Evaluation of Multiple Immunoassay Platforms to Measure the Concentration of a Therapeutic Monoclonal Antibody (TMAb) in Cynomolgus Monkey Serum

Rebecca Crisino, Thomas McIntosh, Jonathan Hicks, Peter Garrett, Alyson McGurrin, and Jennifer Yohrling

Centocor Research & Development Inc., Radnor, Pa, USA

Abstract

Purpose: To evaluate 5 immunoassay platforms for the development of an assay to measure concentrations of a TMAb in cynomolgus monkey serum.

Methods: The following platforms were evaluated: two electrochemiluminescence instruments, BioVeris (BV) and MesoScale Discovery (MSD); a heterogeneous time-resolved fluorescence assay (DELFIA); a luminescence assay, Amplified Luminescent Proximity Homogeneous Assay (AlphaLISA); and a bio-layer interferometry assay using the OCTET platform. All assays except the Octet utilized a double sandwich format with non-competing anti-idiotype mouse monoclonal antibodies to the TMAb as capture and detection reagents. The OCTET utilized a single anti-idiotype antibody in a biosensor format.

Results:

	BioVeris	MesoScale	DELFIA	AlphaLISA	Octet
Platform	Electrochemi-luminescence (fluid)	Electrochemi-luminescence (solid)	Time-Resolved Fluorescence	Luminescence	Optical Biosensor
Instrument	BioVeris M384 Analyzer	MSD Sector Imager 6000	Perkin Elmer - Envision	Perkin Elmer - Envision	ForteBio
Optimal Standard Curve Range	500.00 - 7.81 ng/mL	250.00 - 3.91 ng/mL	250.00 - 3.91 ng/mL	125.00 - 3.91 ng/mL	50,000 - 400 ng/mL
Minimum Required Sample Dilution	1:20	1:10	1:10	1:10	n/a
LLOQ	156.30 ng/mL	39.10 ng/mL	39.10 ng/mL	39.10 ng/mL	500 ng/mL
Dilutional Linearity	1,20,000	1,10,000	1,10,000	n/a	n/a
Average Recovery of Spiked Controls (±SD)	103.3% (± 10.7 %)	107.5% (± 15.9 %)	97.1% (± 8.9%)	120.0% (± 17.6 %)	101.6 % (± 12.9 %)
Intra-Assay Precision	10.7%	14.8%	9.2%	14.7%	12.7%

Conclusions: Five immunoassay platforms were evaluated for development of an assay to measure TMAb drug concentration in cynomolgus monkey serum. The BioVeris assay established a sensitive and robust assay while maintaining a simple homogeneous 1-step format. The MSD and DELFIA platforms showed increased sensitivity compared to the BioVeris but required more than one step. The Octet assay demonstrated acceptable accuracy, but did not produce an assay that was as sensitive as the other formats. The AlphaLISA assay showed promise as a homogeneous format, but had a shorter range of quantification. In this evaluation the BioVeris assay was the simplest format to use, while the MSD and DELFIA formats showed greater sensitivity.

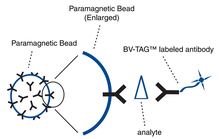
Goal

To evaluate five immunoassay platforms for the development of an assay to measure concentrations of a TMAb in cynomolgus monkey serum.

Methods

- Methods involved in this comparison include the electrochemiluminescent immunoassay (ECLIA) on the M384 analyzer from BioVeris™ Corp., a second ECLIA on the MesoScaleDiscovery (MSD®) instrument, the Dissociation Enhanced Lanthanide FluoroImmunoassay (DELFIA®) platform, and AlphaLISA® platform, both on the EnVision® from PerkinElmer Corp., and a bio-layer interferometry assay using the OCTET® from ForteBio Corp.
- All assays except the Octet utilized a double sandwich format with non-competing TMAb mouse monoclonal antibodies to the TMAb as capture and detection reagents. The OCTET utilized a single antibody in a biosensor format.
- Controls were prepared in cyno monkey serum and interpolated from a standard curve. Each method was tested for accuracy & precision.
- Total error was assessed by running 2 independent determinations (in duplicate) of each control per assay. A total of 6 independent assays over multiple days were run. Total error will be calculated by combining the interbatch precision (coefficient of variance - %CV) and the absolute mean bias (relative error - %RE).

