The MULTIple trials of generating a SINGLE data set

Taking biomarker data through the Clinical Stages

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Presentation Summary

Study investigating the utility and comparability of multiplex and singleplex assays

What are the major analytical differences and considerations?

Do we get the same results from a multiplex and singleplex assay?

Can we use multiplex and singleplex assays seamlessly through the drug development process to measure the same biomarker?

Our historical perspective

Singleplex

- Target engagement/efficacy/ patient stratification
- More confidence, less complexity
- Easier to outsource, validation simple
- Fits with remit: Fewer markers with strong hypothesis

Multiplex

- Translational/exploratory biomarker selection
- Screening/Fishing tool
- Minimal assay qualification
- Cost and complexity prohibitive

The Multiplex shift

Recent experience with multiplex

Request to measure 5 markers in human plasma

- 4 weeks to complete
- Chose multiplex analysis due to time and budget constraints
- MSD V -Plex Pro-inflammatory panel
- Designed and performed tailored package of work
  - Focus on assay precision and limit of quantification

Requirement for validated IL-8 and TNF-Alpha assays

- V-Plex IL-8 assay validated in human serum
- Cross validation of V-Plex TNF-Alpha assay vs. original high sensitivity assay (plasma/sputum)
- Validation according to internal SOP
  - P&A with matrix QCs, LLOQ, Matrix recovery...

✓ **IL-8 & TNF-α both on the 5-plex plate**

✓**Spare plates available**

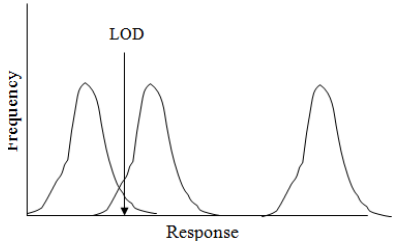
✓**Opportunity to directly compare assays**

What was missing?

What are the key features that may differ between the formats?

Sensitivity/LOD

Differentiation of low concentration analyte from background

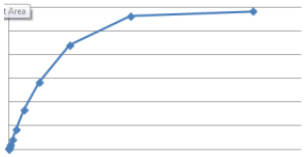


Interference

From other matrix components but also other analyte spots in the well

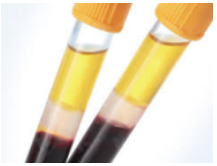
Hook Effect

Is this more prevalent in multiplex assays?



Sample values

The <u>ULTIMATE</u> question:
Do we get the same results for 'real' samples?



Experimental design

- **3 batch study**
  - Each batch run on the 3 assay types on the same day by the same analyst

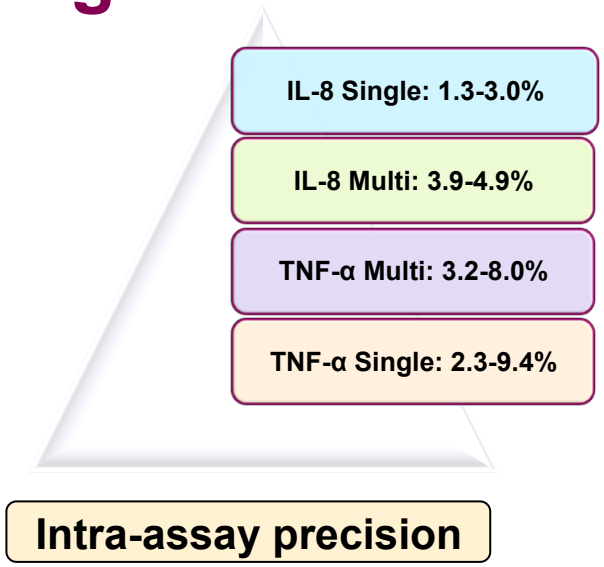
IL-8 singleplex **TNF-α singleplex** **Multiplex 5-plex**

- Bulk sample preparation to minimise pre-analytical variation
- All antibodies added for multiplex plate (5 analytes in total)

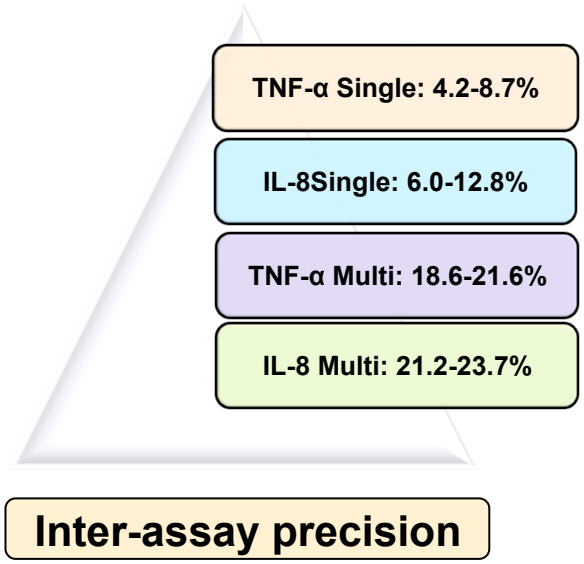
Calibration standards	• Singleplex kit standards (Multi Analyte) • Multiplex kit standard (Multi Analyte) • Alternative source standards (Single Analyte)
Hook Effect	• Up to 50,000 pg/mL (Approx. 100xULOQ) in assay diluent
Sensitivity/Limit of detection	• Diluent vs. HV Serum vs. HV Sputum • n=26 wells of each in same plate
Interference	• (8x) HV Serum + 50 IU/Ml Rheumatoid Factor • (8x) HV Serum + 1000 pg/mL Interleukin-6 • (8x) HV Serum + diluent (control)
Sample Concordance	• Patient serum and LPS sputum samples • No spike addition • Diluted according to likely concentration & DTT effect

