

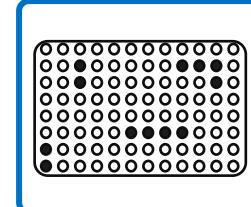
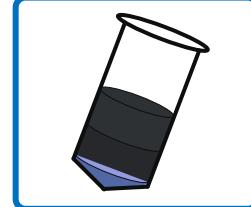
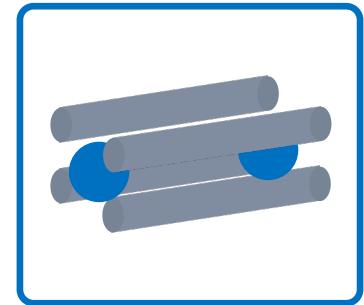
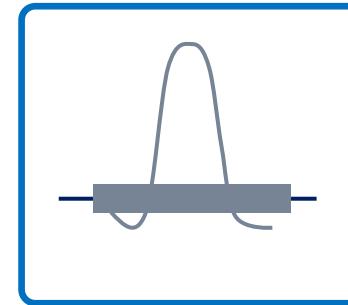
# Enzyme activity assays using LC-MS

**NUVISAN**

14<sup>th</sup> EBF Open Symposium 2021



# Workflow



# 1 Case Report: S-COMT enzymatic assay

# 2 Case Report: RAAS system

# # 1 Case Report: S-COMT enzymatic assay



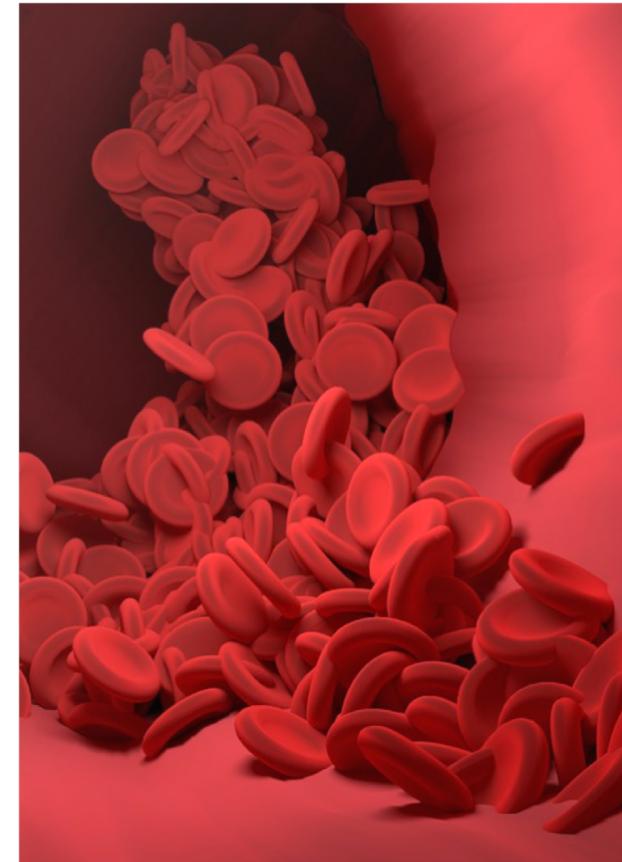
## Catechol-O-Methyltransferase

COMT inactivates catechols by methylation  
(Catecholamine)

Ubiquitously expressed, enhanced in brain, liver,  
placenta, lymphocytes and erythrocytes

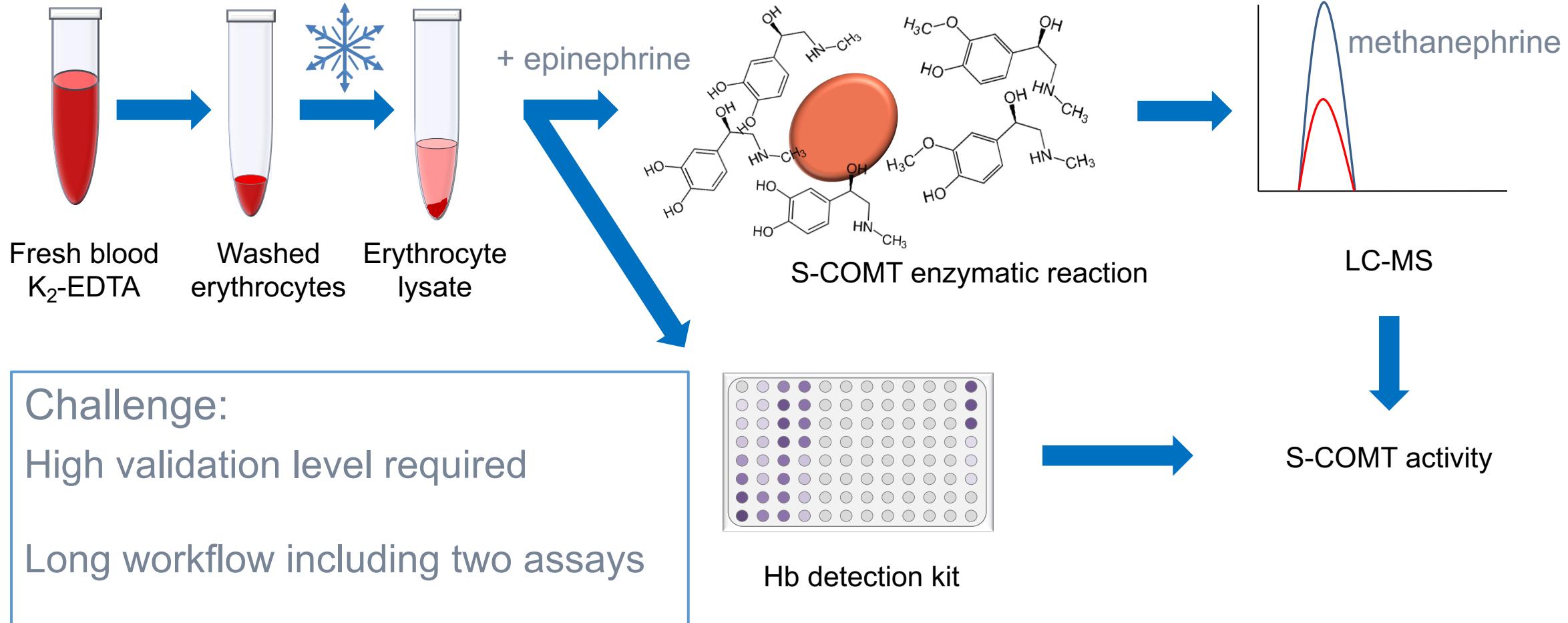
Soluble COMT in cytoplasm

S-COMT inhibitors increase L-Dopa availability  
(Parkinson's disease)



