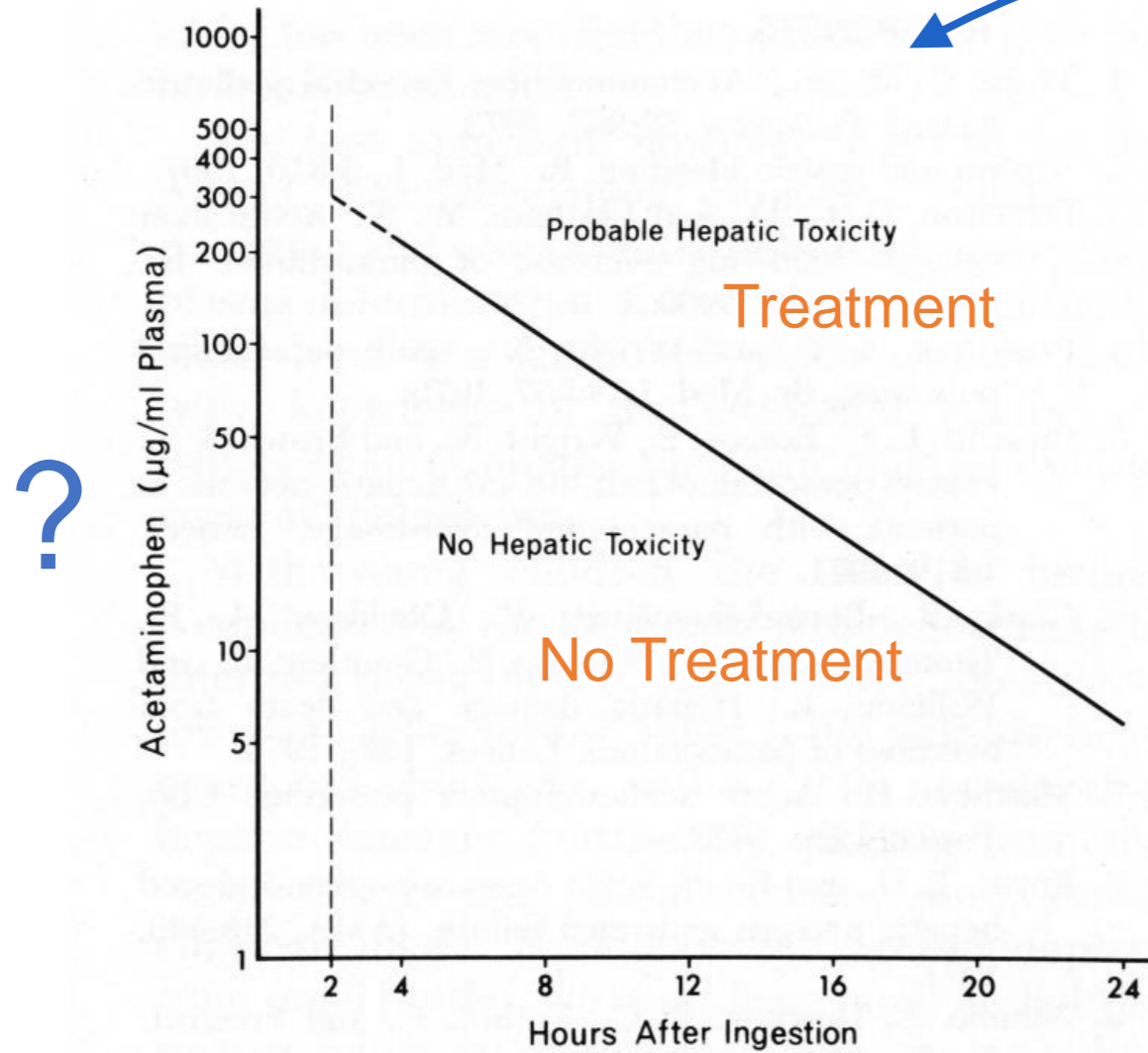


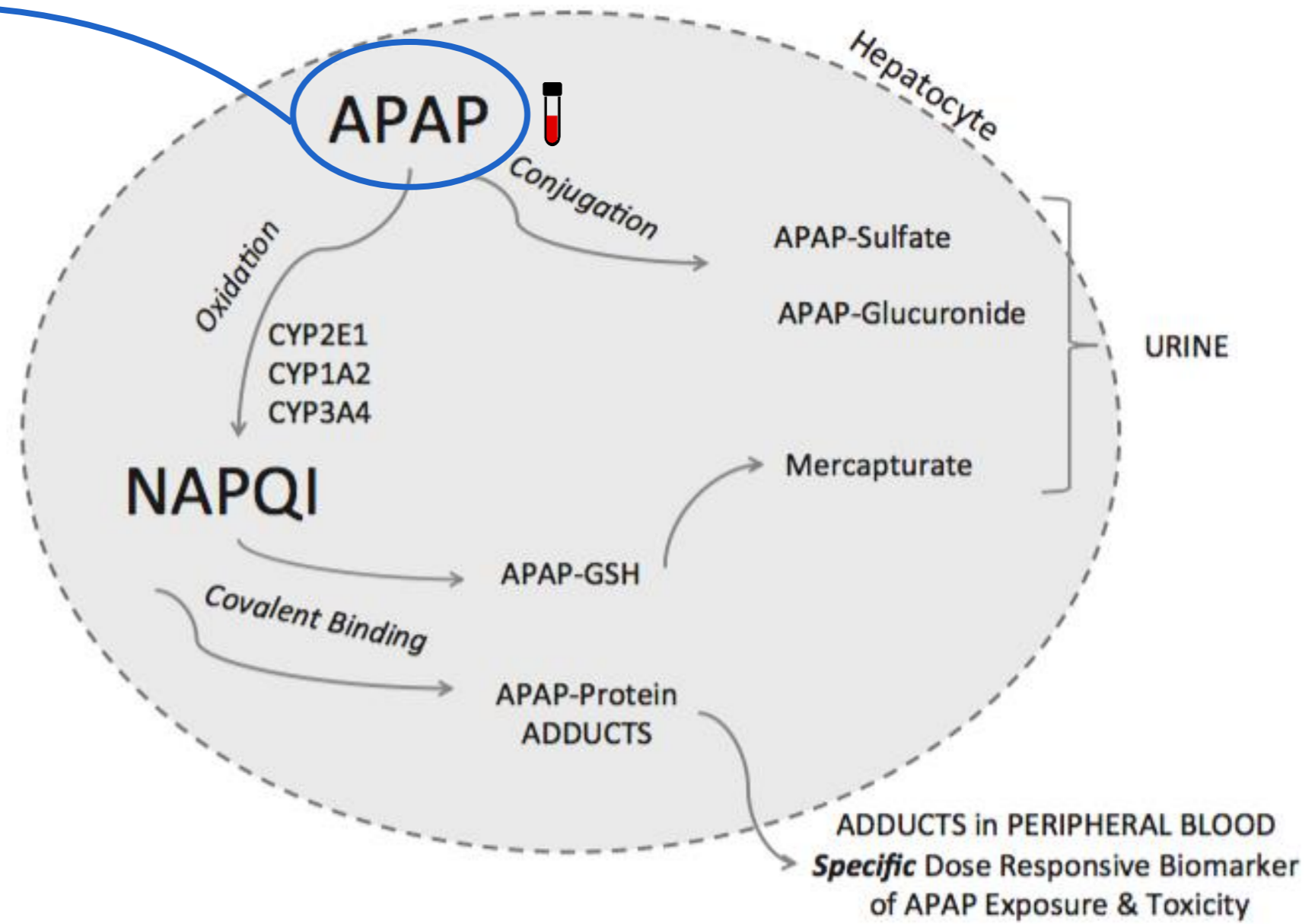
VOLUMETRIC ABSORPTIVE MICROSAMPLING AS AN ALTERNATIVE TOOL FOR ADDUCT-BASED MONITORING OF PARACETAMOL TOXICITY