Results

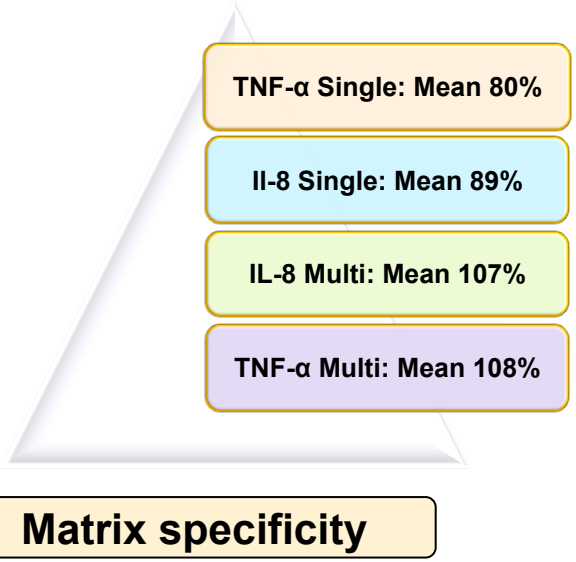
Original assessments



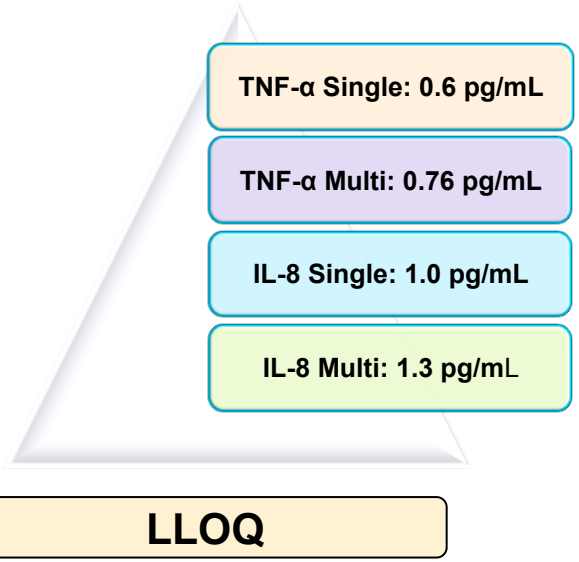
Intra-assay precision



Inter-assay precision

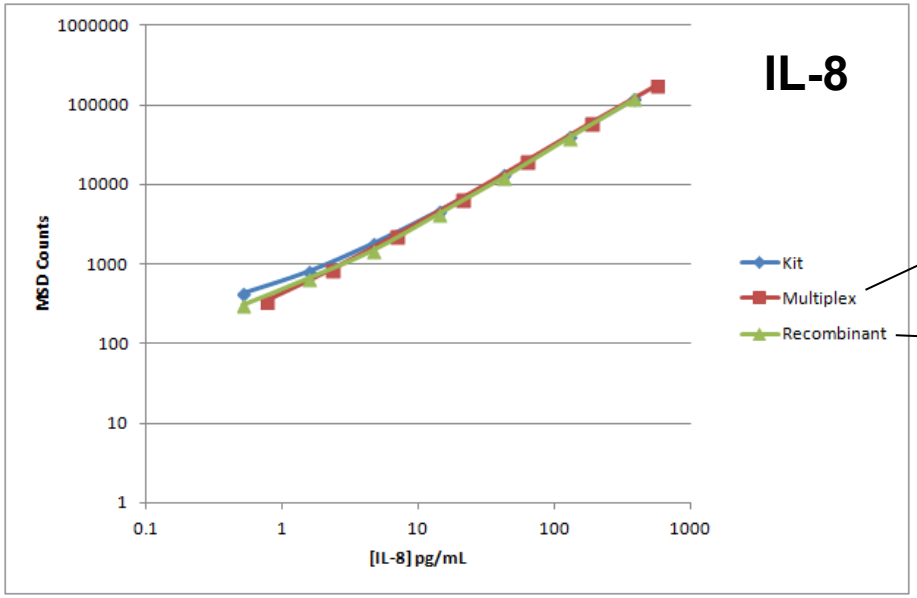


Matrix specificity



LLOQ

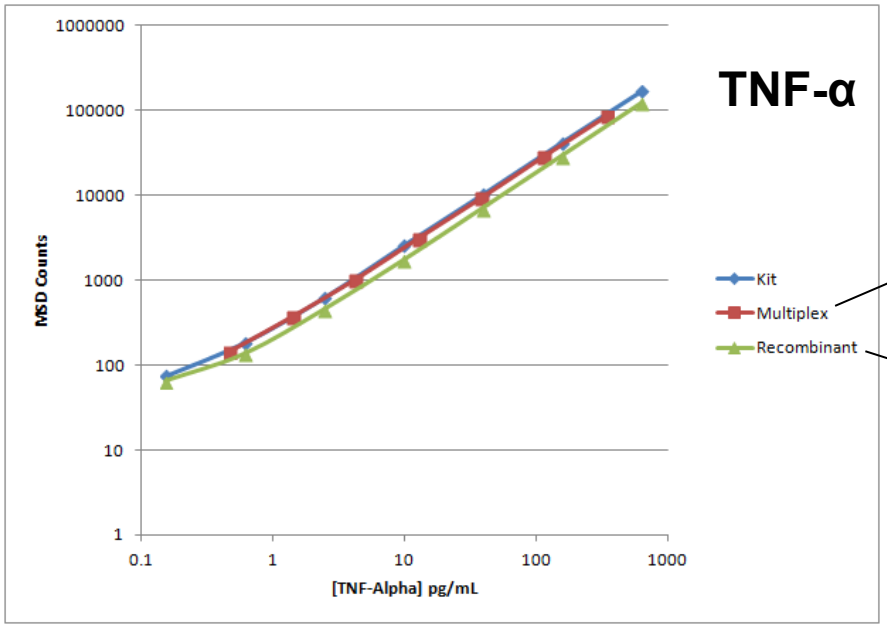
Calibration standards (Singleplex plates)



95% concordance

94% concordance

Multiplex kit: 99% concordance



95% concordance

72% concordance

Multiplex kit: 73% concordance

Interference testing (HV serum)

IL-8

Sample	Control (Single)	Control (Multi)
1	50.7	56.8
2	101	103
3	217	223