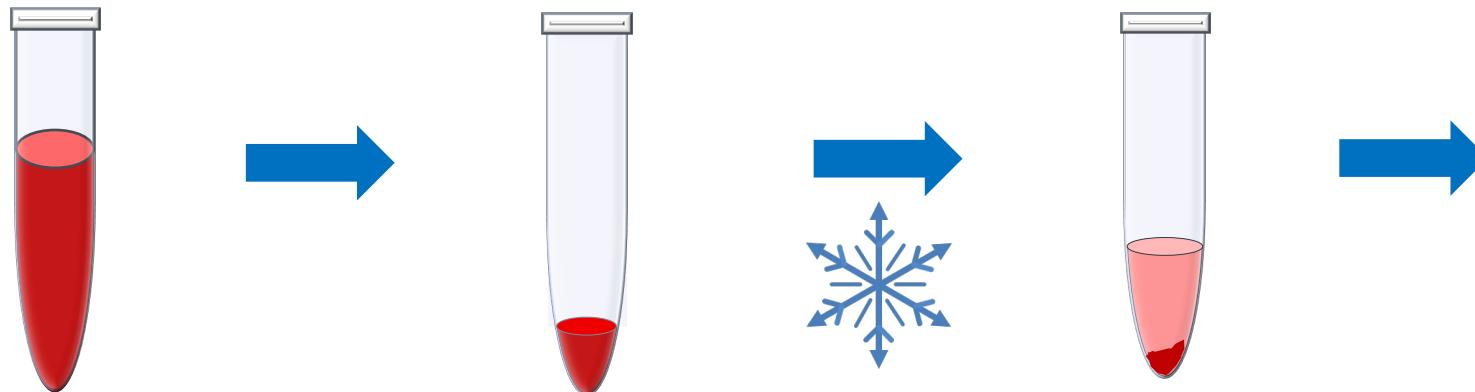


# S-COMT Assay - Workflow





# Hemoglobin Calorimetric Detection Kit



Calibration range 5.96 – 160 mg/mL

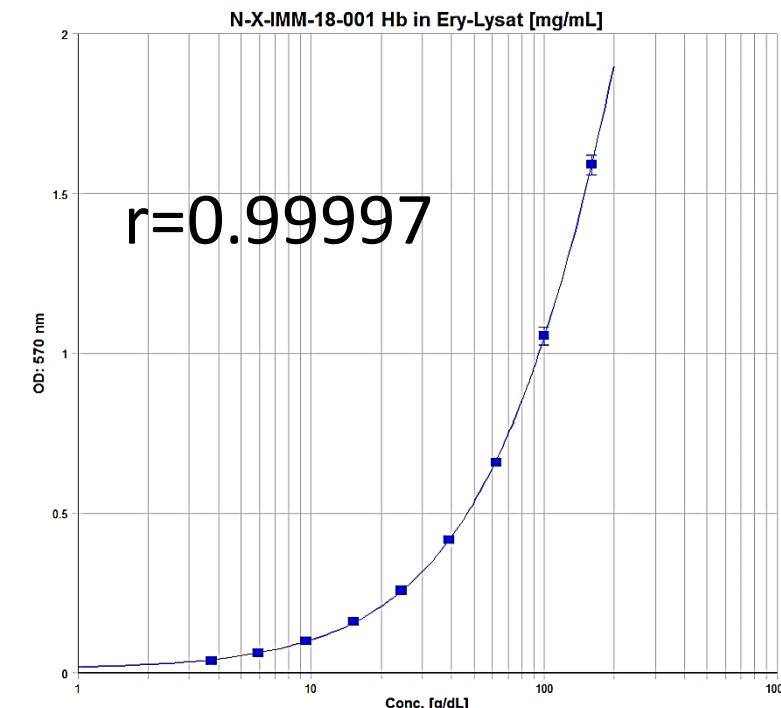
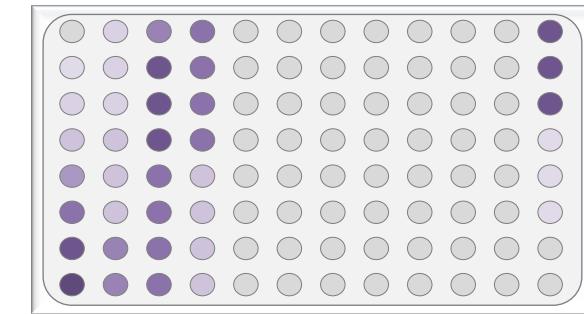
Inter assay precision: < 9 % (surrogate)/ < 10% (lysate)

Intra assay precision: < 6 % (surrogate)/ < 2% (lysate)

