The identification and development of a fit for purpose biomarker assay using the MSD platform

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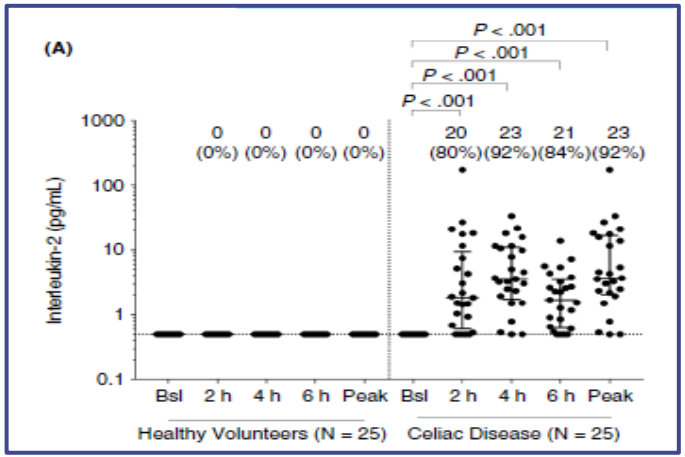
Background information

IL-2 and celiac disease

Celiac disease:

- Celiac disease is caused by the ingestion of dietary gluten and the subsequent activation of gluten specific CD4+ T cells.
- Activated gluten specific CD4+ T cells are implicated in the release of IL-2
- Elevation of serum IL-2 correlate with the severity of symptoms (nausea & vomiting) after gluten exposure
- Elevation in IL-2 serum levels after gluten exposure is limited to celiac disease patients



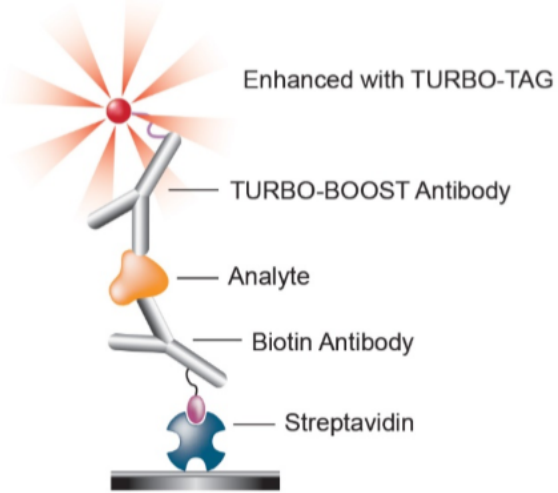
- Previous studies have been unable to detect baseline IL-2 concentrations due to the assay LLOQ (0.5 pg/mL)

Tye-Din, J.A., et al., Alim. Pharm. Ther. 50 (2019) 901-910.

Commercially available MSD platforms

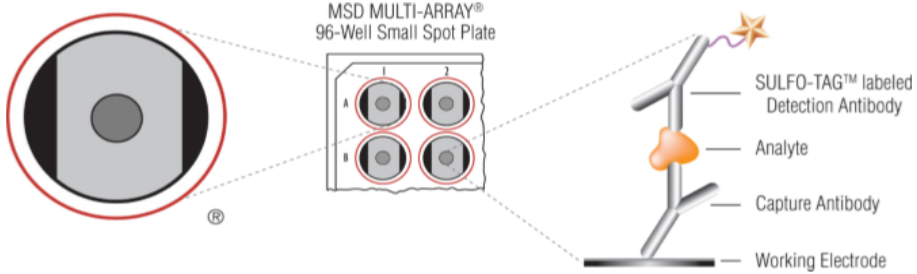
MSD S-plex assay:

- Newly available highly sensitive assay
- Range of assay 47333.3- 11.6 fg/mL*



MSD V-plex assay:

