



Cross-Platform Comparison of IL-6 Immunoassays

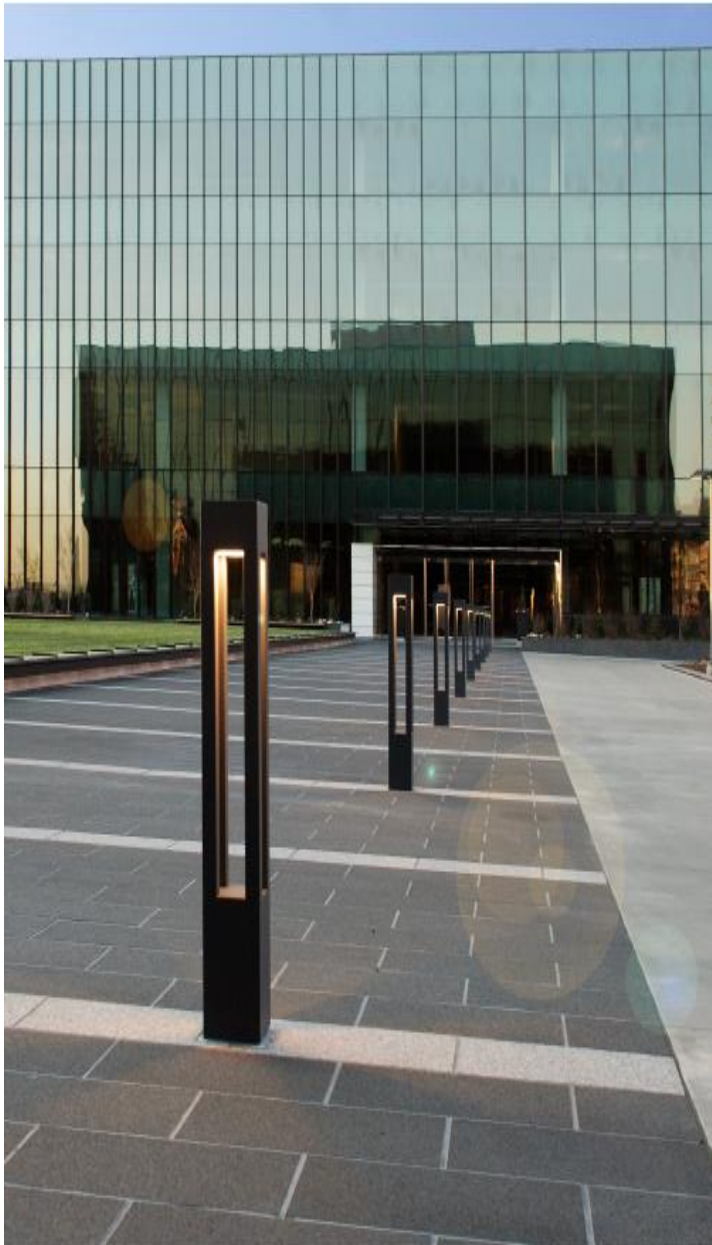
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Vice President of Laboratory Services

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Biomarker World Congress

PBI | Pacific **Biomarkers**
The Value of Expertise



Company Overview

- **Founded in 1989 as spin off from University of Washington Lipid Research Center**
- **Ex-Member of CDC-CRMLN (Cholesterol Reference Method Laboratory Network)**
- **Relationships and collaborations with numerous clinical diagnostic manufacturers**
- **CAP CLIA-certified facilities and processes but also operate to GLP and bioanalytical standards**
- **Menu of over 300 validated assays**
- **Platform agnostic**
- **Established reputation for scientific expertise**
 - **Scientific oversight throughout, from pre-analytical input to data delivery**



Study Objectives

- **Evaluate Commercial IL-6 immunoassays**
 - **Use serum samples from normal healthy donors and inflammatory disease patients.**
 - **Parallelism**
 - **Additional Performance Parameters**
 - **Spike and Recovery**
 - **Functional Sensitivity**
- **Ciraplex Ultra IL-6 US performance**
 - **QC performance**
 - **Clinical Trials**

IL-6 Commercial Immunoassay Kits

Selection of the best immunoassays depends on the intended use and individual requirements