4	3.2	3.98
5	5.42	6.54
6	68	81.5
7	17.5	20.1
8	5.71	6.24

+ IL-6 (1000 pg/mL)

Recovery (Single)	Recovery (Multi)
98	104
97	101
104	100
97	115
108	117
100	89
102	102
81	91
Mean recovery	
98.4	102.4

+ RF (50 IU/mL)

Recovery (Single)	Recovery (Multi)
103	107
98	100
109	109
101	102
106	110
100	87
107	100
82	89
Mean recovery	
100.8	100.5

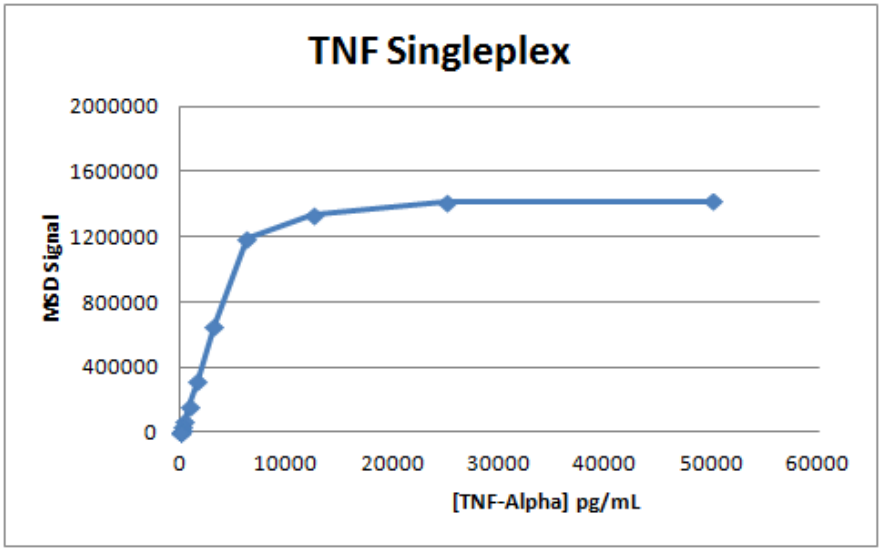
TNF-α

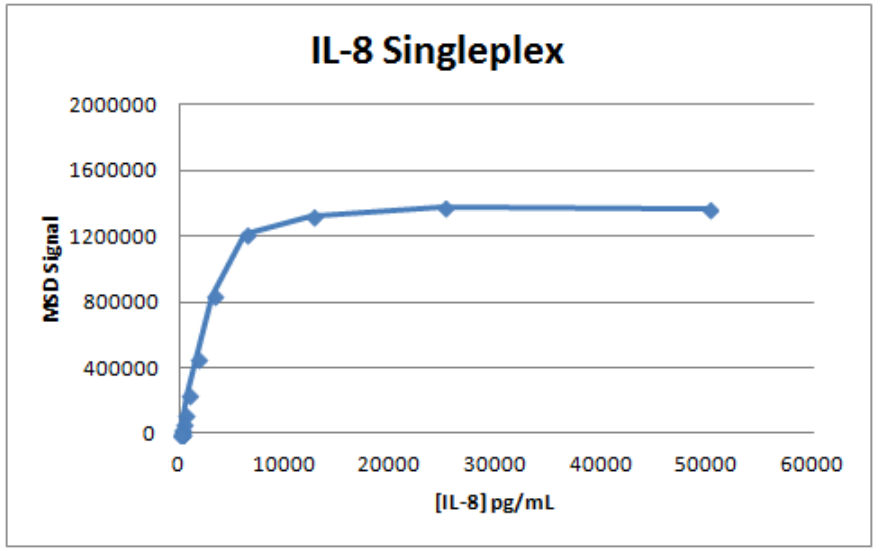
Sample	Control (Single)	Control (Multi)
1	0.326	0.431
2	0.4	0.533
3	0.405	0.383
4	0.732	0.743
5	0.976	1.14
6	1.76	2.05
7	0.74	0.714
8	1.18	1.36