- highly Validated assay
- Range of assay 1420- 0.347 pg/mL*

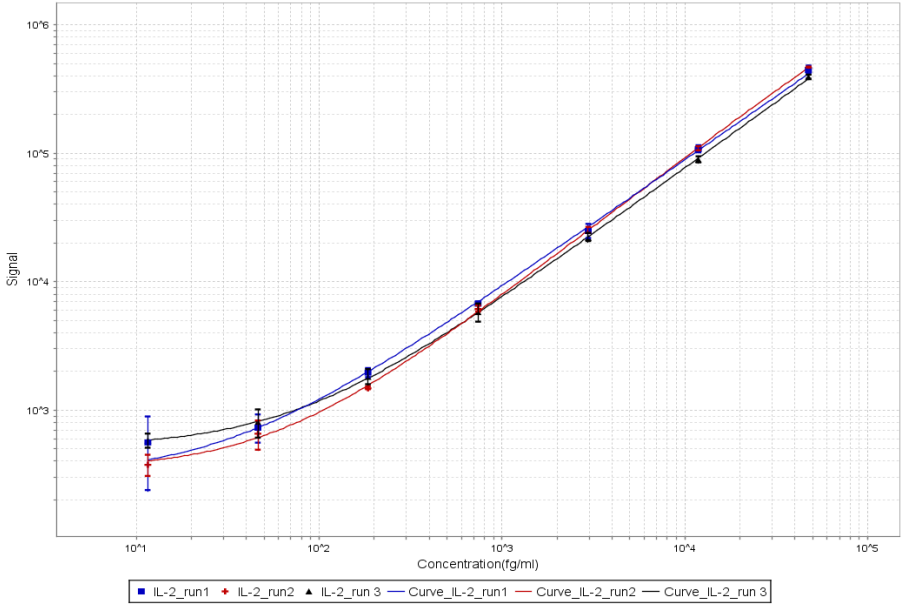


*assay range maybe Lot specific

1) S-PLEX: TrueSensitivity, Simple Execution, MSD, Website: https://www.mesoscale.com/en/products_and_services/assay_kits/s-plex#S-PLEX-Technology. Accessed 26th April 2021
2) MSD MULTI-SPOT Assay System, MSD, Website: https://www.mesoscale.com/~/media/files/product%20inserts/proinflammatory%20panel%201%20human%20insert.pdf. Accessed 26th April 2021

MSD S-plex assay assessment

Calibrator curve precision and accuracy



- Standards 1-5 were within target acceptance criteria (CV≤20%, %recovery within 20%+/- nominal concentration)
- Standards 6&7 were outside target acceptance criteria

	Run 1		Run 2		Run 3	
	% recovery	CV	% recovery	CV	% recovery	CV
Calibrator 6 (46.2 fg/mL)	100	42.6	115	49.9	97.3	67.2
Calibrator 7 (11.6 fg/mL)	241	127	N/A	N/A	94.6	107

S-plex assay assessment

Conclusions

- In our hands we couldn’t reproduce the results demonstrated by MSD:
  - Standards 6&7 consistently did not meet the target acceptance criteria
  - Assay did not meet the required sensitivity
  - Assay was not fit for clinical sample analysis
  - Assay maybe more suitable for exploratory work

MSD V-plex assay

Enhanced sensitivity

Original MSD assay V-plex assay:

– Assay range 1420- 0.347 pg/mL

– 8 point calibration curve using a 1:4 serial dilution

Calibrator	1	2	3	4	5	6	7	8
Original MSD assay (pg/mL)	1420	355	88.8	22.2	5.55	1.39	0.347	0.00

Original MSD assay V-plex assay:

– Assay range 1420- 0.087 pg/mL

– 10 point calibration curve using a 1:4 and 1:2 serial dilution

Calibrator	1	2	3	4	5	6	7	8	9	10
Adapted MSD assay (pg/mL)	1420	355	88.8	22.2	5.55	1.39	0.693	0.347	0.173	0.087