Lisa Delahaye / 22.03.2019 / 5th YSS - Bologna

INTRODUCTION



B.H. Rumack and H. Matthew, *Pediatrics*, 1975



L. James, J.E. Sullivan, and D. Roberts, *Paediatr Child Health*, 2011

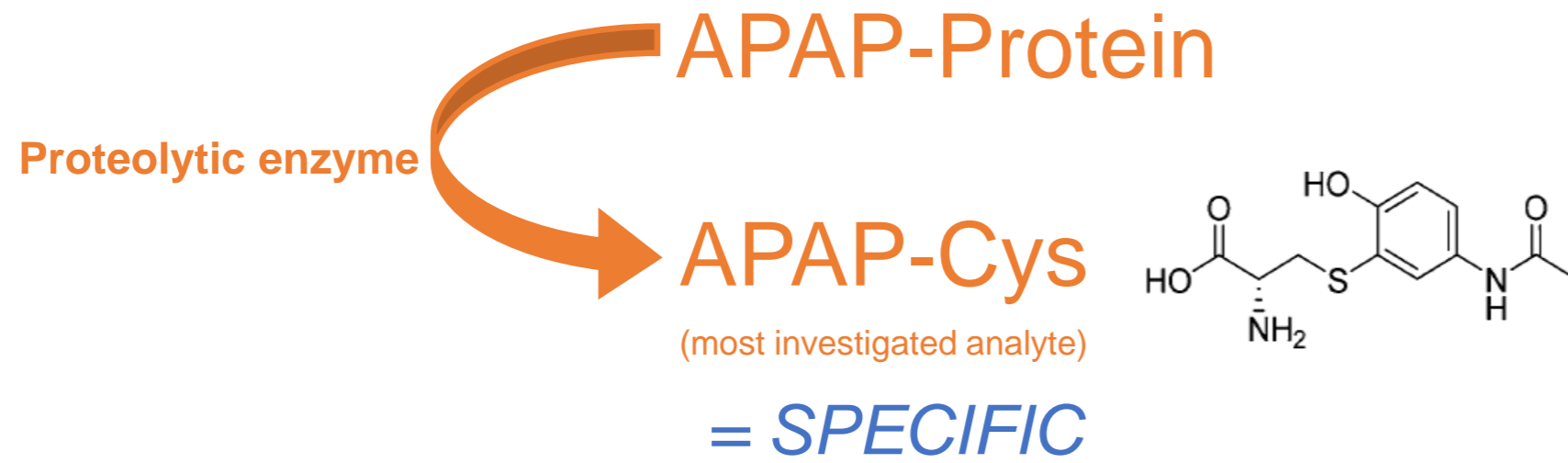
?

↓


~~AST, ALT, INR, ...~~ **NOT SPECIFIC**

INTRODUCTION

Alternative biomarker for paracetamol toxicity: **APAP-Cys**

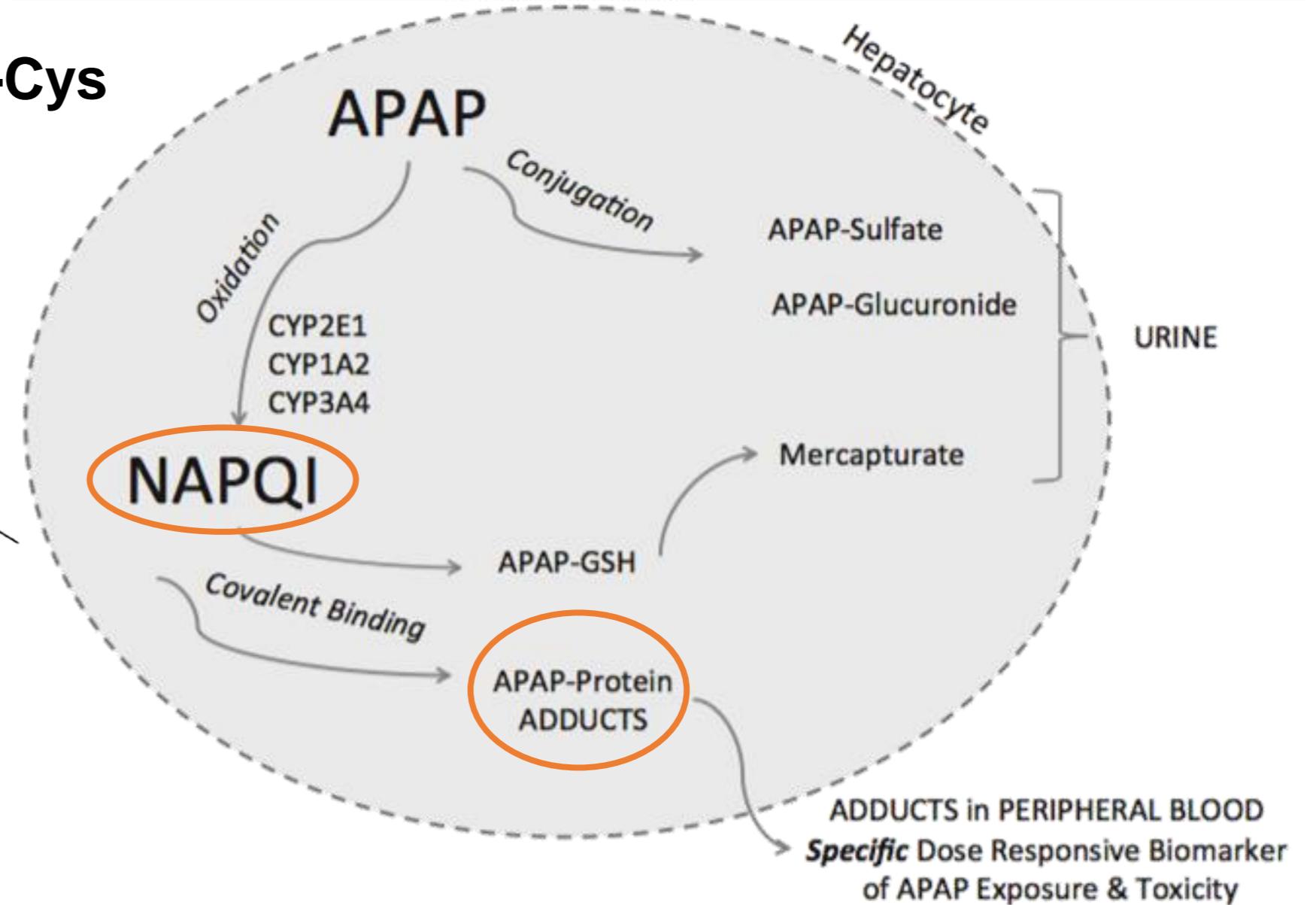


↓

1 μM (plasma) 