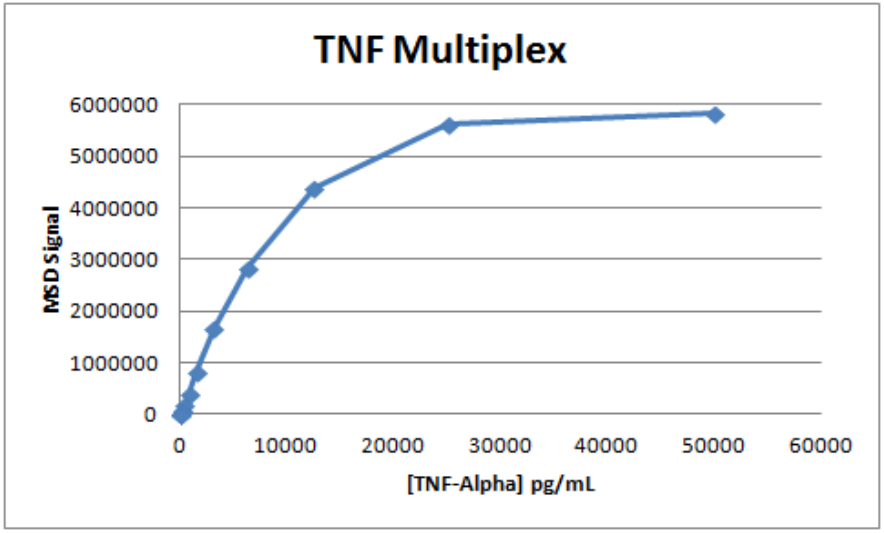
Recovery (Single)	Recovery (Multi)
108	111
125	123
79	108
107	107
113	110
93	95
98	126
110	107
Mean recovery	
104.1	110.9

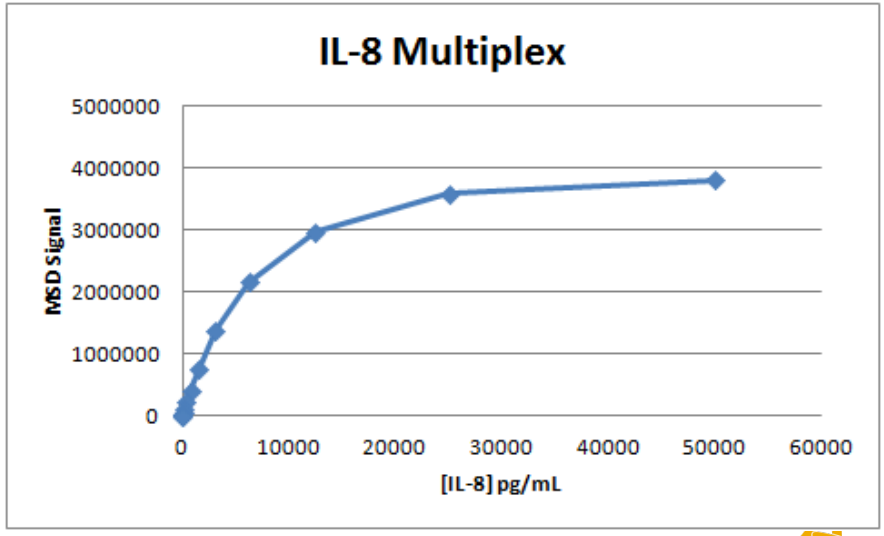
Recovery (Single)	Recovery (Multi)
1215	935
980	583
1173	1099
533	431
504	427
307	268
512	507
381	319
Mean recovery	
700.6	571.1

