
**Lifecycle of a Bioanalytical Assay : Technical and Communication Challenges
From a Real Case Study**

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Bioanalytical Manager - Hoffmann-La Roche

Clinical Context & BA Strategy

Chronology of Events: From Development to Samples Analysis

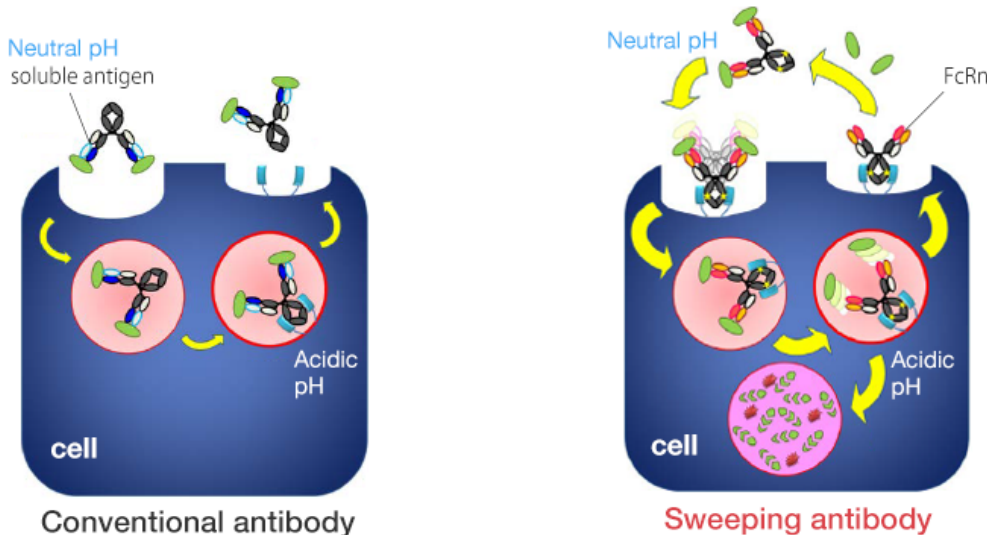
Lessons Learned

Our Strategy at Roche

Clinical Context

- Characterization of the **pharmacokinetics (PK)**, **pharmacodynamics (PD)** and **immunogenicity** for a First in Human study.
- *Our compound of interest is a recycling and antigen sweeping monoclonal antibody (mAb) that binds to its target and thereby blocks its conversion to a mature form.*

Effect of Sweeping Antibody[®] on Soluble Antigen



- Can bind to the antigen only once
- Antigen persists in plasma as an antibody bound form, and antigen accumulates in plasma

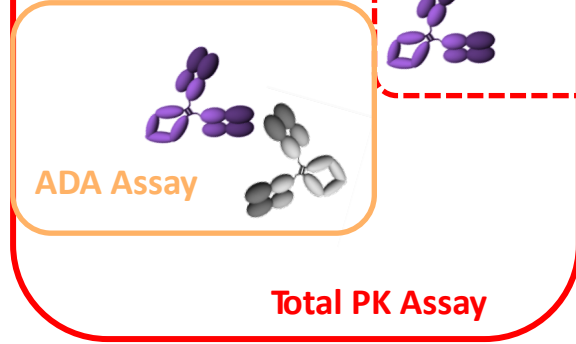
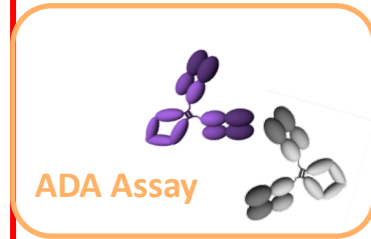
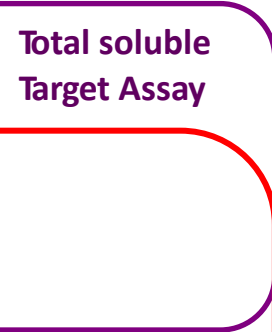
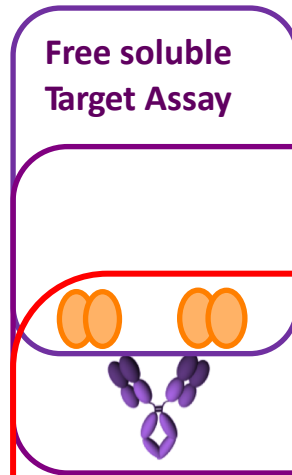
- Can bind to the antigen multiple times
- Can actively degrade antigen
- Can eliminate or sweep antigen from plasma

The Sweeping Antibody[®] is a Recycling Antibody[®] that has been further engineered to bind to FcRn at neutral pH.

Bioanalytical Package : Our Choice



Transformation to mature target



Drug



Soluble dimeric target



Mature target



Anti-drug antibody

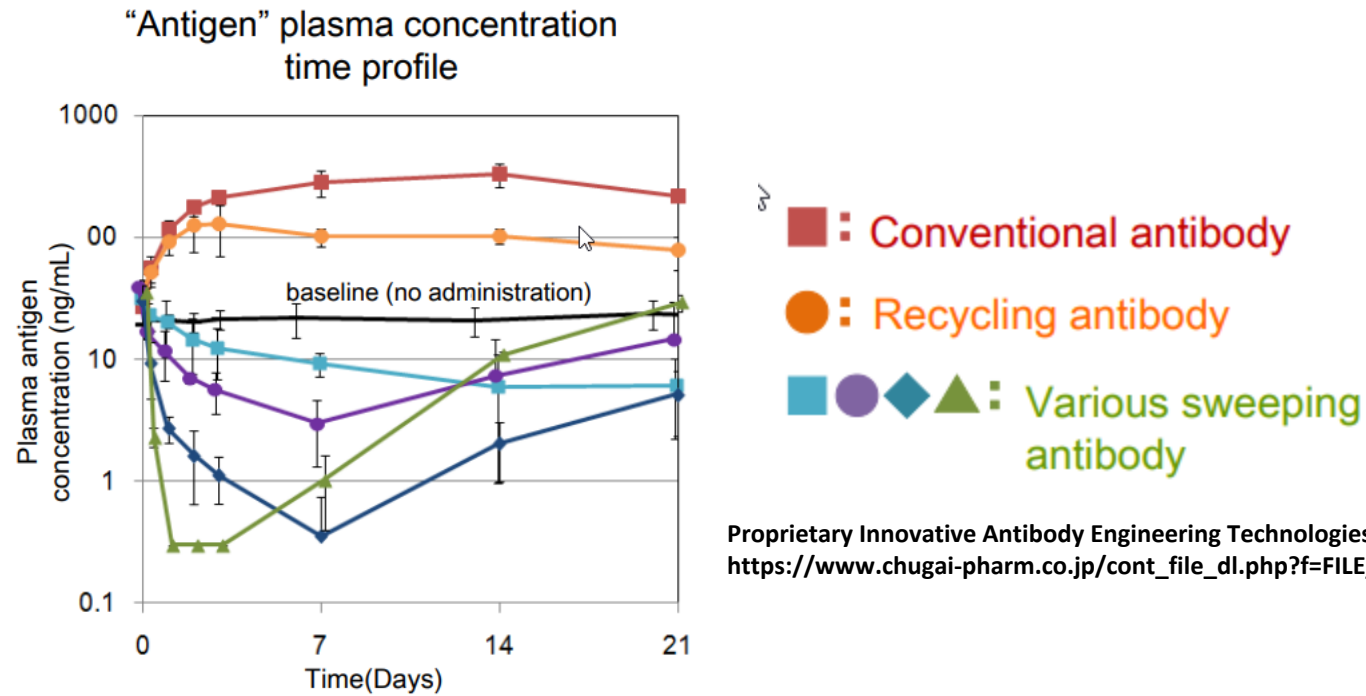
BM or Target assays

PK assay

Immunogenicity

PD Strategy : Why Total & Free assays ?