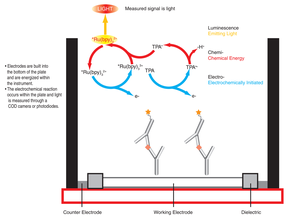
Figure 1. ElectroChemiluminescence (ECLIA) – BioVeris™ Format



Cartoon Adapted from BioVeris™ Assay Development Workshop

In solution, a biotinylated antibody (Ab) was bound to streptavidin-coated paramagnetic beads, which then is bound to the TMAb. A ruthenium labeled Ab bound to the TMAb-biotinylated Ab to form a complex. Beads were collected on an electrode using a magnet and the stimulated electrode excited the ruthenium label, emitting light. Emitted light correlated with the TMAb concentration.

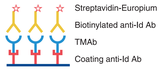
Figure 2. ElectroChemiluminescence (ECLIA) – MSD® Format



Cartoon Adapted from MSD® Technology Platform Brochure

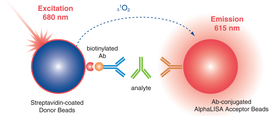
TMAb was bound to biotinylated-Ab which was bound to streptavidin-coated assay plates. A ruthenium labeled Ab was bound to the TMAb-biotinylated Ab to form a complex. An electrode along the bottom of each well was used to excite the ruthenium label and elicited the chemical reaction to emit light signal. Emitted light correlated with the TMAb concentration.

Figure 3. Time Resolve Fluorescence – DELFIA®



Ab bound to plate was used to capture TMAb. Biotinylated-Ab binded to TMAb, followed by streptavidin-Europium binded to the biotinylated-Ab. Enhancement solution was added to release the europium. Free europium forms a highly fluorescent chelate. Amount of fluorescence correlated to TMAb concentration.

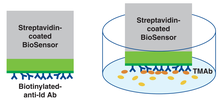
Figure 4. Luminescence – AlphaLISA® Format



Cartoon Adapted from Internal PerkinElmer Report

The TMAb was bound Ab conjugated to acceptor beads. Biotinylated-Ab was bound to drug/acceptor bead complex, which was then bound to streptavidin-coated donor beads. Photo-sensitized donor bead converted ambient O_2 to an excited form of O_2 at 680 nm. Excited O_2 transferred energy to acceptor bead which emitted light at 520 - 620 nm. Emitted light correlated with TMAb concentration.

Figure 5. BioSensor – OCTET® Format



Cartoon Adapted from Internal ForteBio Report

Pre-wetted streptavidin biosensors in PBS, loaded 20 µg/mL biotin-Ab, then established baseline values of assay buffer. TMAb was bound to a new set of sensors for each column.

Table 1. Assay Procedures. Step-by-step description of each procedure

Assay Steps	BioVeris™	MesoScale®	DELFIA®	AlphaLISA®	Octet®
1	dilute TMAb	block streptavidin coated plate with biotinylated Ab @ 1.58 µg/mL	coat plate with 2.5 µg/mL Ab incubate overnight	10 µL Ab-conjugated acceptor beads @ 10 µg/mL + 5 µL TMAb + 10 µL biotinylated Ab @ 3nM, incubate 60 min.	Pre-wet sensors in PBS for 5 min.
2	10 µL TMAb + 190 mL master mix (biotinylated Ab @ 0.25 mg/mL + ru Ab @ 0.50 mg/mL + beads), incubation 2 hours	wash	block, 60 min.	25 µL SA-donor bead @ 30 µg/mL incubation 60 min.	15 µL biotinylated Ab incubated with streptavidin-coated sensors for 15 min.
3	read on BioVeris M834 Analyzer	25 µL TMAb + 25 µL ru Ab @ 7.7 µg/mL, incubation 2 hours	wash	read on EnVision Multi-Label Reader	dilute TMAb, incubate and read 2 minutes per column (~30 min/plate)
4	–	wash	50 µL TMAb incubation 60 min.	–	–
5	–	add 150 µL read buffer	wash	–	–
6	–	read on MSD Sector Imager 6000	50 µL biotinylated Ab @ 1 µg/mL incubation 60 min.	–	–
7	–	–	wash	–	–
8	–	–	50 µL streptavidin-europium @ 10 µg/mL incubation 60 min.	–	–
9	–	–	wash	–	–
10	–	–	enhancement solution	–	–
11	–	–	read on EnVision Multi-Label Reader	–	–

