Transfer of a Generic PK Assay to Different CROs: Challenges, Troubleshooting and Lessons Learned

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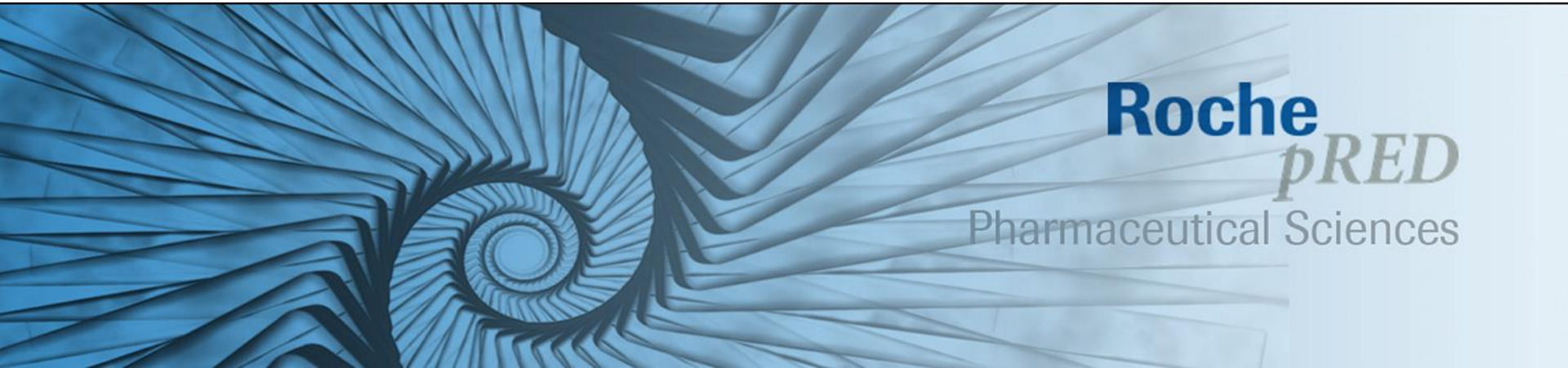


Outline of the Presentation

- Generic PK Assay format
- Strategy and Processes for Assay Transfer
- Case studies
  - Description
  - Challenges, Issues and Troubleshooting
- Lessons learned

Scope of the Presentation

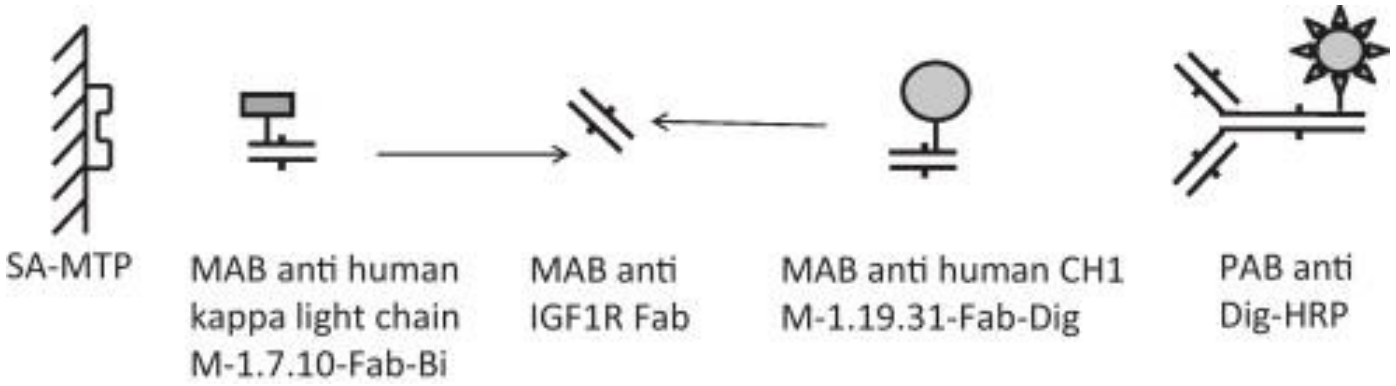
Share our experience with the establishment of a generic PK assay at different CROs

Generic PK Assay Format

- A generic PK assay is routinely applied to provide rapid support for early animal studies across various programs at a time when specific reagent such as anti-idiotype antibodies are not yet available.
- The assay uses 2 monoclonal antibodies binding unique epitopes in the Fab region of human IgG
  - mAb M-1.7.10 directed against the constant domain of the the kappa light chain.
  - mAb M-1.19.31 binds to the constant domain 1 of the heavy chain.
- These antibodies are versatile tools as capture and detection reagents in a generic enzyme-linked immunoassay to quantify human antibodies in animal matrix