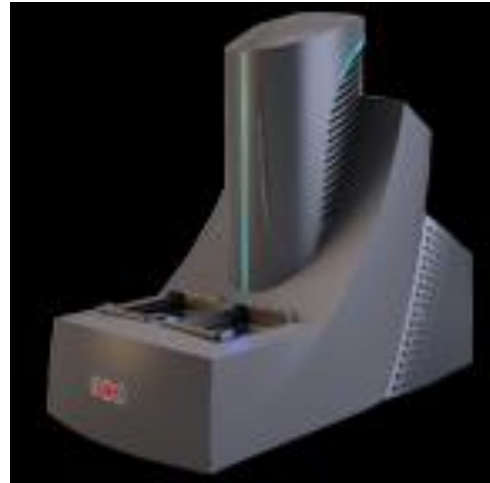
Assay Parameter	R&D Systems hsIL-6 ELISA	Ciraplex ULTRA IL-6 US	MSD IL-6 V-plex	Erenna IL-6
Kit Price	\$	\$	\$	\$\$\$
Length of Test	5 hrs	3.5 hrs	4.5 hrs	18-22 hrs
Assay Format	ELISA	Chemiluminescent	Electrochemi- luminescent	Fluorescent Microparticle
Analytical measureable range	0.56 - 10 pg/mL	0.002 - 114 pg/mL	0.01 - 657 pg/mL	0.01 - 50 pg/mL
# of Standards	8	12	10	12
Sample volume (per well)	100 µL	50 µL	50 µL	75 µL
MRD	NA	1:2	1:2	NA
Sensitivity/LLOQ (assigned)	0.08 pg/mL	0.016 pg/mL	1.58 pg/mL	0.08 pg/mL
Normal Levels	0.5 - 4.92 pg/mL	< 4.5 pg/mL	0.16 - 27.2 pg/mL	< 5.4 pg/mL

Platforms for IL-6 Immunoassay Evaluation



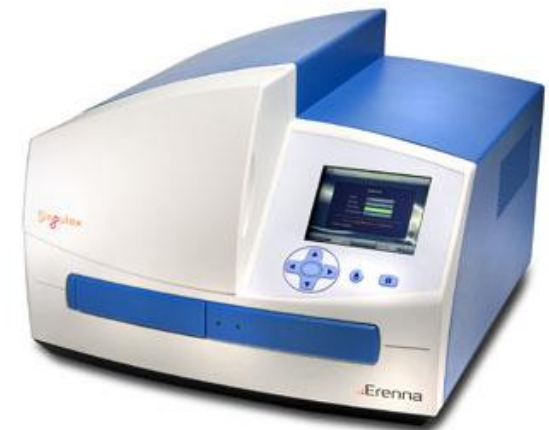
Aushon Cira

- *Broad RoQ (3+ Log)*
- *Multiplexing*
- *Exploratory analysis*
- *Fit-for-purpose*
- *Open platform*



MSD QuickPlex

- *Well-established*
- *Versatile menu*
- *Multiplexing*
- *Large-scale screening*



Singulex Erenna

- *Ultra-low concentration*
- *PK/PD for biologics*
- *Proprietary assay transfer*
- *Open platform*

Standard Curves and Quality Controls

Ciraplex ULTRA US IL-6 High Control above Erenna ULOQ and Low control below MSD LLOQ

IL-6 Assay Standards

STD	Aushon (pg/mL)	MSD (pg/mL)	Erenna (pg/mL)
11	114.000		50
10	38.000		16.67
9	12.667	657	5.56
8	4.222	164.25	1.85
7	1.407	41.06	0.62
6	0.469	10.27	0.31
5	0.156	2.57	0.15
4	0.052	0.64	0.08
3	0.017	0.16	0.04
2	0.006	0.04	0.02
1	0.002	0.01	0.01
0	0	0	0

PBI in-house IL-6 Matrix Controls

QC level	Aushon		MSD		Erenna	
	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV
QC Low	0.25	1.8	0.09	29.0	0.12	4.9
QC Med	6.44	5.2	5.10	7.2	4.45	14.2
QC High	63.70	4.8	50.46	16.8	77.18	63.1