- **Aim of Total PD assay** : The drug has sweeping properties. The total soluble target assay should confirm this “sweeping” to show concentrations decrease compared to baseline.



- **Aim of the Free PD assay** : As endogenous level of soluble target allows it, development of a free PD assay was chosen to prove the target engagement and evaluate the amount of active soluble target still available.

Main challenge is to have really sensitive PD assays

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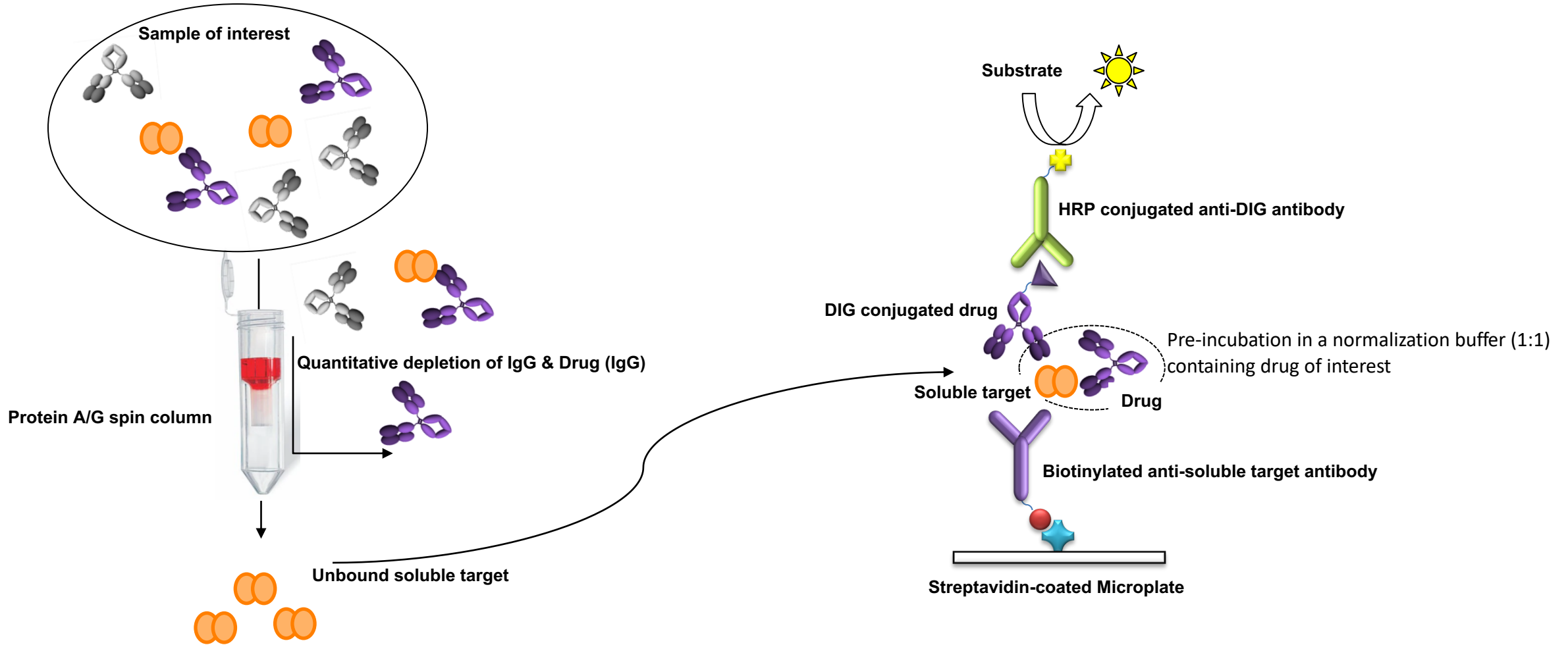
Our Strategy at Roche

PD Assays - Development Plan

- Setup Total and Free soluble target assay with **high sensitivity**.
- If possible **independent from high sensitive assay platforms**.
- **Combined Assay Protocol** for Total and Free assay to narrow down complexity in validation and reagent supply.
- Request to **analyze placebo samples in both assay**.

