Navigating through the composite source of biomarker assay variability

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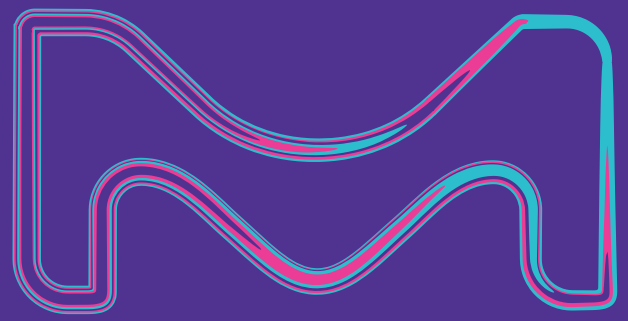
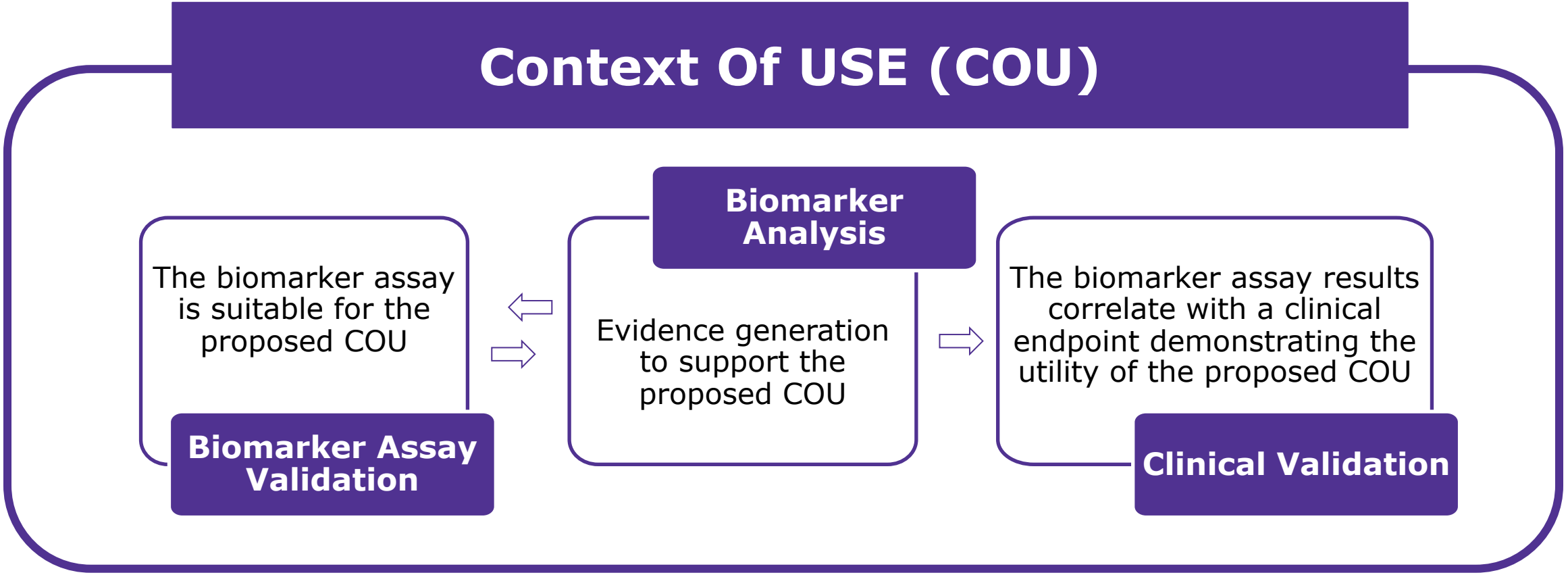