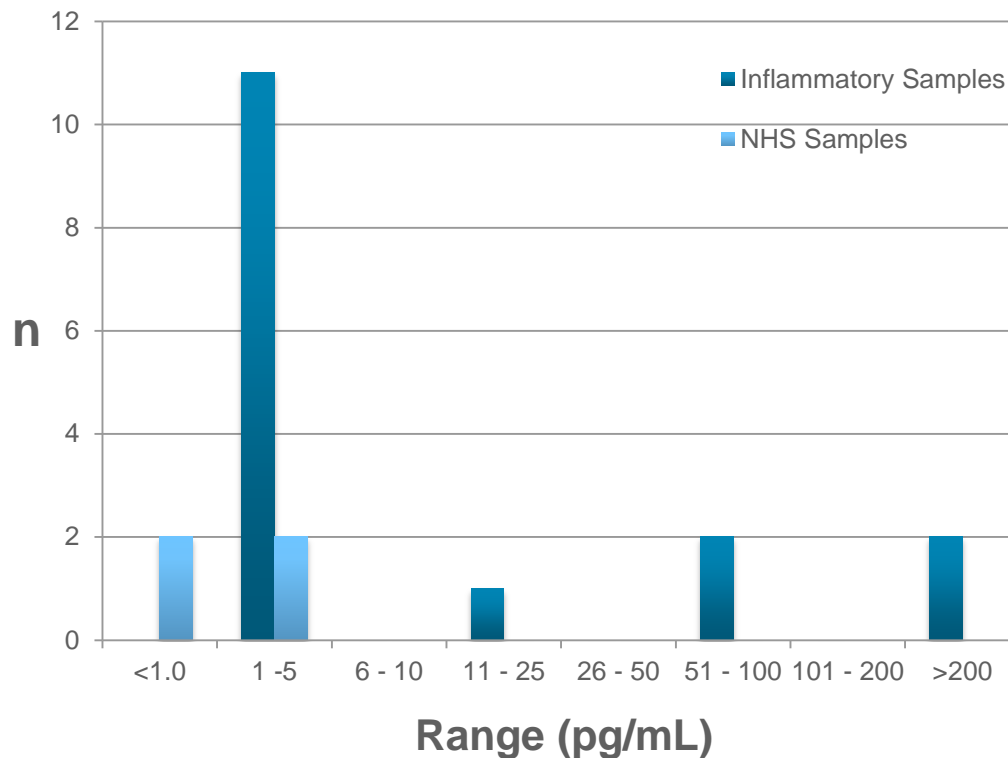
~ LLOQ region

Elevated IL-6 values in 5 of 16 inflammatory samples

UC = ulcerative colitis CR = Crohn's Disease PS = Psoriasis RA = Rheumatoid Arthritis

Sample ID	Aushon (pg/mL)	Erenna (pg/mL)	MSD (pg/mL)
UC 1	13.225	6.51	6.82
UC 2	1.684	1.83	1.4
UC 3	2.104	0.81	0.57
CR 1	1.065	0.52	0.43
CR 2	3.323	2.23	1.44
CR 3	1.336	1.31	0.83
PS 1	0.528	0.37	0.36
PS 2	1.473	1.6	1.13
PS 3	2.320	1.14	0.84
RA 1	2.702	2.67	1.94
RA 2	2.745	1.40	0.82
RA 3	1.551	1.28	2.52
Sepsis 5160	77.488	124.17	41.68
Sepsis Pool 1	614.915	>50	261.59
Sepsis Pool 2	86.788	80.99	39.87
Sepsis Pool 3	300.481	>50	116.64
NHS 1	0.848	0.49	0.42
NHS 2	3.338	2.78	1.87
NHS 3	2.087	2.58	1.01
NHS 4	2.764	2.46	1.76