Plan taking into account future assays transfer to a preferred CRO partner

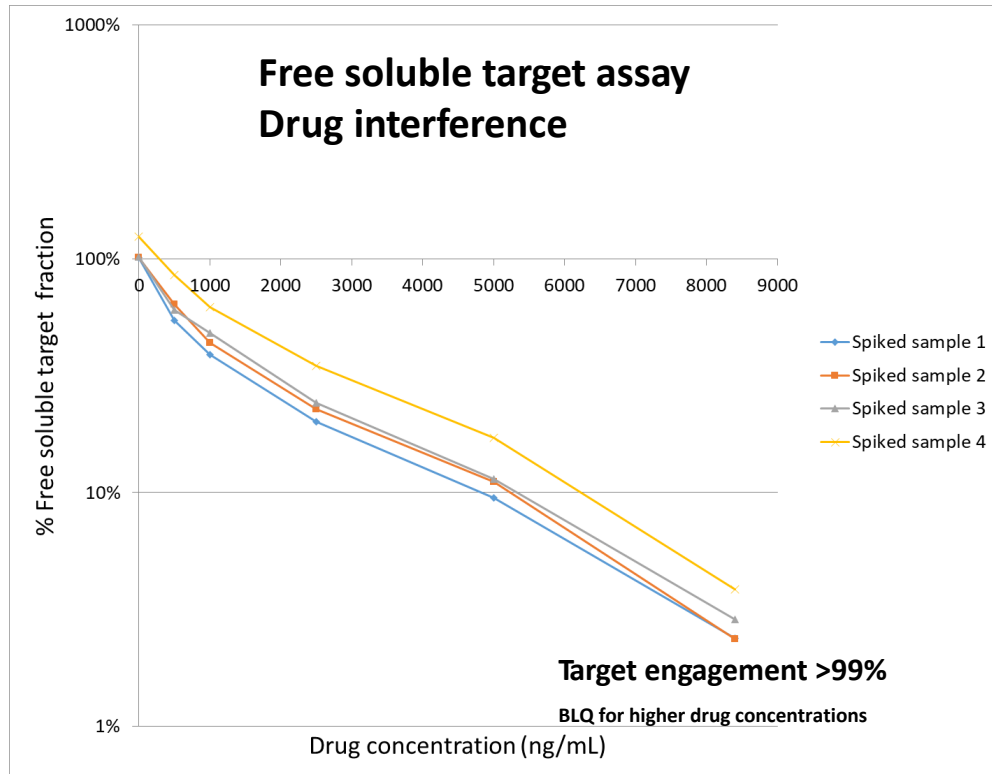
PD Assays - A combined approach



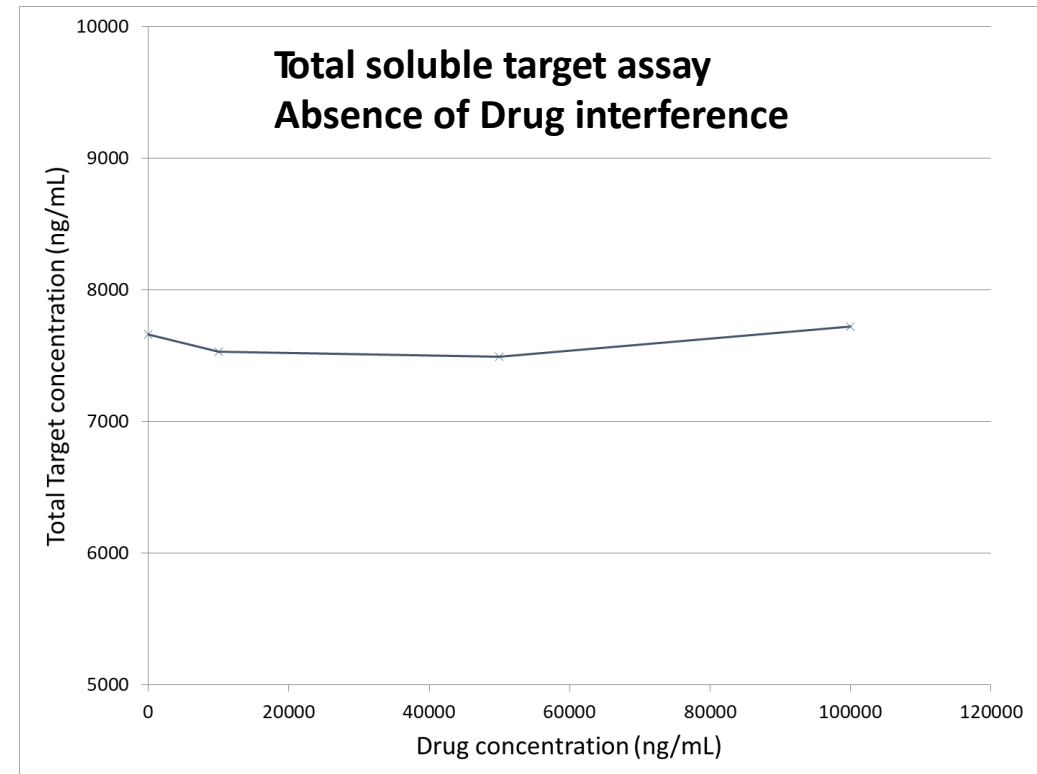
PRE-TREATMENT

ASSAY FORMAT

PD Assays – Performance Comparison

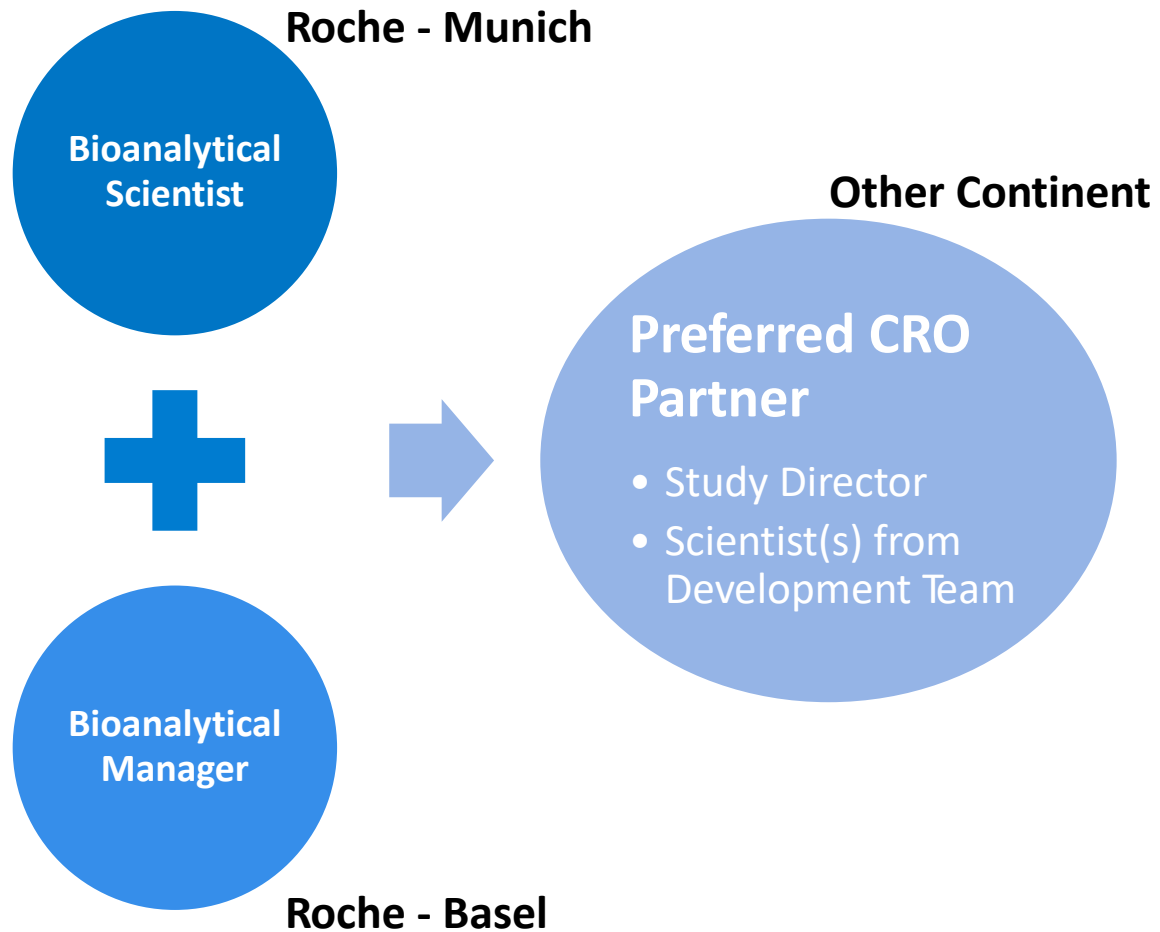


LLOQ : 60.0 pg/mL



LLOQ : 240 pg/mL

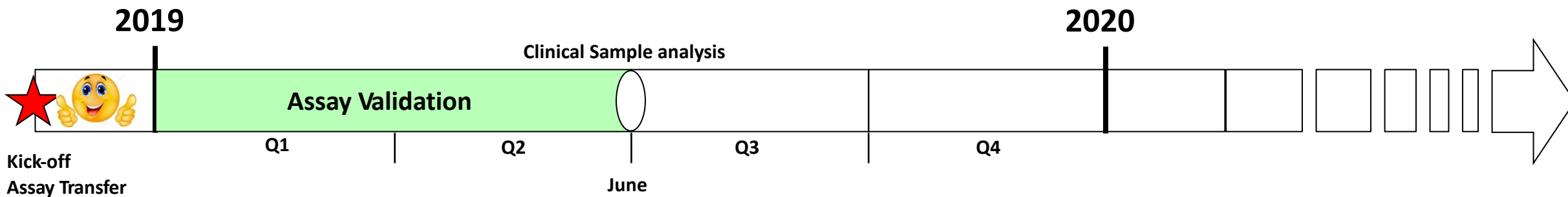
PD Assays - Transfer to a Preferred CRO Partner



- Preferred CRO Partner located on a **different continent**
- **Test procedure** and **Pipetting scheme** were shared prior to the kick-off meeting
- **Kick-off Meeting** organized with the preferred CRO partner via TC with :
 - Description and explanation of the method and critical points to consider
 - Data from assay development/qualification shown (but not shared)
 - Discussion about technical points