Results

Table 2. BioVeris™ Spike/Recovery Data

TMAb Conc. (ng/mL)	Dilution	Set # 1 Adj. Result (ng/mL)	Set # 1 % Recovery	Set # 2 Adj. Result (ng/mL)	Set # 2 % Recovery	Set # 1 Adj. Result (ng/mL)	Set # 1 % Recovery	Set # 2 Adj. Result (ng/mL)	Set # 2 % Recovery
375000	1000	407394.65	108.64	395901.872	105.57	427393.323	113.97	453180.891	120.85
100000	1000	95086.63	95.09	102880.171	102.88	106602.182	106.60	110011.966	110.01
20000	1000	18375.5	91.88	19612.75	98.06	23118.182	115.59	22235.415	111.18
10000	20	12364.74	123.65	11992.475	119.92	12610.77	126.11	12722.121	127.22
156.3	20	185.31	39.01	169.063	108.17	185.842	118.90	189.078	120.97

TMAb Conc. (ng/mL)	Dilution	Set # 1 Adj. Result (ng/mL)	Set # 1 % Recovery	Set # 2 Adj. Result (ng/mL)	Set # 2 % Recovery	Set # 1 Adj. Result (ng/mL)	Set # 1 % Recovery	Set # 2 Adj. Result (ng/mL)	Set # 2 % Recovery
500000	1000	585522.57	117.10	593319.81	118.66	568514.98	177.87	564292.45	176.54
375000	1000	300962.28	80.26	319631.65	85.24	309484.45	320.45	309335.26	320.27
100000	1000	78146.52	78.15	96578.07	96.58	82271.39	21139.68	97520.19	25057.86
475	20	422.45	88.94	389.18	81.93	452.66	417.51	421.73	388.98
156.3	20	131.35	84.04	108.42	69.37	142.90	131.80	117.27	108.16

Mean % Recovery: 103.20%

Mean Std. Dev.: ± 10.7%

Mean % CV: 10.70%

Controls were prepared in pooled cyno serum and diluted in 1×PBS-1%BSA-0.5%Tween-20 (with 15% pooled serum for all dilutions over MRD) and tested in duplicate over multiple days.

Table 3. BioVeris™ Dilutional Linearity Data

TMAb Conc. (ng/mL)	Dilution	Individual 1 Adj. Result	Individual 1 % Recovery	Individual 2 Adj. Result	Individual 2 % Recovery	Individual 3 Adj. Result	Individual 3 % Recovery
250,000	100	211339.69	84.54	221042.681	88.42	216650.238	86.66
250,000	1,000	215497.159	86.20	220388.13	88.16	229545.325	91.82
250,000	5,000	225634.458	90.01	227727.225	91.09	222950.744	89.18
250,000	20,000	197100.748	78.84	216590.473	86.64	222125.006	88.85
5,000,000	100	157920.443	3.16	167844.349	3.36	179824.421	3.60
5,000,000	1,000	3373826.374	67.48	3106114.529	62.12	3067667.167	61.35
5,000,000	5,000	4795865.734	95.92	4847875.342	96.96	4304098.069	86.08
5,000,000	20,000	5779137.197	115.58	4846437.854	96.93	4105416.201	82.11

Controls were prepared in individual lots of cyno serum and diluted in 1×PBS-1%BSA-0.5%Tween-20 with 15% pooled serum) and tested in duplicate in a single assay.

Table 4. BioVeris™ Accuracy & Precision Data

Characteristic	Statistic	QC1 200.0 mg/mL	QC2 20.0 mg/mL	QC3 2.0 mg/mL	QC4 0.20 mg/mL
# Results	N	12	12	12	12
Accuracy	Mean Bias (%RE)	1.3	17.4	3.4	22.4
Precision	Intrabatch (%CV)	4.6	6.6	3.8	5.8
	Interbatch (%CV)	6.3	6.6	5.6	7.7
Accuracy + Precision	(Mean) + Interbatch	7.58	24.05	9.00	30.08

BioVeris® Mean Assay % Bias = 11.1%; BioVeris® Mean Inter-Assay % CV = 6.6%; BioVeris® Total Assay Error = 17.68%

Controls were prepared in pooled cyno serum and diluted in 1×PBS-1%BSA-0.5%Tween-20 (with 15% pooled serum for all dilutions over MRD) and tested in duplicate over multiple days.