MERCK

Biomarker qualification



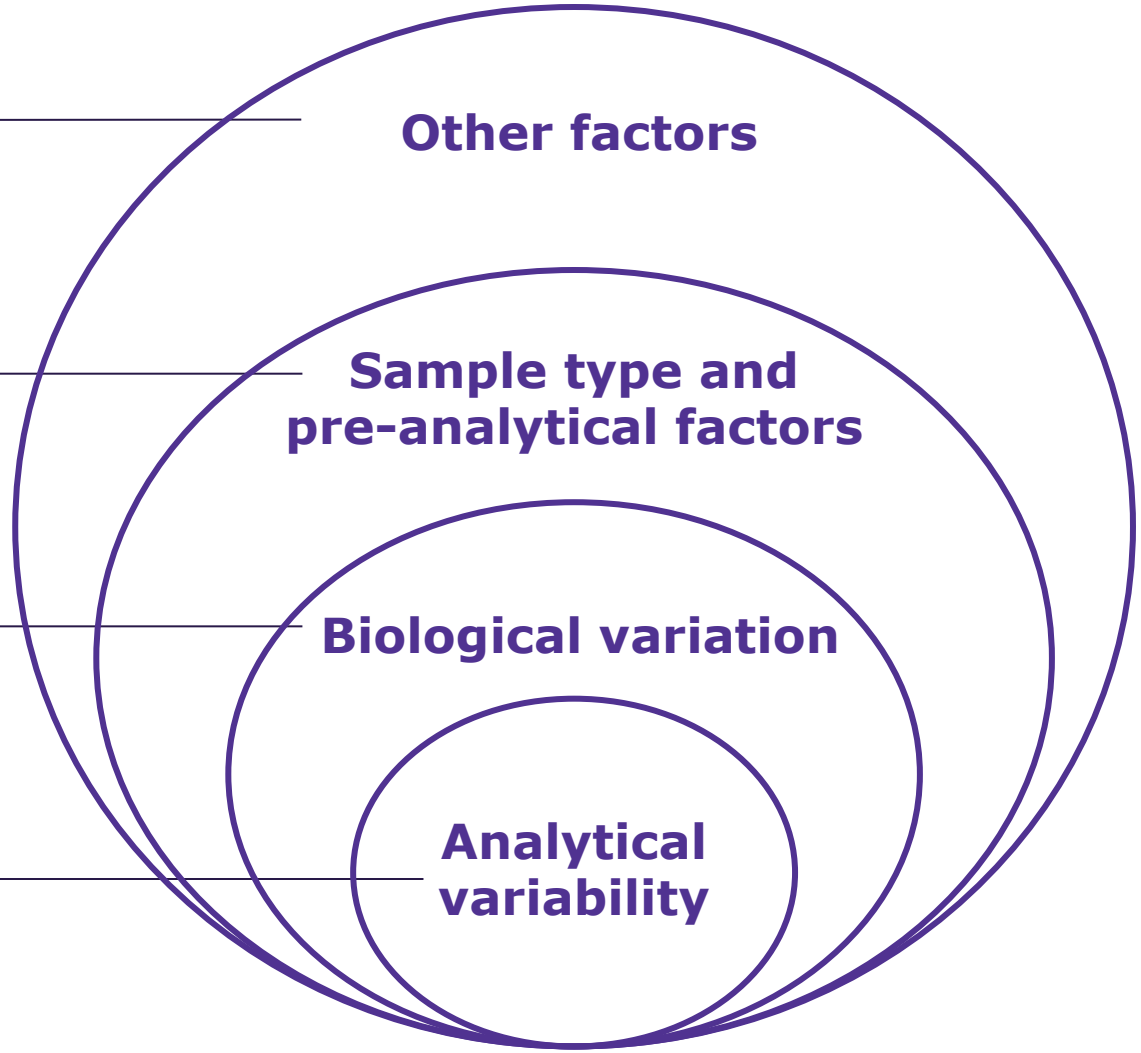
The composite source of variability of a Biomarker assay

Consider and evaluate changes over time and space (e.g. critical reagent management and inter-lab analyses)

Select the right specimen (e.g. blood, tumor) and consider its pre-analytical factors

Investigate and know the modulation of the biomarker of interest in healthy and diseased population

Establish and control the performance characteristics of the biomarker assay



Biological variation (BV)

- **Within-subject BV**, random fluctuation of the BM of interest around homeostatic setting points