Sensitivity ✓

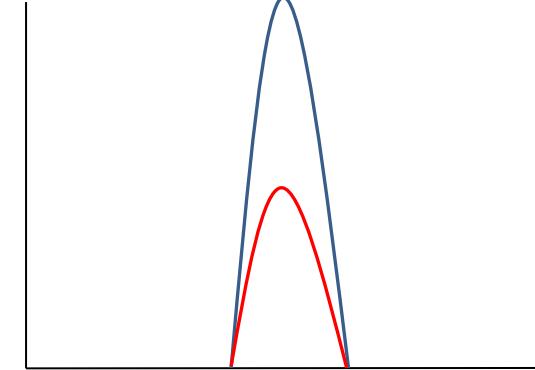
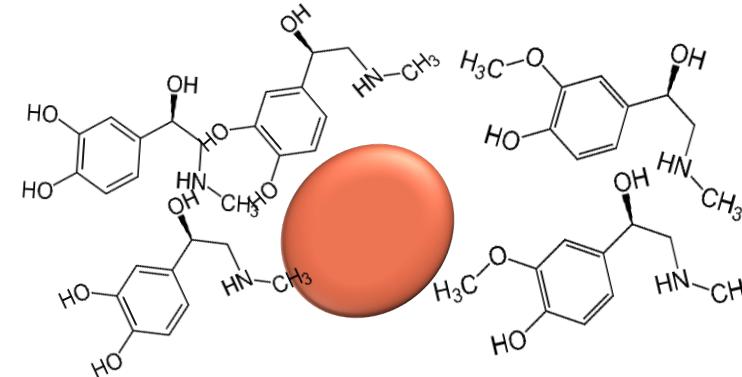
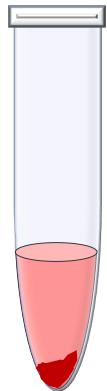
Selectivity (10 different sources) ✓

Stability: 6 days (4°C/RT), 3 month (-20 °C/-75°C)





# S-COMT Activity Determination



Calibration range: 1- 150 ng/mL

Intra-/Interrun, Recovery, Selectivity, Sensitivity, Matrix Effect ü

Stability S-COMT activity 6h on ice and 112d -75 °C

Reproducibility S-COMT activity CV <15%



## S-COMT Assay Application

Routine application:

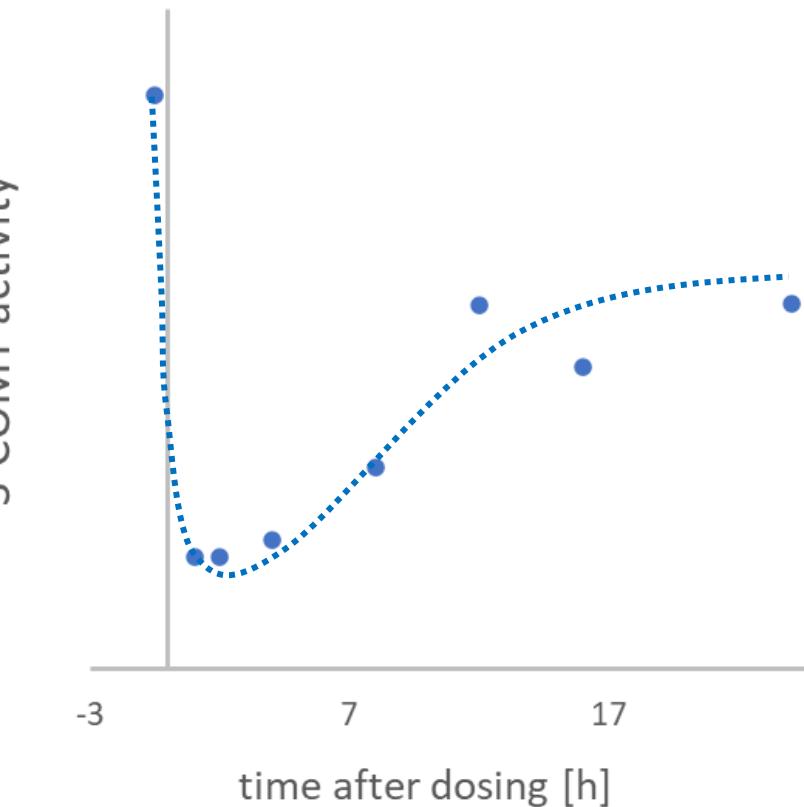
>2600 Subject samples analyzed

> 50 runs

Good precision (CV < 10 %)

Minimal BIAS (< 5%)