Hook Effect

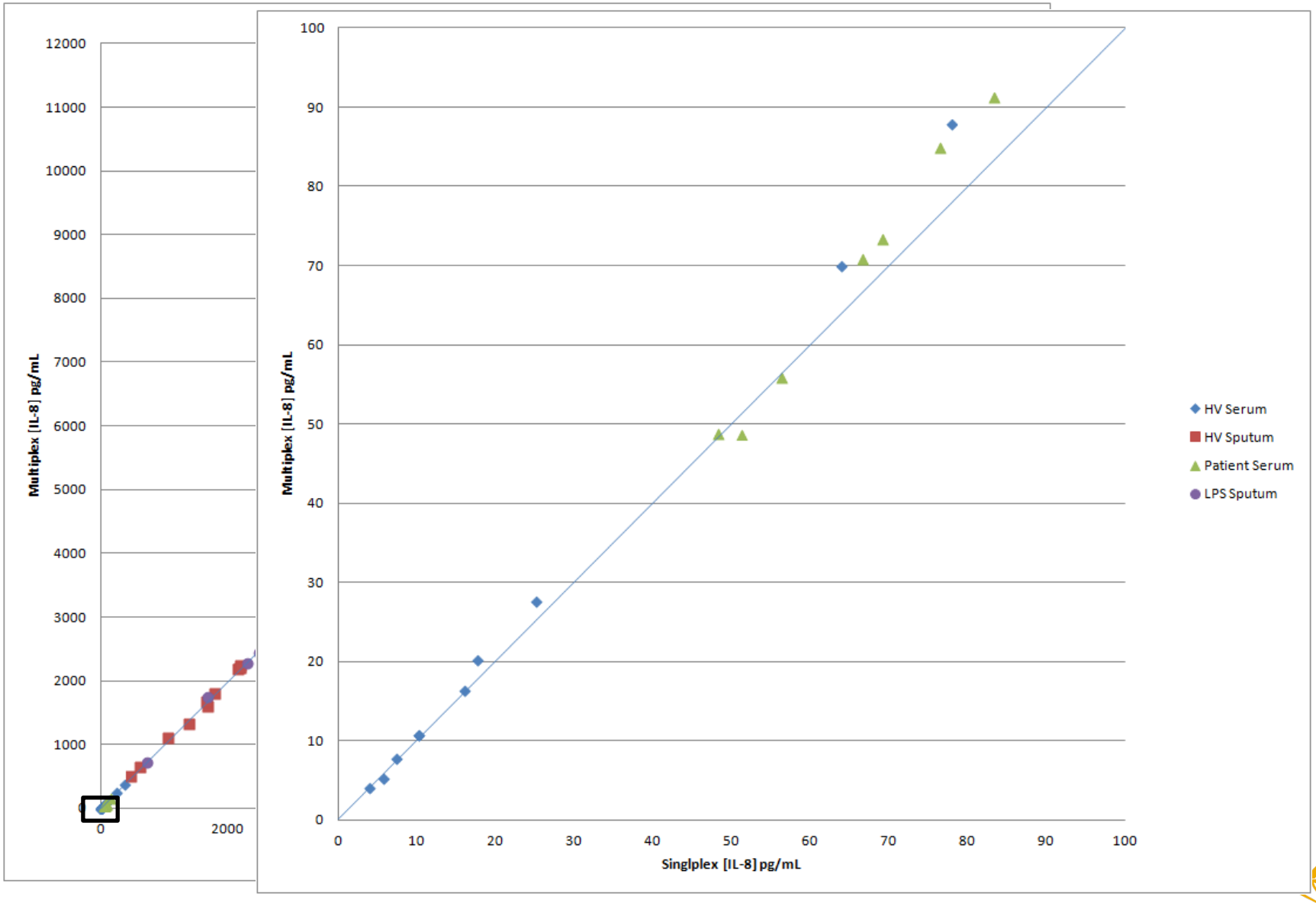

TNF Singleplex
MSD Signal
2000000
1600000
1200000
800000
400000
0
0
10000
20000
30000
40000
50000
60000
[TNF-Alpha] pg/mL


IL-8 Singleplex
MSD Signal
2000000
1600000
1200000
800000
400000
0
0
10000
20000
30000
40000
50000
60000
[IL-8] pg/mL