- **Between-subject BV**, overall variation of BM of interest from the different subjects‘ setting point

✓ **Essential for the correct design of the BM assay**
✓ **Essential for the correct interpretation of results**

Biological Variation and Magnitude of Biomarker Response to the Treatment are strictly linked to the analytical assay performance

- A biomarker assay takes into account:
  - the within- and between-subject variation
  - the magnitude of biomarker response to the treatment
  - the required analytical performance

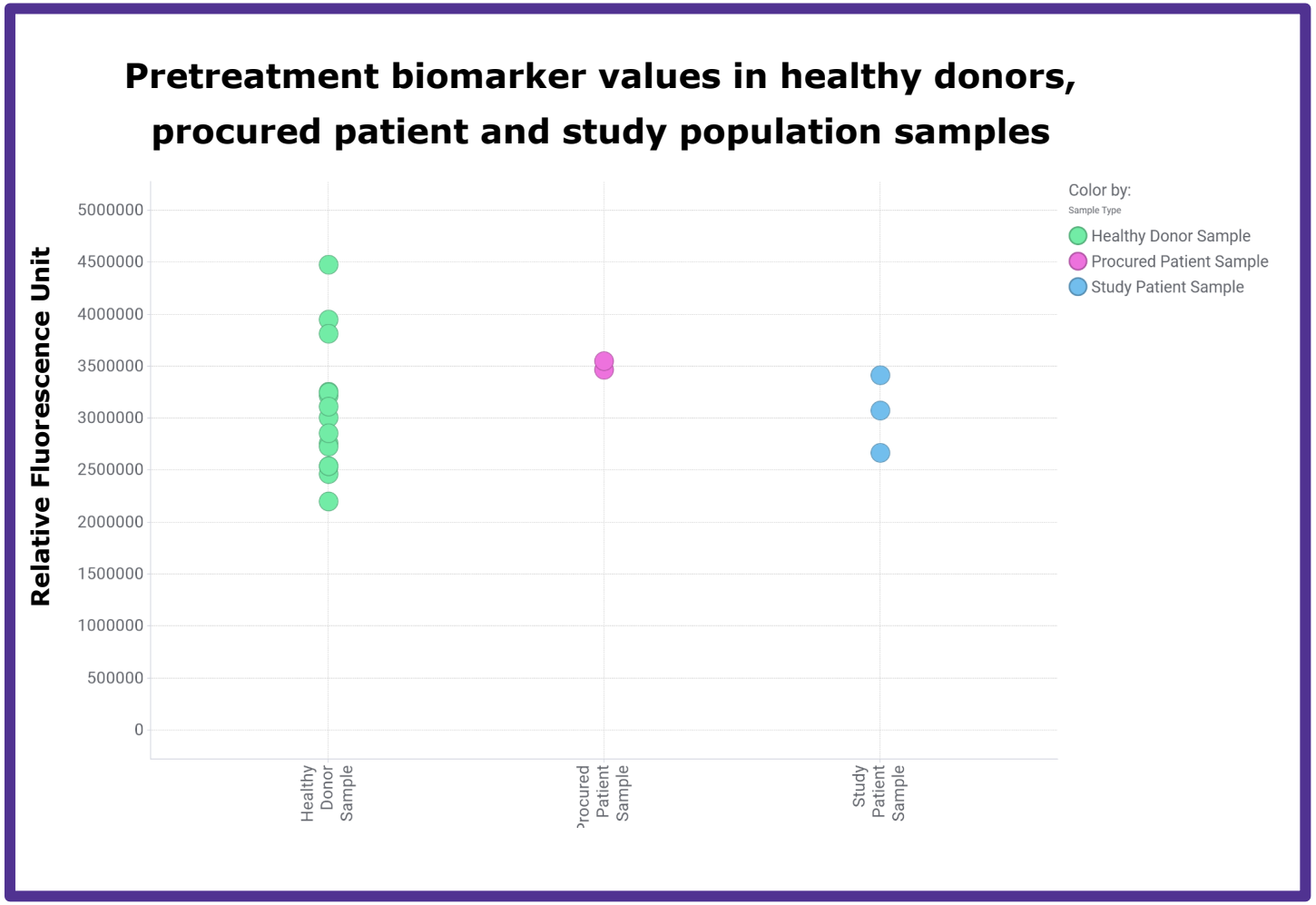
Scenario		Analytical Requirements	Biomarker selected
Worst-case	• Large biological variability • Low magnitude of biomarker response to the treatment		
Best-case	• Small biological variability • High magnitude of biomarker response to the treatment		