S-COMT inhibition profile

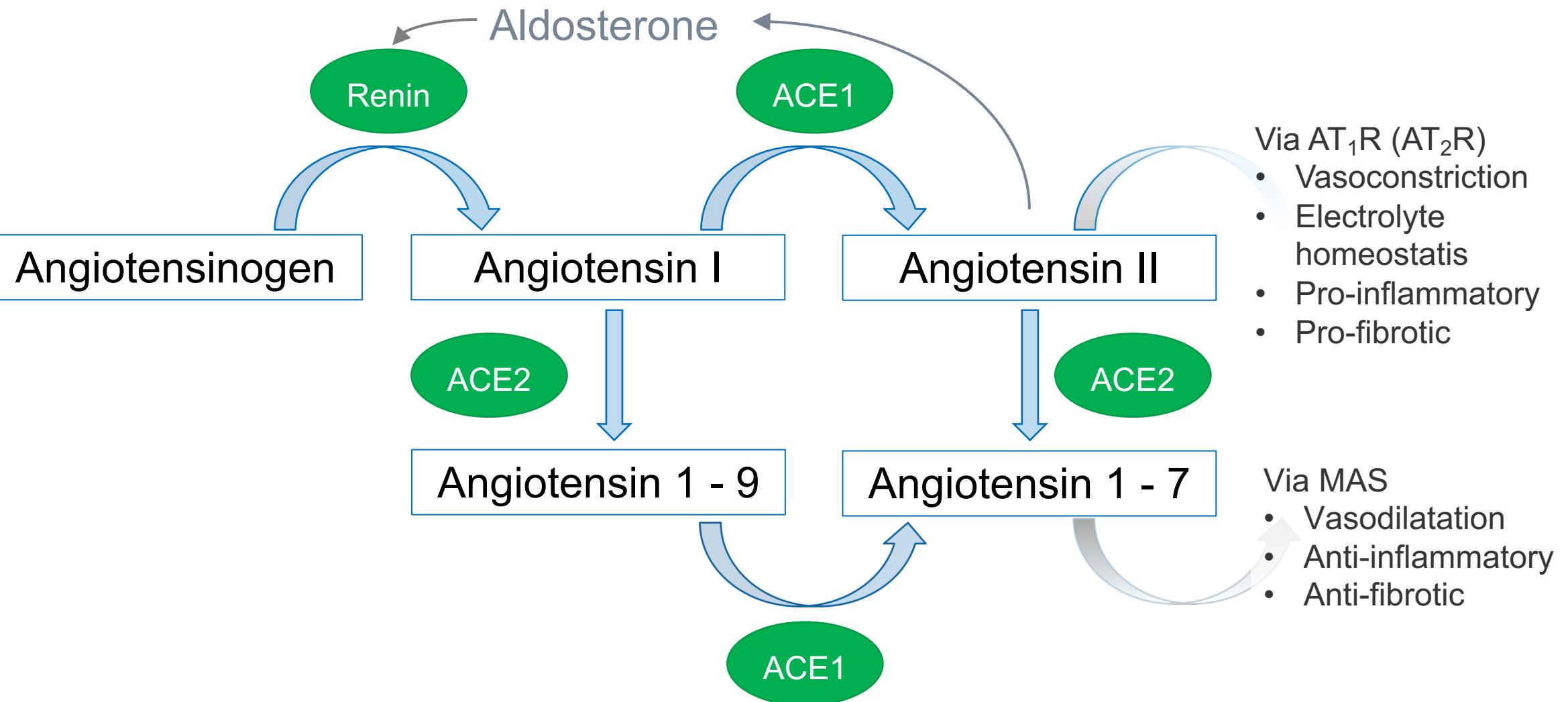




## #2 Case Report: RAAS



# Renin-Angiotensin-Aldosterone System (RAAS)

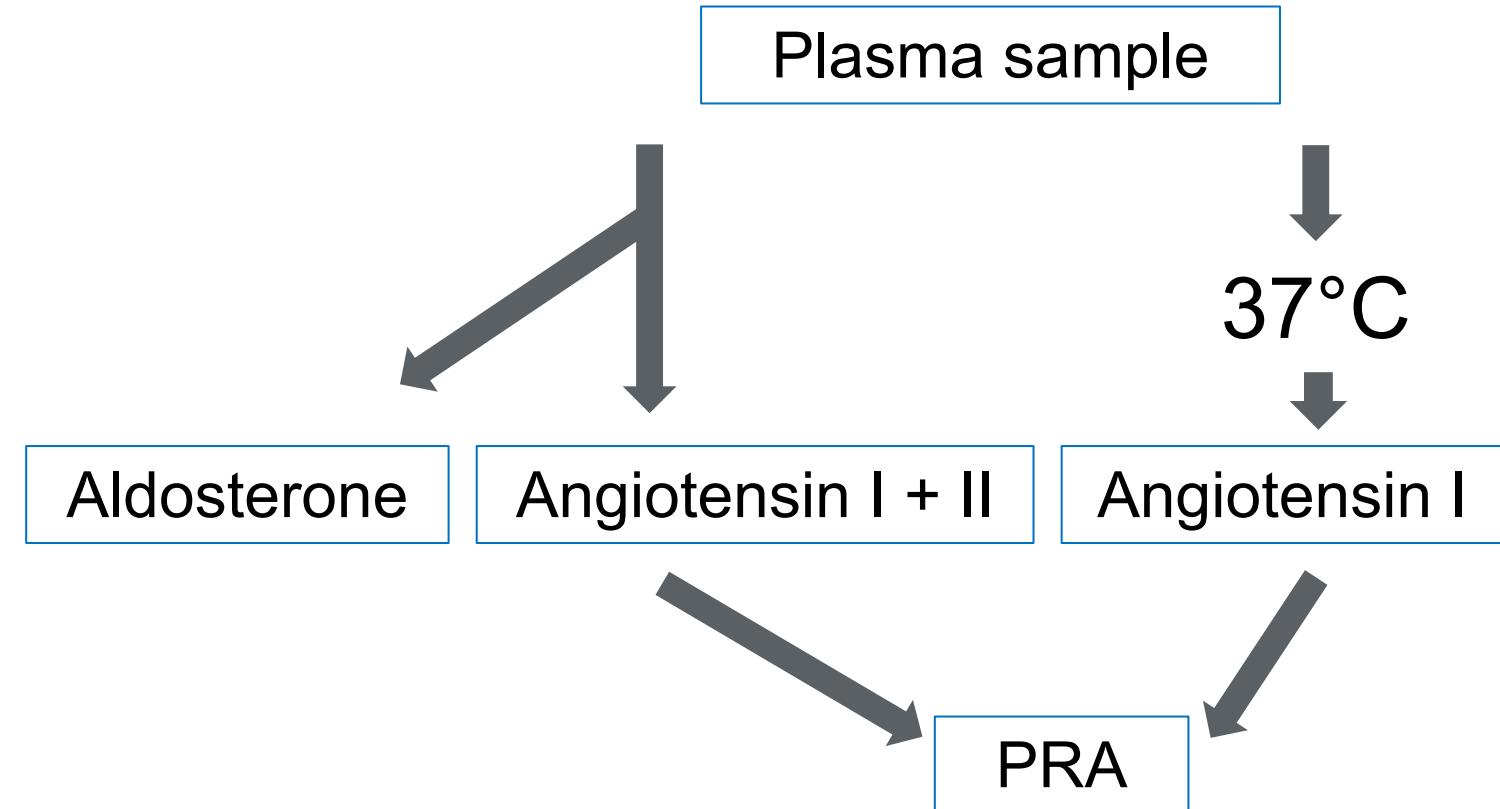




# Assay Setup and Challenge

Sensitivity:  
Very low LLOQs  
required

PRA:  
Substrates and  
enzymes present  
in sample





## Sample Preparation

Target LLOQs (250 µL plasma):

