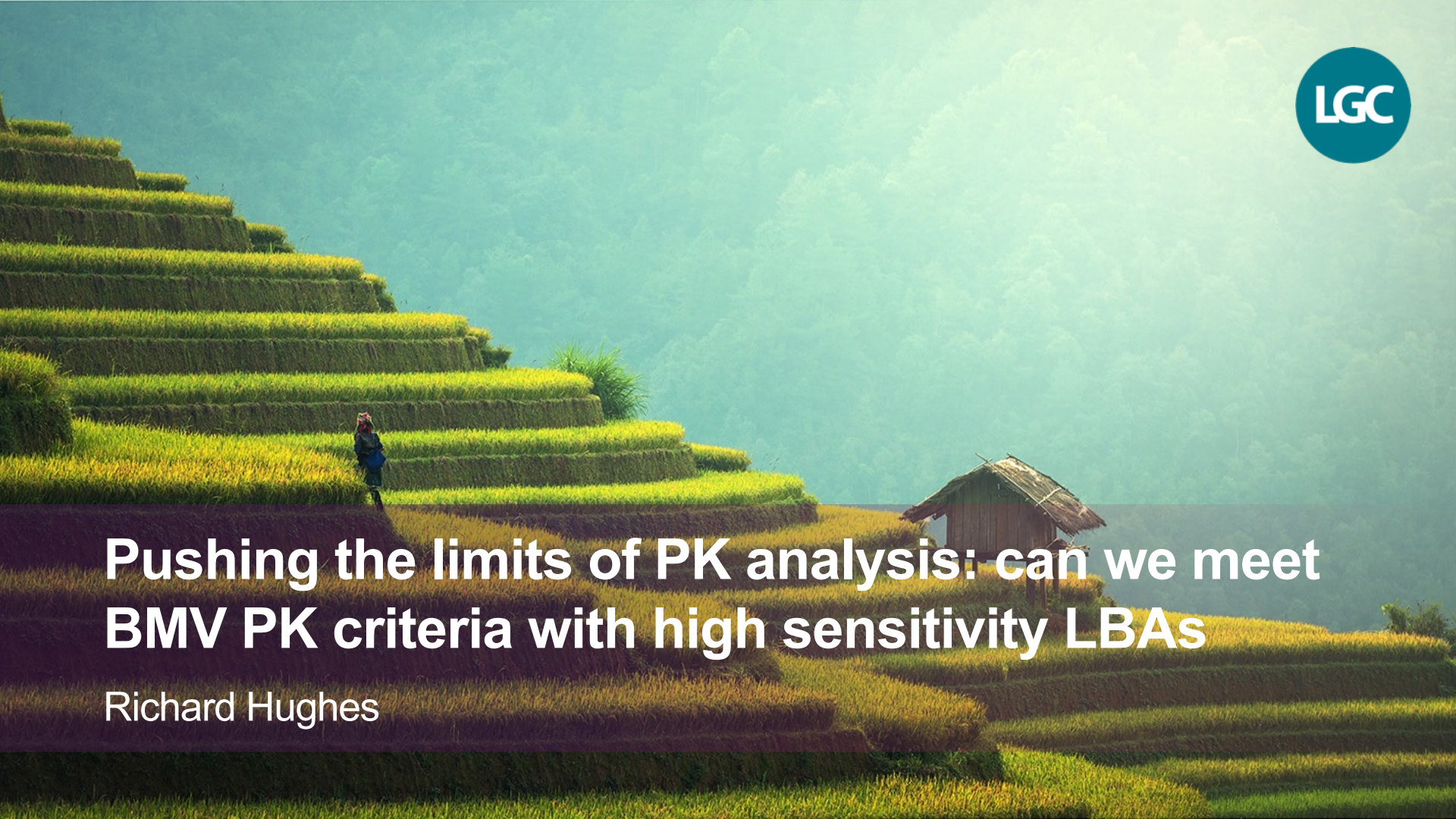


Pushing the limits of PK analysis: can we meet BMV PK criteria with high sensitivity LBAs

