LC-MS/MS assay transfer: a journey through the method cross-validation challenges

Alessio Maiolica, PhD

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NOVARTIS

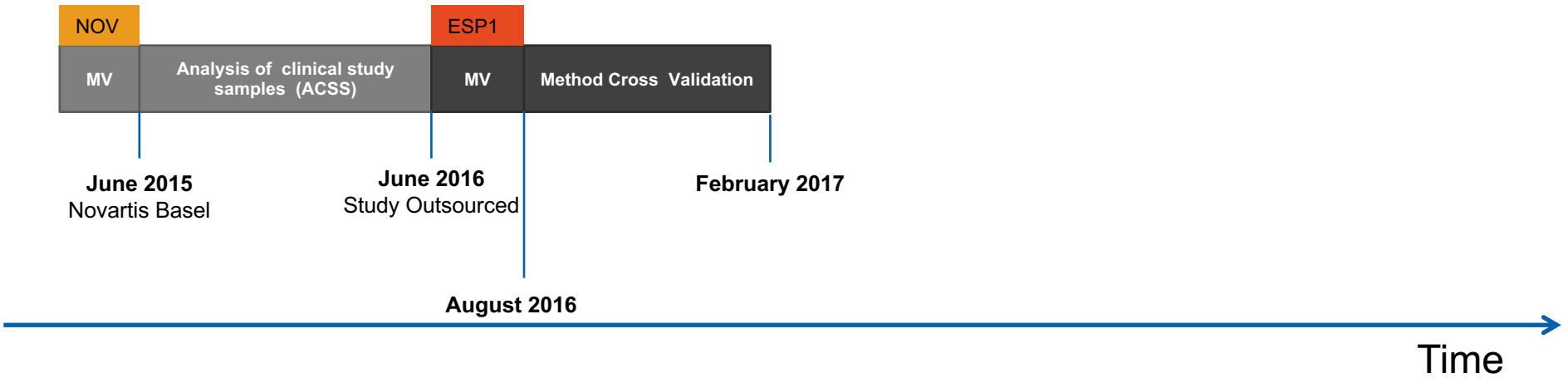
Agenda

Case study: Small molecule LC-MS/MS method cross-validation between 3 bioanalytical sites

- Issues faced
- Troubleshooting performed
- Robustness tests
- Impact of analytical methods variability on the PK

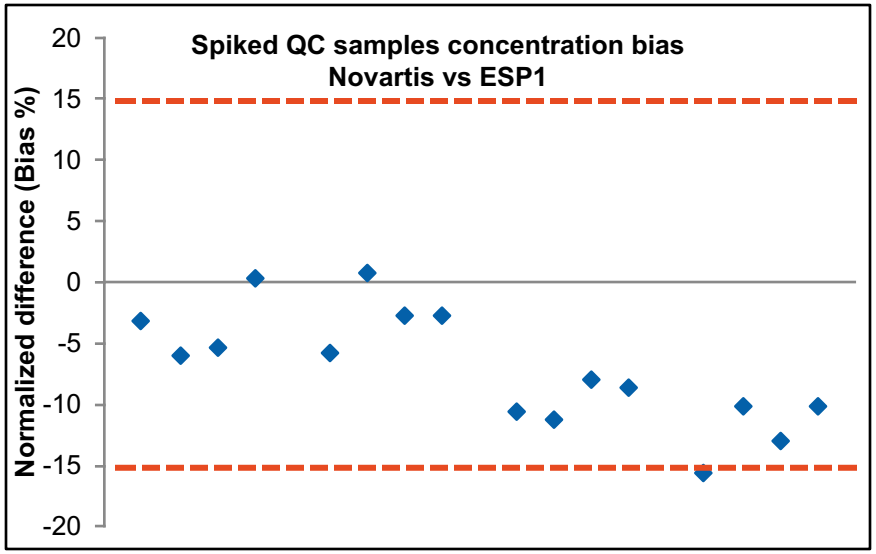
References:
Xiaohui Xu et al., Fit-for-purpose bioanalytical cross-validation for LC–MS/MS assays in clinical studies, Bioanalysis (2013) 5(1), 83–90
Stephen White et al., The quest for assay robustness across the life cycle of a bioanalytical method, Bioanalysis (2015) 7(7), 815–824
Alexandra Georgiou et al., An inter-laboratory transfer of a multi-analyte assay between continents, Bioanalysis (2015) 7(7), 825–831