Successful Assay Transfer

Plan & Timelines

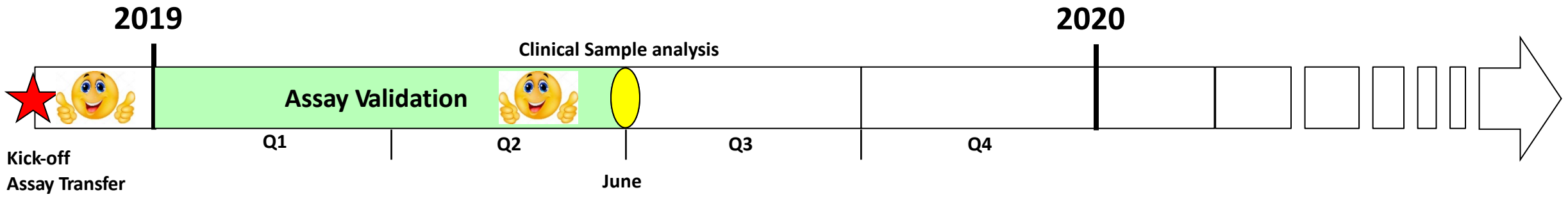


Assay Validation



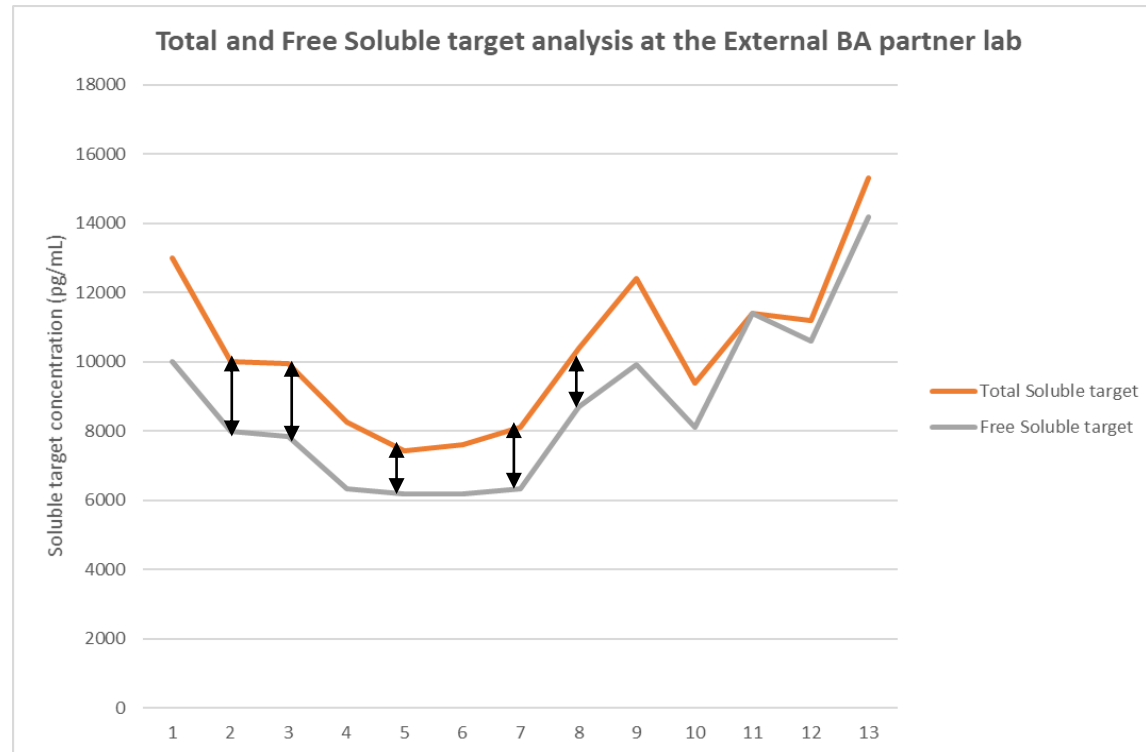
Validation Parameter	Validation Results
Calibration range	60.0 pg/mL to 28000 pg/mL
LLOQ	60.0 pg/mL
ULOQ	28000 pg/mL
Precision	Intra-assay precision: 1.9 to 11.7% Inter-assay precision: 2.7 to 19.4%
Accuracy	Intra-assay accuracy: 5.7 to 14.2% Inter-assay accuracy: -26.5 to 13.6%
Drug Interference	50.0 µg/mL drug interferes with endogenous QCs
Short and Long term stability	24h at RT 42 days at -20°C and -70°C

Plan & Timelines



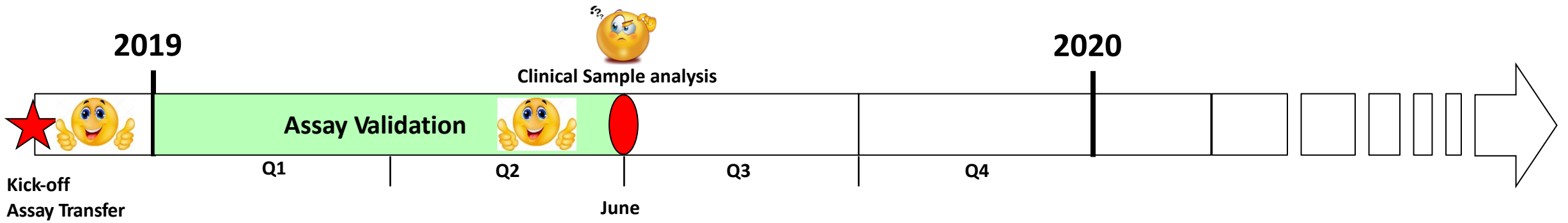
Samples analysis : The importance of Placebo samples

- First samples analysis... First questions...

