F·star THERAPEUTICS an invoX company

A Direct Assay Comparison Between the MSD V-PLEX® and S-PLEX® Proinflammatory Panel 1

Does improved sensitivity equal an improved assay?

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Why Did We Want to Perform This Comparison?

- F-star Therapeutics is a clinical stage biotechnology company developing tetravalent bispecific antibodies in the fight against cancer.
- To support biomarker discovery, an exploratory biomarker study was planned to identify cytokines that may be affected in patients dosed with bispecific antibodies.

- MSD offers the “proinflammatory panel 1” which measures up to 10 human cytokines that are important in inflammatory responses, immune system regulation and are implicated in several disorders including cancer.
- The proinflammatory panel 1 is provided on the V-PLEX® and S-PLEX® platforms but which is most appropriate?

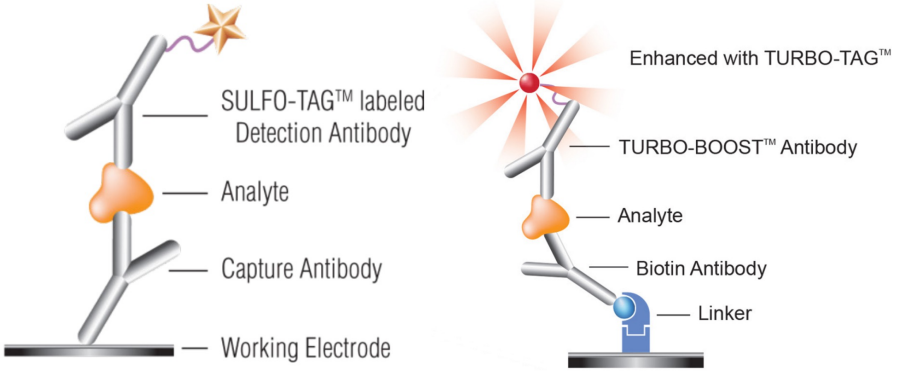
The aims of the investigation were to:

1. Reveal possible limits of quantification for both kits in F-star labs
2. Assess kit suitability by analyzing samples from healthy and key oncology indications
3. Identify whether the data generated are comparable between kits

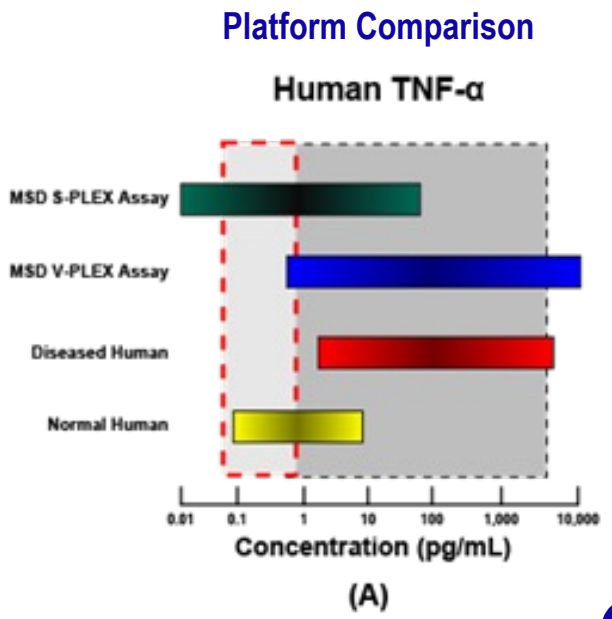
- This purpose of this presentation is to look at performance of each kit as a whole and will not break down the details of each individual analyte.

Platform Comparison

- The V-PLEX panel offers a validated platform with proven specificity providing “guaranteed limits of **quantification**”
- The S-PLEX panel offers an ultrasensitive platform that reduces “the lower limit of **detection** by 10- to 1000-fold”



V-PLEX Proinflammatory panel 1	S-PLEX Proinflammatory panel 1
2-Step Method (Analyte-Detection)	5-Step Method (Capture, Block+Analyte, TURBO-BOOST, Enhance, Detection)
Pre-coated Plates	Plates coated manually
Sub- to low pg/mL detection limit	fg/mL detection limit
10 Analytes	9 Analytes



Intra- and Inter- Assay Precision and Relative Accuracy

- Standard curves were prepared following the recommended procedure (7-point curve, 4-fold serial dilution) and run on two occasions.
- The precision (%CV) and relative accuracy (%RE) were averaged across the whole assay range for all analytes.