Adapted MSD V-plex assay

Assessment parameters

- Method assessment for the adapted MSD V-Plex assay was performed across 4 different runs across different days
- **Assay parameters assessed:**
  - Calibration curve precision and accuracy (Limit of detection, lower and upper limits of quantification)
  - Intra assay precision and accuracy
  - Inter assay precision and accuracy
  - Parallelism (demonstrate minimum required dilution and selectivity)
  - Stability (freeze thaw, 24 hours, 2 hours)

Adapted MSD V-plex assay

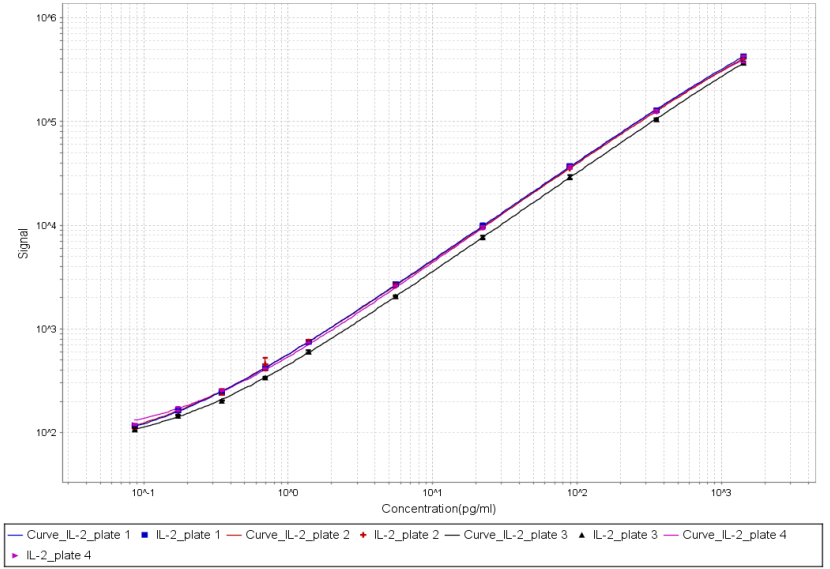
Assessment parameter: Calibrator curve precision and accuracy

- Calibrator precision and accuracy was calculated over all 4 method assessment runs (all individual runs met acceptance criteria)

Calibrator (pg/mL)	Mean	%CV	% RE
1420	1433.10	4.51	-0.92
355	346.90	2.58	2.28
88.7	89.45	2.16	-0.79
22.19	22.14	2.68	0.21
5.55	5.60	1.85	-1.04
1.39	**1.41**	**2.93**	**-1.44**
0.693	**0.72**	**8.04**	**-3.16**
0.347	**0.34**	**4.64**	**2.49**
0.173	**0.17**	**6.77**	**-0.11**
0.087*	**0.08**	**7.63**	**3.22**

* Values shown are calculated over 3 analytical runs due to analyst error

Average LOD: 0.059 pg/mL



	Target Acceptance Criteria
Standard curve precision/accuracy	Precision: ≤ 20% CV (≤ 25% at LLQ, HLQ), Accuracy: ±20% RE (±25% at LLQ, HLQ),

Adapted MSD V-plex assay

Assessment parameters: Intra- and Interassay precision and accuracy

Inter-assay P&A

	RVC1	RVC2	RVC3	RVC4	RVC5	EVC1
Nominal* (pg/mL)	390.00	39.80	3.46	3.46	3.46	N/A
mean	402.55	37.98	3.08	3.17	2.92	24.48
%CV	4.56	6.48	5.90	9.13	6.08	7.04
%RE	-3.22	4.57	10.88	8.29	15.71	N/A
Total error	1.34	11.05	16.78	17.42	21.79	N/A

*dilution factor applied

Intra-assay P&A

	RVC1	RVC2	RVC3	RVC4	RVC5	EVC1
Nominal* (pg/mL)	390.00	39.80	3.46	3.46	3.46	N/A
mean	396.84	36.73	2.91	3.02	2.76	23.90
%CV	**2.92**	**4.88**	**4.54**	**9.04**	**5.29**	**6.88**
%RE	**-1.75**	**7.72**	**15.87**	**12.60**	**20.26**	**N/A**
Total error	**2.40**	**11.38**	**17.86**	**17.12**	**21.92**	**N/A**