Figure 6. Graphed BioVeris™ Standard Curve Data

Standard curve data for all total assessment experiments.

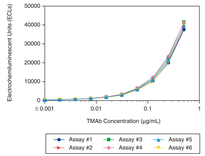


Table 5. MSD® Spike/Recovery and Dilutional Linearity Data

TMAb Conc. (ng/mL)	Adj. Result (ng/mL)	Dilution	% Recovery	TMAb Conc. (ng/mL)	Adj. Result (ng/mL)	Dilution	% Recovery
100,000	107074.84	1000	107.07	500	495.41	2	99.08
100,000	113519.89	5000	113.52	500	524.33	5	104.87
100,000	120389.72	10000	120.39	500	527.20	10	105.44
50,000	40047.41	100	80.09	100	98.65	2	98.65
50,000	57747.56	1000	115.50	100	111.10	5	111.10
50,000	67888.90	10000	135.78	100	120.88	10	120.88
10,000	10721.01	100	107.21	50	49.39	1	98.78
10,000	11900.34	500	119.00	50	53.50	2	107.00
10,000	12985.59	1000	129.86	50	60.34	5	120.69
5000	3821.67	10	76.43	10	10.86	1	108.58
5000	5009.55	100	100.19	10	12.06	2	120.60
5000	5906.14	500	118.12	10	11.90	5	118.97
1000	572.14	2	57.21	5	5.46	1	109.20
1000	976.41	5	97.64	5	5.50	2	109.90
1000	1066.15	10	106.62				

Controls were prepared in pooled cyno serum and diluted in 1×PBS-1%BSA and tested in duplicate.

Table 6. MSD® Accuracy & Precision Data

Characteristic	Statistic	QC1 200,000 ng/mL	QC2 20,000 ng/mL	QC3 20,000 ng/mL	QC4 200 ng/mL
# Results	N	12	12	12	12
Accuracy	Mean Bias (%RE)	-26.9	-12.6	-19.4	-10.3
Precision	Intrabatch (%CV)	5.9	2.2	3.6	2.8
	Interbatch (%CV)	5.9	6.1	5.3	7.6
Accuracy + Precision	(Mean) + Interbatch	32.79	18.70	24.67	17.95

MSD® Mean Assay % Bias = -17.3%; MSD® Mean Inter-Assay % CV = 6.2%; MSD® Total Assay Error = 23.53%

Controls were prepared in pooled cyno serum and diluted in 1×PBS-1%BSA) and tested in duplicate over multiple days.

Figure 7. Graphed MSD® Standard Curve Data

Standard curve data for all total assessment experiments.

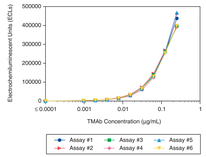


Table 7. DELFIA® Spike/Recovery Data

TMAb Conc. (ng/mL)	Dilution	Pool Adj. Result	Pool % Recovery	Individual 1 Adj. Result	Individual 1 % Recovery	Individual 2 Adj. Result	Individual 2 % Recovery	Individual 3 Adj. Result	Individual 3 % Recovery	Individual 4 Adj. Result	Individual 4 % Recovery
2500	10	2106.60	84.26	2750.07	130.55	2751.87	130.63	2654.84	126.02	2694.61	127.91
1250	10	1230.02	98.40	952.21	12.37	118.42	9.63	144.49	11.75	151.67	12.33
156.3	10	153.24	98.04	84.41	55.08	57.17	37.31	85.29	55.66	81.14	52.95
78.1	10	81.64	104.53	84.21	103.15	51.57	63.17	80.49	98.59	81.24	99.51
39.05	10	38.19	97.80	35.48	92.90	11.61	30.39	36.38	95.25	40.47	105.98