V-PLEX Intra-assay curve performance		
	%RE	Replicate %CV
Average	100.8	4.7
Min	63.1	0.0
Max	141.7	49.4

S-PLEX Intra-assay curve performance		
	%RE	Replicate %CV
Average	100.3	13.5
Min	43.9	0.0
Max	163.9	124.8

- The precision of the recovered concentrations was calculated across the two runs and averaged across all analytes

V-PLEX Inter-assay precision	
Mean %CV	Max %CV
4.6	34.0

S-PLEX Inter-assay precision	
Mean %CV	Max %CV
10.0	69.6

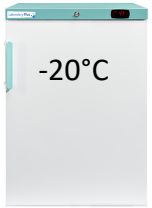
- Overall, the V-PLEX assay demonstrated superior intra- and inter-assay relative accuracy and precision on average across all analytes included in this assay range.
- Precision and relative accuracy could be improved by setting an appropriate assay range.

Potential areas for increased assay variability

Potential reasons for increased variability in the S-PLEX assay:

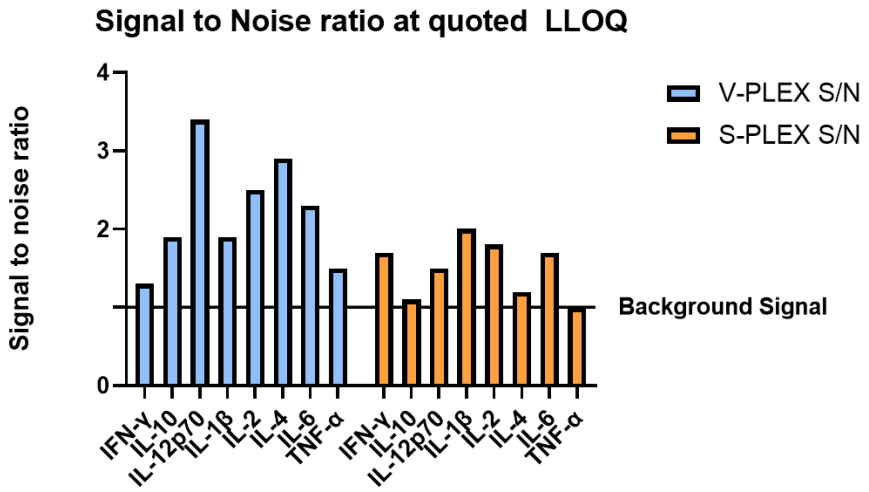
- More complex assay procedure
- More complex reagent storage and thawing process

	V-PLEX	S-PLEX
Assay Procedure	1. Sample addition (2Hr) 2. Detection Addition (2Hr)	1. Coat plate (1Hr) 2. Block solution + Analyte addition (1.5Hr) 3. Detection Addition (1Hr) 4. Enhance Solution (30Min) 5. Detect Solution (1 Hr)
Reagent Storage	-20°C / +4°C / RT	**-80°C** / -20°C / +4°C / RT
Reagent Thawing	RT / 24°C Water Bath	RT / 24°C Water Bath / **Wet Ice**



Performance at quoted limits of quantification

- MSD provide a certificate of analysis, which specifies the lower limit of quantification (LLOQ) for each analyte in that kit.
- The Signal to Noise (S/N) ratio was calculated at each quoted LLOQ.
- The V-PLEX assay typically produced a higher S/N ratio at these quoted limits.
- The variation in S/N over two runs was also lower on the V-PLEX platform.
- Highlights the importance of verifying the assay performance within your own lab.

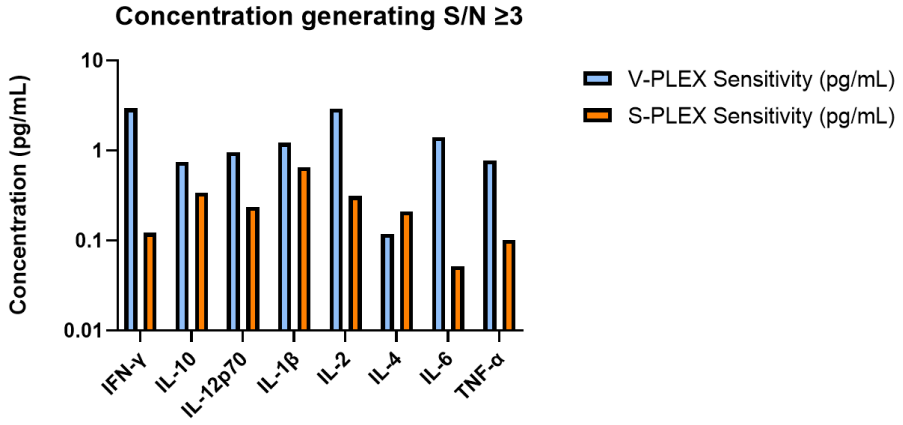


Average S/N at quoted LLOQ		
Platform	S/N at LLOQ	%CV
V-PLEX	2.50	15.4
S-PLEX	1.59	20.7

Achieved Limits of Quantification

- An extended standard curve was prepared across the original assay range but using a 2-fold serial dilution.
- An LLOQ was assigned to the concentration generating a minimum Signal to Noise ratio (S/N) of 3.