- The stringency of the criteria applied to the assay analytical performance depends on the proposed COU

CASE STUDIES

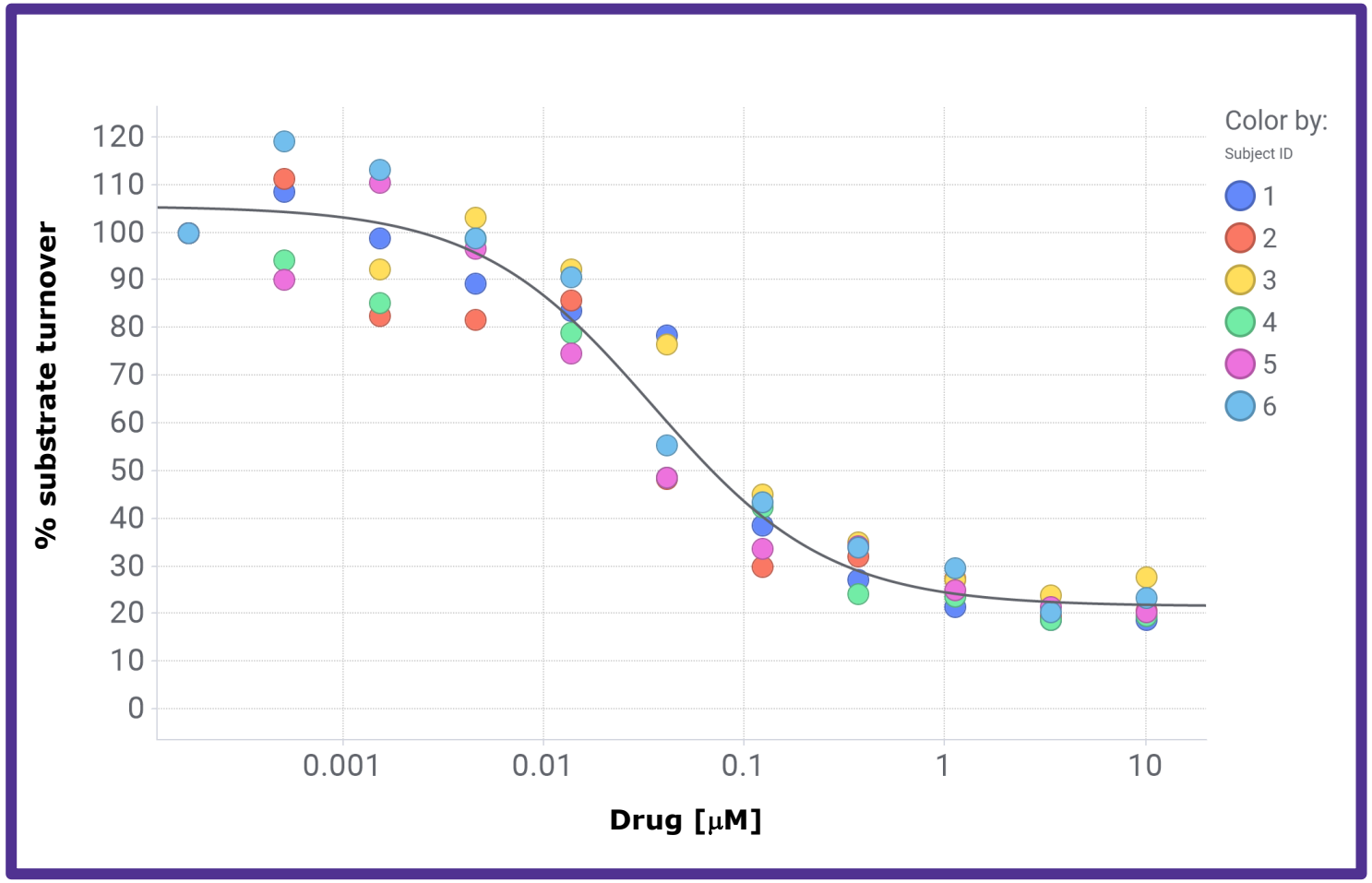
- COU:pharmacodynamic biomarker used as an indicator of the intended activity of the drug in a global Phase 1 dose escalation study
- Biomarker of interest: intracellular protein expressed mainly in immune cells
    - Biomarker unstable at frozen conditions
    - Sample Type selected: freshly collected PBMC
    - Biomarker analysis to be conducted within a short timeframe from sample collection
    - Assay to be validated in two laboratories:
        - Full validation conducted in EU lab
        - Partial validation in US