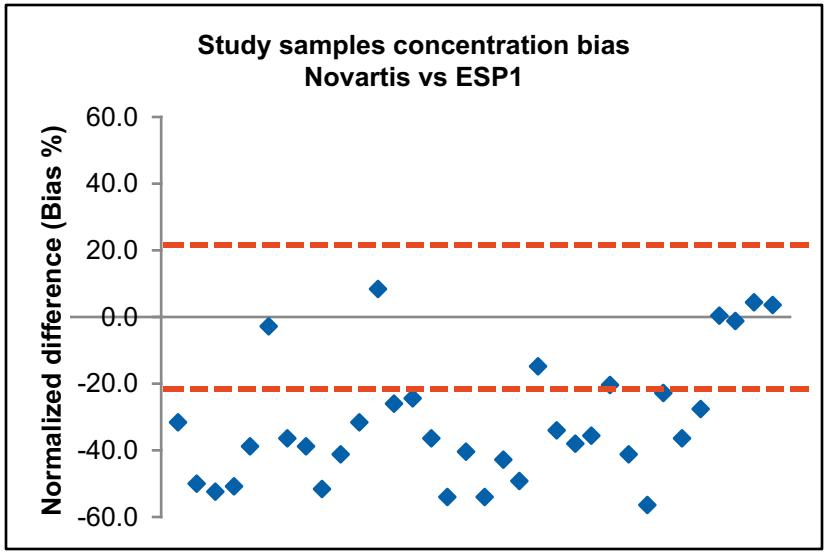
Summary of BA activities
-First in man study-



Per EMA and FDA guidelines during the course of a study, incurred and spiked samples should be analyzed and cross checked between the involved laboratories when clinical study samples are measured by 2 or more laboratories.

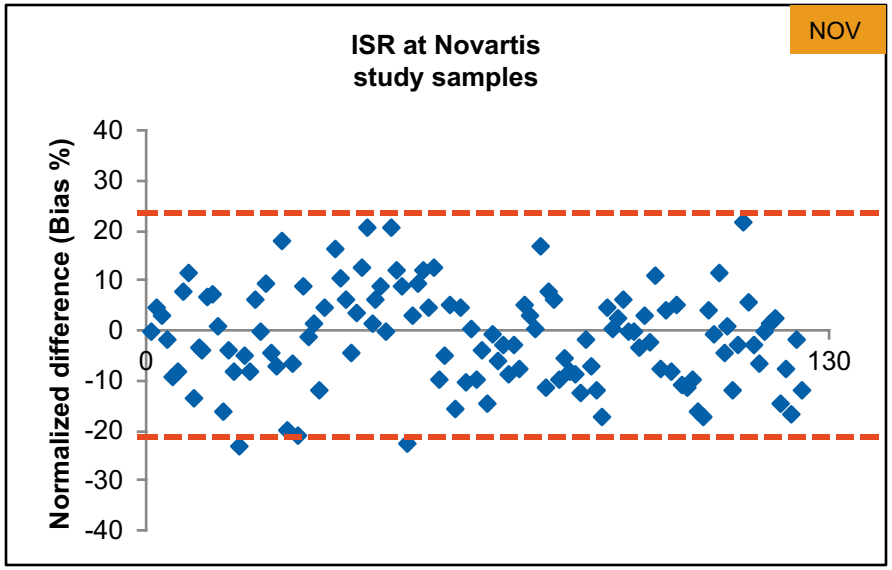
Results: Method cross comparison
-Spiked QC samples and clinical samples-