=

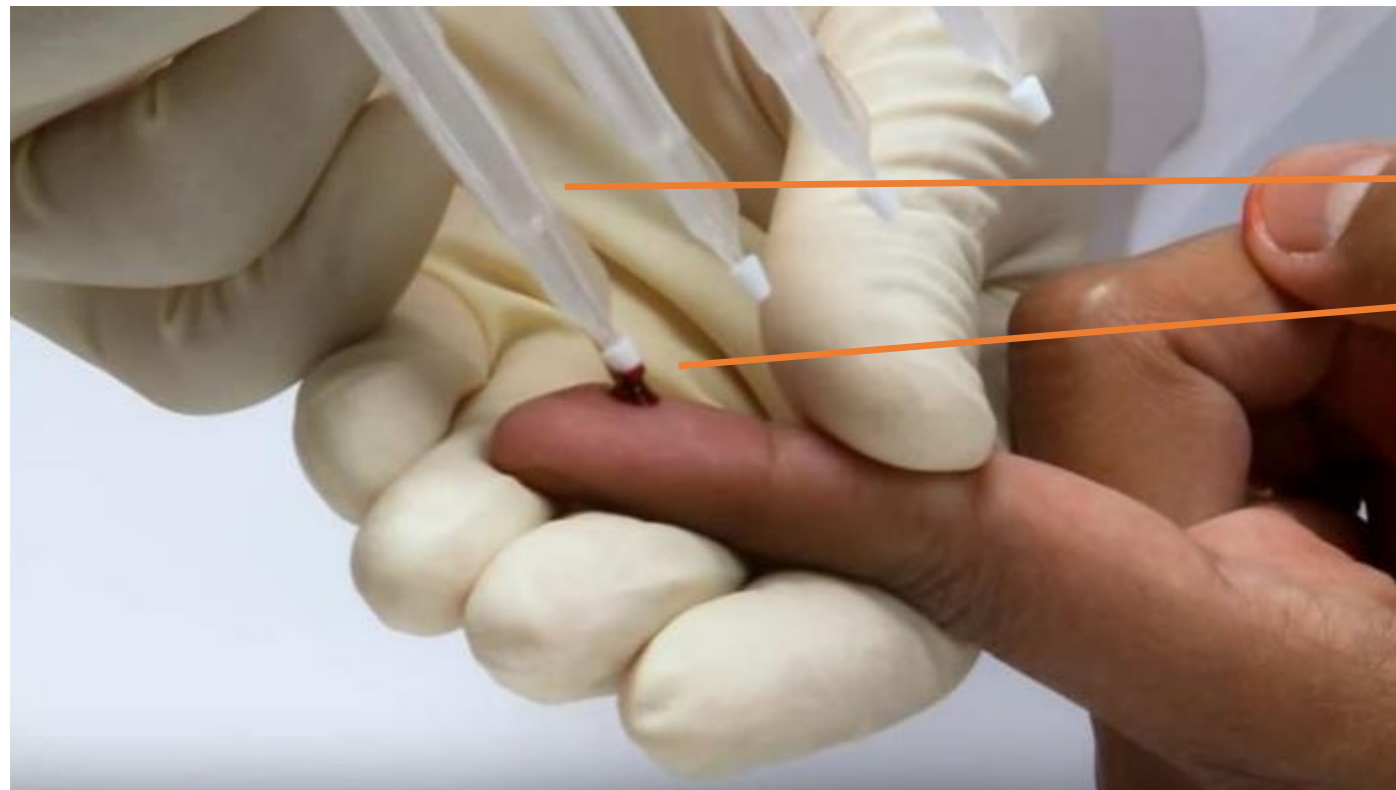
**Cut-off for treatment
with NAC**



L. James, J.E. Sullivan, and D. Roberts, *Paediatr Child Health*, 2011

MICROSAMPLING: VAMS

Pediatric patient
population
+
ICU setting



Volumetric Absorptive Microsampling (Mitra™)

→ Plastic handle

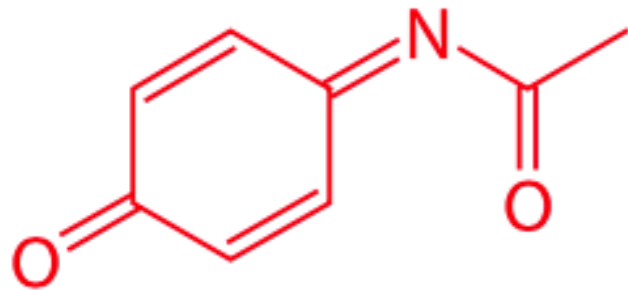
→ Polymeric tip

Alternative for dried blood spots (DBS)

<https://www.neoteryx.com/microsampling-blog/msacl-2016-dried-blood-sampling>

APAP-CYS: HOW TO TACKLE THE FREE CYSTEINE ISSUE?

NAPQI



Free cysteine
in blood, GSH,...



