Isotyping Therapeutic Protein Immunogenicity in an Automated Multiplexed Assay

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Introduction:

The formation of Anti-drug antibodies (ADAs) are a concern for both drug efficacy and safety. Isotyping the ADA response provides further characterization of the immune response to a novel therapeutic during both pre-clinical and clinical studies. In this automated assay, the drug of interest was printed in a planar microarray and the isotypes of the ADA response were measured in a single well by a cocktail of differentially labeled secondary antibodies (anti-IgG, A and M). Species specific reporter cocktails have been developed for pre-clinical animal studies (rat, mouse, rabbit, monkey) or human ADA testing.

Materials and Methods:

Adnectin-Fc fusion protein was printed on epoxysilane glass plates at 250 µg/mL, cured, blocked and assayed. Samples (monkey, mouse, rat or rabbit) were prepared in 1X PBS + 1% BSA at various dilutions to measure the binding of anti-Adnectin-Fc fusion IgG, IgM and IgA antibodies. After incubation for 1 hour and washing the plates, the G/A/M reporter cocktail was incubated for another hour. After a final wash, plates were scanned (Sensovation) and spot finding algorithms were applied for mean fluorescent intensity result reporting.

For detection limit and drug tolerance, increasing levels of matrix were added up to neat (100%) anti-adnectin-deficient plasma. Detection limits and drug tolerance thus reflect in vivo drug detection conditions.

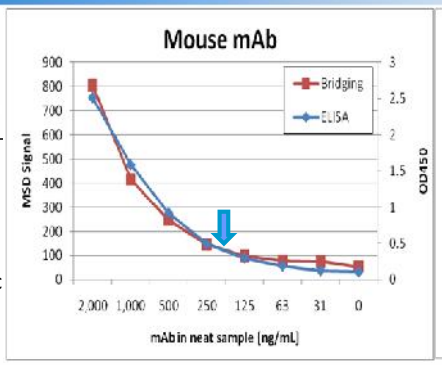
Measurement of ADAs in drug-treated animal samples was conducted at 1:100 sample dilution to endpoint titration. Specificity cutpoints were based on mean of 10 normal species-specific sera + 3 standard deviations.

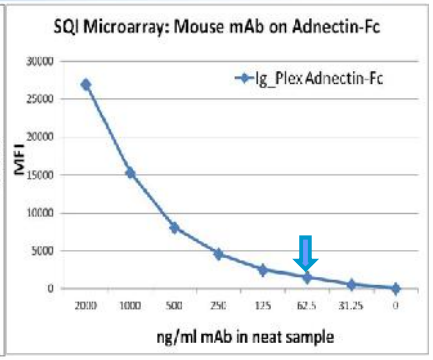
For drug tolerance and ADA specificity studies, purified Adnectin-Fc was spiked into samples containing monoclonal, polyclonal or ADA at doses up to 100 µg/mL.

Results:

Data for detection sensitivity, drug tolerance, assay specificity and clinical accuracy are presented with comparisons to ELISA and Meso Scale Discovery bridging assays. For agreement, the SQI multiplex assay was compared to a G+M total ELISA, and the additional IgA results added. 50% (*) of rat IgM results were indeterminate at the specificity cutpoint. For specificity confirmation with high dose Adnectin-Fc fusion, over 90% inhibition was seen at the 1:100 test dilution. Reproducibility of polyclonal samples between 6 runs for the microarray assay was typically < 15% CV.

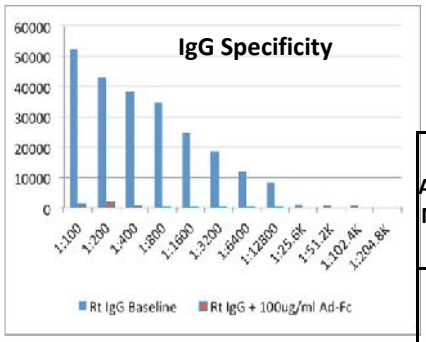
Conclusions: Sensitivity and drug tolerance of the SQI assay is equivalent to or better than other assays while providing isotyping and automation. The preclinical crossover animal study showed a high percentage of data agreement with ELISA with additional isotype data available. SQI Ig Plex assay reproducibility study shows low CV's from multiple print runs.





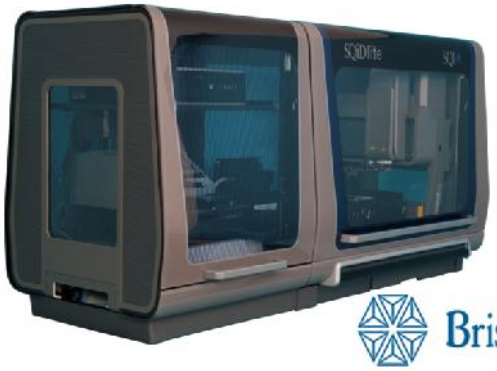
Anti-Adnectin-Fc Fusion	LOD (Monoclonal)	Drug Tolerance (Polyclonal)
ELISA	125 ng/mL	200 ng/mL @ 1:64K
MSD Bridging Assays	500 ng/mL	0.10 ng/mL @ 1:160
SQI	62.5 ng/mL	250 ng/mL @ 1:128K

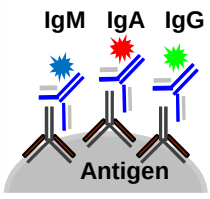
Species	Monkey		Mouse		Rat	
Isotype	IgG/M	IgA	IgG/M	IgA	IgG/M	IgA
% Positive Agreement	100 (8/8)	8/8	80 (8/10)	8/10	50 * (5/10)	1/10
% Negative Agreement	100 (9/9)	9/9	87.5 (7/8)	4/8	100 (8/8)	8/8
% Overall Agreement	100	NA	83.3	NA	72.2	NA



REPRODUCIBILITY (n=72 replicates)	Sample	Pab 2 1:200	Pab 2 1:800	Pab 2 1:256K	Pab 2 1:512K	Pab 6 1:400
Avg. MFI	IgA	7993	3987	944	635	9431
	IgG	62465	63866	59613	40432	62779
	IgM	35733	27002	5693	3161	23383
% CV	IgA	14.2	11.9	14.7	17.8	21.7
	IgG	2.7	6.8	6.8	12.5	2.9
	IgM	14.3	14.7	14.7	11.8	11.3

Automated SQiDlite System for Immunogenicity





SQI

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