The Challenges of Developing and Validating Biomarker assays

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Introduction

Focus of presentation

- Difference between endogenous and recombinant analyte
  - Stability
  - Drug interference

- Determination of Acceptance criteria
  - Context of Use
  - Project timelines

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

Endogenous vs recombinant analyte

Use of recombinant analyte:

- Required for early stage antibody screening
  - Removes the effect of matrix
  - Low endogenous analyte levels
- Required for calibration curves and QC's
  - Analytical range

Difference between endogenous and recombinant analyte:

- Recombinant analyte raised *in vitro*
- SNPs
- Post translational modifications (Glycosylation, oxidation, acetylation)

Endogenous Vs recombinant analyte- Stability

Case study one- Analyte X

Assay information:

- PD Biomarker assay on the MSD platform for a FTIH study
- Assay range 69714-34 fg/mL
- To cover the analytical range VC's were a combination of pooled human serum and spiked pooled human serum

Validation control	Nominal Concentration (fg/mL)*	Make up
VC1	94	Endogenous analyte
VC2	234	Endogenous analyte
VC3	2022	Approx. 1500 fg/mL recombinant spike
VC4	17011	Approx. 27000 fg/mL recombinant spike
VC5	46484	Approx. 60000 fg/mL recombinant spike

*Nominal concentration determined from pre-validation P&A runs

Endogenous Vs recombinant analyte (Stability)

Case study one- Analyte X

Freeze thaw stability

3 Freeze/Thaw cycles

VC	Nominal concentration (fg/mL)	BCC (fg/mL)	% Recovery
VC1	94	85	90.4
VC2	234	233	99.6
VC3	2022	1951	96.5
VC4	**17011**	**35784**	**210.5**

Bench top stability

2 hour RT stability

VC	Nominal concentration (fg/mL)	BCC (fg/mL)	% Recovery
VC1	94	74.5	79.3
VC2	234	196	83.8
VC3	2022	1997	98.8
VC4	**17011**	**27596**	**162.2**

24 hour RT stability

VC	Nominal concentration (fg/mL)	BCC (fg/mL)	% Recovery
VC1	94	87	92.6
VC2	234	246	105.1
VC3	2022	1676	82.9
VC4	**17011**	**41952**	**246.6**

Conclusions & learnings:

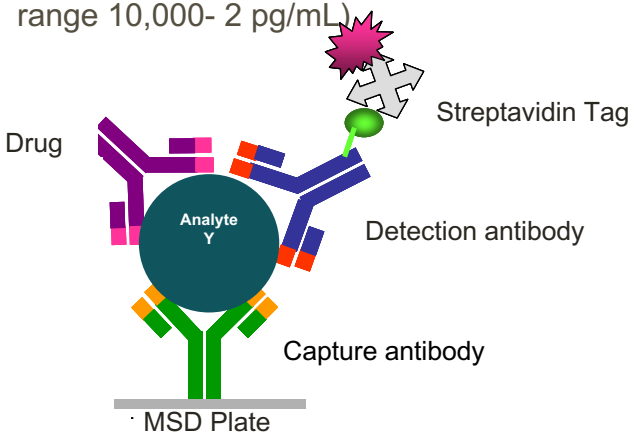
- Recombinant analyte X behaves differently to endogenous analyte X
- Not always feasible to perform stability assessments in validation which cover the analytical range.
- Is it more appropriate to monitor stability using study samples?

Endogenous Vs recombinant analyte (drug interference)

Case study two- Analyte Y

Assay background:

- Total target engagement biomarker assay on the MSD platform for a FTIH single escalation dose study
- Assay was required to be highly sensitive (Assay range 10,000- 2 pg/mL)



Initial Method development results:

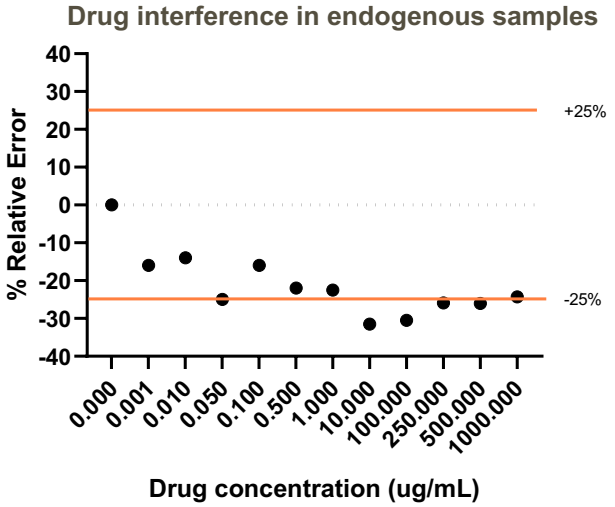
- Commercial antibodies were screened to find a pair which could detect total analyte Y
  - Surrogate matrix was spiked with recombinant analyte Y and incubated with and without drug

ECL Units with No drug present	ECL units with drug present	%Recovery
1488	1545	103%