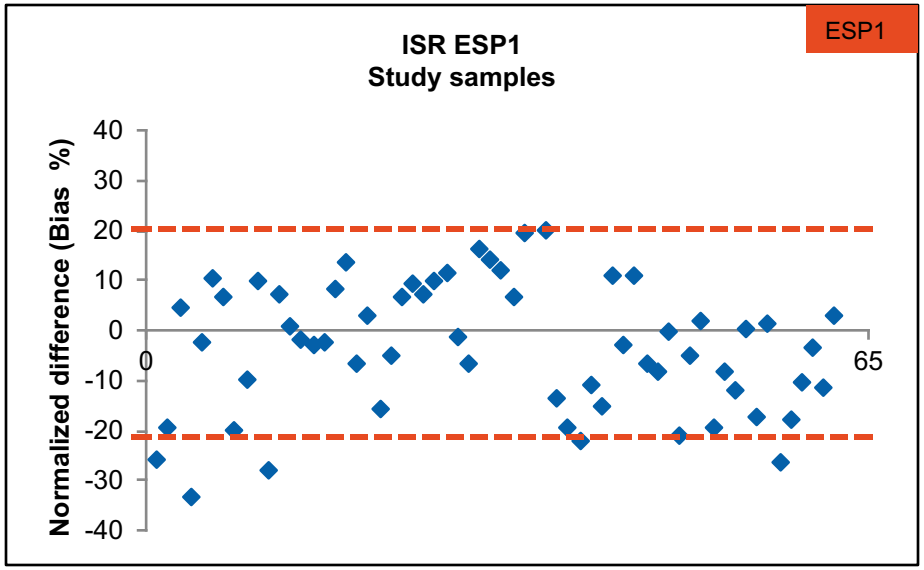




- **Spiked QC samples Acceptance criteria:** at least 2/3 of the data within ± 15% of the normalized difference. **Cross-check: OK**
- **Clinical study samples Acceptance criteria:** at least 2/3 of the data within ± 20% of the normalized difference. **Cross-check : Failed**

ISRs Methods reproducibility assessment -Clinical samples-





- **ISR assessments demonstrate methods reproducibility both at Novartis and ESP1 sites**

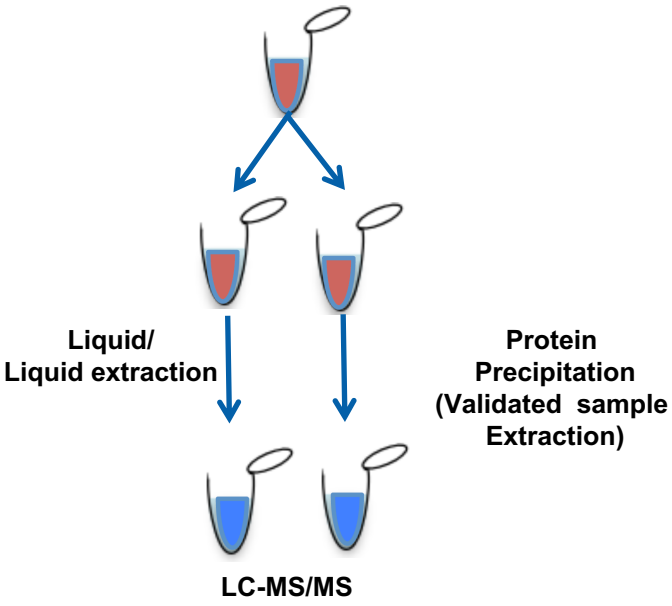
What went wrong?

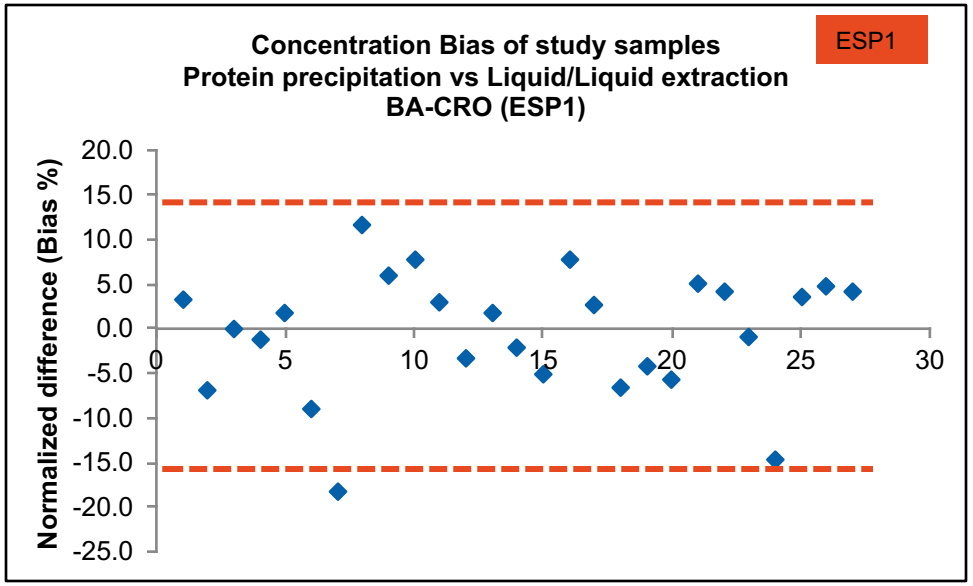
	Novartis Basel	BA-CRO ESP1
Validation	OK	OK
Long term stability	Fulfilled	Fulfilled
Stock solution stability	Fulfilled	Fulfilled
Selectivity, (N-Oxide)	Fulfilled	Fulfilled
Sample processing	Consistent between the two laboratories	
Chromatography	Consistent between the two laboratories	
Mass spectrometer	API 5000	API 6500
UPLC	Symbiosis	Shimadzu
Samples	Concentrations consistent between primary and back up samples	
ISR	Passed acceptance criteria in both laboratories	

We don’t know !