Distribution of Ciraplex ULTRA IL-6 levels in Serum Samples

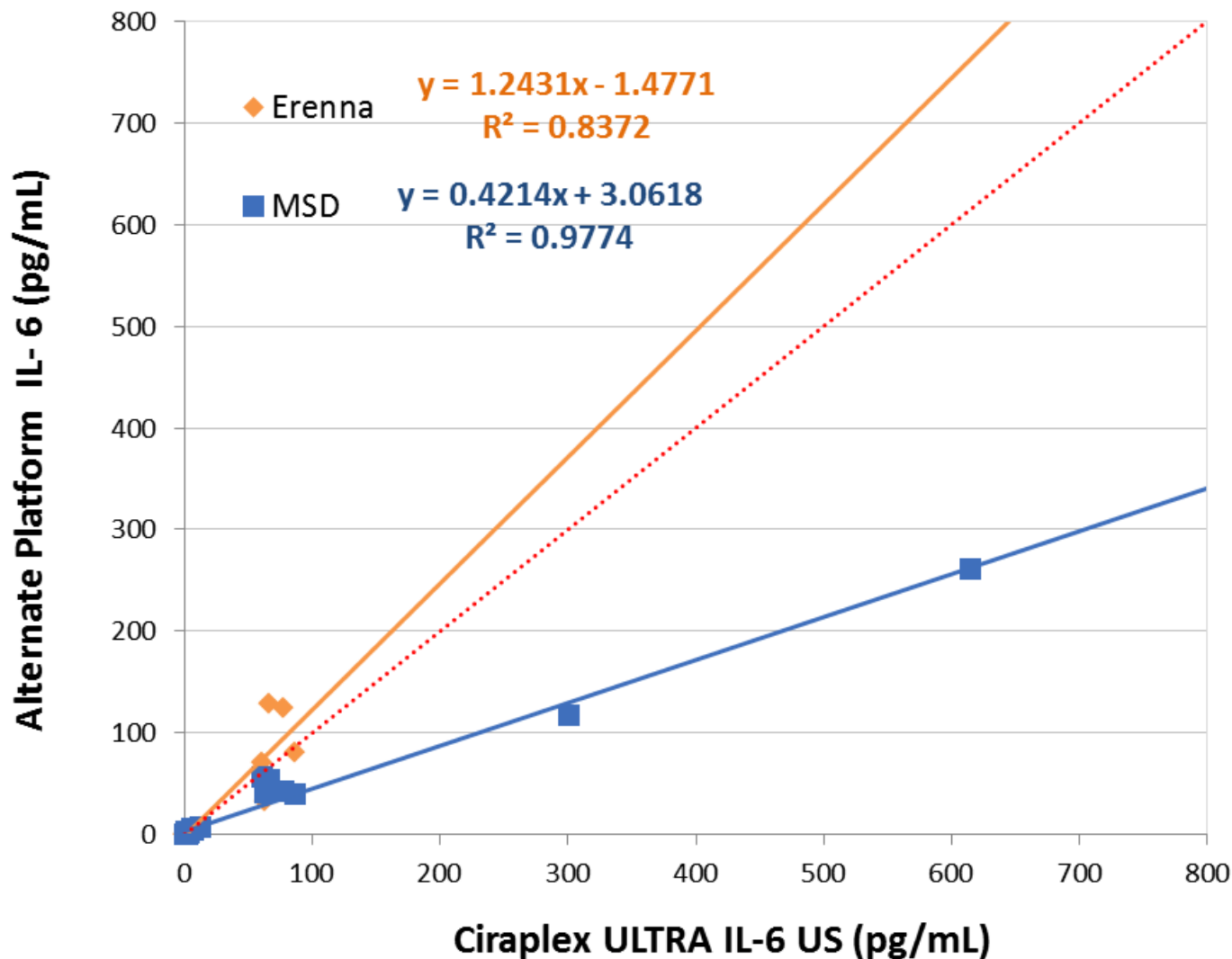


QCL = 0.25 QCM = 6.50 QCM = 65.00

>ULOQ %CV >20% <LLOQ

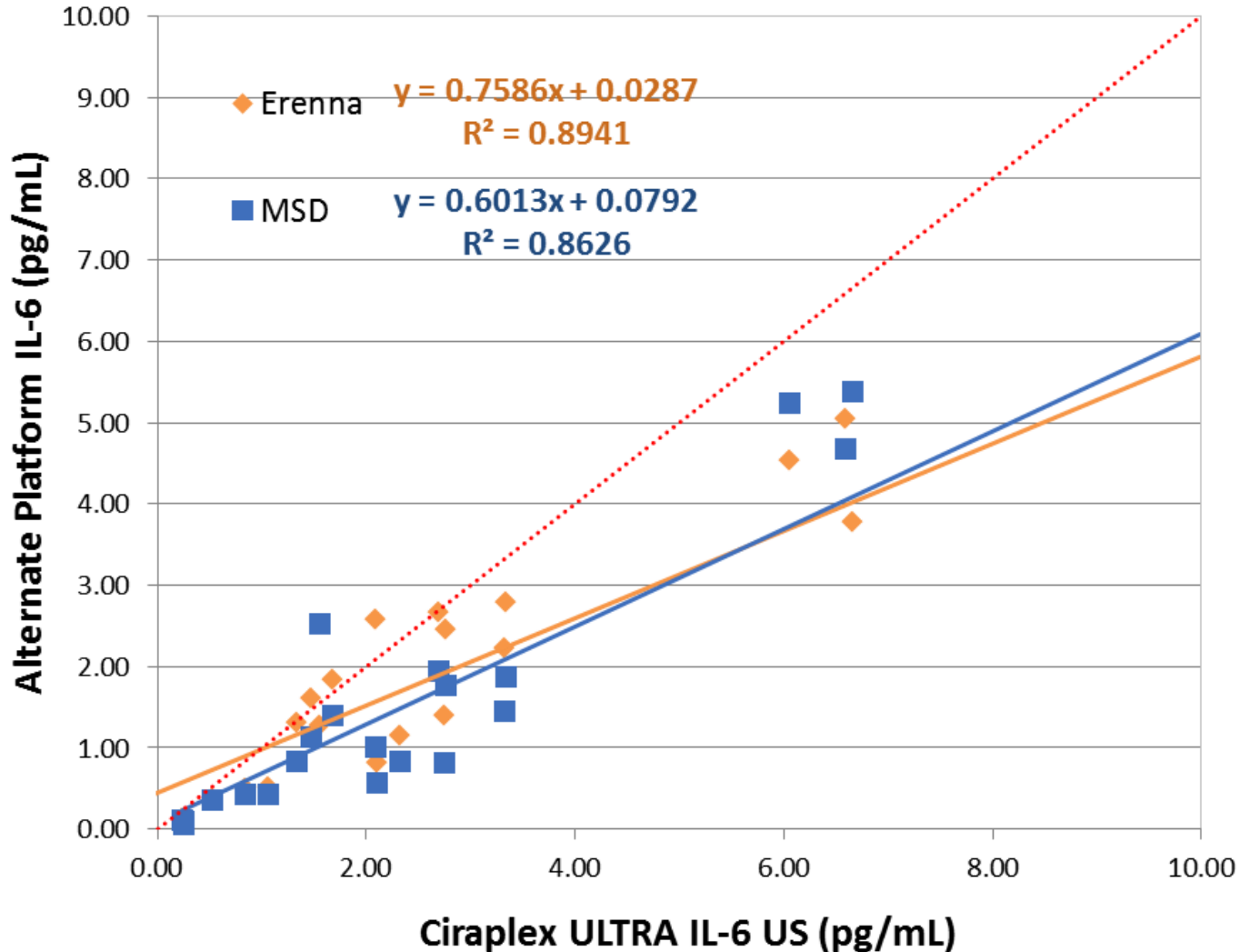