~~APAP-Cys^{Free}~~

- High concentration
- Large inter- and intra-individual variability

Cys residues in proteins

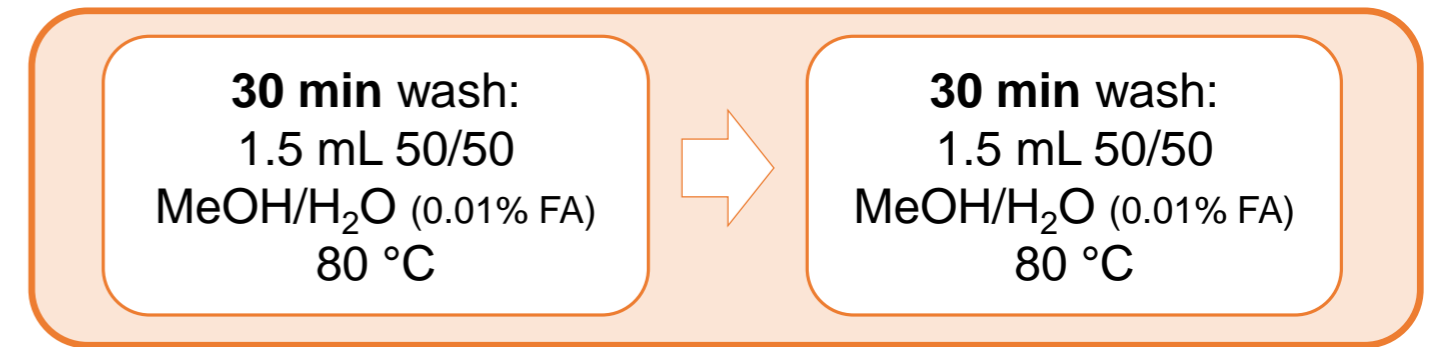


APAP-Protein

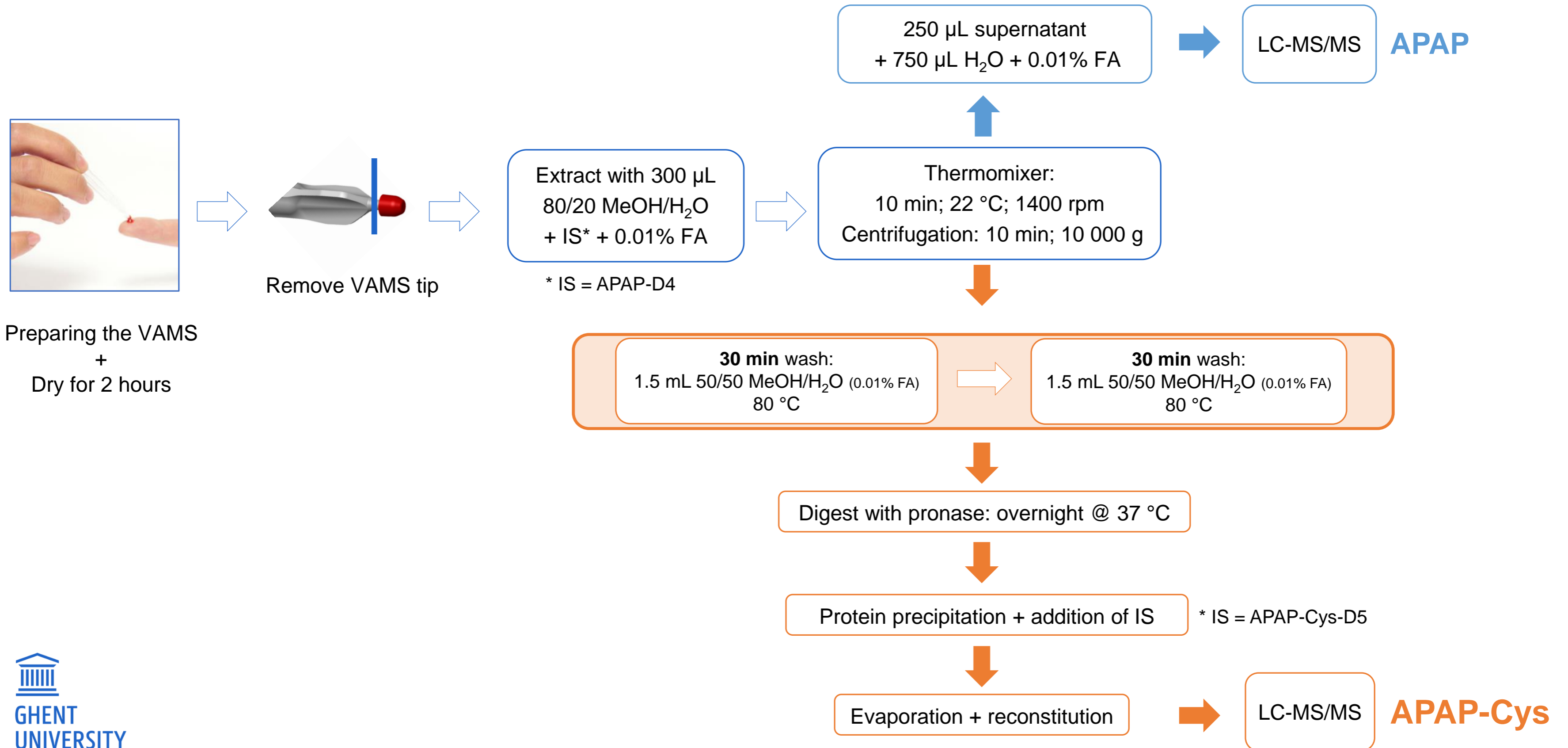
Digestion with pronase



APAP-Cys^{Prot}



SAMPLE PREPARATION PROCEDURE





VALIDATION

Calibration:

Range: 250 ng/mL to 25 µg/mL

Calibration model: linear with 1/x² weighting

Selectivity:

No interferences detected

No carry over detected

Meets acceptance criteria ✓

Accuracy and precision:

| QC Level | Intra-day precision (%RSD) | Total precision (%RSD) | Accuracy (% bias) |
|----------|----------------------------|------------------------|-------------------|
| LLOQ | 5.56% | 9.50% | -1.5% |
| Low | 7.76% | 10.11% | -5.5% |
| Medium | 5.83% | 7.54% | -6.5% |
| High | 6.56% | 8.81% | -7.3% |

Meets acceptance criteria ✓

Dilution integrity:

| Dilution factor | Accuracy (% bias) | Precision (%RSD) |
|-----------------|-------------------|------------------|
| 5 | 0.75% | 6.9% |

Meets acceptance criteria ✓

Matrix effect:

| QC level | Absolute | IS compensated | %RSD |
|----------|----------|----------------|-------|
| Low | 39.8% | 91.3% | 8.40% |
| High | 42.6% | 94.6% | 4.17% |