Method robustness assessment -sample extraction-

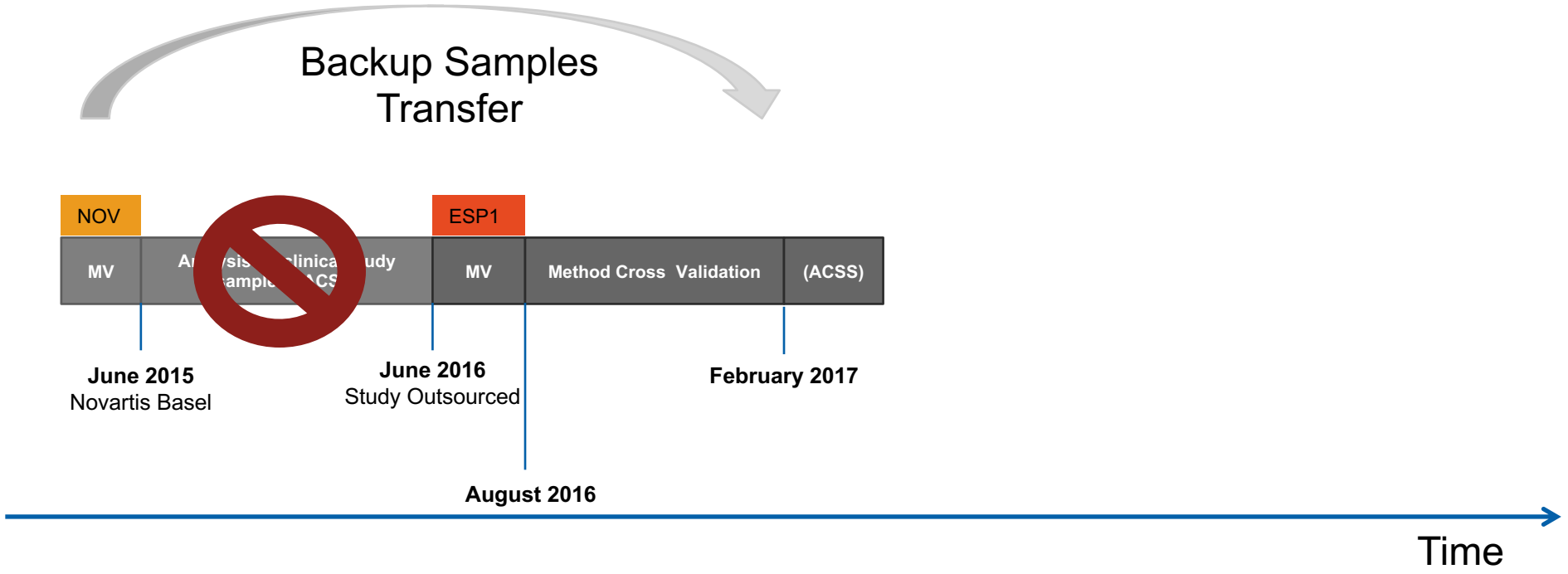
- The calculation of pharmacokinetic parameters for a drug assumes that the concentrations measured in clinical samples are reliable.