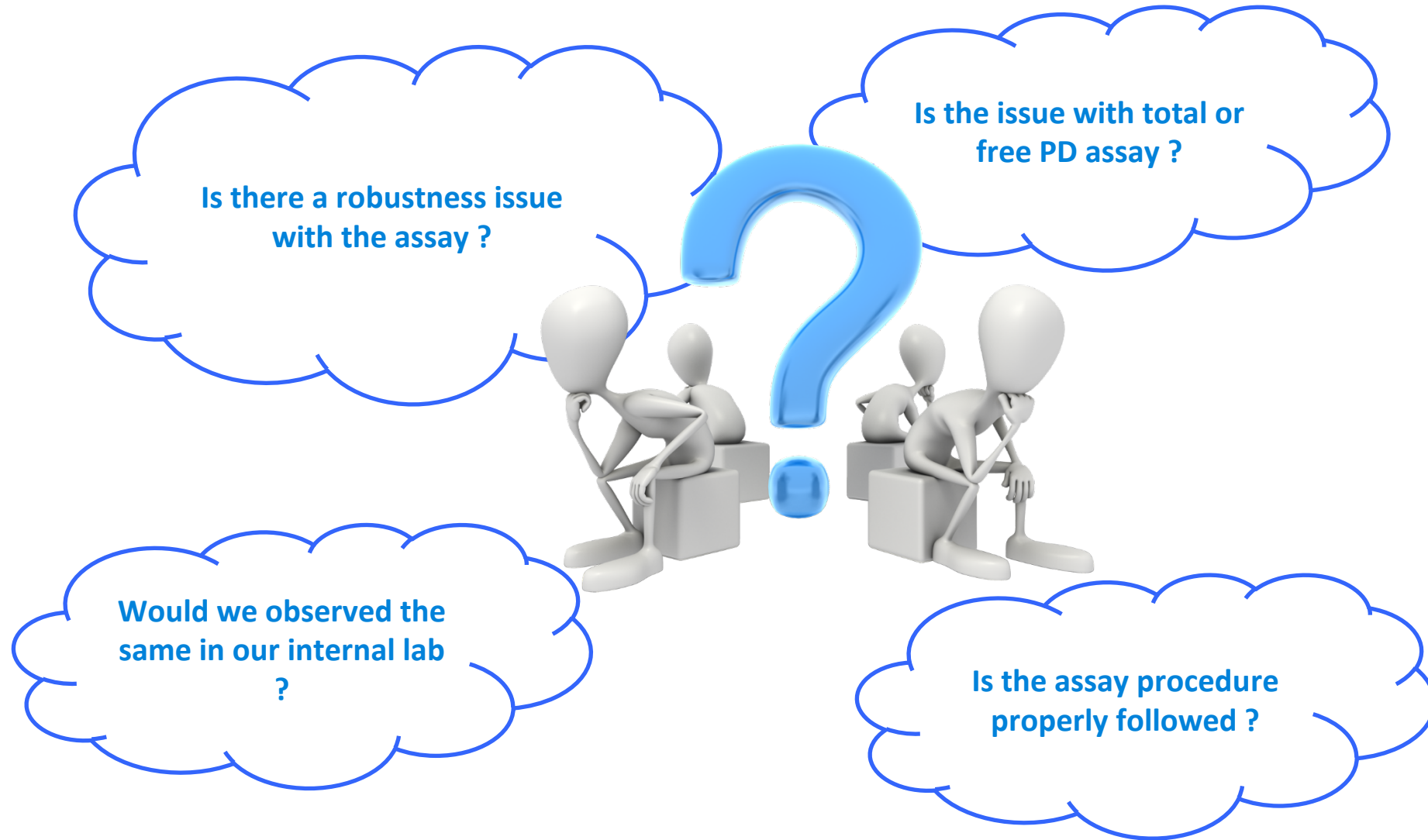


Under-estimation of the level of soluble target measured in the Free assay

Plan & Timelines



Troubleshooting : Rational Approach



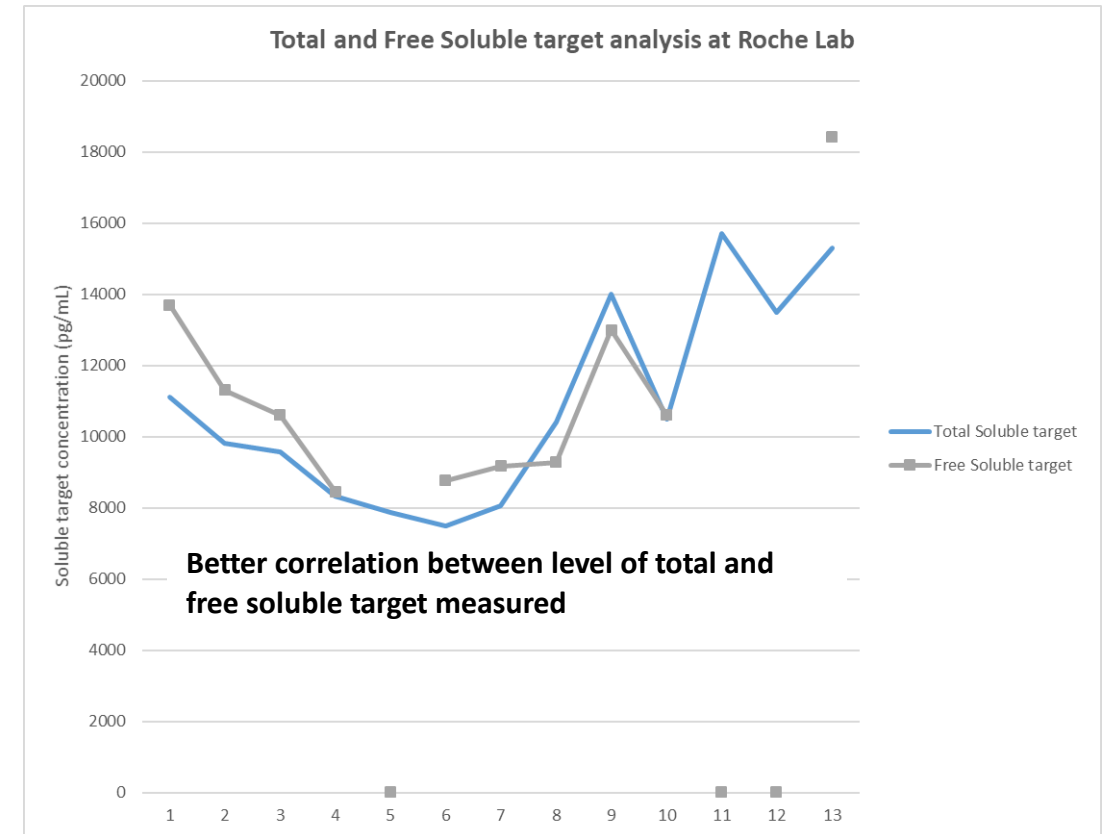
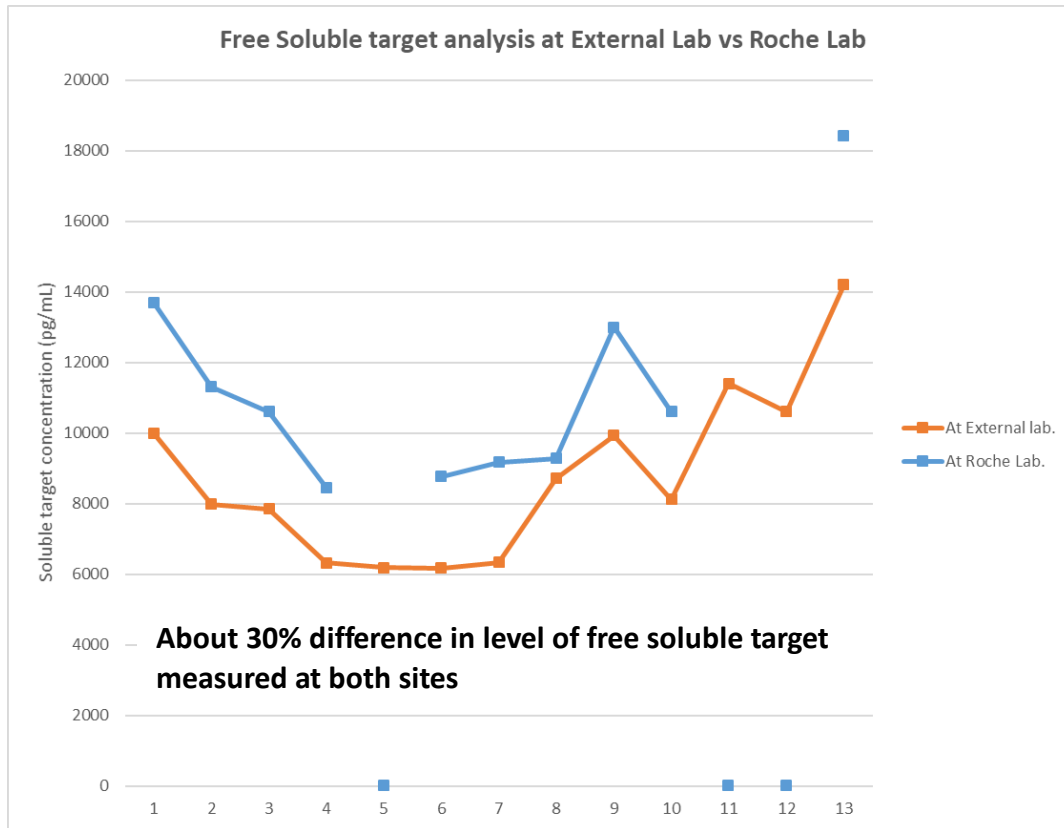
Troubleshooting : First answers