Platform Correlation Analysis - Total

Better correlation with MSD ; better value agreement with Erenna although some values > Erenna ULOQ



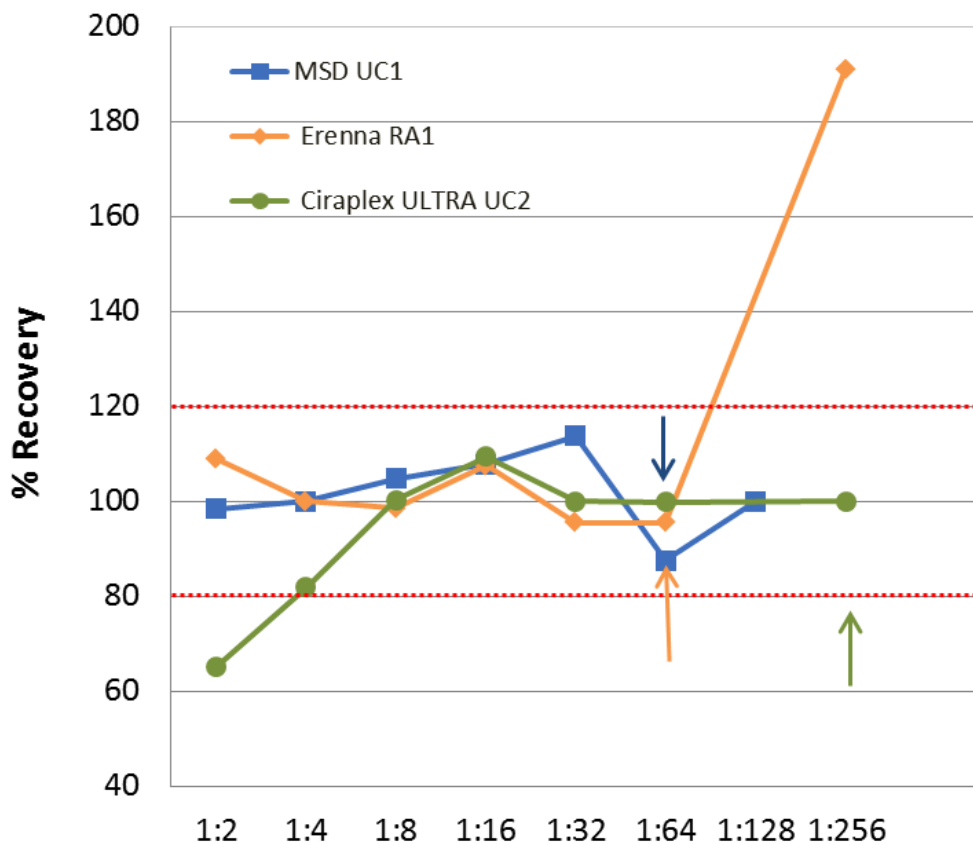
Platform Correlation Analysis (< 10 pg/mL)