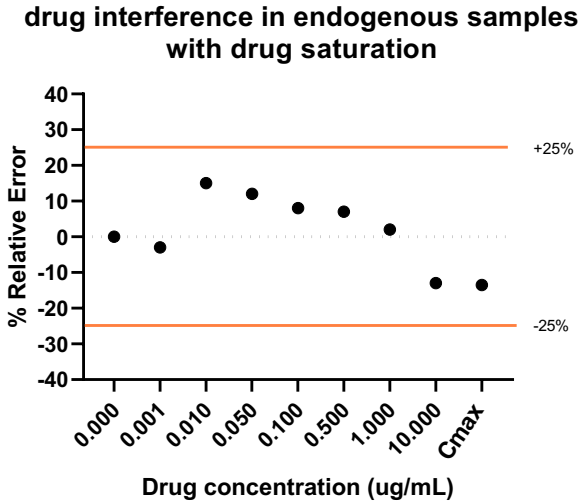
- Method development and optimization continued using the above antibody pair

Endogenous Vs recombinant analyte (drug interference)

Case study two- Analyte Y



Drug saturation



How could this unacceptable drug interference be solved?

- Use of different buffers
- Use of different MSD plate types
- Different capture and detection antibody

Conclusion: Saturating the assay with drug reduced drug interference

Learnings: Importance of introducing endogenous samples as early on as possible in method development

Endogenous Vs recombinant analyte

Key learnings:

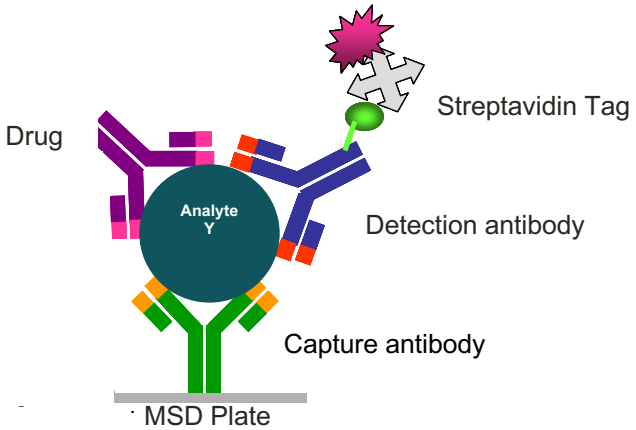
- Importance of introducing endogenous analyte as soon possible during method development
- Impact of reagent availability
- Impact of study time lines

Acceptance criteria

Case study 2- Analyte Y

Assay background:

- Total target engagement biomarker assay on the MSD platform for a FTIH single escalation dose study
- Assay was required to be highly sensitive (assay range 10,000- 2 pg/mL)
- Calibration curve was prepared using recombinant analyte in surrogate matrix
- To cover the analytical range VC's were combination of pooled human serum and spiked pooled human serum



Acceptance criteria

Case study two- Analyte Y

Initial Method development:

- Echo 525 acoustic dispenser was used prepare calibration curve
- Addition of reagents and samples were added to the assay plate manually.
- assay precision and accuracy were acceptable

Validation A:

- Precision and accuracy VC's was unacceptable

VC	%RE	%CV
1 (LLOQ)	1.72	28.01
2	1.82	15.91
3	-5.19	17.88
4	-7.55	17.56
5	-13.01	22.82
6 (ULOQ)	-14.16	31.45

- The precision and accuracy was causing other validation parameters to fail

Further method development

- Method was fully automated to try and improve the precision and accuracy.
- Micropro 300 was used to stamp reagents and samples onto the assay plate

Acceptance criteria

Case study two- Analyte Y

Validation B:

- Automation had resulted in the calibrators having very tight %CV and %RE across the assay range

Calibrator	1 (ULOQ)	2	3	4	5	6	7	8	9 (ULOQ)
%CV	3.91	3.36	4.11	0.23	6.03	0.69	2.67	6.50	4.99
%RE	1.00	-1.67	-3.60	3.00	6.00	3.33	-7.20	-2.00	6.50

- Validation showed higher level of variability in the QC's than our target acceptance criteria (<25% %CV, %RE)

QC	1 (rep 1)	1 (rep2)	2 (rep 1)	2 (rep 2)	3 (rep 1)	3 (rep 2)	4 (rep 2)	4 (rep 1)
%CV	10.55	2.51	2.90	6.73	2.27	0.94	8.03	2.65
%RE	**38.35**	-4.13	18.90	2.44	**27.73**	3.18	**27.04**	2.97

- Could dilute samples to remove matrix effect however this would increase the LLOQ

Acceptance criteria

Case study two- Analyte Y

Validation showed wider acceptance criteria than target Precision and accuracy was required

Option 1: Restart Method Development and validation

- Could the precision and accuracy be improved
- Would Project timelines allow for re-developing the assay

Option 2: Expand acceptance criteria

- Will increasing acceptance criteria impact the overall data
- Is the target acceptance criteria feasible in a sensitive assay

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Questions