- At Roche Lab., simulation of potential «failures» (modification of pH values and dilutions...) on the assay:
 - **Assay is really robust.**
- **Total soluble target** assay cross-analysis between Roche BA lab and Preferred CRO partner :
 - 8 selected healthy volunteers coming from Roche BA lab:
 - **100% samples within +/- 30% difference**
 - 26 study samples (1 placebo + 1 treated patient):
 - **81% samples within +/- 30% difference (69% within +/- 20% difference)**

Assay is robust and Total PD analysis is consistent between both labs

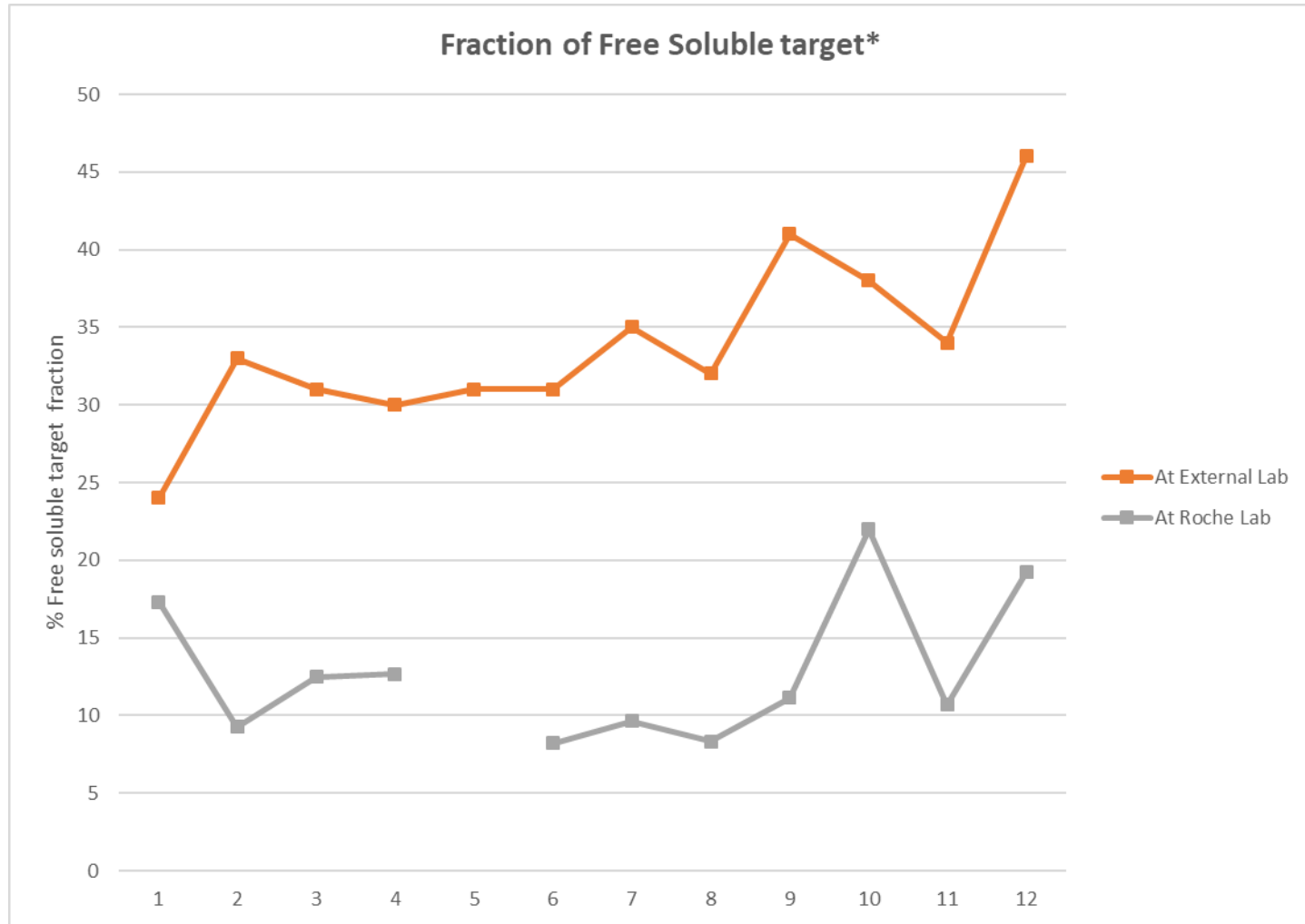
Troubleshooting : Focus on Free PD Assay

Placebo patient



Troubleshooting : Focus on Free PD Assay

Treated patient



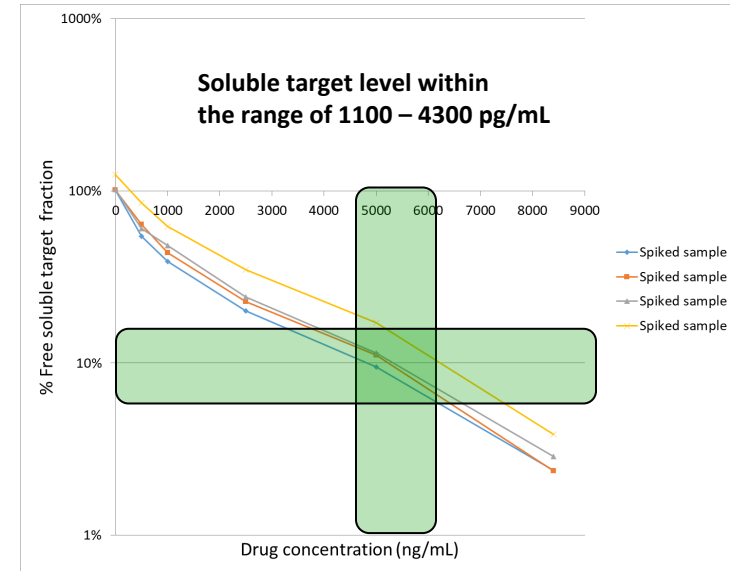
Fraction of free soluble target is overestimated by around 20% at the preferred CRO partner

* Based on level of total soluble target measured

Troubleshooting : Focus on Free PD Assay

From Assay development, we know...

Spiked level of drug [ng/mL]	Fraction of Free soluble target (Mean %)
1000	~ 50
2500	~ 25
5000	~ 12
8400	~ 3



From the Clinical study, we get...