Achieved Sensitivity at F-star using extended Standard Curve			
Analyte	**V-PLEX Sensitivity (pg/mL)**	**S-PLEX Sensitivity (pg/mL)**	**Fold-Difference**
IFN-γ	2.97	0.123	24.1
IL-10	0.754	0.339	2.2
IL-12p70	0.961	0.237	4.1
IL-1β	1.22	0.656	1.9
IL-2	2.95	0.313	9.4
IL-4	0.119	0.211	0.6
IL-6	1.42	0.052	27.3
TNF-α	0.780	0.102	7.6



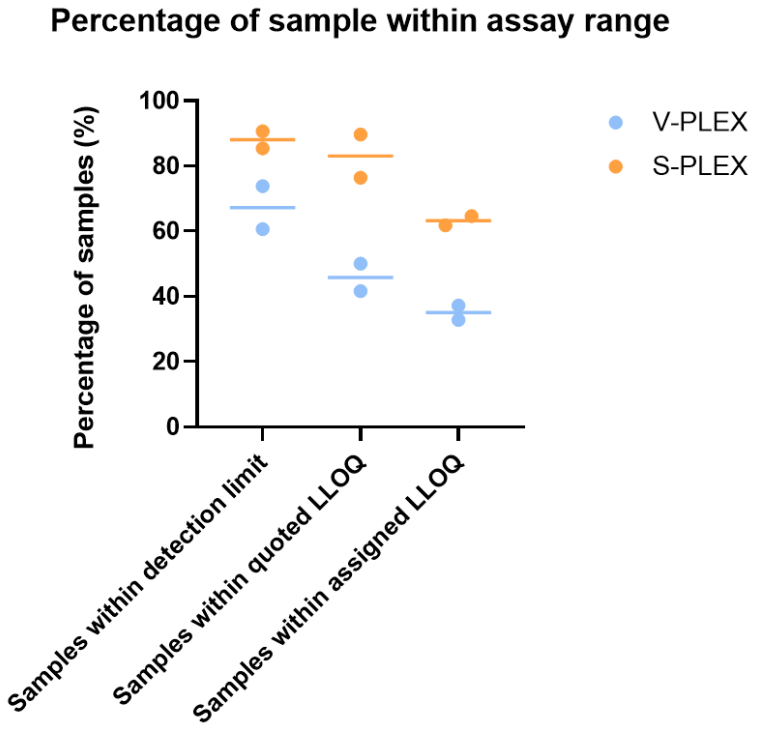
- While the S-PLEX assay platform did not meet the quoted sensitivity limits, it demonstrated up to a 27-fold increase in sensitivity over the V-PLEX assay platform.
- Dependant on what is set as the minimum S/N for reliable quantification.

Number of samples in assay range

- A panel of 31 human serum samples from healthy individuals and individuals from key oncology indications were sourced commercially.
- All samples were analysed on two occasions following MSDs recommended procedure.

	Mean % of Samples in Range	
	V-PLEX	S-PLEX
Within Detection Limit	67.2	88.0
Within Quoted LLOQ	45.8	83.0
Within Assigned LLOQ	35.0	63.2

- On both occasions, more samples were within the assigned assay range using the S-PLEX assay (63%) compared to the V-PLEX assay (35%).

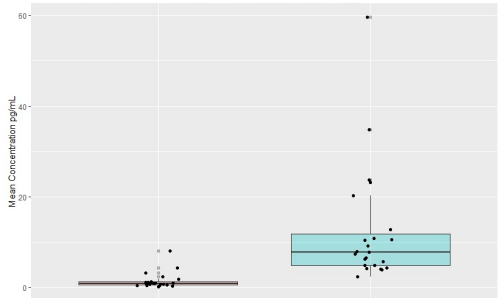


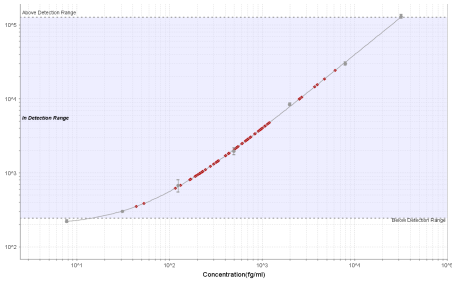
Comparability of quantified results

- Samples which were quantifiable on both kits were compared to see how they differed across kits.
- Recovered concentrations varied greatly with no clear trend between analytes.
- Differences may arise from where results fall on the calibration curve.