Case Study – Biological Variation and Magnitude of biomarker response to the treatment 1/2



- A relatively small between-subject variability is observed in Healthy Donor samples (n=15) before treatment (CV% ~20)
- Procured Patient (n=2) and Study Patient (n=3) samples data is within the range of the Healthy Donor sample data.

Case Study – Biological Variation and Magnitude of biomarker response to the treatment 2/2



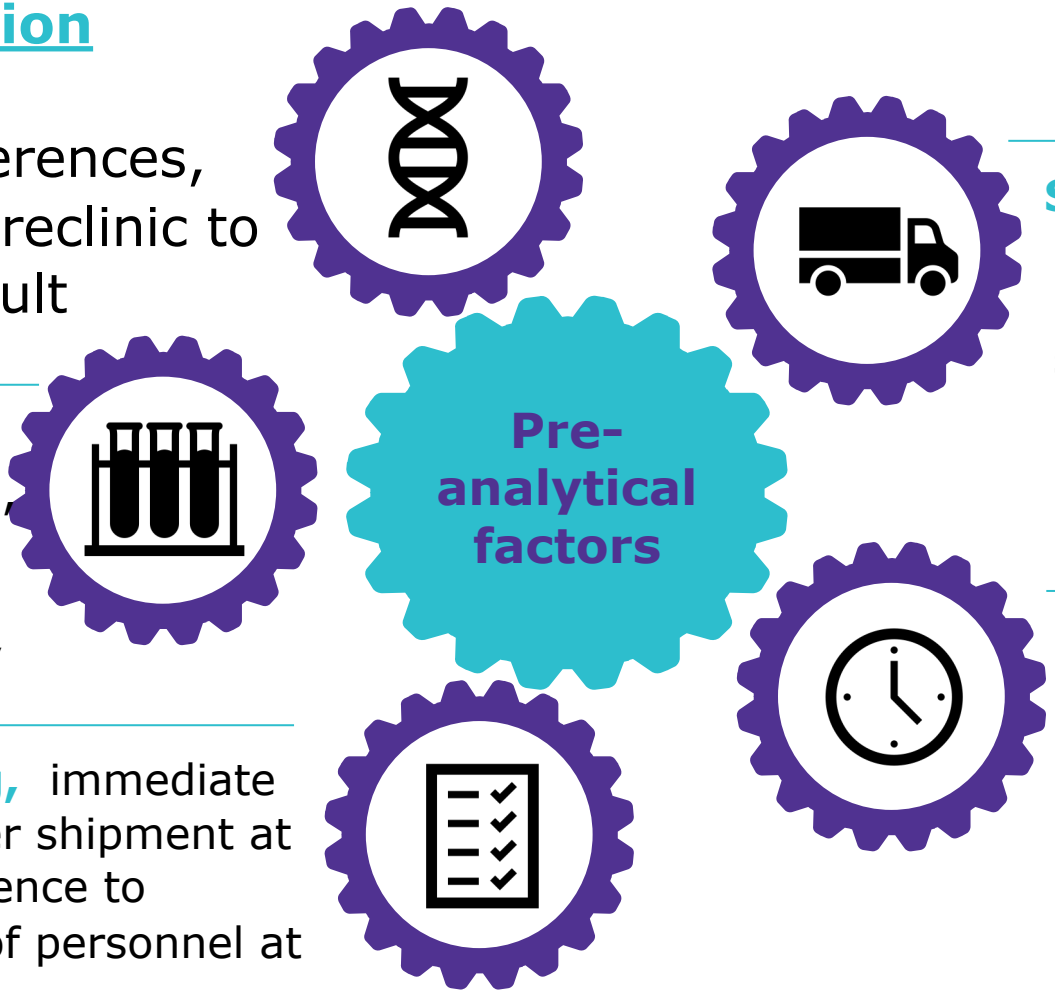
- Between-subject variability reduced in response to the treatment with the drug
- The magnitude of biomarker response to the treatment increased at increasing drug concentrations.
- The BM assay was considered suitable for the proposed COU applied in the clinical study