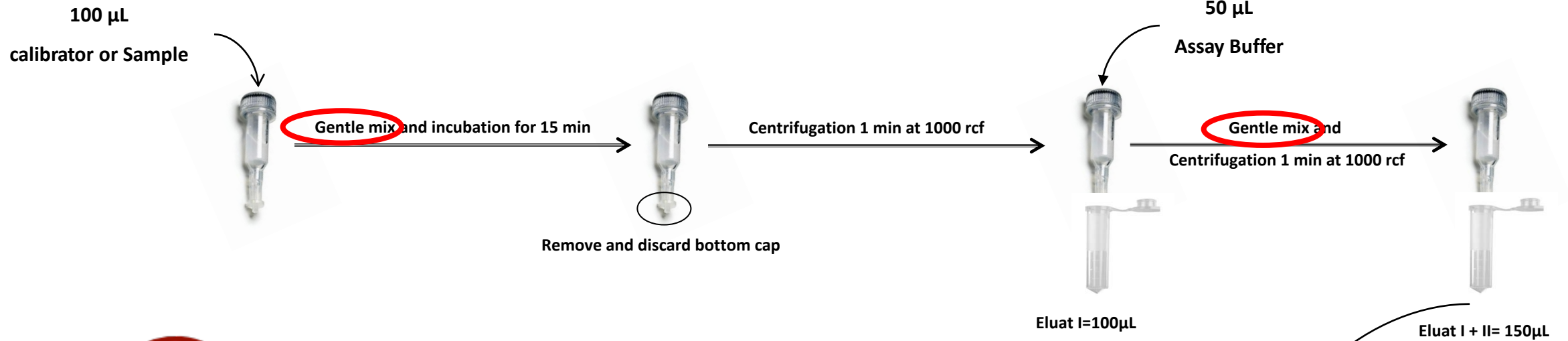
Samples	Drug level measured with PK assay (ng/mL)	Fraction of Free soluble target (%) measured at	
		Roche Lab	Preferred CRO Partner
4	5920	12	31
12	5060	11	34
13	4900	19	46

Free assay works as expected at Roche Lab.

Troubleshooting : Visit at our Preferred CRO Partner

- **2 Days visit**
 - Day 1 : Observation of the assay procedure performed at the CRO lab.
 - Day 2 : If required, Roche Scientist will perform the assay
- After Day 1, the issue was immediately identified :
 - Our preferred CRO partner used a different interpretation of the mixing procedure for the spin column :
«Samples were added to the spin column and gently mixed by hand (i.e. not inverted nor mixed with vortex) prior the 15 minutes incubation step and centrifugation. 50 µL of the assay buffer was added similarly and then centrifuged».

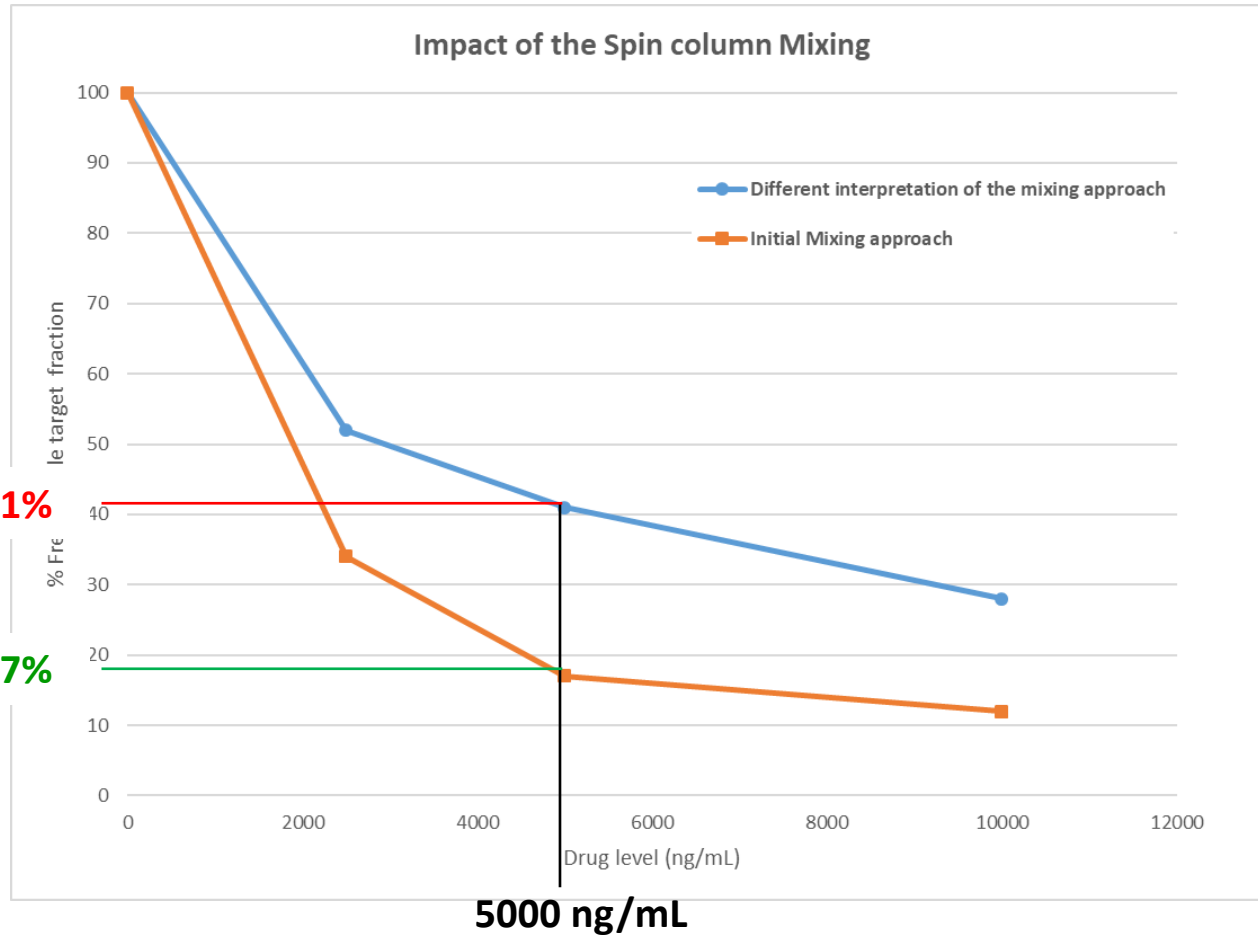
PD Assays - Pre-Treatment step Procedure



- Cap place back tightly
- Mix with vortex
- Loosen cap before centrifugation

IgG not fully depleted from sample

Troubleshooting : Visit at our Preferred CRO Partner



Spiked level of drug [ng/mL]	Fraction of Free soluble target (Mean %)
1000	~ 50
2500	~ 25
5000	~ 12
8400	~ 3

The different mixing procedure interpretation generated a partial IgG depletion

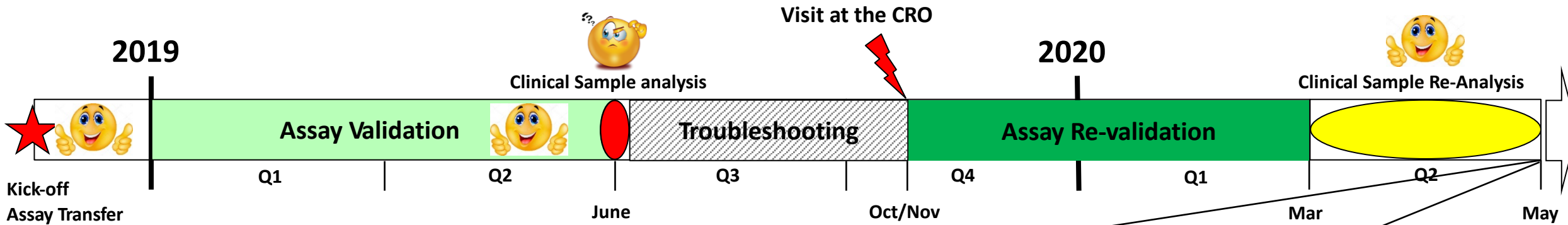
Troubleshooting : Conclusion

A rational approach was used to evaluate the situation :

- The issue was not due to a lack of robustness of the assay
- The Total PD assay performed the same way at both labs
- The Free PD assay gave different results at both labs
- **Visit at the preferred CRO lab helped to identify the different pre-treatment procedure followed**
- The issue would have been difficult to identify without a visit on site

Small “detail”... Big impact

Impact : Delay on Clinical Samples Analysis



Data comparison from placebo & pre-dose samples analysed with free & total PD assays:

- 236 data compared
- **175 data within acceptance criteria* (74%)**
- 61 data outside acceptance criteria

*% difference between both values $\leq \pm 30\%$

Clinical Context & BA Strategy

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Our Strategy at Roche

Lesson Learned : Communication is KEY!