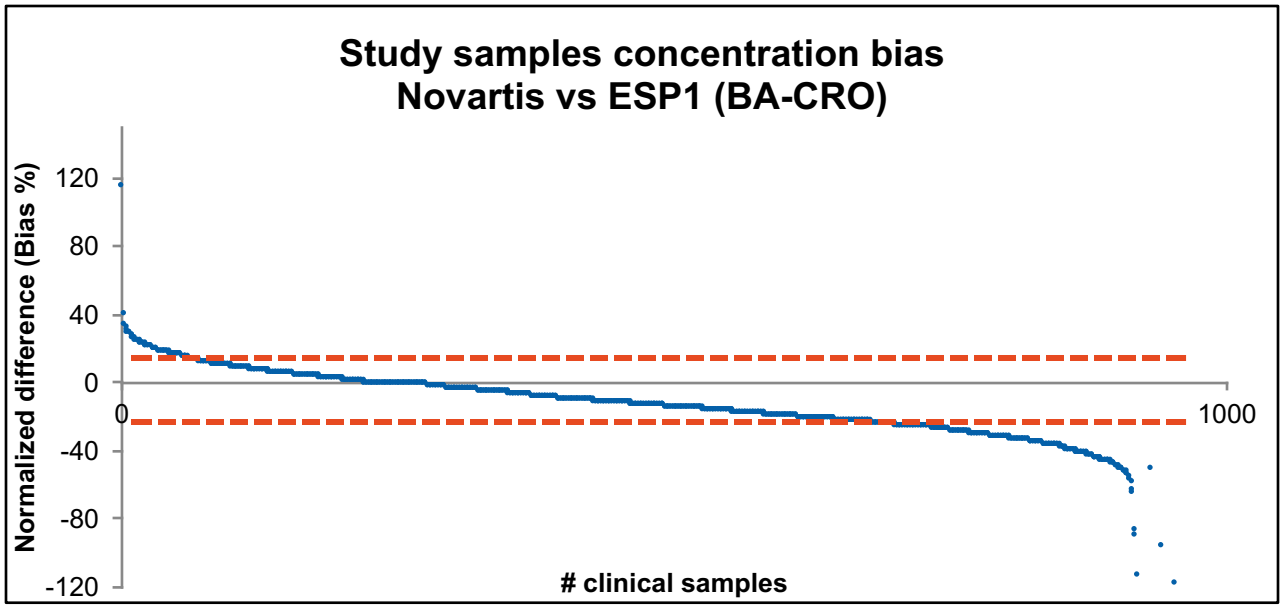


- **The concentration measured in clinical samples were confirmed by a different sample extraction strategy**

Summary of BA activities -First in man study-

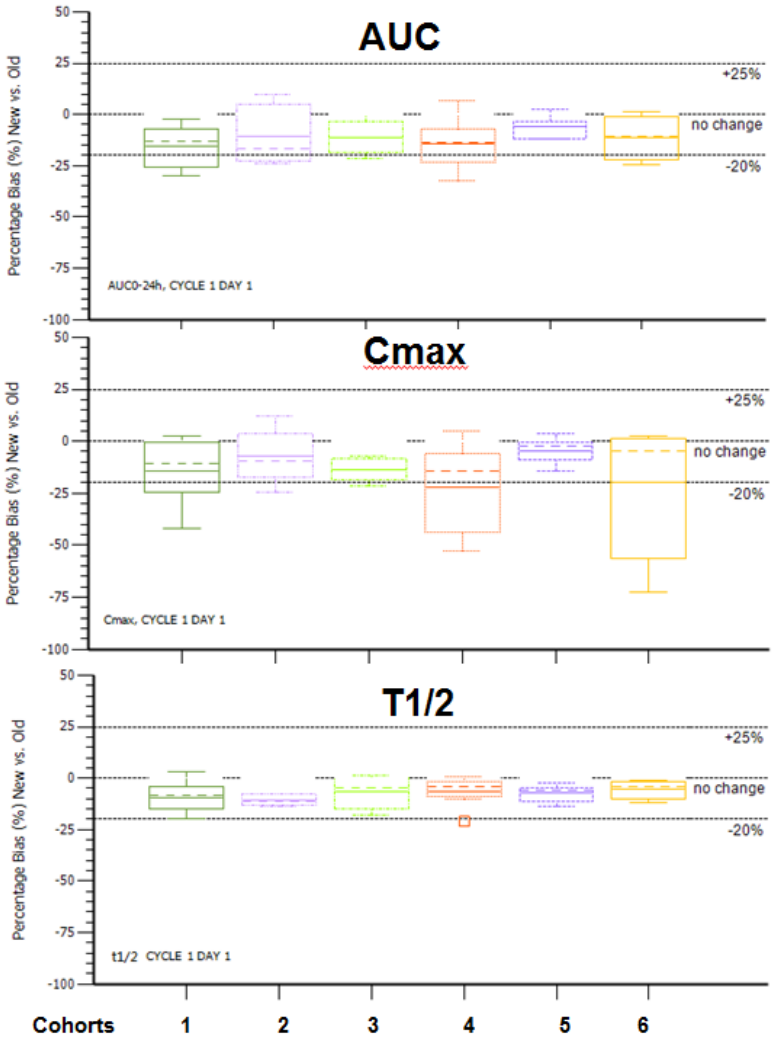


Concentration bias of re-measured samples at the ESP1



- 61% of the concentration measured are within acceptance criteria (±20% of normalized concentration bias).