Aldosterone: 25 pg/mL

Angiotensin I: 10 pg/mL

Angiotensin II: 10 pg/mL

Challenges:

Interfering signal in Aldosterone mass trace

Fractionation on SPE

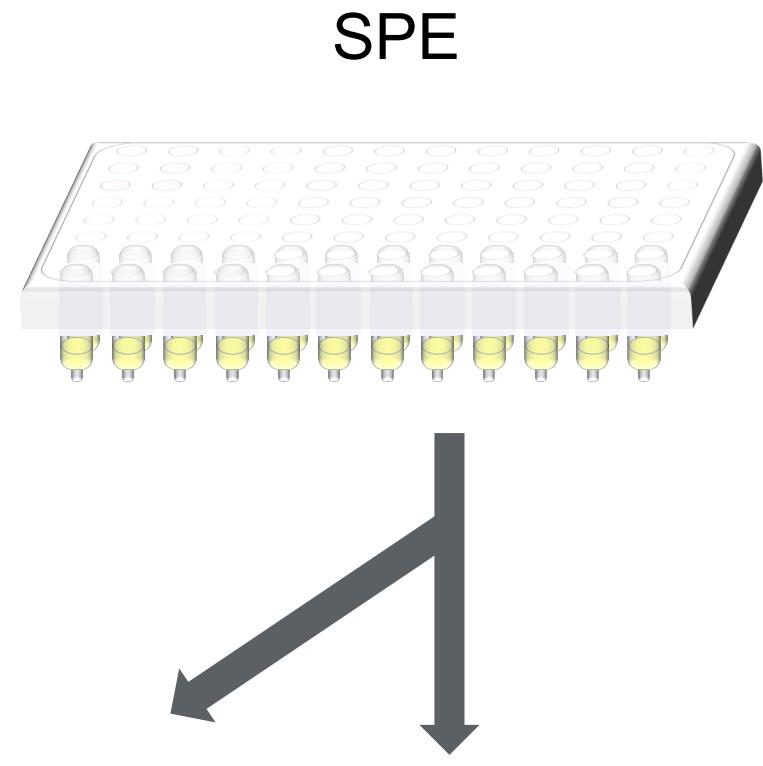
Low LLOQs

µElution plate

Keeper substance DMSO for Aldosterone

Mobile phase aging effect

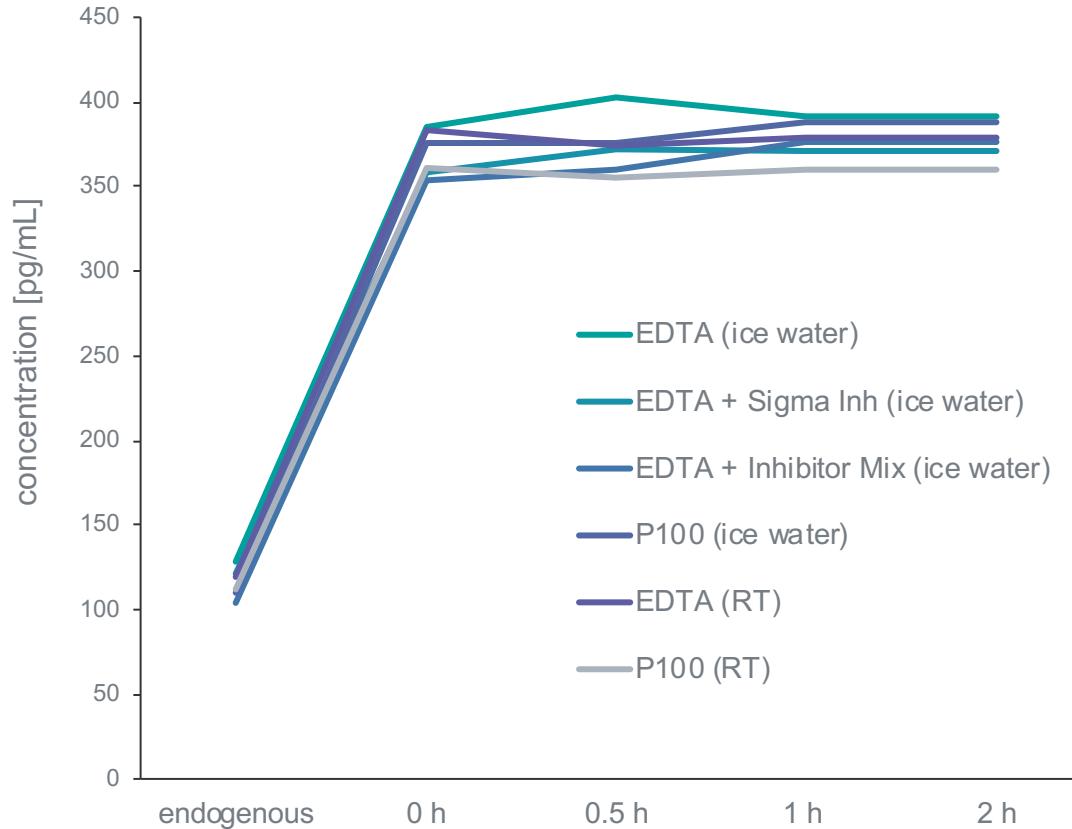
Add bicarbonate buffer