Hct range: 19.1%-57.3%

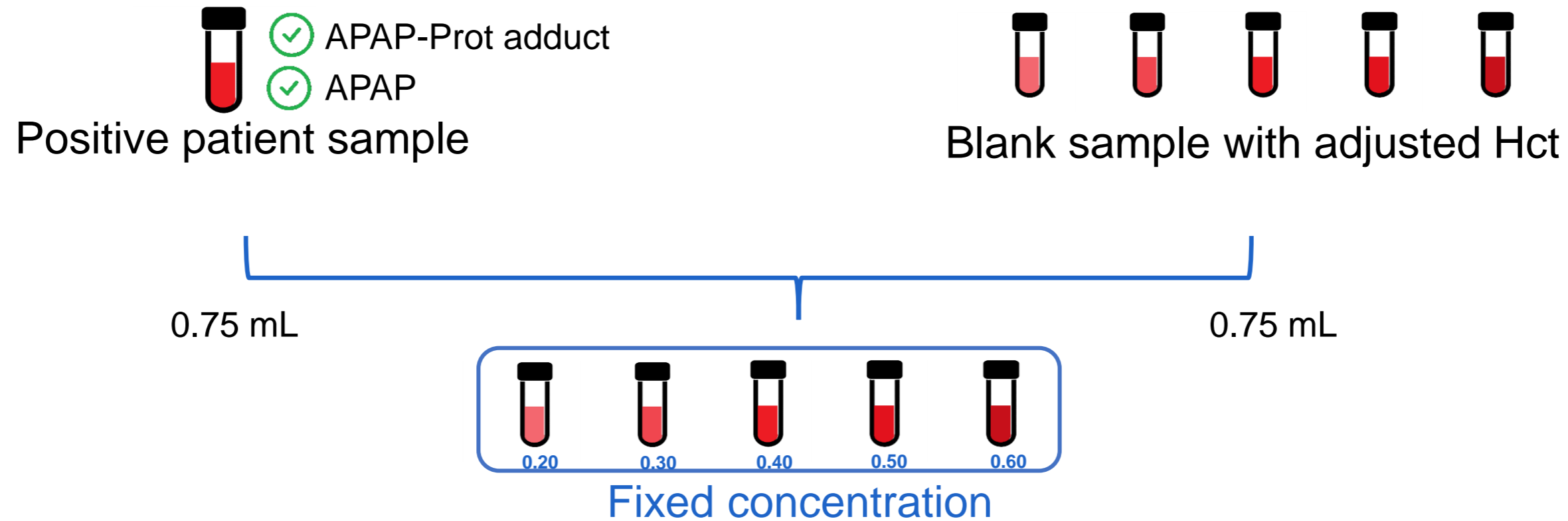
Meets acceptance criteria ✓

VALIDATION: RECOVERY AND HEMATOCRIT EFFECT

Spiking of APAP-Protein adduct is not possible

Blood to plasma ratio $\neq 1$

👉 Mix positive patient blood sample with blank blood with adjusted Hcts

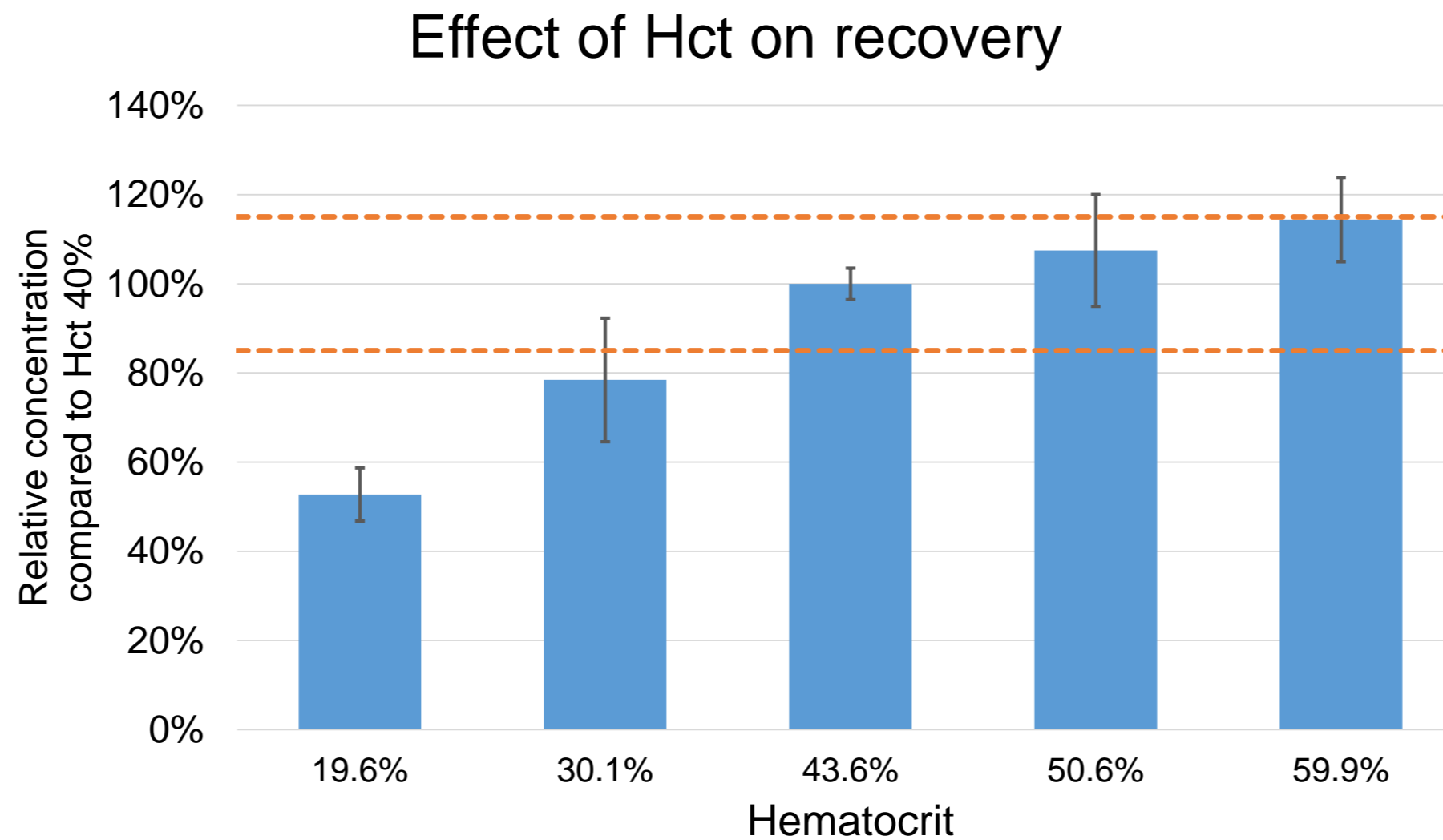


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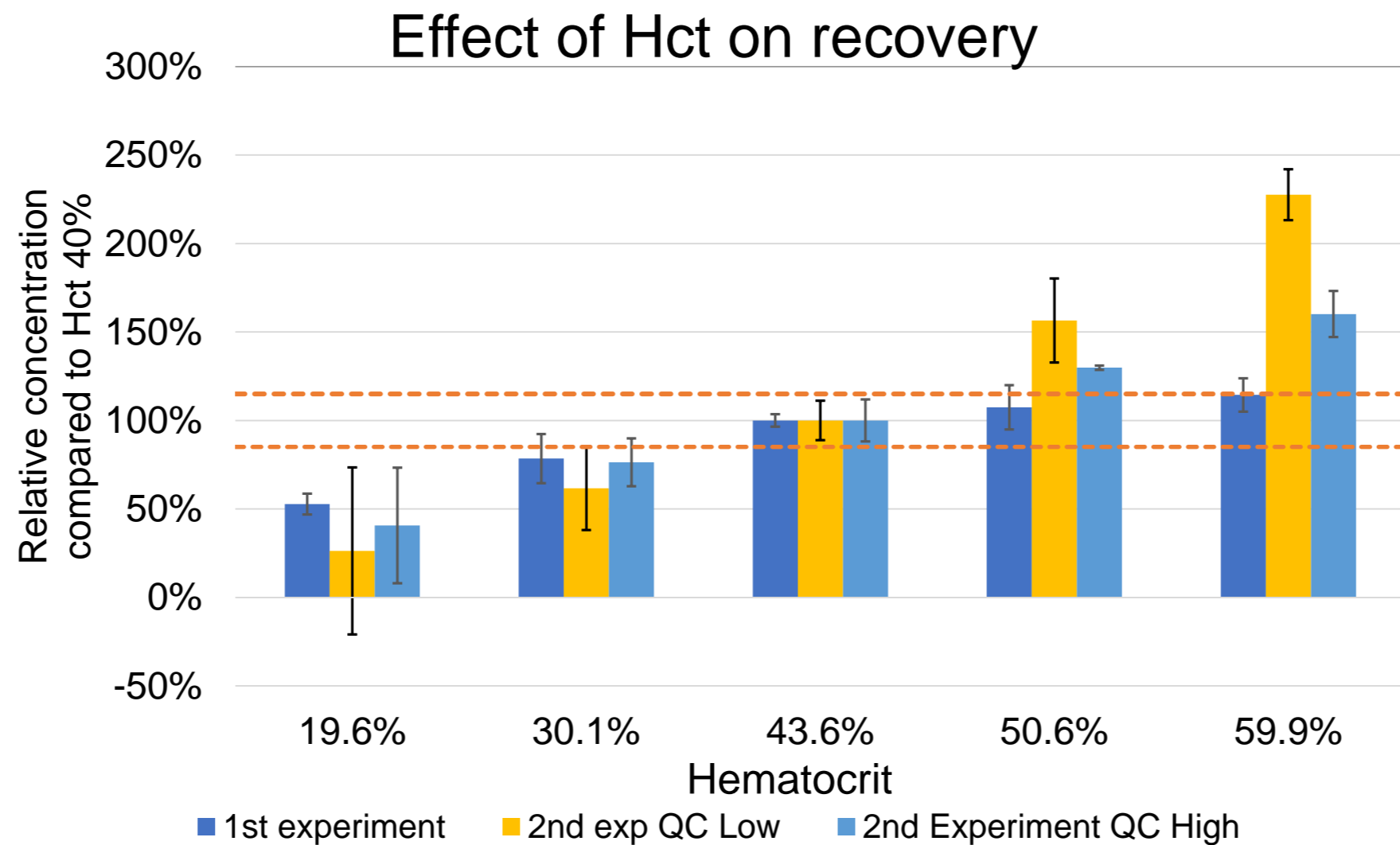


