Validation of a flow cytometric assay to detect antibodies to a novel cell therapy for immunogenicity assessment

POSTER #229

Jing Yu, Amanda Hays, Lynn Kamen, Michelle Miller
BioAgilytix, 2300 Englert Drive, Durham, NC 27713

BioAgilytix

PURPOSE

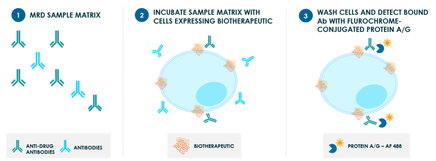
Genetically engineered cell therapies, specifically CAR (chimeric antigen receptor)-based cell therapies, are successful treatments in immune oncology. Now, alternative engineered cell-surface proteins, including cytokines, immune-evasion proteins, and other immunologic complexes show therapeutic promise. Immunogenicity to these engineered proteins is a safety concern during clinical studies. Thus, it is important to develop robust assays for measuring potential immune responses to these novel therapeutics. Herein, a cell-based, flow cytometric assay was validated to assess humoral immunogenicity to the engineered cell-surface protein in human serum. A cell-based assay was warranted, as the engineered cell-surface protein could not be purified for use in a standard ligand binding assay.

METHOD

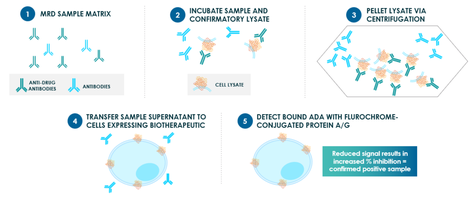
For this cell-based assay, K562 cells were transduced to express the engineered protein (referred to as the "drug"). Serum samples were incubated with the transduced K562 cells to allow binding of specific antibodies against the drug. Anti-drug antibodies were detected with Protein A/G conjugated with Alexa Fluor 488. Fluorescence intensity was measured via flow cytometry and normalized against negative controls. The fluorescence intensity correlates with the detection of antibodies to the drug in the sample. To confirm specificity of the signal (i.e., confirmatory assay), serum samples were exposed to transduced K562 cell lysate. Antibodies specific to the drug were sequestered by the lysate. Following centrifugation, the sample supernatant was transferred onto the transduced K562 cells. A decrease in assay signal indicates specificity. Samples were determined to be positive or negative based on statistically derived cut points.

ASSAY PRINCIPLE

Screening Assay



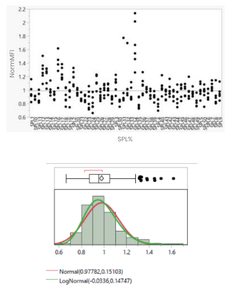
Confirmatory Assay



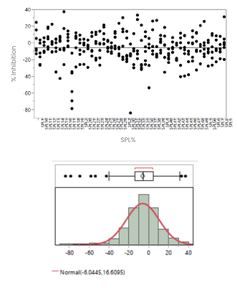
RESULTS

Cut-Point Determination

Screening Cut-Point



Confirmatory Cut-Point



Intra- and Inter-Assay Precision

Inter Assay (Screening)

Analyst/Date	HPC (5000 ng/mL)		MPC (1000 ng/mL)		LPC1 (100 ng/mL)		LPC2 (200 ng/mL)		LPC3 (300 ng/mL)	
	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)
KH 21Apr20	17.4	5.4	5.63	9.4	1.90	7.3	1.95	8.2	1.91	1.0
	17.8	8.7	5.57	0.2	1.94	11.9	1.86	3.9	1.86	0.3
SeBa 21Apr20	20.0	13.4	6.22	12.9	2.08	12.4	2.17	18.2	2.04	16.2
	18.8	9.8	5.89	11.2	2.07	7.0	1.84	10.1	1.87	8.6
KH 22Apr20	20.0	4.0	5.67	0.1	2.10	2.2	2.05	3.7	2.14	2.6
	20.2	9.4	6.06	4.5	2.03	0.3	1.98	12.0	1.93	7.6
SeBa 28Apr20	17.1	10.7	5.84	17.0	2.12	11.8	2.13	9.3	2.15	5.0
	14.6	25.9	4.49	11.0	1.72	12.1	2.00	12.3	1.71	1.0
SeBa 29Apr20	17.4	30.1	4.83	20.4	1.76	15.3	1.86	13.0	1.75	14.8
	15.6	7.2	4.31	8.3	1.69	7.5	1.83	3.5	1.69	5.6
KH 28APR20	22.2	1.7	7.43	7.1	2.38	1.0	2.34	1.0	2.26	7.8
	22.6	3.1	7.24	11.4	2.51	9.8	2.61	10.9	2.42	0.5
Overall Mean	18.6		5.77		2.02		2.05		1.98	
Overall CV (%)	13.1		16.5		12.2		11.4		11.5	