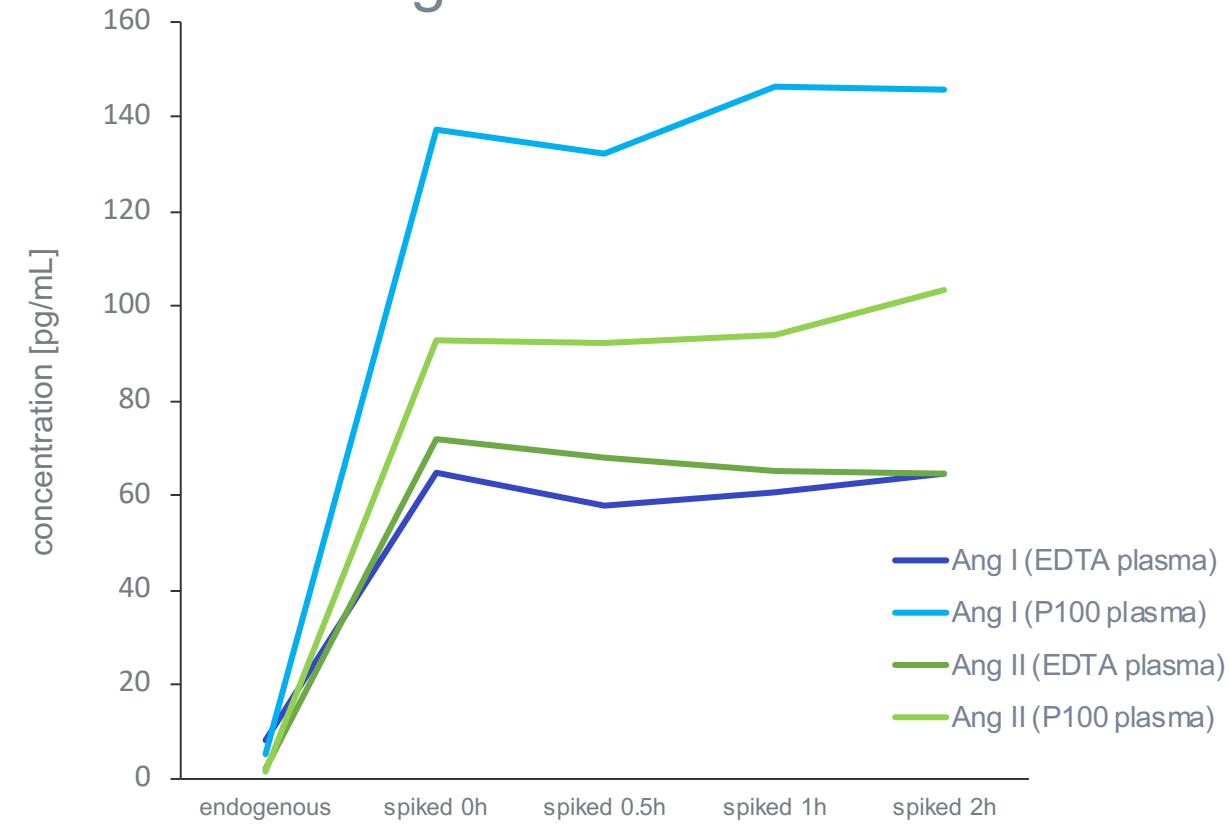


# Whole blood stability

## Aldosterone



## Angiotensins I + II on ice

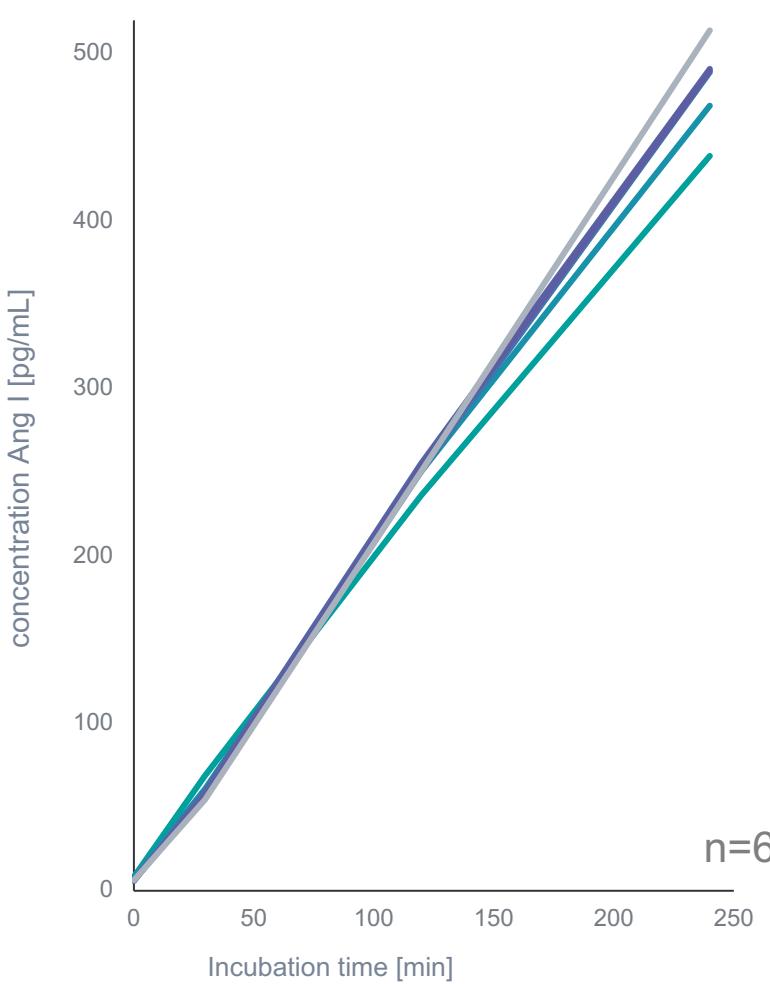


Tested inhibitors in plasma: EDTA, PMSF, soybean trypsin inhibitor, Sigma inhibitor mix, Bertin inhibitor mix, P100  
 → no inhibitor (mix) guarantees stability at RT, all conditions are stable in ice water