TMAb Conc. (ng/mL)	Dilution	Individual 1 Adj. Result	Individual 1 % Recovery	Individual 2 Adj. Result	Individual 2 % Recovery	Individual 3 Adj. Result	Individual 3 % Recovery	Individual 4 Adj. Result	Individual 4 % Recovery
2500	20	2682.27	107.29	2615.50	104.62	2641.90	105.68	2495.00	99.80
156.3	20	147.57	11.81	115.63	9.25	140.35	11.23	151.53	12.12
78.1	20	82.14	52.55	40.37	25.83	88.26	56.47	83.16	53.21
78.1	10	84.71	108.46	39.77	50.92	80.51	103.09	90.67	116.09
39.05	10	38.71	99.13	12.53	32.09	40.10	102.68	44.09	112.91

TMAb Conc. (ng/mL)	Dilution	Pool Adj. Result	Pool % Recovery
5000	20	4737.24	94.74
2500	20	2515.55	100.62
1250	20	1347.29	107.78
156.3	20	156.12	99.88
78.1	20	85.53	109.51
156.3	20	180.77	115.66
156.3	20	124.52	79.67
156.3	20	126.46	80.91
156.3	20	156.75	100.29
156.3	20	160.83	102.90
156.3	20	160.39	102.62

Controls were prepared in pooled or individual lots of cyno serum and diluted in TBS (with 5% pooled serum for all dilutions over MRD) and tested in duplicate over multiple days.

Table 8. DELFIA® Dilutional Linearity Data

TMAb Conc. (ng/mL)	Dilution	Individual 1 Adj.Result	Individual 1 % Recovery	Individual 2 Adj.Result	Individual 2 % Recovery
250000	10000	214531.83	85.81	202411.94	80.96
250000	1000	227057.92	90.82	218743.84	87.50

TMAb Conc. (ng/mL)	Dilution	Individual 3 Adj.Result	Individual 3 % Recovery	Individual 4 Adj.Result	Individual 4 % Recovery
250000	10000	215376.96	86.15	226996.70	90.80
250000	1000	225099.53	90.04	210375.05	84.15

Controls were prepared in pooled cyno serum and diluted in TBS (with 5% pooled serum for all dilutions over MRD) and were tested in duplicate in a single assay.

Table 9. DELFIA® Accuracy & Precision Data

Characteristic	Statistic	QC1 200.0 mg/mL	QC2 20.0 mg/mL	QC3 2.0 mg/mL	QC4 0.20 mg/mL
# Results	N	12	12	12	12
Accuracy	Mean Bias (%RE)	3.9	0.8	-3.5	-7.1
Precision	Intrabatch (%CV)	9.8	7.0	2.9	1.3
	Interbatch (%CV)	9.8	8.6	4.7	2.3
Accuracy + Precision	(Mean) + Interbatch	13.70	5.39	8.28	9.36

DELFIA® Mean Assay % Bias = 3.8%; DELFIA® Mean Inter-Assay % CV = 6.4%; DELFIA® Total Assay Error = 10.20%

Controls were prepared in pooled cyno serum and diluted in TBS (with 5% pooled serum for all dilutions over MRD) and tested in duplicate over multiple days.

Figure 8. DELFIA® Graphed Standard Curve Data

Standard curve data for all total assessment experiments.

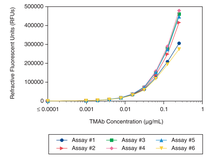


Table 10. Summary of AlphaLISA® Control Data

TMAb Conc. (ng/mL)	Dilution	Result (ng/mL)	Mean Result (ng/mL)	Adjusted Result (ng/mL)	% Recovery
100000	500	84.12	84.664	Range?	n/a
		85.21			
75000	500	23.17	23.168	Range?	n/a
		23.17			
500	10	6.54	6.618	Range?	n/a
		6.69			
500	5	1.16	1.144	Range?	n/a
		1.13			
100	5	0.41	0.399	120.7	120.70
		0.39			
50	5	0.18	0.156	58.83	117.66
		0.13			
25	5	0.01	0.009	24.821	99.28
		Range?			
12.5	10	0.08	0.076	83.625	669.00[1]
		0.07			
12.5	5	Range?	Range?	17.783	n/a
		Range?			
6.25	10	0.02	0.02	59.598	n/a

[1] *outlier, not used in average % recovery or average % CV calculations*

n/a = not applicable; Average % Recovery = 119.98 %; Average % CV = 14.68 %

Controls were prepared in pooled cyno serum and diluted in 1×PBS-1%BSA-0.1%Tween-20-0.05% Dextran with 20% pooled serum and were tested in duplicate in a single assay.