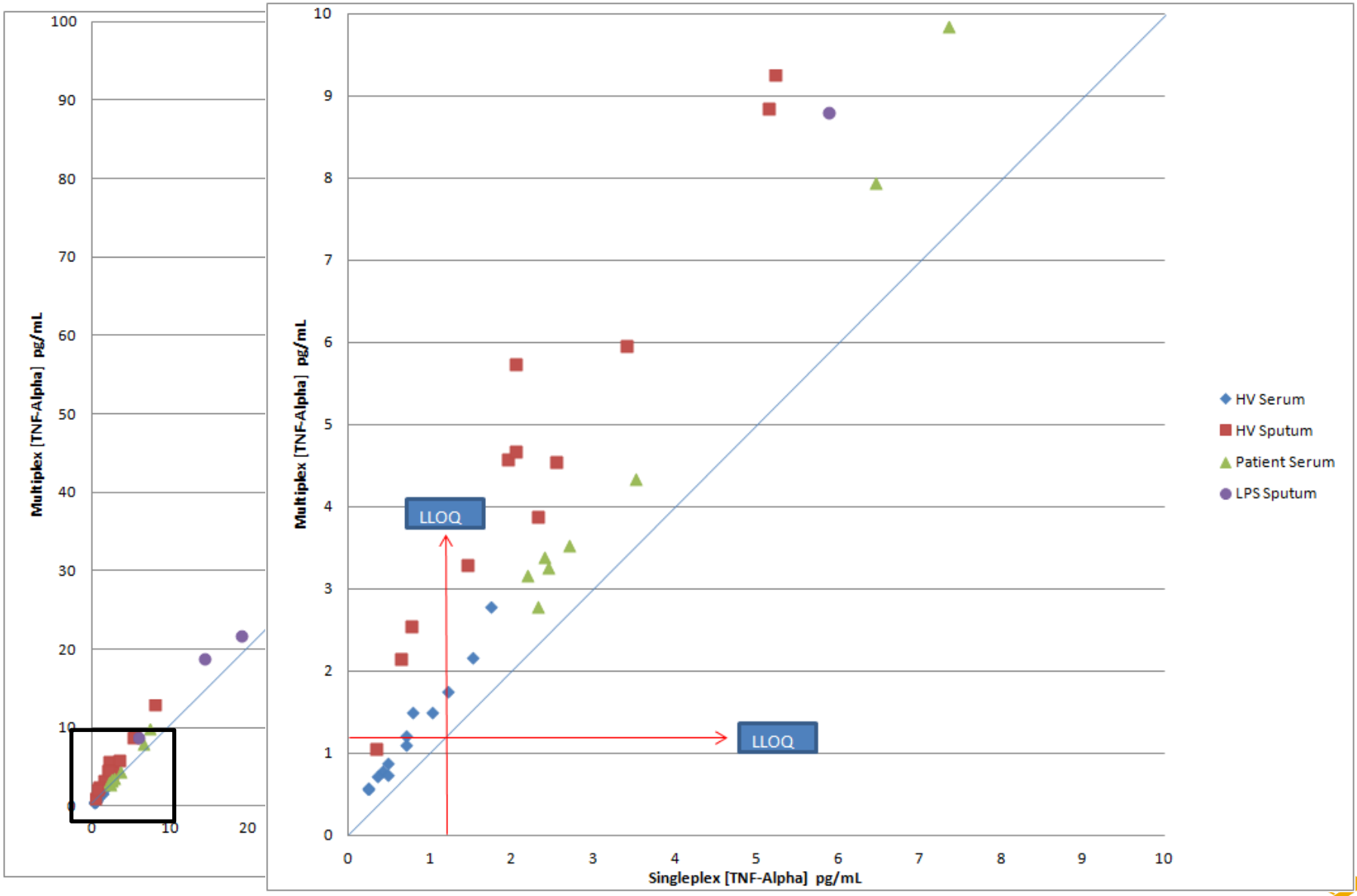

TNF Multiplex
MSD Signal
6000000
5000000
4000000
3000000
2000000
1000000
0
0
10000
20000
30000
40000
50000
60000
[TNF-Alpha] pg/mL


IL-8 Multiplex
MSD Signal
5000000
4000000
3000000
2000000
1000000
0
0
10000
20000
30000
40000
50000
60000
[IL-8] pg/mL