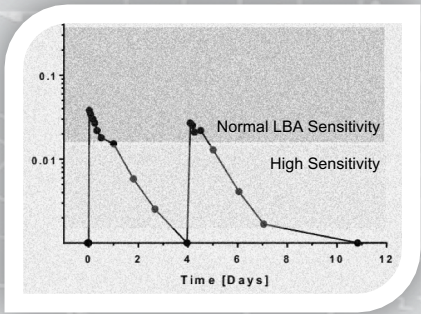
Richard Hughes

Pushing the limits of PK analysis: can we meet BMV PK criteria with high sensitivity LBAs

- Requirements for new modalities and ways of working
- Enabling technologies
- Defining the right strategy

Requirements and Challenges

- **Requirements to improve PK sensitivity due to biological MoA**
  - New modalities…bi-specifics, tri-specifics, BiTes, VHH…
  - highly potent molecules with effects observed at low exposures
  - Route of administration
  - More complete picture of PK profile



- **Patient-centric microsampling**
  - Extraction or elution from the collection device often results in a dilution effect
  - Further additional dilution may be required to reduce matrix effects.

- **PK acceptance criteria for precision and accuracy**
  - Irrespective of technology platform
  - Sensitivity despite minimal sample volume availability (pre-clinical)
  - Sensitivity in study population e.g. healthy vs disease (Clinical)
  - Sensitivity in testing high sample numbers, potentially across multiple sites

Weighing up the options

µg/mL ng/mL pg/mL fg/mL

ng/mL – fg/mL range
Fast run times (2-3 expt / day)
many avenues to explore in method development to maximise sensitivity
More time consuming to switch formats around

µg/mL – pg/mL range, various options for solid phase
Easy to switch formats around
Effective with high matrix concentrations
Fast method development (2-3 expt / day)

µg/mL – pg/mL range
Easy to switch formats & simpler for free/total assays
Method development can take time (1 expt / day)

Spotlight on the assay parameters that can determine sensitivity

Quanterix Simoa

- Bead conjugation chemistry
- On-bead capture concentration
- Biotin linker
- Bead number
- Detection concentration
- Matrix concentration
- Galactosidase concentration
- Diluent type
- 2-step or 3-step method
- Instrument Cadence

Gyrolab

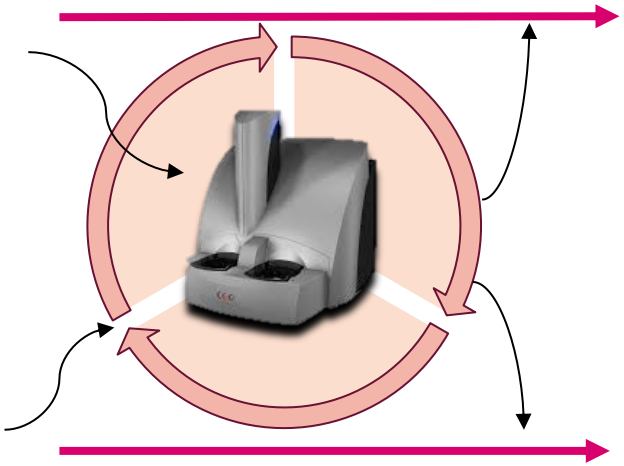
- Biotin linker
- Capture and Detection concentration
- Matrix concentration
- Diluent type
- CD type
- Instrument method

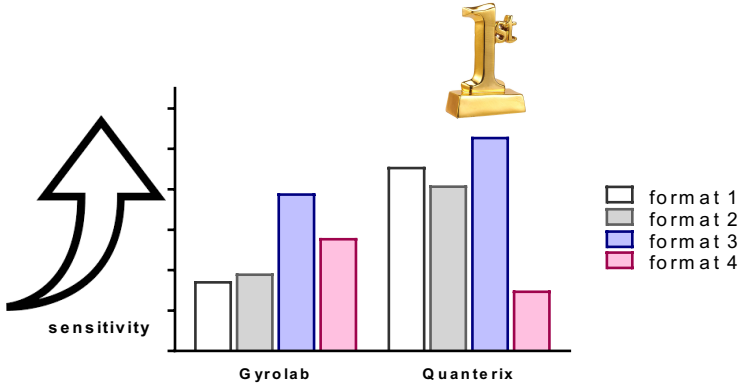
MSD

- Capture and Detection concentration
- Matrix concentration
- Diluent type
- Plate type (maybe?)