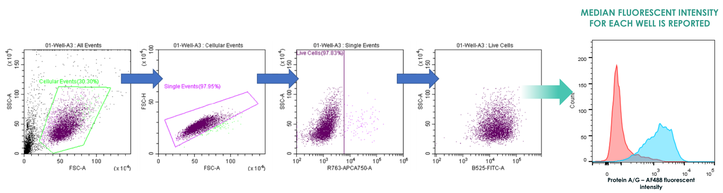
Intra Assay (Screening)

Replicate	HPC (5000 ng/mL)		MPC (1000 ng/mL)		LPC1 (100 ng/mL)		LPC2 (200 ng/mL)		LPC3 (300 ng/mL)	
	NormMFI Mean)	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)	NormMFI Mean	Duplicate CV(%)
1	17.6	34.1	3.88	3.5	2.36	6.0	2.07	5.6	1.84	4.4
2	16.2	28.1	4.11	4.7	2.34	1.5	2.18	0.8	1.84	8.5
3	15.7	11.7	4.16	2.2	2.23	3.5	2.10	2.3	1.68	10.1
4	15.7	11.5	4.41	17.2	2.30	3.5	1.90	13.4	1.85	7.9
5	15.1	11.8	4.36	5.0	2.30	0.4	1.98	0.1	1.79	8.5
6	15.5	19.0	4.34	7.7	2.26	1.8	2.19	3.0	1.77	2.8
Overall Mean	16.0		4.21		2.30		2.07		1.80	
Overall CV (%)	5.5		4.8		2.1		5.5		3.7	

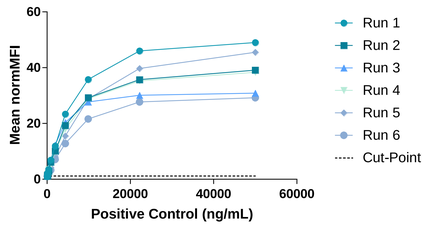
Validation Summary Table

Validation Parameter	Experimental Design	Results
Cut-Points	Cut-points were established with 50 treatment-naïve normal serum samples. The confirmatory cut-point was determined using the same 50 treatment-naïve samples pre-incubated with the K562 cell lysate.	The screening cut-point was statistically determined as 1.26 (normalized value). The confirmatory cut-point, expressed as % inhibition, was determined to be 30.1%.
Sensitivity	Assay sensitivity was defined as the concentration of surrogate positive control antibody that intersected with cut point values following assessment of serial dilutions.	The assay sensitivity was determined to be 57.1 ng/mL (screening assay) and 90.0 ng/mL (confirmatory assay)
Selectivity	Ten individual lots of normal serum were unspiked and spiked with low (200 ng/mL) and high (5000 ng/mL) concentrations of the SPC.	90% of unspiked individuals met acceptance criteria. 100% of individuals spiked with a low level of PC and all individuals spiked with a high level of PC were above the cut point.
Specificity	Specificity was assessed in the screening format and confirmatory format using pooled normal human serum spiked at high (5,000 ng/mL) and low (200 ng/mL) levels of a non-specific antibody.	The low spiked sample tested below the cut point. Although one of four high spiked samples tested above the screening cut point, all samples tested below the confirmatory cut point.
Intra- and Inter-Assay Precision	High, Mid, and Low PCs were evaluated as six independent replicates on a single plate (intra) and on six independent runs (inter).	The CV for each level of QCs analyzed was less than or equal to 30%.

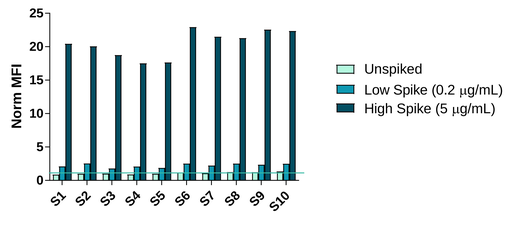
GATING STRATEGY



Sensitivity



Selectivity



CONCLUSIONS

- We developed and validated a robust, cell-based assay for the detection of antibodies to an engineered, cell-surface protein.
- This assay is an example of a robust, cell-based assay for the detection of antibodies to an engineered, cell-surface protein.
- This assay format can be used to assess humoral immunogenicity in human samples when the engineered protein cannot be expressed in a soluble, recombinant form for use in a standard ligand binding assay.