Impact of the concentration Bias on key PK parameters (NVS vs ESP1)

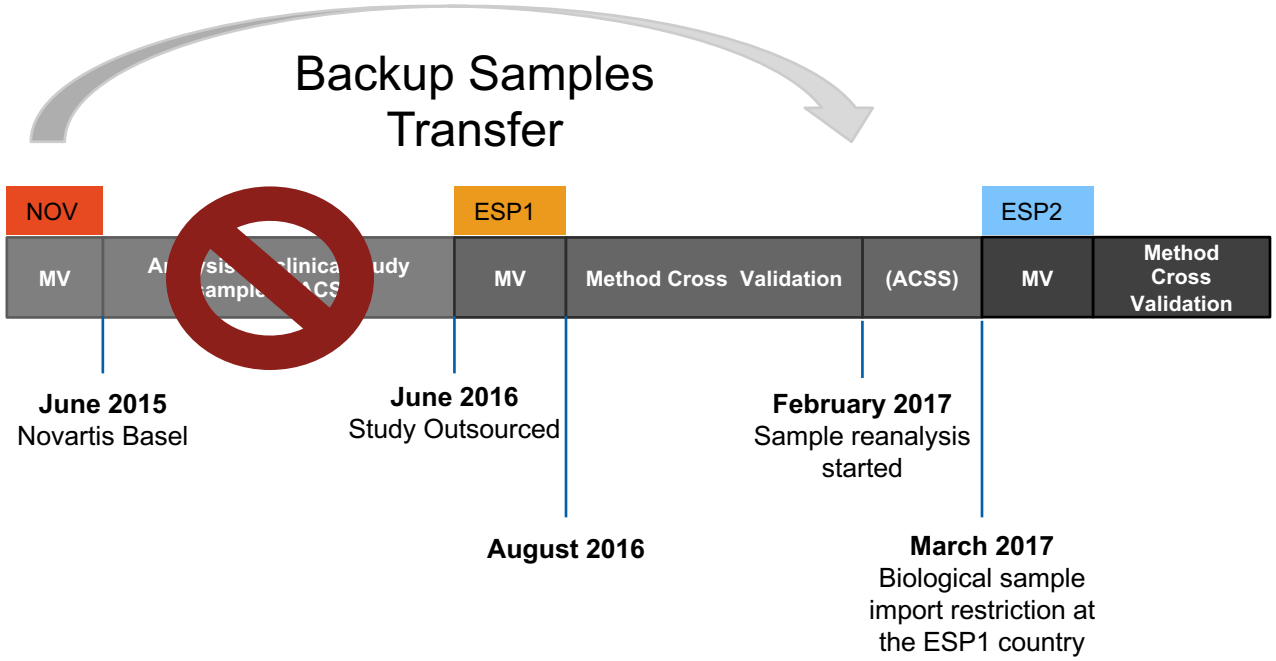


Graphs Courtesy of Yi (Gary) Gu, PKS Novartis

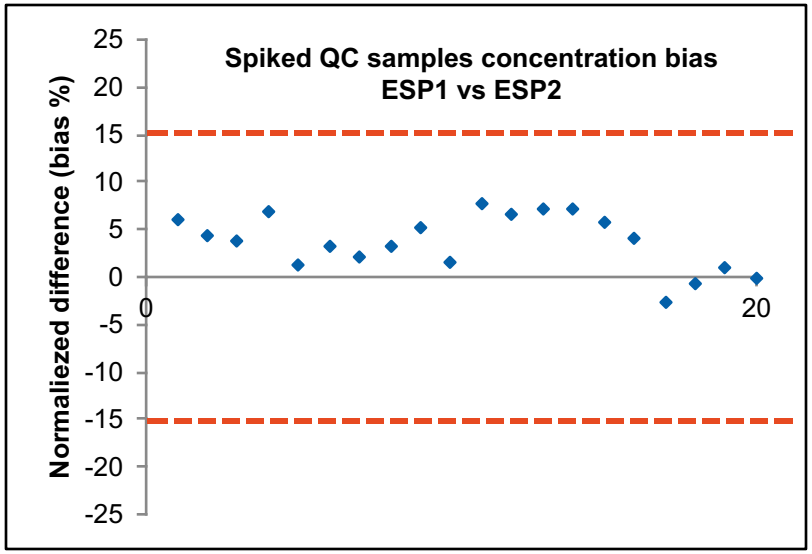
NIBR, PK Sciences

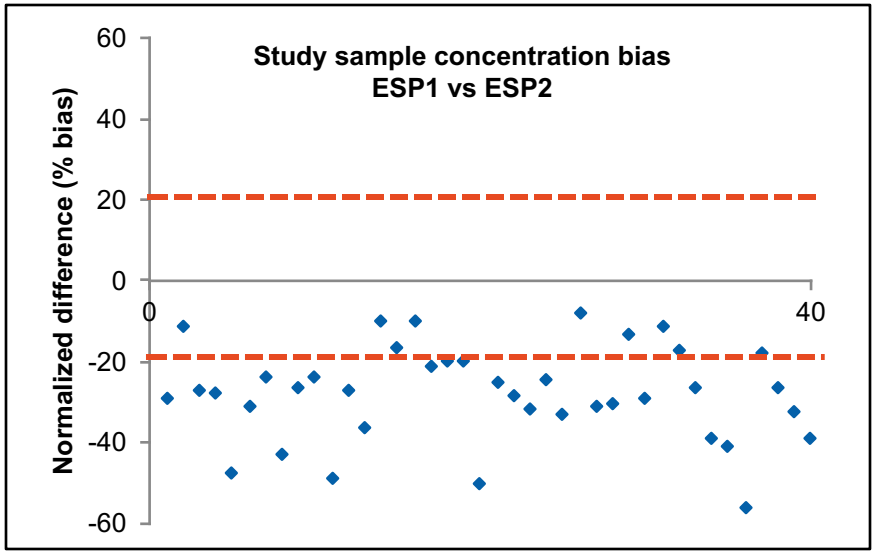
PK Parameters	Effects of the methods bias
AUC T1/2	Not of clinical significance
Cmax	In some individuals and for some cohorts of clinical significance
Impact on the clinical strategy	----------
Dose escalations	Not impacted
Recommended Phase II Dose	Possibly impacted
Food effect cohort samples measured between the two BA sites	PK results of difficult comparison