We need an assay with <100 pg/mL sensitivity

Method development was platform agnostic with multiple options for capture/detection including various anti-idiotypes and drug target



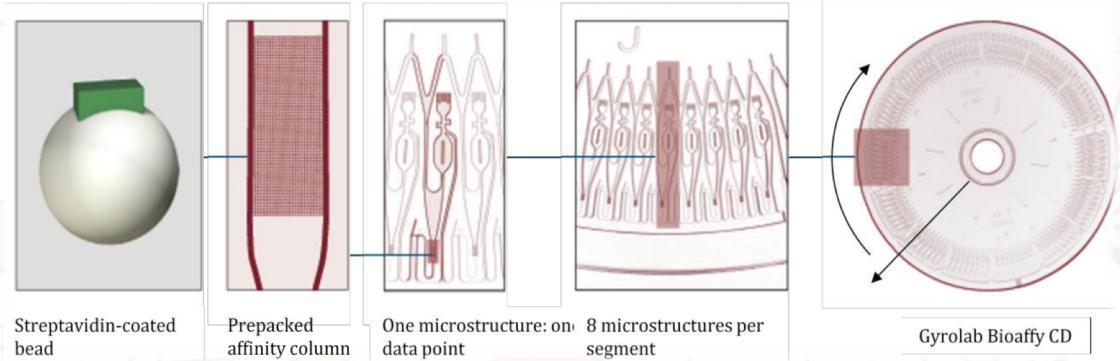


Can we save time and start developing methods across multiple platforms simultaneously….

Gyrolab

- Biotin linker
- Capture and Detection concentration
- Matrix concentration
- Diluent type
- CD type
- Instrument method

Coinciding with this case study, Gyros released the new more sensitive CD – Bioaffy 4000 CD

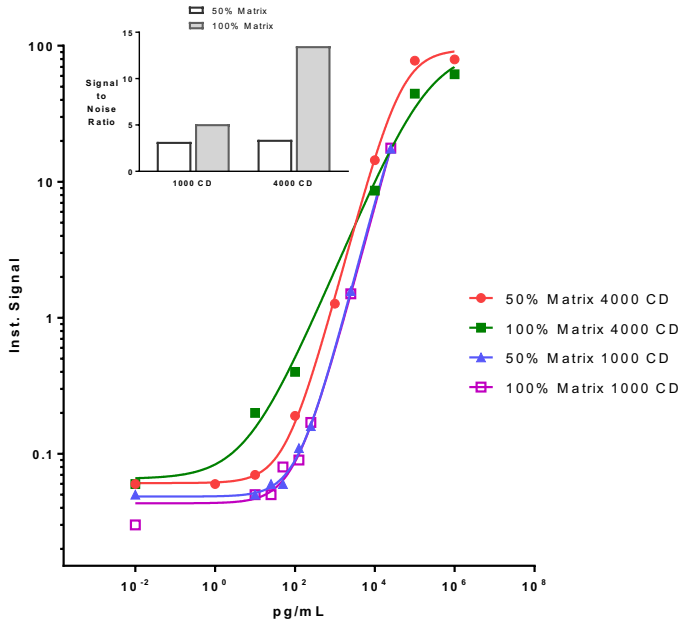


We needed an assay with <100 pg/mL sensitivity,
so can we get around having to have a matrix dilution?

Can we save time and start developing methods across multiple platforms simultaneously….

Gyrolab

- Biotin linker
- Capture and Detection concentration
- Matrix concentration
- Diluent type
- CD type
- Instrument method



Can we save time and start developing methods across multiple platforms simultaneously….