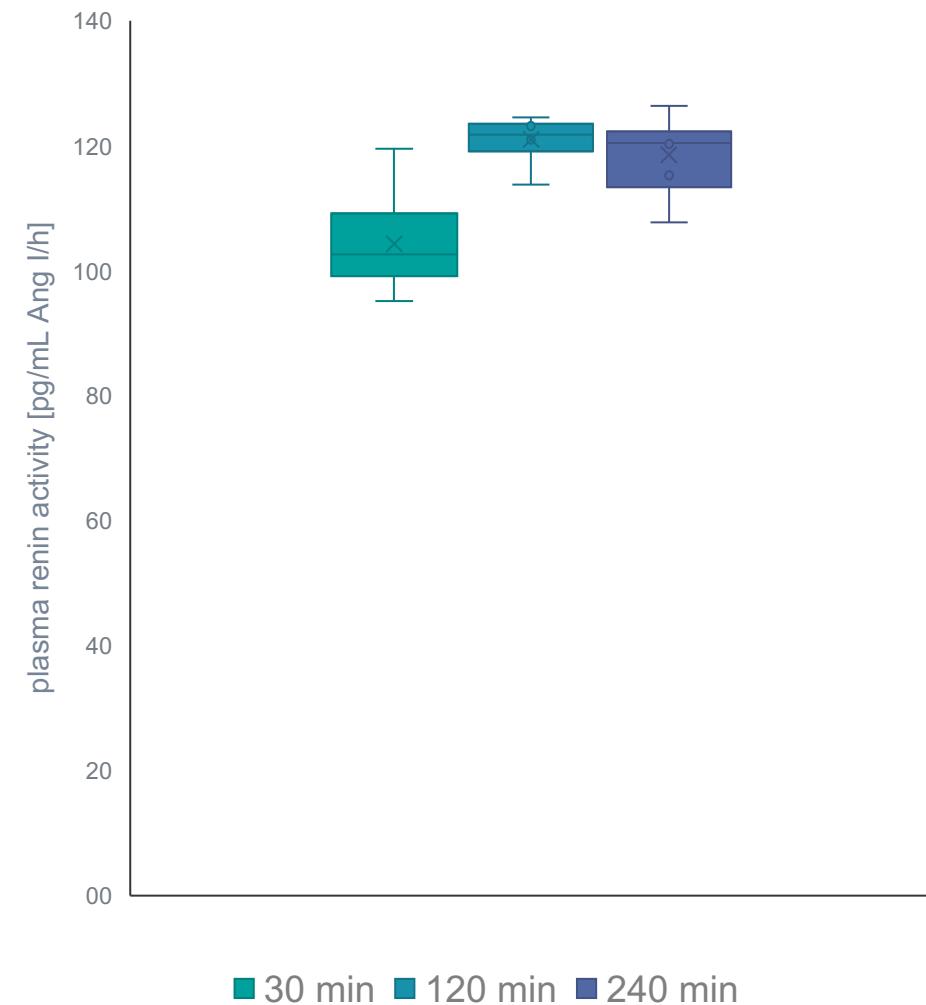


# Plasma renin activity

Angiotensin I concentration at 37 °C



PRA Plasma source 3



## PRA stability:

- 2h ice water ✓
- 2h RT ✓
- 1x & 3x FT ✓

## LTS:

- up to 233 d (-20 °C) ✓
- up to 381 d (-75 °C) ✓

## Reproducibility:

Interday: CV  $2.6 \pm 1.1\%$

Intraday: Bias  $6.6 \pm 0.2\%$



## Sample Preparation

Target LLOQs (250 µL plasma):