VALIDATION: RECOVERY AND HEMATOCRIT EFFECT

Spiking of APAP-Protein adduct is not possible

Blood to plasma ratio $\neq 1$

👉 Mix positive patient blood sample with blank blood with adjusted Hcts



RECOVERY – HCT ISSUE

Hypotheses for cause of Hct-effect

- ② Effect related to VAMS-sampling?
- ② Decreased elimination of APAP-Cys^{Free} in higher Hcts?
- ② Loss of proteins in wash step?
- ② Matrix effects?
- ② Instability/Adsorption during sample prep?
- ② *In vitro* formation of APAP-Cys in blood?
- ② ...

RECOVERY – HCT ISSUE

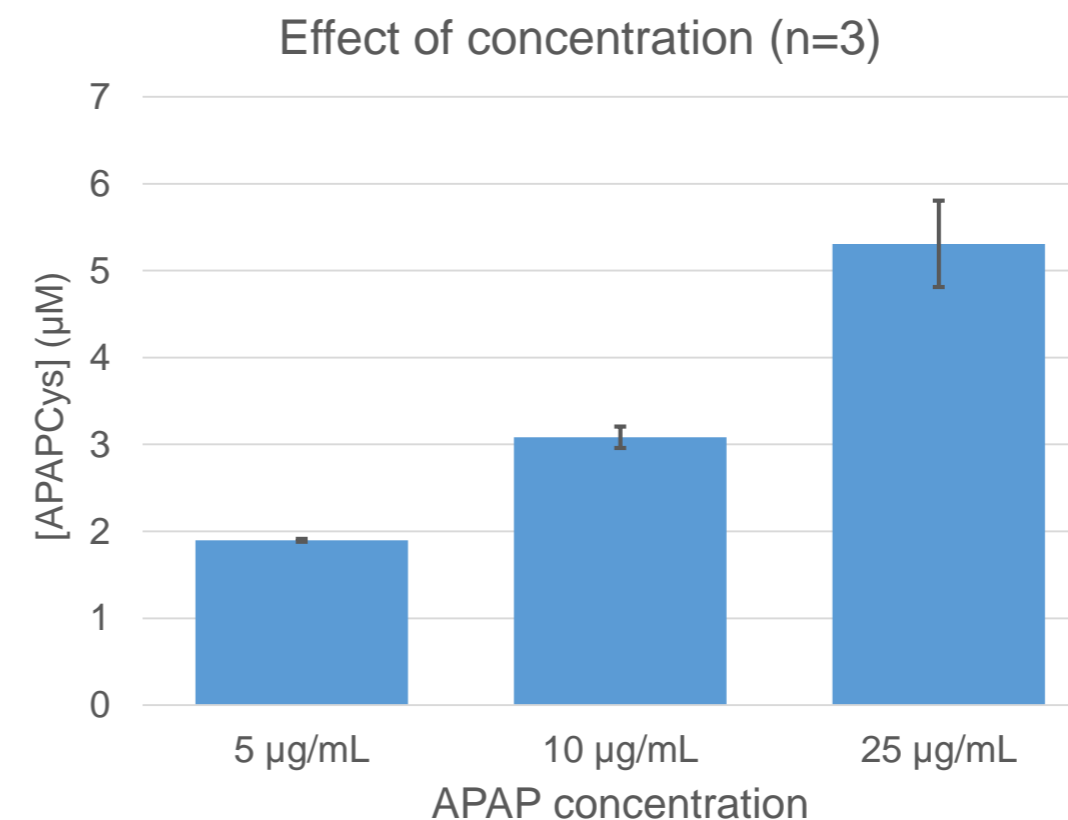
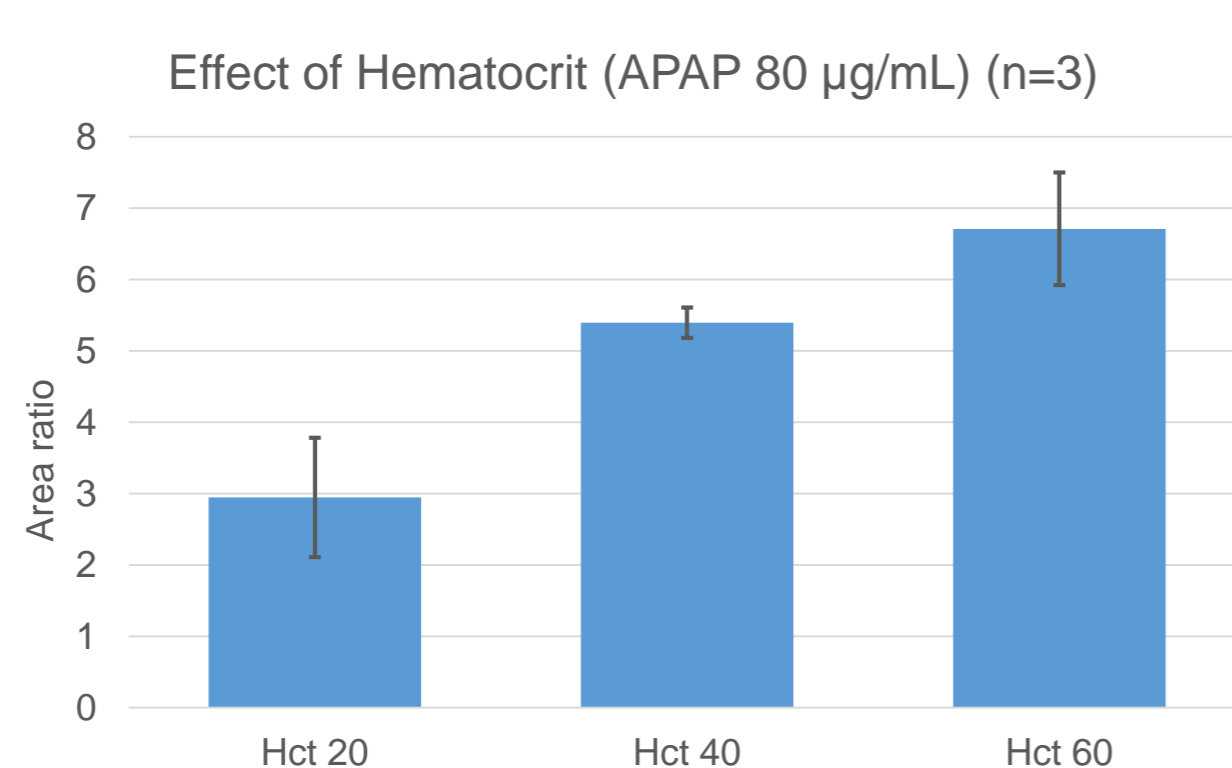
Hypotheses for cause of Hct-effect

- ✗ Effect related to VAMS-sampling?
- ✗ Decreased elimination of APAP-Cys^{Free} in higher Hcts?
- ✗ Loss of proteins in wash step?
- ✗ Matrix effects?
- ✗ Instability/Adsorption during sample prep?
- ⊛ ***In vitro* formation of APAP-Cys in blood?**
- ⊛ ...