Between what I think, what I want to say, what I believe I say, what I say, what you want to hear, what you believe to hear, what you hear, what you want to understand, what you think you understand, what you understand...They are ten possibilities that we might have some problem communicating. But let's try anyway...

Bernard Werber

WWW.STOREMYPIC.COM

better way

Many information to share during an assay transfer. Focus should be made on critical aspects:

- **Identify all critical steps, potential weaknesses** of the BA method during development
- **Describe and report details** in relevant documents (development report, slides...)
- Be as **precise** as possible during Assay transfer initiation
- **Spend time** to explain and **follow up** to be sure the implementation is successful
- In this specific case, it would have been important to **include cross-evaluation of QCs samples** as part of the assay transfer
- Ideally, a **face to face transfer** is recommended but probably more and more challenging... (alternative could be video recording ?)

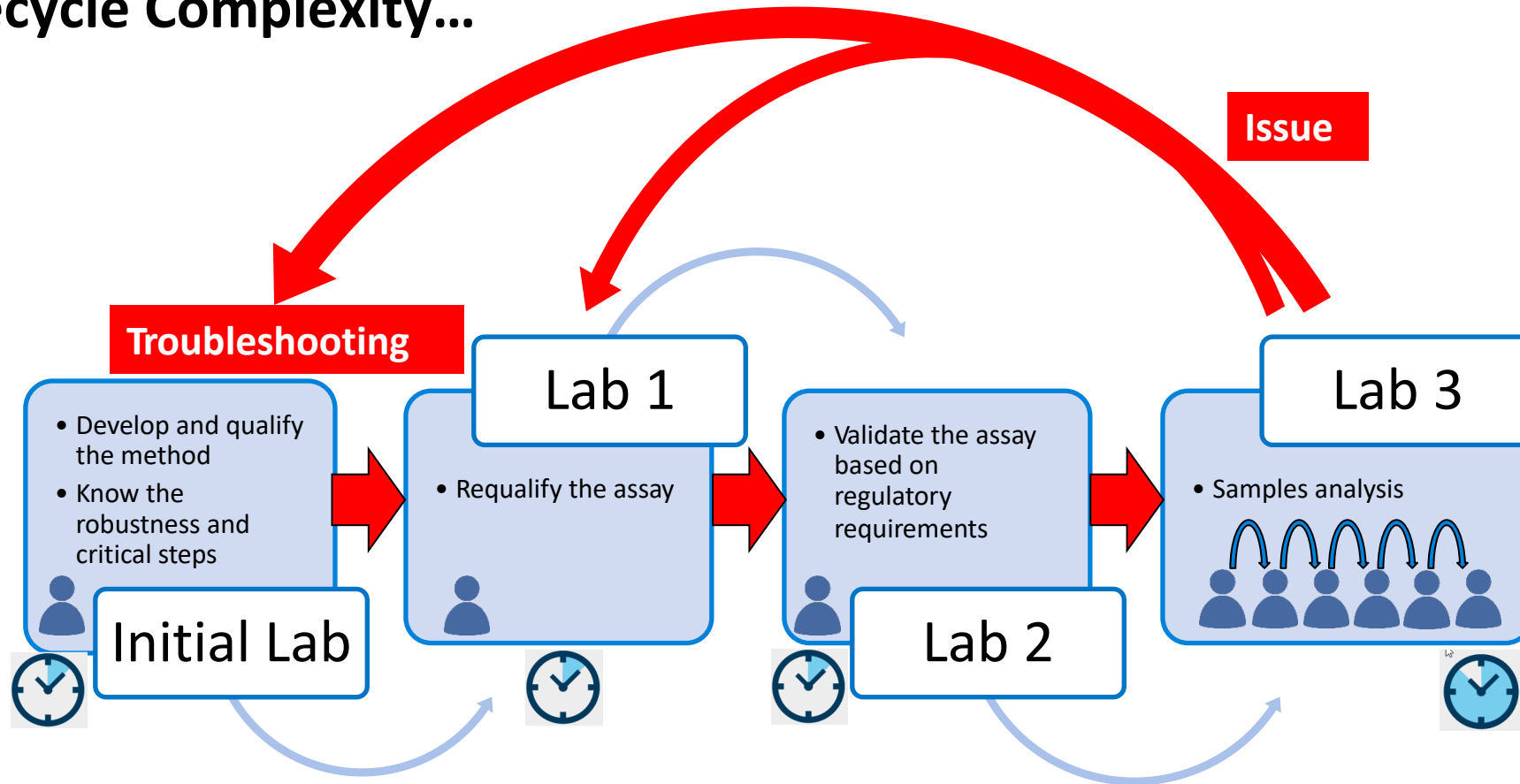
Never assume the exact same procedure will be followed by different operators.

Assay transfer is only one step of the assay life cycle...

Lesson Learned : Interpretation can be different...



Assay Lifecycle Complexity...



Assays can be used on a long period of time :

- Different labs/companies
- Different operators

Key elements :

- To have a good and deep understanding of the assay
- Consider the first assay transfer as critical
- Clear and standardized transfer/training procedure

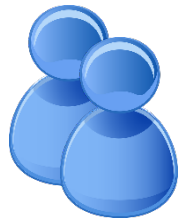
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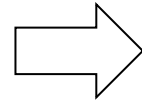
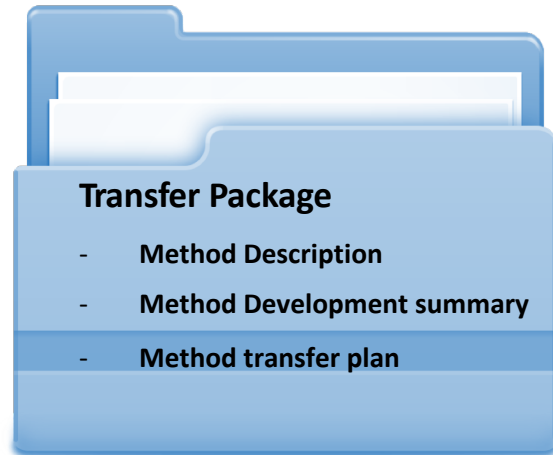
Our Strategy at Roche

Our Assay Transfer Strategy at Roche



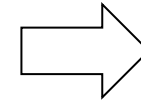
Bioanalytical Scientists

Development and Qualification of BA methods



Kick-Off Meeting

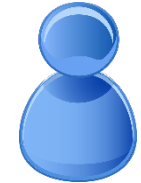
- technical discussion (critical steps, relevant questions...)
- lines of communication
- timelines
- acceptance criteria for a successful assay transfer
- planned experiments
- planning of the required reagents



Assay Transfer

- Regular meeting
- Data review
- Go/no GO decision to move to Validation

LABORATORY



Point of Contact

Project Manager and/or Principal Scientist and/or Lab associate...



Bioanalytical Manager

Full regulatory BA support for the all project lifecycle

Acknowledgments

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Jasna Canadi,
Matthew Barfield

External CRO Partner

To our different contacts for their great collaboration and openness

***Doing now what patients need
next***