Sample type and Pre-analytical factors

Sample type selection
Expression level, heterogeneity, interferences, transferability from preclinic to clinic, pediatric vs adult

Sample Collection, modality (e.g. venipuncture, guided biopsy), draw order, timing, fasting status, sample quality and quantity

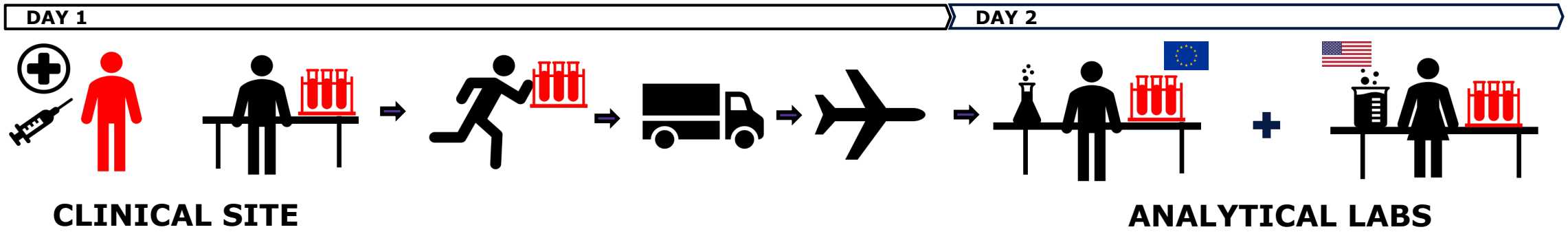
Sample processing, immediate at clinical site or after shipment at analytical lab, adherence to procedure, training of personnel at CLS



Sample Logistic, punctual sample pick-up and delivery, controlled shipment conditions (RT vs frozen)