Summary of BA activities -First in man study-



Results: Method cross comparison -Spiked QC samples and clinical samples-





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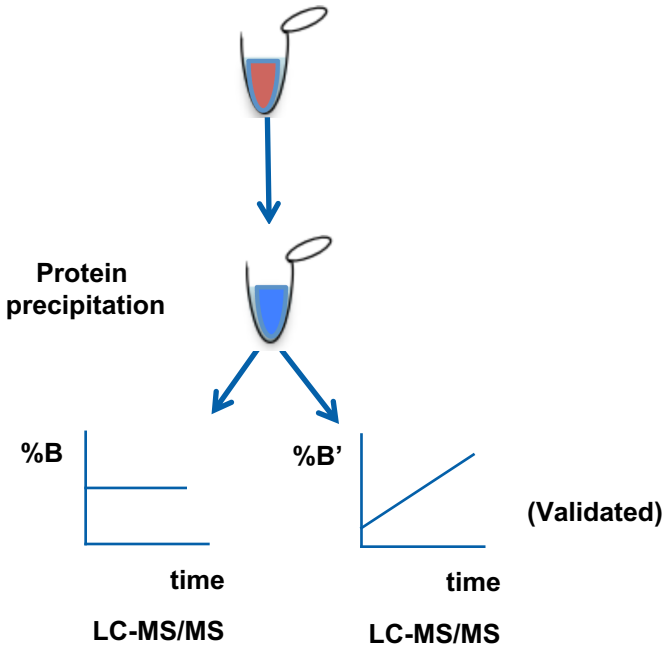
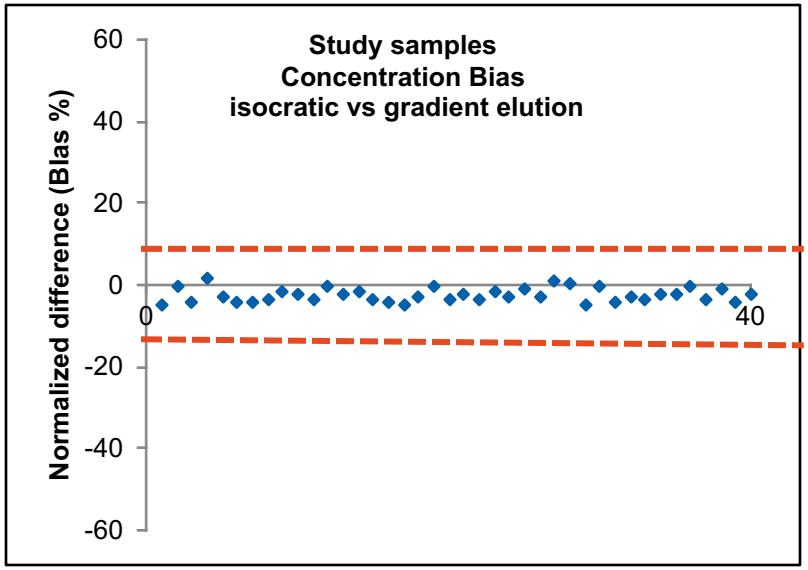
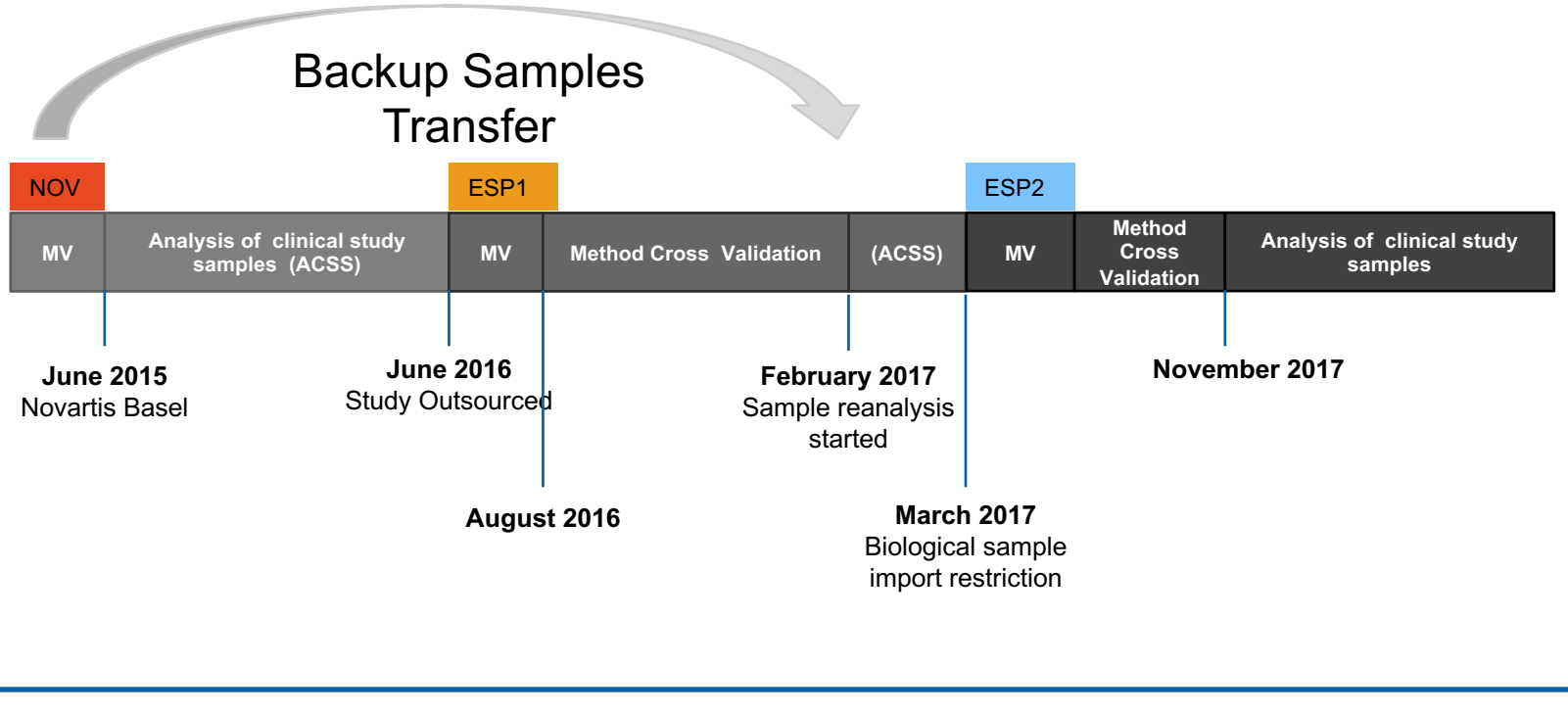
Method robustness assessment -Selectivity assessment-





- The concentration measured in clinical samples were confirmed by a different chromatographic set up

BA activities to support a first in man study



Conclusions/lessons learned

- Method transfer between different laboratories is the “optimal” robustness test.
- Method robustness assessment should be designed early on during the drug development program.
- Study samples should be used for the method robustness assessment.
- The effect of methods bias should be assessed in the context of the PK objectives of a clinical program.

Thank you!