MSD and Erenna correlate with each other and have 25-40% lower values than Ciraplex ULTRA

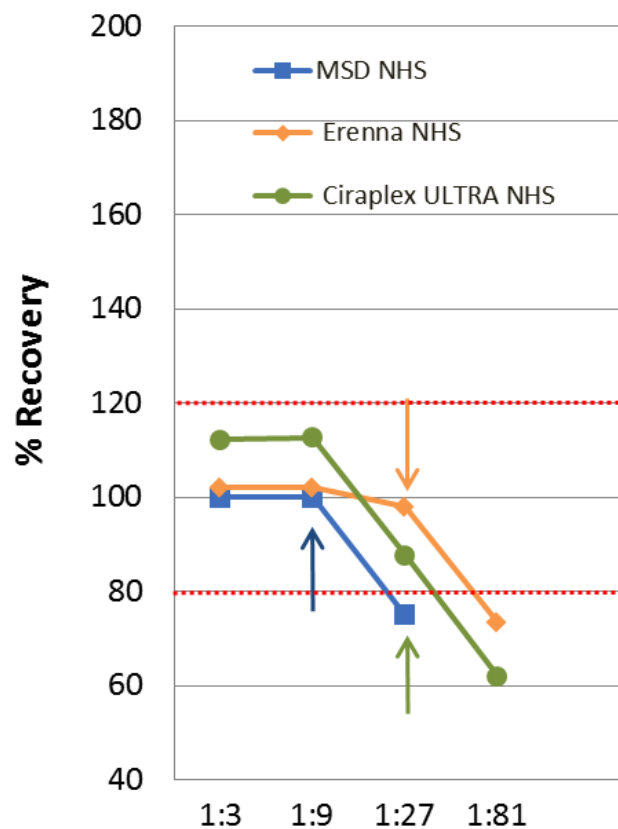


All three platforms exhibit good Parallelism to the LLOQ

Diseased Serum Samples



Normal Serum Samples



Arrow indicates value nearest LLOQ

Functional Sensitivity – LLOQ Comparison

Ciraplex ULTRA IL-6 US exhibits best IL-6 sensitivity

MSD V-plex (IL-6 pg/mL)					
Sample	Rep 1	Rep 2	Rep 3	Mean	%CV
LLOQ 1	0.32	0.33		0.33	2.2
LLOQ 2	0.17	0.19		0.18	7.9
LLOQ 3	0.08	0.08	0.13	0.10	29.9
LLOQ 4	0.03	0.04	0.06	0.04	35.3
LLOQ 5	0.01	0.01	0.06	0.03	108.3
LLOQ 6	0.01	0.01	0.04	0.02	86.6