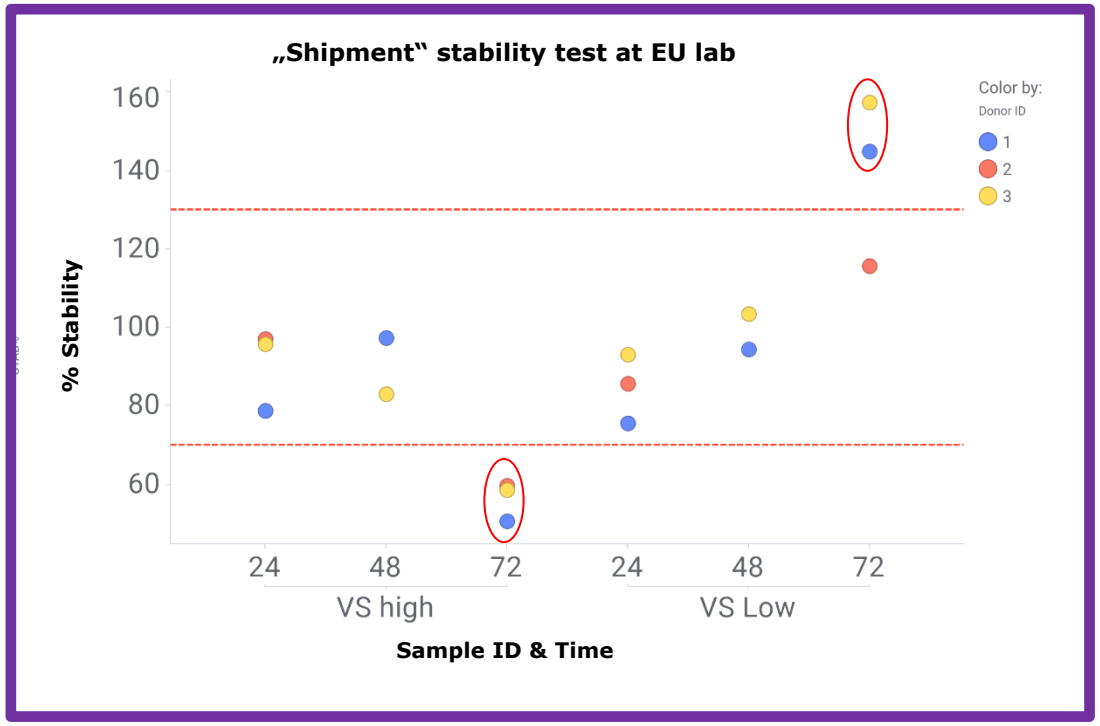
Sample stability and storage, short-term and/or long-term stability, temperature conditions (RT, frozen, liquid nitrogen).