Sample concordance: IL-8

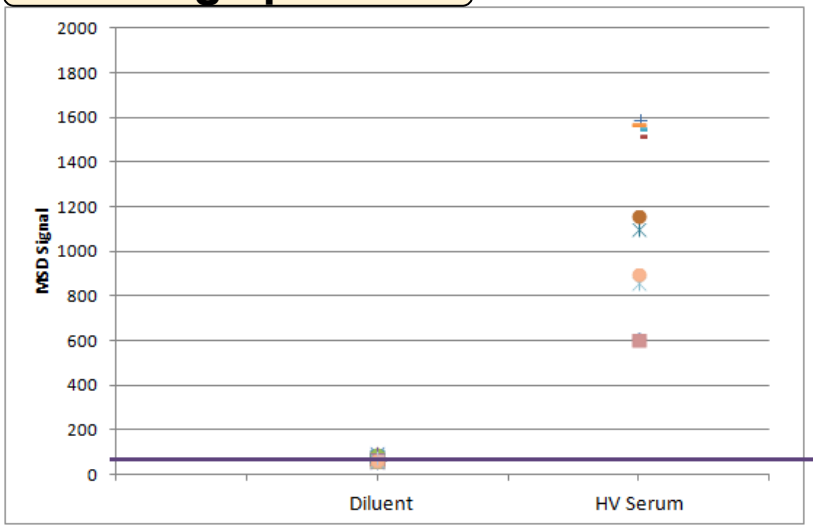


Sample concordance: TNF-Alpha



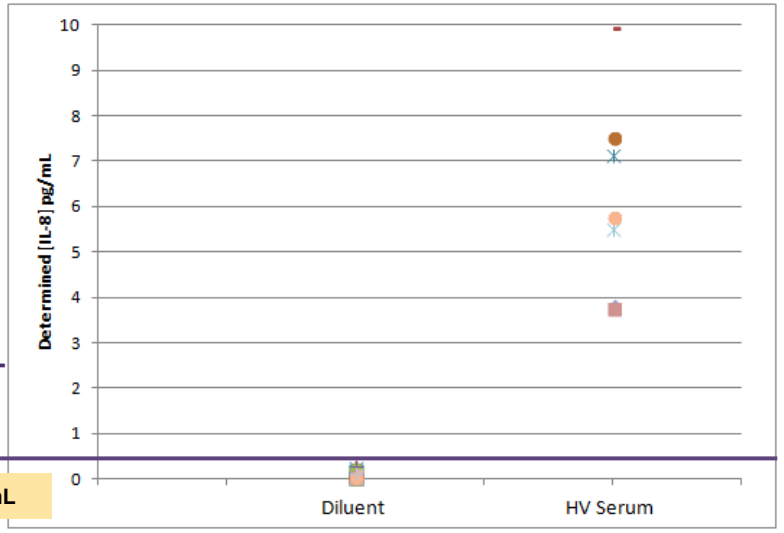
Sensitivity/Limits of detection: IL-8

Singleplex



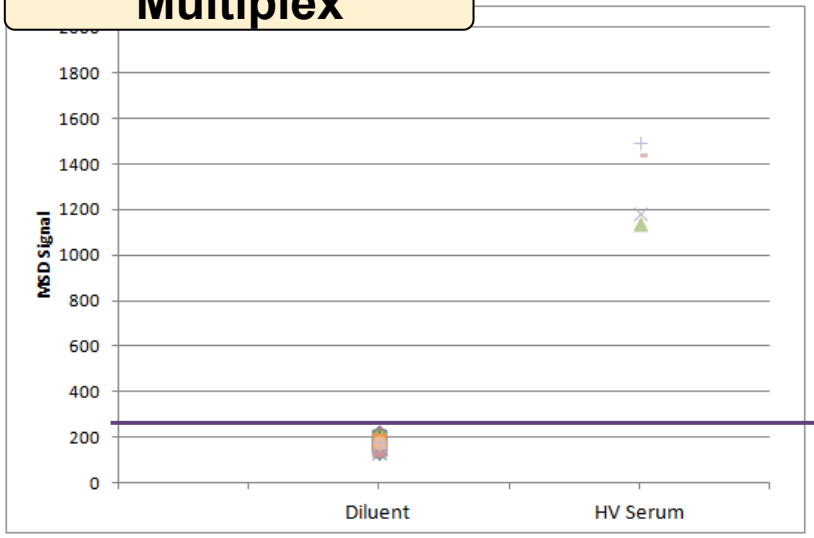
Diluent Mean + 2SD

96.4



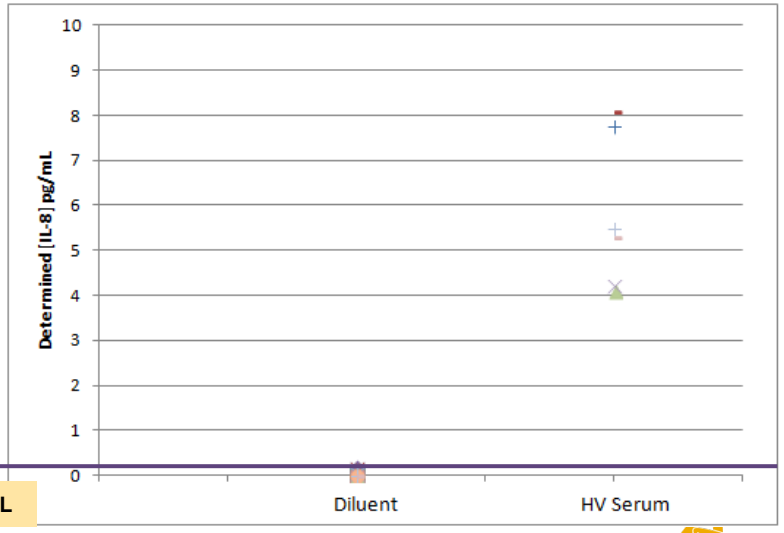
0.27 pg/mL

Multiplex



Diluent Mean + 2SD

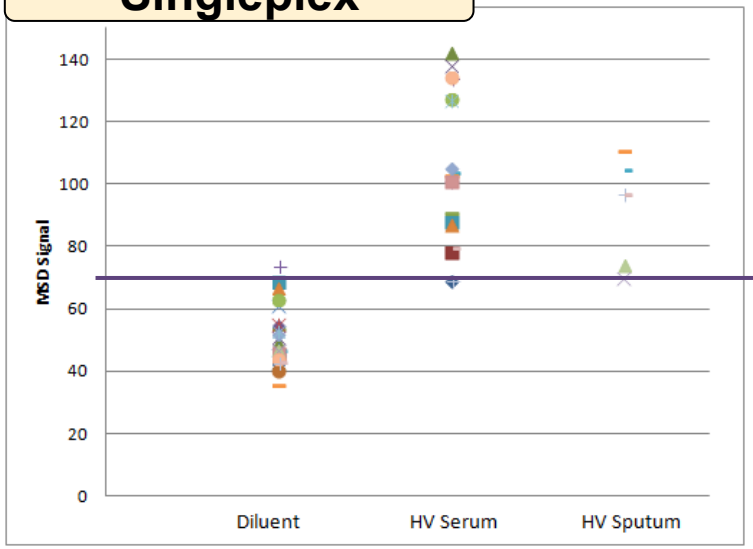
229



0.204 pg/mL

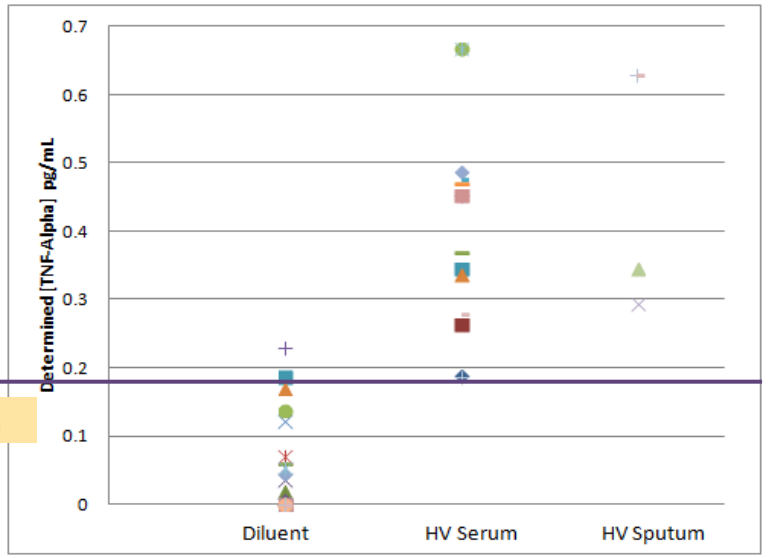
Sensitivity/Limits of detection: TNF-Alpha