LLOQ = **0.086** pg/mL

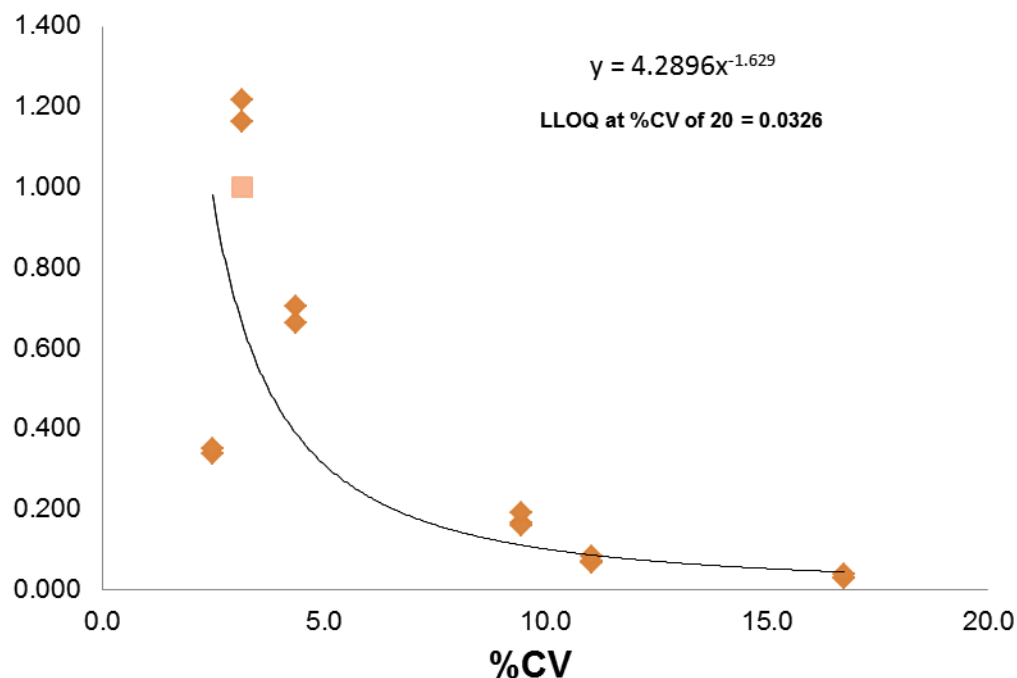
Erenna IL-6 (pg/mL)					
Sample	Rep 1	Rep 2	Rep 3	Mean	%CV
LLOQ 1	0.48	0.42		0.45	9.43
LLOQ 2	0.21	0.19		0.20	7.07
LLOQ 3	0.13	0.10	0.18	0.14	29.57
LLOQ 4	0.08	0.05	0.15	0.09	54.98
LLOQ 5	0.04	0.03	0.05	0.04	25.00
LLOQ 6	0.02	0.02	0.02	0.02	0.00

LLOQ = **0.124** pg/mL

Ciraplex ULTRA IL-6 US (pg/mL)					
Sample	Rep 1	Rep 2	Rep 3	Mean	%CV
LLOQ 1	1.217	1.164		1.191	3.1
LLOQ 2	0.703	0.661		0.682	4.4
LLOQ 3	0.337	0.349		0.343	2.5
LLOQ 4	0.166	0.159	0.190	0.172	9.5
LLOQ 5	0.068	0.070	0.083	0.074	11.1
LLOQ 6	0.028	0.028	0.037	0.031	16.8