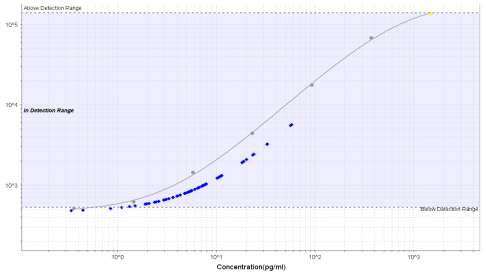
- Recovered concentrations on the V-PLEX platform are close to the lower limit of the assay.
- Recovered concentrations on the S-PLEX platform lay on the linear range of the curve.

Comparability of recovered IFN-γ concentrations





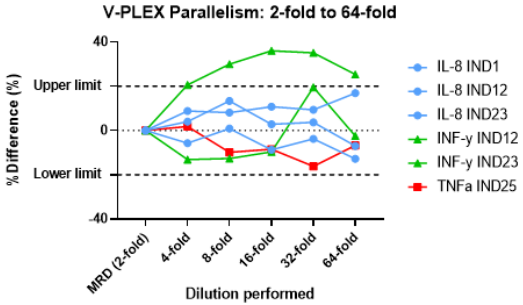
Range: 31.7 pg/mL – 0.007 pg/mL

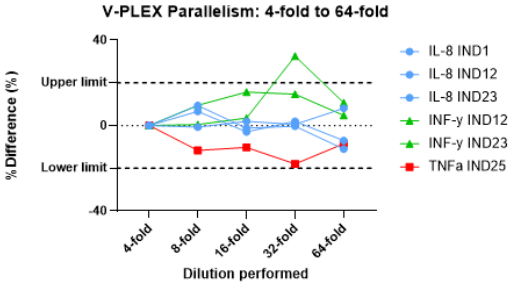


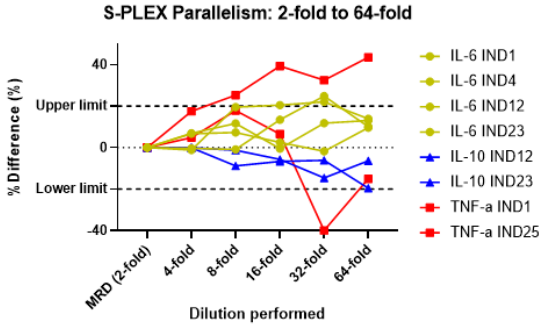
Range: 1480 pg/mL – 0.36 pg/mL

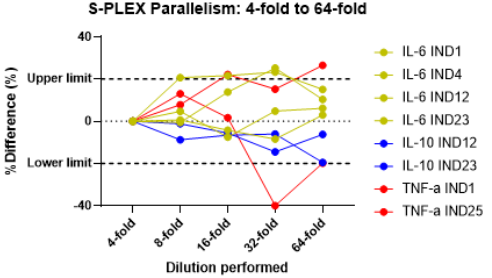
Reliability of quantified results

- Previously quantified samples were selected based on high expression of a single or multiple analytes. Samples were then diluted up to 64-fold.
- The % difference of diluted samples was calculated against the result generated at MRD (2-fold)
- Both V-PLEX and S-PLEX assays demonstrated parallelism up to a 64-fold dilution in assay diluent.
- In both kits parallelism could be improved with a minimum 4-fold dilution.









Conclusion

- The sensitivity quoted for the S-PLEX® platform could not be achieved at F-star but did provide improved sensitivity over the V-PLEX® platform.
- S-PLEX® platform was able to quantify up to 28% more results when analysing a panel of 31 human serum samples.
- While the recovered concentrations between platforms varied, the parallelism assessment did show reliability of the results against the calibrator material on both platforms.
- While the V-PLEX® platform was less sensitive, it showed improved precision and relative accuracy and good S/N at the quoted limits of quantification.

- Potential benefits of the S-PLEX® platform are dependent on the context of use of the assay with consideration of the expected change in samples.