Singleplex



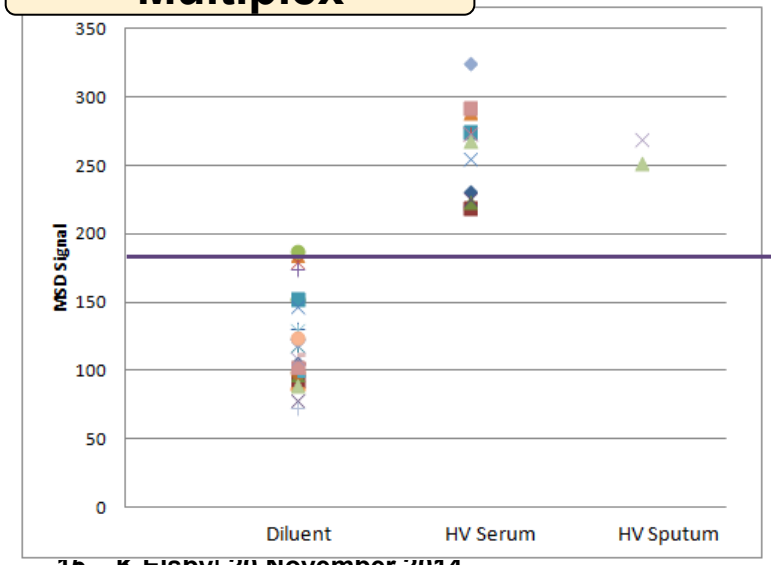
69.4

Diluent Mean + 2SD



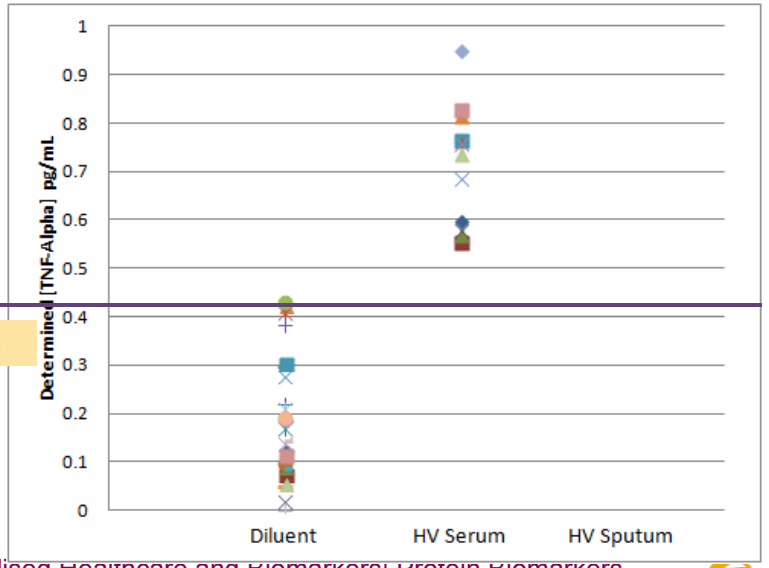
0.18 pg/mL

Multiplex



188

Diluent Mean + 2SD



0.44 pg/mL

Discussion

What are the major analytical challenges and considerations?

- Complexity of assessing interference from numerous sources
- Consider ranges for all analytes and proximity to LLOQ
- Understand the precision requirements of the assay and impact if different results are generated from multiplex to singleplex
- Need to discriminate the validation carried out versus intended use e.g. BM fishing, Informative in clinic or Decision making in clinic

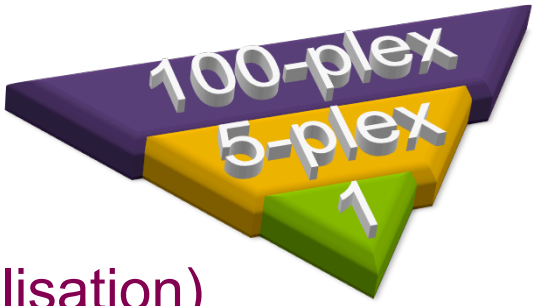
Do we get the same results from a multiplex and singleplex assay?

- *Yes*: Sensitivity, interference, calibration standards, mid/high concentration analytes (IL-8)
- *No*: Low concentration analytes (TNF-Alpha), small differences magnified at the limits (Cal std/DTT effect?)

Can we use multiplex and singleplex assays seamlessly through the drug development process to measure the same biomarker?

- Yes but need to understand where data can or cannot be compared (continuity)
- Clear strategy around normalisation of data/assays if required

Thoughts for the future...



- Can we make the transition easier?
  - Use of same platform
  - Use of same calibration standards (normalisation)

- Do we even need to move to singleplex? Do multiplex assays have a utility in later phase work?
  - Potential for exploratory markers
  - Could small (2-plex) assays offer advantages (time, cost, sample volume)

- Assay validation/qualification:
  - What factors need extra consideration?
  - Is there a basic minimum package of work?.... EBF TT-49

Acknowledgements

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ANY QUESTIONS?