Aldosterone: 25 pg/mL

Angiotensin I: 10 pg/mL

~~Angiotensin II: 10 pg/mL~~

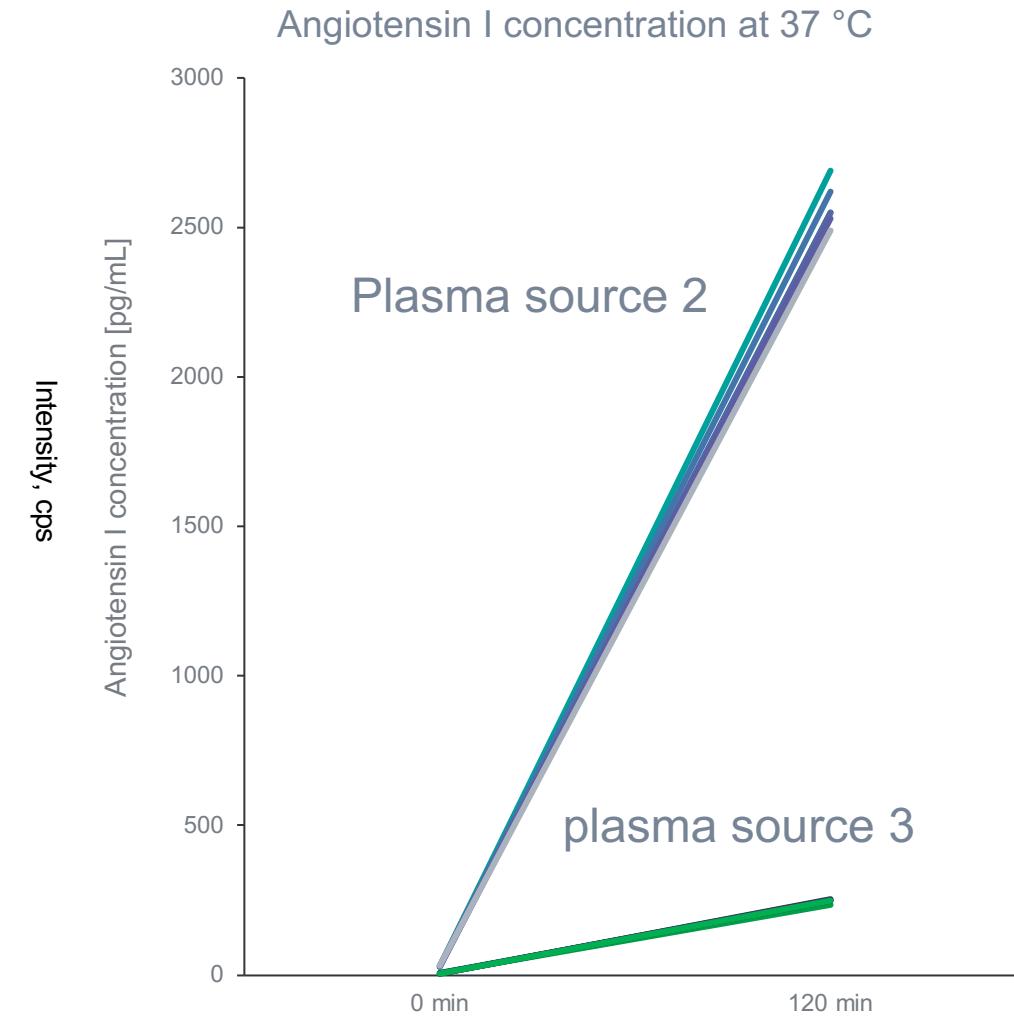
2 pg/mL

PRA of different sources:

Great biological variability -> incubation time adaption

PRA with different operators:

Great variability with  $\pm 60\%$  bias for same plasma source



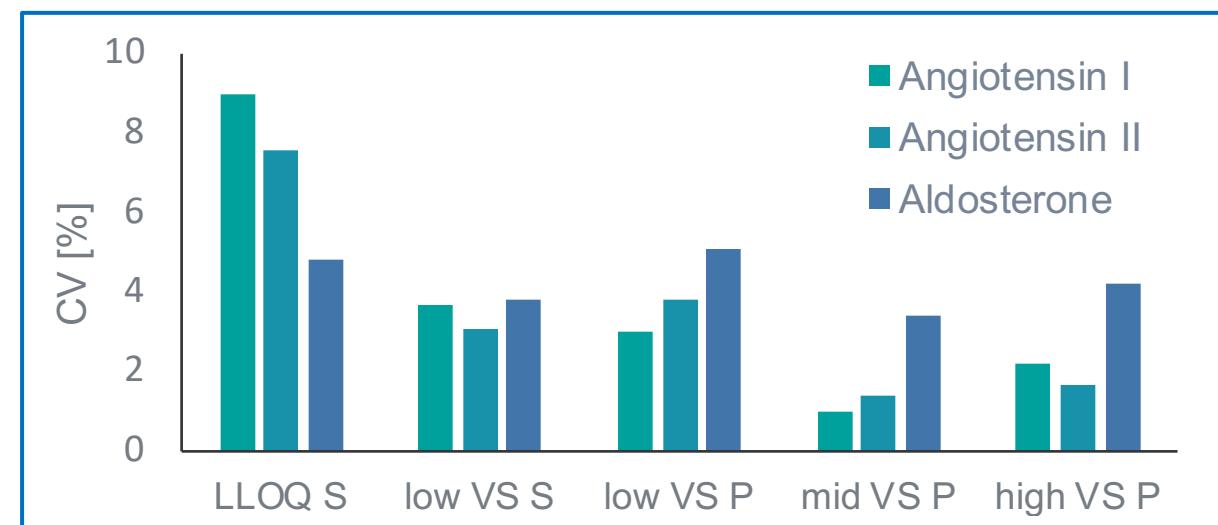
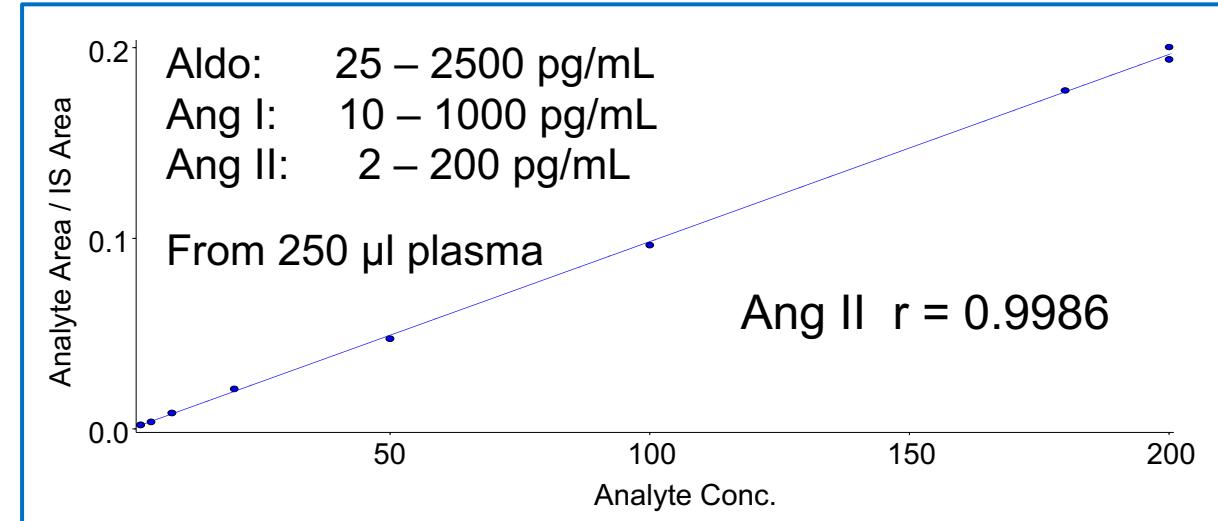


# RAAS Assay Performance



Easy handling for high sample number

Reproducible heating / constant temperature





## Summary

- Normalisation required?  
Careful implementation and validation ensures reproducible results
- How can the stability of enzyme activity, substrates and potential other analytes be ensured?
- Tight control of enzymatic reaction conditions required!
- Knowledge about endogenous levels and biological variability helpful!

Thank you

Bioanalytics Neu-Ulm....

... and the people who turn science into Life Science:

Peter Huber

Thomas Stolz

Annika Schmoll

Judith Mast