HCT-EFFECT – ADDITIONAL FORMATION APAP-CYS

Can APAP present in blood cause (additional) formation of APAP-Cys?

- 👉 Spike APAP to blank blood
- 👉 Analyse samples for presence of APAP-Cys

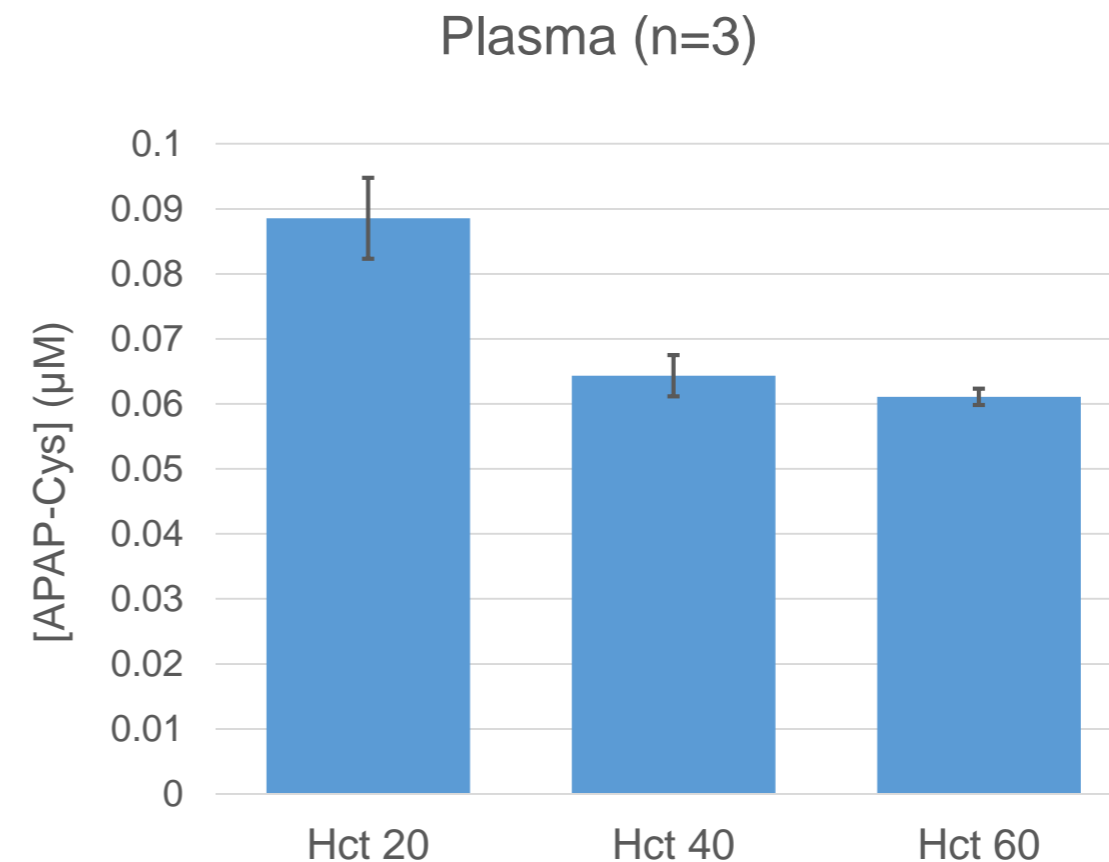
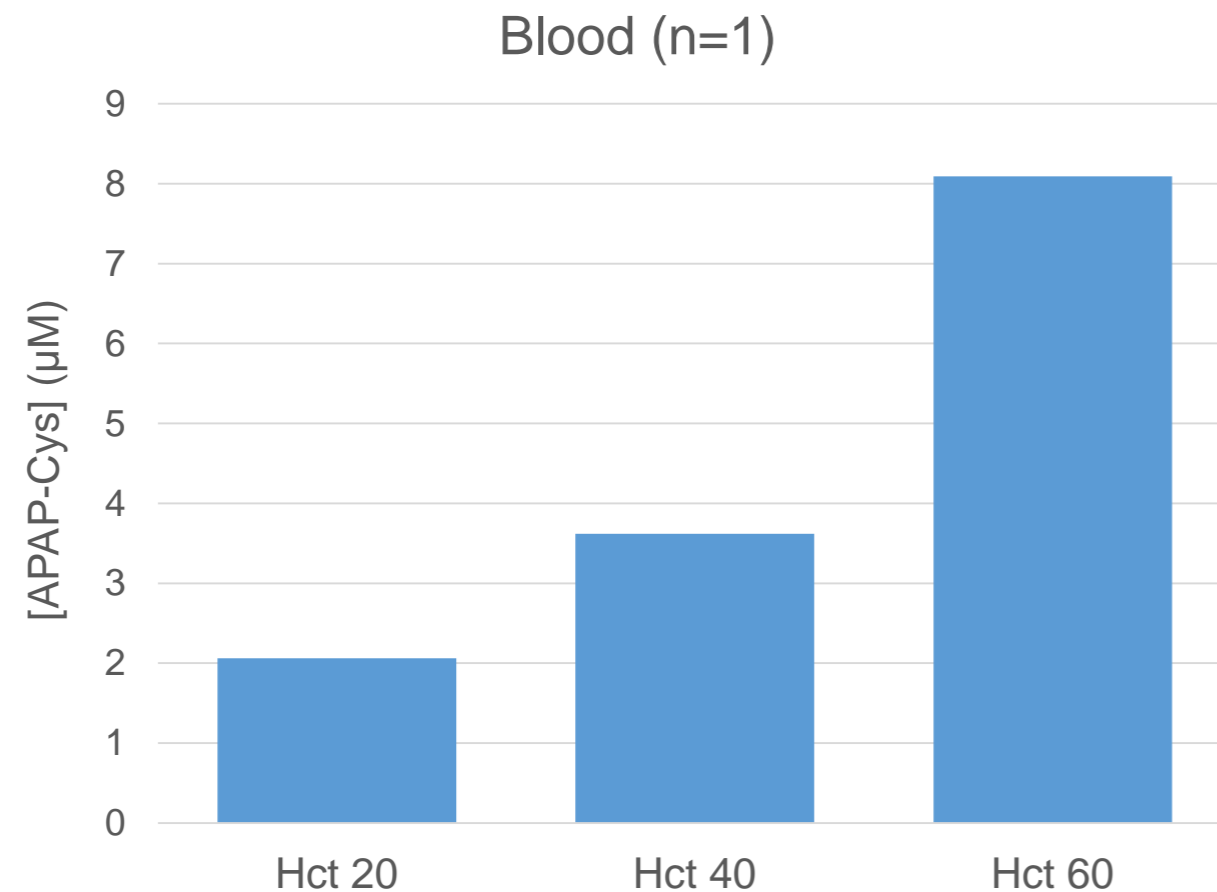


- 💧 APAP-Cys^{Prot} detected after spiking APAP to blank blood
- 💧 Increasing concentrations of APAP-Cys found with increasing Hct
- 💧 Increasing concentrations of APAP-Cys found with increasing [APAP]

HCT ISSUE: FORMATION OF APAPCYS

Increase of [APAP-Cys] in blood after Hct adaptation of authentic patient sample

👉 Is this increase also detected in corresponding plasma samples?