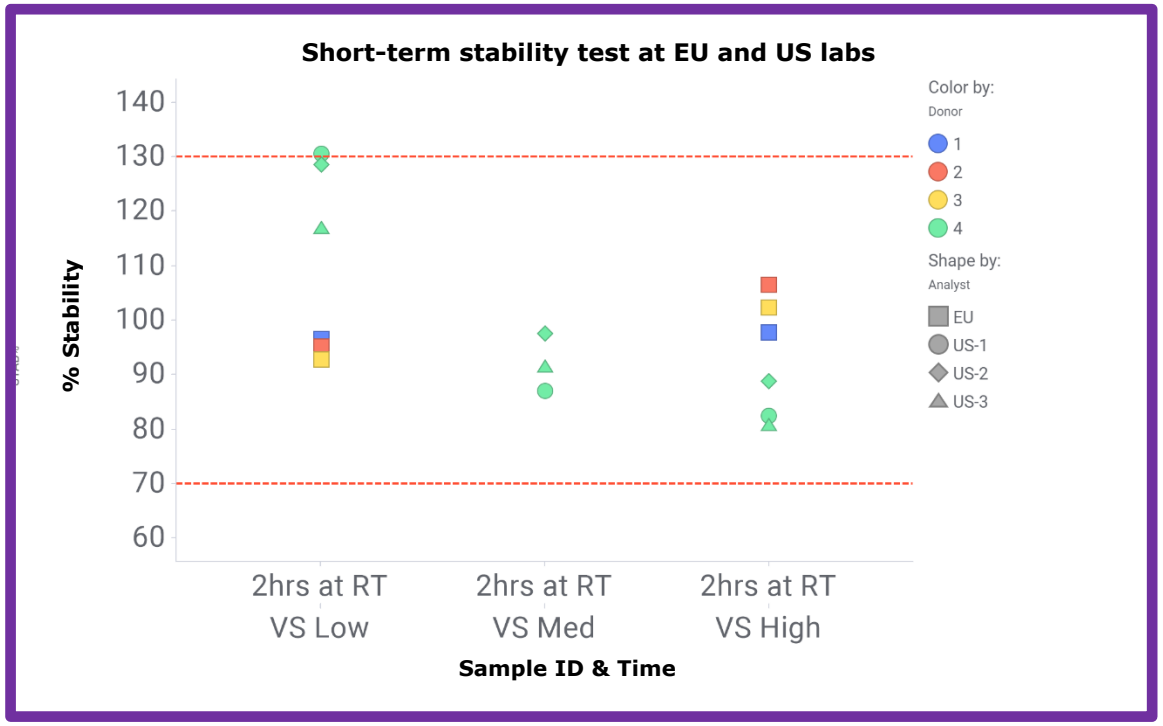
Case Study – Pre-analytical factors 1/3



Collection & Processing	Logistic	Stability & Storage
• Sample procedure established to ensure minimal processing at clinical site(s) • Final sample processing at the analytical labs (EU & US)	• Sample stability mimicking shipment conditions tested at 24, 48 and 72 hours • Introduction of temperature data logger to monitor temperature effects	• Lack of sample stability at frozen conditions requires sample disposition after analysis • Short-term stability assessed allowing one additional re-test in case of first analytical run failure

Case Study – Pre-analytical factors 2/3



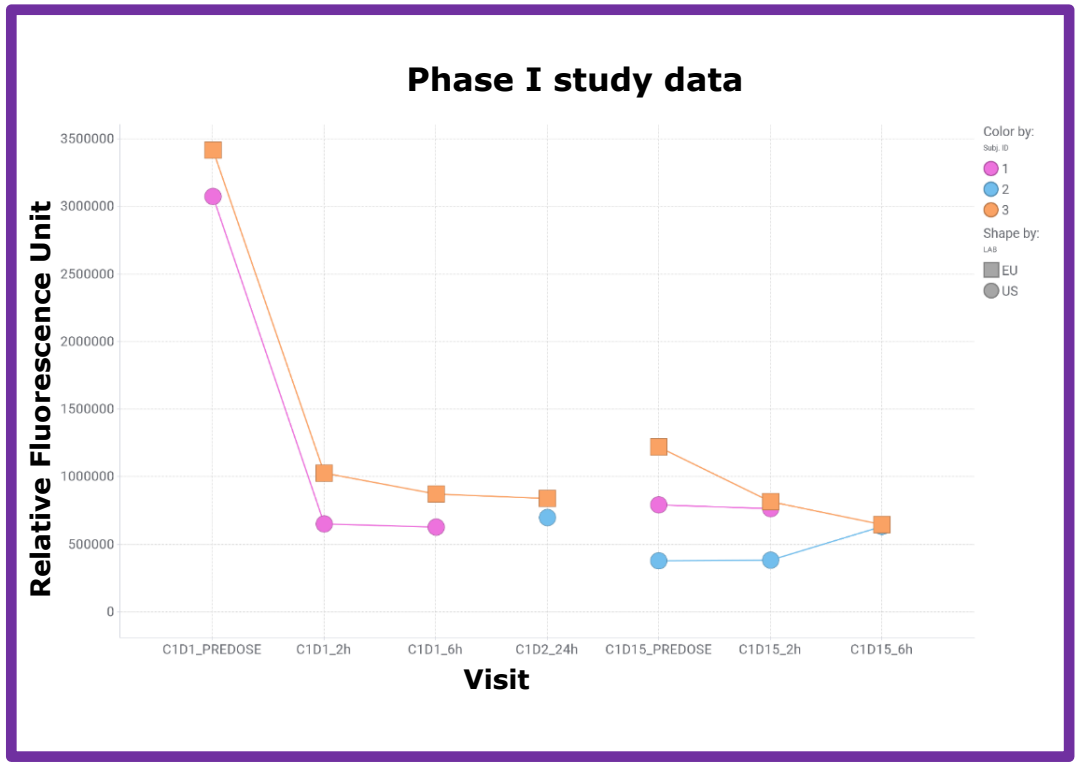


VS, Validation Sample

Pre-processed sample stability observed up to 48 hours

Processed Sample Stability confirmed up to 2 hours at RT

Case Study – Pre-analytical factors 3/3



- The composite source of variability kept under control
- Consistent data generated between labs
- The biomarker assay and the biomarker analysis support the proposed COU

Conclusions

- Identifying and investigating the different components of the composite source of the biomarker assay variability is essential:
  - To work under known and controlled settings
  - To avoid conditions that weaken the linkage of the biomarker to its clinical significance
  - To build a biomarker assay suitable for the proposed COU