Stubenrauch. K et al. J. Pharm. Biomed. Anal. 49 (2013), pp 208 – 215

Generic PK Assay Format



From Stubenrauch. K et al. J. Pharm. Biomed. Anal. 49 (2013), pp 208 – 215

- Classical sequential ELISA: using biotinylated mAb 1.7.10 (capture) and mAb 1.19.31 labelled with Digoxigenin (detection)
- Readout: Colorimetric signal
- **Very sensitive to contamination with human IgG !**

Assay Transfer Strategy

The assay is qualified at Roche, then transferred to the external laboratory

Roche provides

- Critical reagents
  - Reference standard
  - Streptavidin coated plates
  - Biotinylated and digoxigenin labelled antibodies
  - Buffer
- Analytical method SOP
- Technical and scientific support

External laboratory provides

- Other reagents
  - Matrix
  - Anti-Dig POD
- Scientific and technical expertise

CASE STUDY 1

Generic PK assay applied to the determination of a bi-specific antibody in Cynomolgus Serum

Assay Transfer and Validation

Performance at 2 different CROs

Regulated Bioanalysis

Case Study 1: Assay Qualification at Roche

Assay Qualification Summary	
Matrix	Serum
Species	Cynomolgus
Analyte	Bi-specific antibody
MRD	100
calibration range (ng/mL)	10 - 640
QCs (ng/mL)	LLOQ: 10
	LQC: 30
	MQC: 120
	HQC: 480
Precision	Intra-assay < 10%
	Inter-assay < 15%
Accuracy (%RE)	Intra-assay: + 6.2% to - 3.3%
	Inter-assay: + 4.0% to - 10%

Case Study 1: Assay transfer to CRO 1

Issues	Troubleshooting
▪ High background ▪ Desired sensitivity could not be achieved ▪ Issue with selectivity ▪ Lack of reproducibility (recovery in individual matrices)	▪ Review of analytical procedures and methodology ▪ Use of matrix from biologic naïve animals ▪ Washers/shakers

Resolution

- Matrix: Use of biologic naïve animals resolved the “high background” issue.
- However, occasional “spottiness” still observed

Case study 1: Assay Validation at CRO1

	Roche	CRO 1
Matrix	Serum	
Species	Cynomolgus	
Analyte	Bi-specific antibody	
MRD	100	
Quantification range (ng/mL)	10 - 640	20 - 640
QCs (ng/mL)	LLOQ: 10	LLOQ: 20
	LQC: 30	LQC: 50
	MQC: 120	MQC: 120
	HQC: 480	HQC: 480
		ULOQ: 640
Precision	Intra-assay < 10%	Intra-assay : 1.06% to 2.53%
	Inter-assay < 15%	Inter-assay : 3.81% to 6.51%
Accuracy (%RE)	Intra-assay: + 6.2% to - 3.3%	Intra-assay : -10.0% to -3.33%
	Inter-assay: + 4.0% to - 10%	Inter-assay : -15.0% to -6.50%

- Successful validation with increased LLOQ
- Acceptable assay performance of the assay during sample analysis
- The assay is still subject to “spottiness” and is now run in a dedicated pre-clinical laboratory with dedicated washers and sets of pipettes

Case Study 1: Assay transfer to CRO 2

Issues

- High background,
- Desired sensitivity could not be achieved
- Lack of reproducibility (recovery in individual matrices, dilution linearity…)
- Unacceptable P&A at lower and upper end of the calibration curve

Troubleshooting

Step 1:

- Review of analytical procedures and methodology
- Washers / pipettes
- Matrix: different lots of serum pools tested

Step 2

- Exchange of Quality Controls and calibration standards samples

Step 3

- Visit of CROs scientist to development laboratory

Resolution

- Careful handling of reagents and material used (washer, pipettes, matrix)
- Change of matrix pool
- **Sufficient improvement to start assay validation but with truncated calibration range**

Case study 1: Assay Validation at CRO 2

	Roche	CRO 2
Matrix	Serum	
Species	Cynomolgus	
Analyte	Bi-specific antibody	
MRD	100	
Quantification range (ng/mL)	10 - 640	20 - 320
QCs (ng/mL)	LLOQ: 10	LLOQ: 20
	LQC: 30	LQC: 60
	MQC: 120	MQC: 120
	HQC: 480	HQC: 240
		ULOQ: 320
Precision	Intra-assay < 10%	Intra-assay: 5.68% to 12.7%
	Inter-assay < 15%	Inter-assay : 11% to 21.9%
Accuracy (%RE)	Intra-assay: + 6.2% and - 3.3%	Intra-assay : -2.4% to 5.89%
	Inter-assay: + 4.0% and - 10%	Inter-assay: -12.6% to 9.05%
Comment		Selectivity and dilution linearity failed

- The assay was deemed not suitable for sample analysis of GLP Tox study
- The cause for lack of assay robustness is still not clear