Figure 9. Summary of AlphaLISA® Standard Curve Data

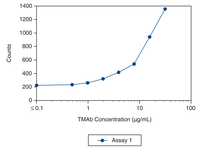


Table 11. Summary of OCTET® Control Data

PBS with 10% pooled cyno serum				
TMAb Concentration (ng/mL)	Result (ng/mL)	Mean Result	% Recovery	% CV
25.00	24.80	24.9	99.2	0.6
25.00	24.99			
5.00	4.90	4.64	98.08	8.0
5.00	4.38			
1.00	1.04	1.06	104.2	2.1
1.00	1.07			
0.50	0.62	0.62	123.33	1.5
0.50	0.63			
PBS alone				
TMAb Concentration (ng/mL)	Result (ng/mL)	Mean Result	% Recovery	% CV
25.00	27.410	27.25	109.64	0.7
25.00	27.160			
5.00	5.340	5.24	106.89	2.8
5.00	5.140			
1.00	0.800	0.80	79.77	0.3
1.00	0.800			
0.50	0.460	0.44	91.78	6.4
0.50	0.420			

Controls were prepared in pooled cyno serum and diluted in PBS alone or PBS with 10% pooled serum and were tested in duplicate in a single assay.

Figure 10. Summary of OCTET® Standard Curve Data

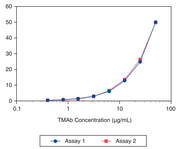


Table 12. Overall Platform Evaluation

	BioVeris™	MesoScale®	DELFIA®	AlphaLISA®	Octet®
Platform	Electrochemiluminescence (fluid)	Electrochemiluminescence (solid)	Time-Resolved Fluorescence	Luminescence	Optical Biosensor
Instrument	BioVeris M384 Analyzer	MSD Sector Imager 6000	PerkinElmer - Envision	PerkinElmer - Envision	ForteBio
Optimal Standard Curve Range	500.00 - 7.81 ng/mL	250.00 - 3.91 ng/mL	250.00 - 3.91 ng/mL	125.00 - 3.91 ng/mL	50,000 - 400 ng/mL
Minimum Required Sample Dilution	1:20	1:10	1:10	1:10	n/a
LLOQ	156.30 ng/mL	39.10 ng/mL	39.10 ng/mL	39.10 ng/mL	500 ng/mL
Dilutional Linearity	1,20,000	1,10,000	1,10,000	n/a	n/a
Average Recovery of Spiked Controls (±SD)	103.3% (± 10.7 %)	107.5% (± 15.9 %)	97.1% (± 8.9%)	120.0% (± 17.6 %)	101.6 % (± 12.9 %)
Intra-Assay Precision	10.70%	14.80%	9.20%	14.70%	12.70%

Summary of Results

	Pros	Cons
BioVeris®	• homogeneous assay • high throughput, wide dynamic range	• discontinued • high matrix effect (requires 1:20 MRD), long read time (15 min./plate)
MesoScale®	• largest dynamic range, low matrix effect (low MRD), short read time (1 min./plate)	• low throughput (3.5 hrs/assay), reagent cost (~$50/plate)
DELFIA®	• most precise and accurate assay, wide dynamic range, low matrix effect (low MRD), short read time (1 min./plate)	• low throughput (4 hrs/assay + overnight coating)
AlphaLISA®	• semi-homogeneous assay, low sensitivity, short read time (1 min./plate)	• high inter-assay variability, reagent cost (~$135/plate)
Octet®	• short assay time (~30 min./assay)	• poor sensitivity

Conclusion

Five immunoassay platforms were evaluated for determining the concentration of a TMAb in cynomolgus monkey serum. Both the MSD® and the DELFIA® platforms are comparable in robustness and sensitivity to the BioVeris™ method. The AlphaLISA® platform shows some promise but continued development is required. The OCTET® platform does not appear to be suitable for measuring less than 400 ng/mL of TMAb at stage of development.

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