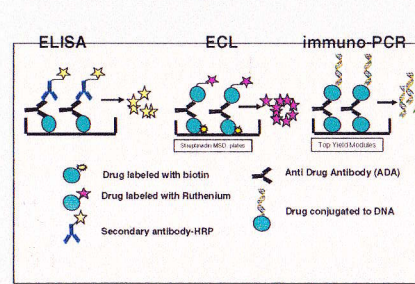
Overall Concordance 90%

CONCLUSIONS Acknowledgement

The i-PCR assay for detection of anti-drug antibodies were developed using i-PCR technology on Inperceptor platform.
 The study demonstrated an assay sensitivity of <100 pg/mL monoclonal antibody equivalent.
 Detection of ADA was possible in the presence of an over ~250-1000 fold excess of the free drug without any sample treatment compared to drug tolerance of 1:1 (ADA:free drug) ratio in ELISA.
 The assay can be utilized for detection of anti-drug antibodies in the presence of high amount of circulating drug. Further work is in progress.

Acknowledgement
 Biogenidec
 Meena Subramanyam
 Paula Hochman
 Chimera Biotec
 Mark Spengler
 Jan Detmers

Technology Comparison



	ELISA	ECL	i-PCR
Sample Volume	100µL	100µL	30µL
Sensitivity Using mAb	70 ng/mL	20 ng/mL	40 µg/mL
Drug Tolerance (mAb:Drug)	1:1	1:40	1:1000
Sample Pre Treatment (Acid Dissociation)	Increased background	Increased background	NA
Matrix Interference	+++	+	NA