Gyrolab

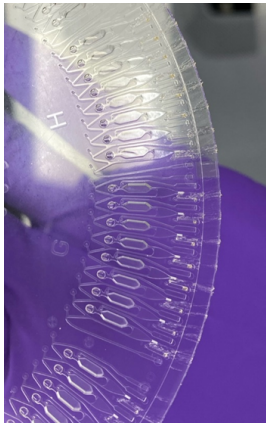
- Biotin linker
- Capture and Detection concentration
- Matrix concentration
- Diluent type
- CD type
- Instrument method

4000 CD, 100% matrix	*S/N*	*%CV*
25pg/mL	<3	>10
50pg/mL	>3	<10
75pg/mL	>3.5	<10
100pg/mL	>5	<10
150pg/mL	.7	<10

5 levels of QC prepared in pooled matrix met PK BMV acceptance criteria for P&A

Can we save time and start developing methods across multiple platforms simultaneously….

ID	Signal
Ind 1	-0.210
	-0.210
Ind 2	-0.207
	-0.203
Ind 3	-0.202
	-0.209
Ind 4	0.003
	-0.204
Ind 5	-0.118
	1.223
Ind 6	2.714
	1.597



	Pooled matrix spike		Individuals
	2 in 3	1 in 2	1 in 2
Diluent 1	75pg/mL	100-150pg/mL	0% pass
Diluent 2	Neg	Neg	NA
Diluent 3	Neg	100-150pg/mL	0% pass
Diluent 4	150pg/mL	100-150pg/mL	NA

- Assay not selective!
  - Saw mixture of over and under-recovery in individuals

Can we save time and start developing methods across multiple platforms simultaneously….

ID	Signal
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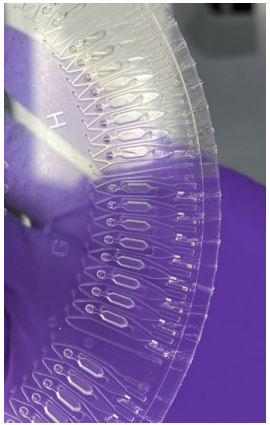

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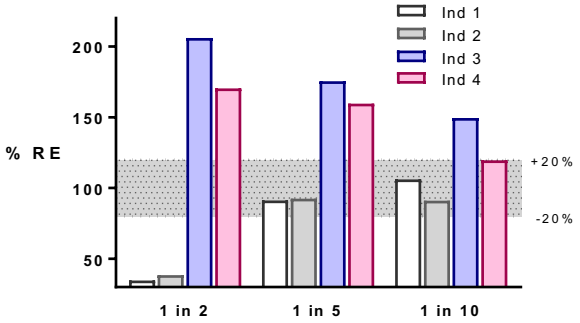
Diluent 4	150pg/mL	100-150pg/mL	NA

- Assay not selective!
  - Saw mixture of over and under-recovery in individuals
- What matrix dilution is needed to remove these matrix effects?



Sensitivity is driven by selectivity

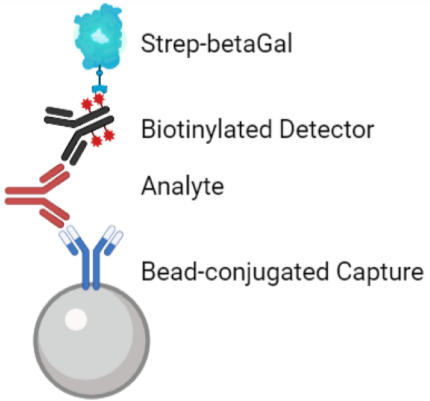
Can we achieve selectivity on the HD-X?

Method development was taking place simultaneously on both Gyrolab and the Quanterix, which quickly led to….

Can we achieve selectivity on the HD-X?

Quanterix

- Bead conjugation chemistry
- On-bead capture concentration
- Biotin linker
- Bead number
- Detection concentration
- Matrix concentration
- Galactosidase concentration
- Diluent type
- 2-step or 3-step method
- Helper Beads
- Instrument Cadence



		Bead numbers					
	Diluent	Diluent 1			Diluent 2		
	Matrix (%)	100%	50%	25%	100%	50%	25%
pg/mL	0	NaN	1132	NaN	2147	4700	9210
	100	622	933	856	2578	8441	11462
	500	NaN	NaN	744	3189	10494	13096
	1000	4109	NaN	NaN	2846	10445	11335
	10000	628	1167	2298	2771	7304	10048