LLOQ = **0.0326** pg/mL

Ciraplex Ultra IL-6 US Graph



Platform Comparison Summary

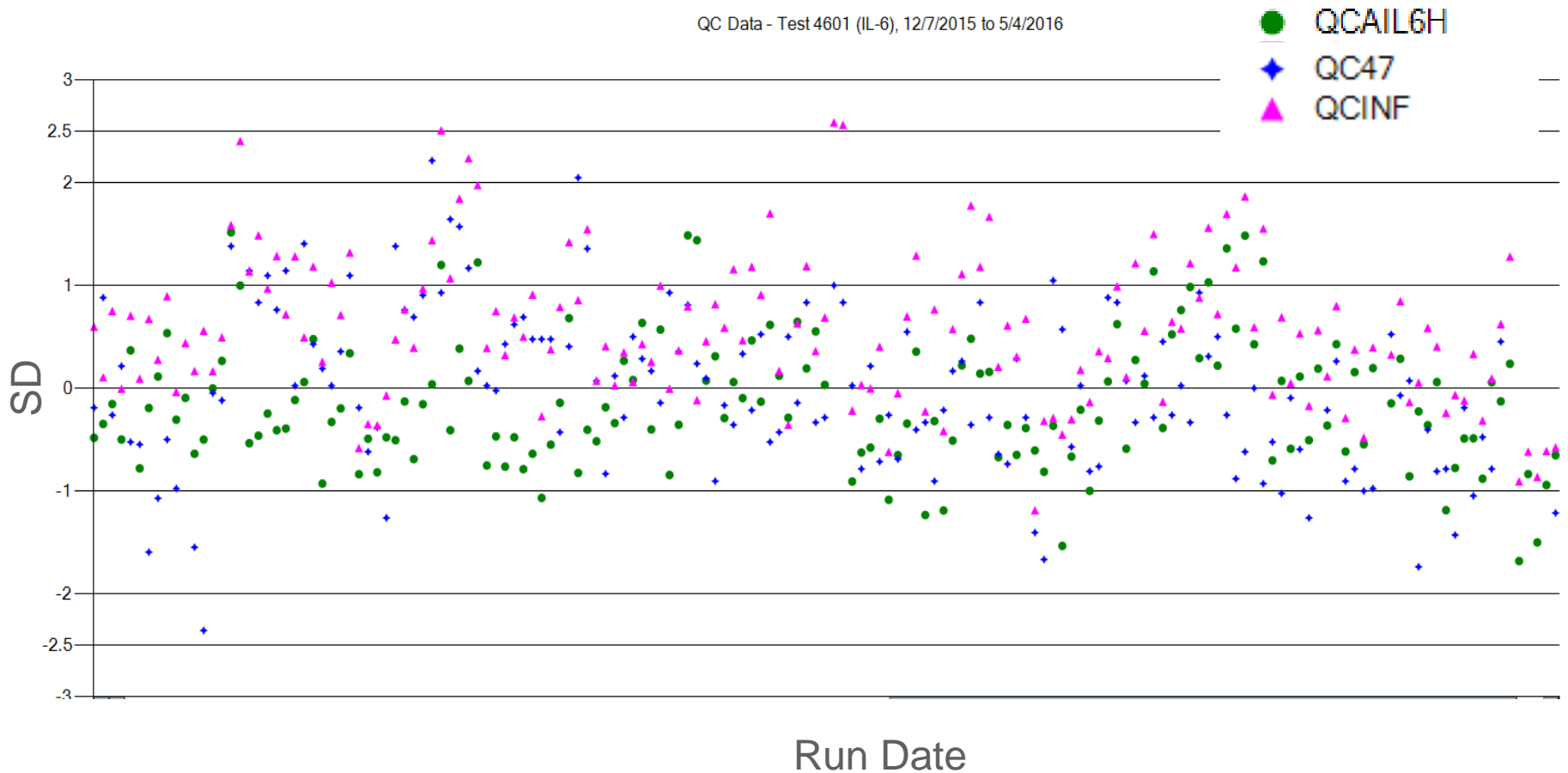
- Alternative to classic R&D systems ELISA?
 - Ciraplex ULTRA and MSD V-plex cover several logs of IL-6 values
 - Erenna issues with duplicate CV failures, low throughput, and high kit costs makes it a less attractive
- Ciraplex ULTRA IL-6 US values
 - 40 and 60% higher than MSD V-plex when IL-6 > 100 pg/mL and < 10 pg/mL, respectively
 - 25% higher than Erenna platform
- Parallelism
 - Parallelism acceptable to LLOQ for NHS and inflammatory samples for all platforms
 - Ciraplex ULTRA parallelism at 1:4 MRD is better than 1:2
- Accuracy – Spike Recovery
 - Recovery better at low concentrations for Ciraplex ULTRA while Erenna and MSD V-plex yields better recoveries at higher concentrations
- Sensitivity
 - Ciraplex ULTRA IL-6 US has best sensitivity with LLOQ @ 0.016 – 0.03 pg/mL
 - MSD LLOQ better than listed in kit insert; close to ~ 0.1 pg/mL, but below Ciraplex low QC

Ciraplex ULTRA IL-6 Performance in Clinical Studies

Ciraplex ULTRA IL-6 US performance range reduces repeat rate for clinical trial studies

- **Clinical Studies**
 - *Over 2500 samples*
 - *Collected over a year span*
 - *As many as 24 plates (~750 samples) analyzed per day*
- **IL-6 observed values**
 - Range from 0.246 – 376.384 pg/mL
 - Detects changes in patients over 2-3 logs
 - > ULOQ (228 pg/mL); N = 1
 - < LLOQ (0.016 pg/ml); N = 2
 - > 10 pg/mL; N = 324
 - Mean IL-6 value = 4.832; Median IL-6 values = 2.848

Consistent Performance of the Ciraplex ULTRA IL-6 US over time and across clinical studies



Ciraplex ULTRA IL-6 US is the right choice for clinical trial studies

- Practical Considerations
 - Price is right
 - Short incubation times
 - Small sample volume
 - Amenable to automation, so high throughput
- Performance
 - Excellent sensitivity - able to detect 100% normal samples and levels below normal
 - Range is high enough to detect most inflammatory samples except maybe sepsis patients.
 - Can detect changes in IL-6 values in patients over several logs
 - Recovery is good where it counts; at the low end
 - 1:4 MRD may improve performance even more
- Kits do not include assay controls which may be an issue for some labs

Acknowledgements

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Any Questions?

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