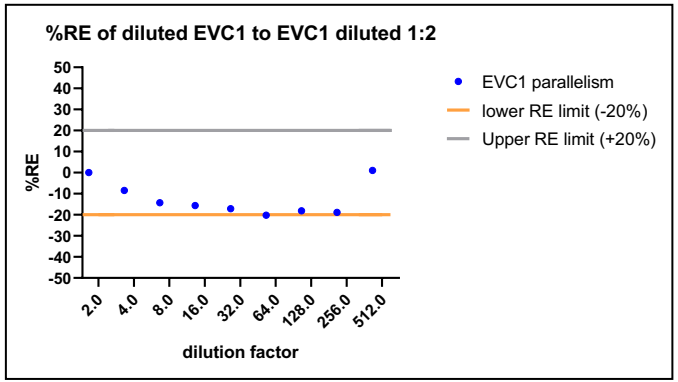
*dilution factor applied

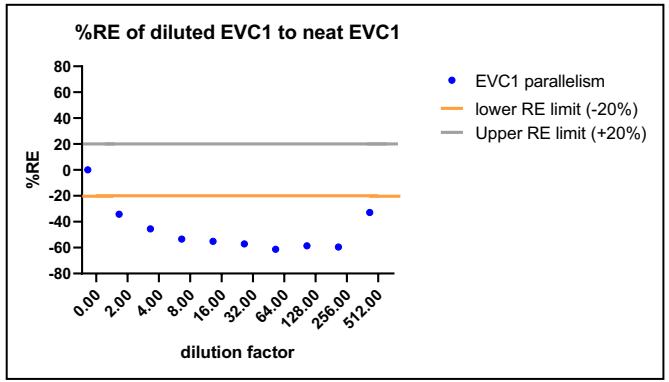
	Target Acceptance Criteria
Precision	≤ 20% CV (≤ 25% at LLQ, HLQ)
Total error	Total error of all VC's should be <30%.
Accuracy	Within ±20% RE (±25% at LLQ, HLQ),

Adapted MSD V-plex assay

Assessment parameters: Parallelism

- Samples were diluted using a 2 fold serial dilution to below the assay LLOQ (1:512)
- Parallelism demonstrated selectivity and no matrix interference
- MRD Was confirmed to be 1:2 as suggested by MSD
- Samples do not back calculate to the neat concentration





	Target Acceptance Criteria
Selectivity	Minimum of 80% of samples within ±20% RE (±25% at LLQ, HLQ)

Adapted MSD V-plex assay

Assessment parameters: Stability

2 Hour bench top stability:

Sample type	Calc. Conc. Mean	%RE
EVC1	26.76	0.75
2 hour bench top	26.56	

24 hour stability at +4°C

Sample type	Calc. Conc. Mean	%RE
EVC1	26.76	0.34
24 hour +4°C	26.67	

Freeze thaw stability:

One freeze thaw cycle consists of storing the EVC at -80°C for at least 16 hours and thawing it unassisted at room temperature .

Sample type*	Calc. Conc. Mean	%RE
EVC1	26.76	N/A
F/T 1	26.37	1.47
F/T2	27.22	-1.69
F/T3	27.82	-3.95

* EVC1 is stored at -80 thus undergoes 1 F/T before use. Therefore EVC1 and F/T1 received the same treatment.

– Endogenous IL-2 remained stable throughout all stability assessments

	Target Acceptance Criteria
Stability	VC tested within ±20% Difference compared to actual concentration.

Conclusions

- Based on the data produced in our hands:
  - Not all commercially available assays are suitable for clinal sample analysis
  - Possible to adapt a commercially available assay to make it fit for purpose