CASE STUDY 2

Generic PK assay applied to the determination of human antibodies in Cynomolgus and mouse matrix

Assay Establishment at CRO

Performance on 2 different platforms

Discovery Bioanalysis

Case Study 2: Assay transfer to CRO

The generic PK assay is qualified at the CRO for different analyte/matrix (Discovery)

Issues

- High background
- Desired sensitivity could not be achieved
- Lack of reproducibility

Troubleshooting

- Review of analytical procedures and methodology
- Handling of material (tubes, tips, pipettes)
- Washers/shakers

Resolution

- Work performed in a sterile environment, with sterile tubes, tips.
- Washing steps performed manually

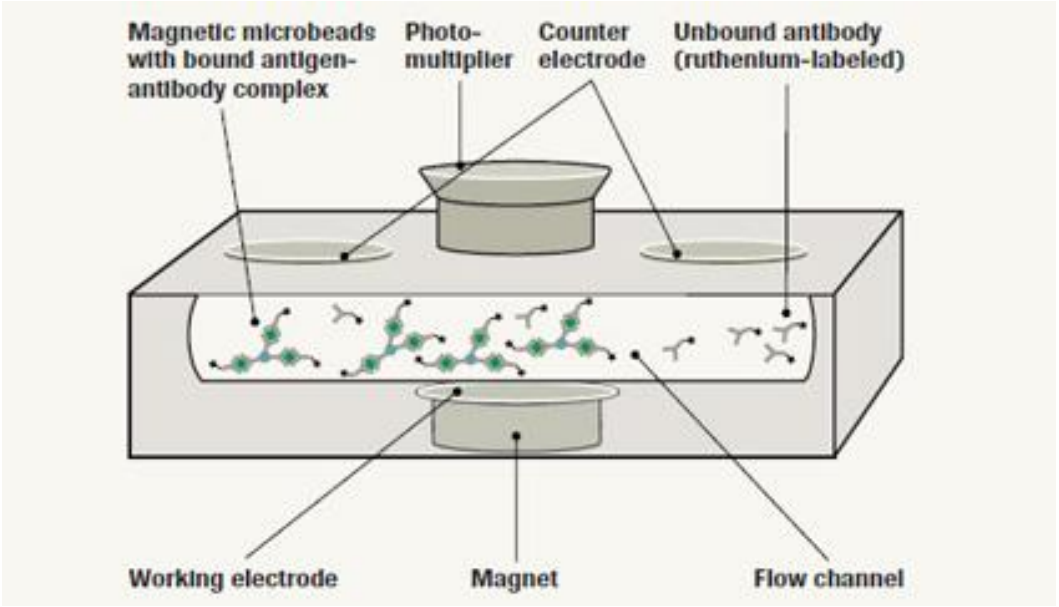
Case Study 2: Assay performance at CRO

ELISA performance overview		
Species	Cynomolgus	Mouse
Matrix	Serum	Serum
Analyte	Analyte 1	
MRD	10	50
calibration range (ng/mL)	0.95-62.5	4.75- 312.5
LLOQ (ng/mL)	0.95	4.75

- However, occasional “spottiness” still observed
- The assay is adapted to an automated platform

Case Study 2: Assay Performance on an Automated Platform

The assay has been adapted and transferred to a Roche Elecsys 2010 analyzer. The detection is based on the ECL technology.



Case Study 2 Assay performance on automated platform

Automated assay performance overview		
Species	Cynomolgus	Mouse
Matrix	Serum	Serum
Analyte	Analyte 2	
MRD	20	50
calibration range (ng/mL)	13.8-30000	125 - 75000
LLOQ (ng/mL)	13.8	125

- Lower sensitivity on the automated platform but higher dynamic range (ECL detection)
- Higher throughput and decreased risk of contamination
- Increased assay robustness

Conclusion

- A generic PK Assay is used to support regulated and non-regulated animal studies.
- The assay is very sensitive to contamination with human IgG and requires careful handling of material and reagents
- During assay implementation at various CROs, very similar issues have been observed leading to poor assay performance.
- In some cases, the issues could be resolved by performing the assay in dedicated laboratories with dedicated material (pipette, washers..) and reagents
- In another case, the assay was transferred in a completely automated platform

Lessons learned

- Assay transfer strategy: review of the documentation provided to the external laboratory at the time of the transfer.
- Kick off TC with CRO and development team to discuss procedures and protocol and clarify any points if needed
- Transfer of reagents such as matrix or spiked samples (QCs and calibration standards) as well
- Careful selection of the matrix used in the assay (from biologic naïve animals).
- Dedicated instruments, materials, reagents (laboratories)
- “Dry run” when working with a new CRO

➢ **Extensive and open communication between external laboratory and sponsor on a <u>scientific level</u> is key**.

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