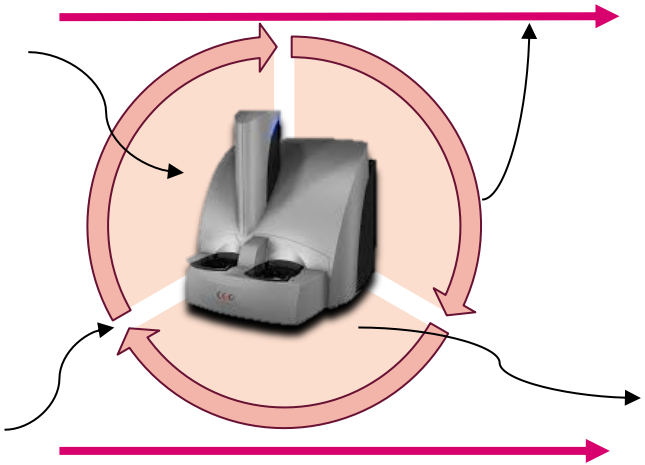
NaN = Not calculable

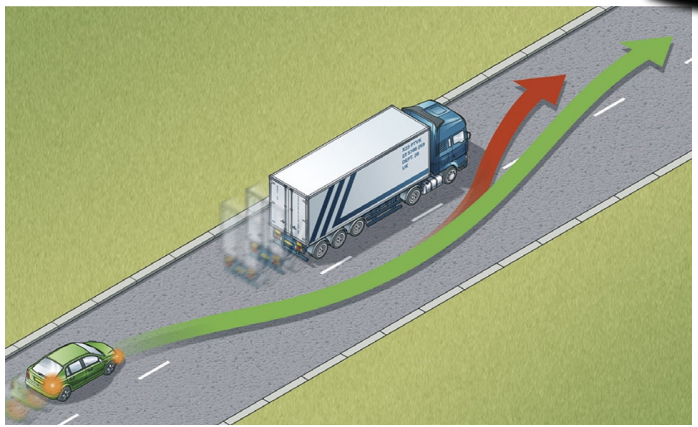
Once again, 5 levels of QC prepared in pooled matrix met PK BMV acceptance criteria for P&A

	Ind 1	*Ind 2*	*Ind 3*	*Ind 4*	
LQC	11.5	8.4	3.7	1.0	%CV
250 pg/mL	-5.5	**60**	**-73.2**	**34.5**	%RE
LLoQ	9.8	**30.3**	BLQ	21.8	%CV
100 pg/mL	-6.1	**-47.8**	BLQ	24.4	%RE

In this example, an underdog won the race

Method troubleshooting was frequently performed using the MSD that eventually the gains in sensitivity were outweighed by the need to have a validated assay up and running





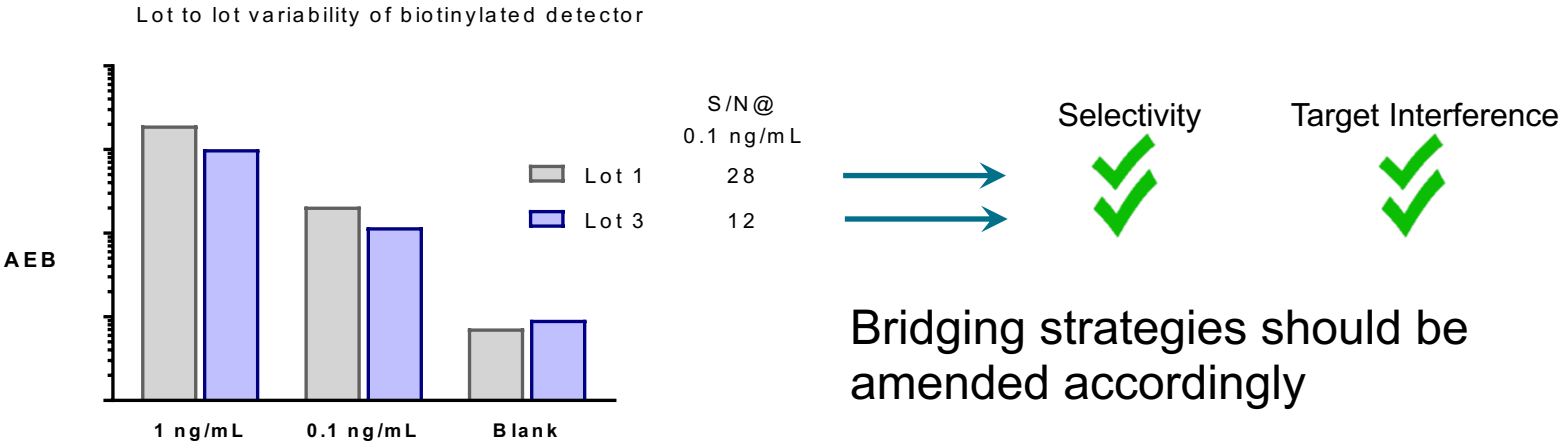
Quanterix Simoa can solve selectivity issues with a better signal to noise….

	MRD	S/N @ 100 ng/mL	Selectivity ok?
Gyrolab 4000 CD	2	1.66	No
Quanterix HD-X	4	4.4	Yes

Quanterix Simoa can solve selectivity issues with a better signal to noise….but….

	MRD	S/N @ 100 ng/mL	Selectivity ok?
Gyrolab 4000 CD	2	1.66	No
Quanterix HD-X	4	4.4	Yes

S/N can be increased but you really need to watch the critical reagent and conjugation.



Bridging strategies should be amended accordingly

How about two assays?

In cases where a validated PK assay already exists, but additional sensitivity is required

Do we need a complete validation of a new, more sensitive assay?

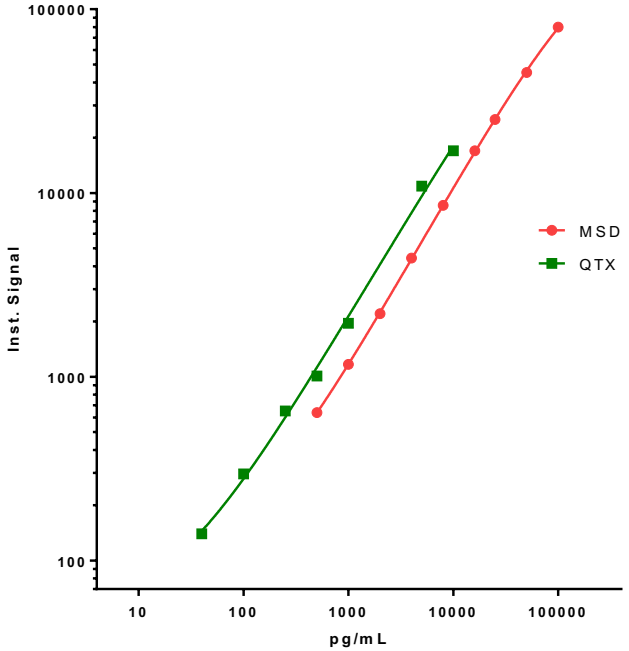
4.3. Cross validation

Where data are obtained from different methods within and across studies or when data are obtained within a study from different laboratories, applying the same method, comparison of those data is needed and a cross validation of the applied analytical methods should be carried out.

The extent of validation should be on a case-by-case basis

- Existing format, *same* platform?
  Existing format, *different* platform??
  Different format, *different* platform???

- Do the calibration ranges overlap?
- Should stability be restarted at the new QC concentrations??



Conclusions and key points

High sensitivity platforms can deliver on robust PK assays at <1 ng/mL

The complexity of method development *de novo* does mean a longer process.
A clearer strategy is to consider a platform such as the HD-X as a *transfer* instrument – and actually work with a simpler system to develop the format initially.

Selectivity drives sensitivity

High sensitivity has the potential to influence or amplify any matrix or target interference so test selectivity as soon as possible.

Conjugated critical reagents

Bridging strategies should be dictated by key validation parameters, assay format and underlying biology
Additional tools such as LC/MS and SDS-PAGE to characterise can be invaluable

Two assay strategies

There is a need for careful consideration around the parameters that will be tested to effectively demonstrate that both assays are equivalent.

Thank you for listening

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