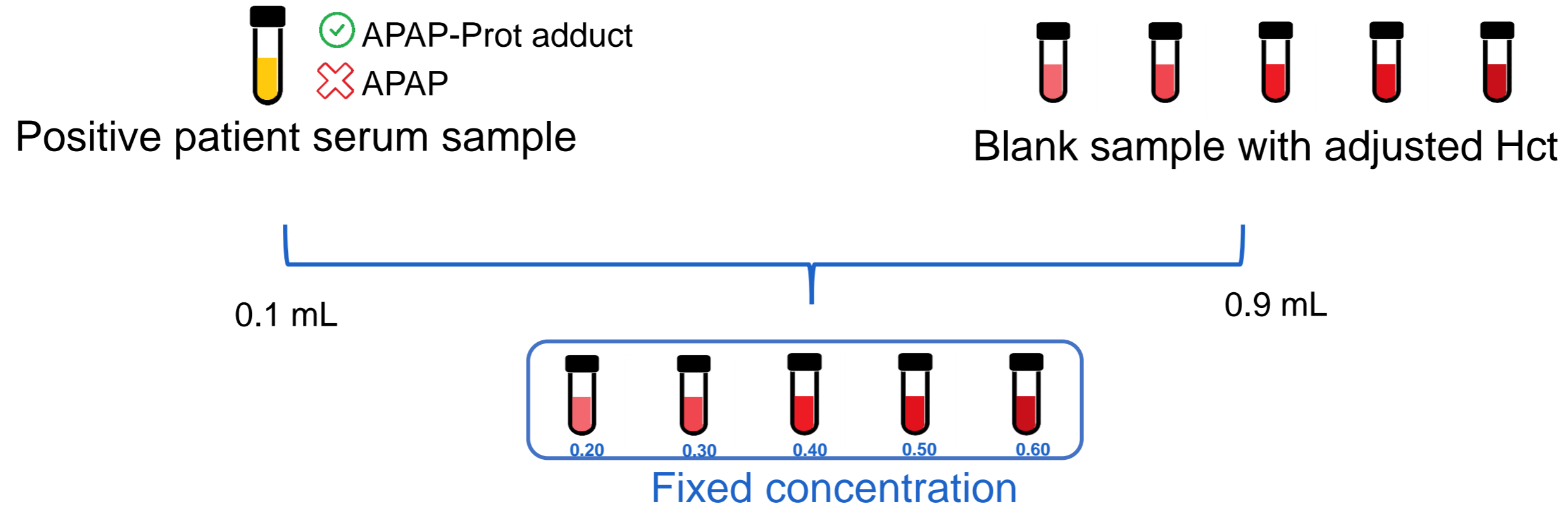
No increase of [APAP-Cys] with Hct in corresponding plasma samples

👉 Formation of additional APAP-Prot (mainly) in RBC fraction

VALIDATION: RECOVERY AND HEMATOCRIT EFFECT

Reperformed recovery/hematocrit experiment

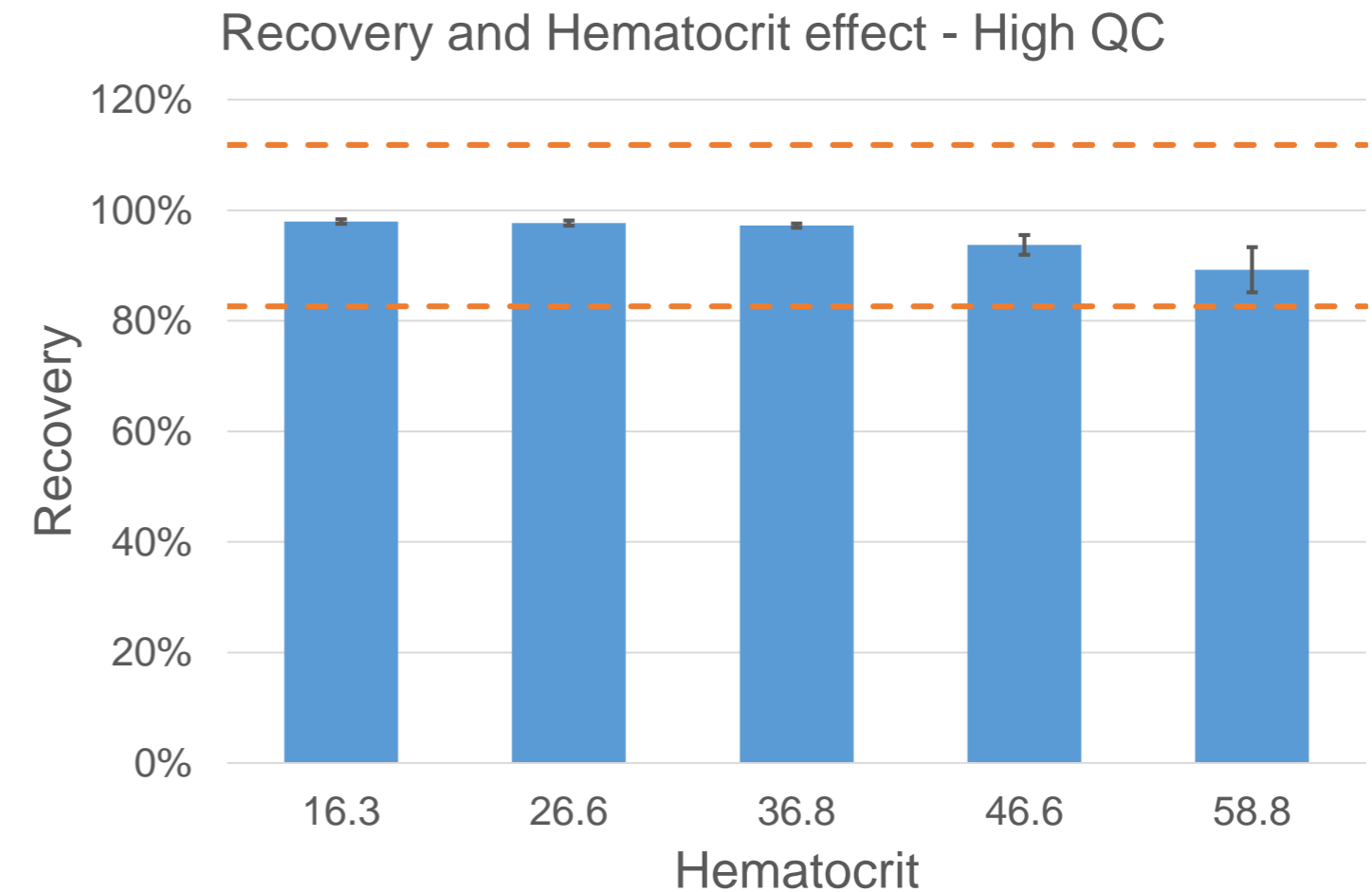
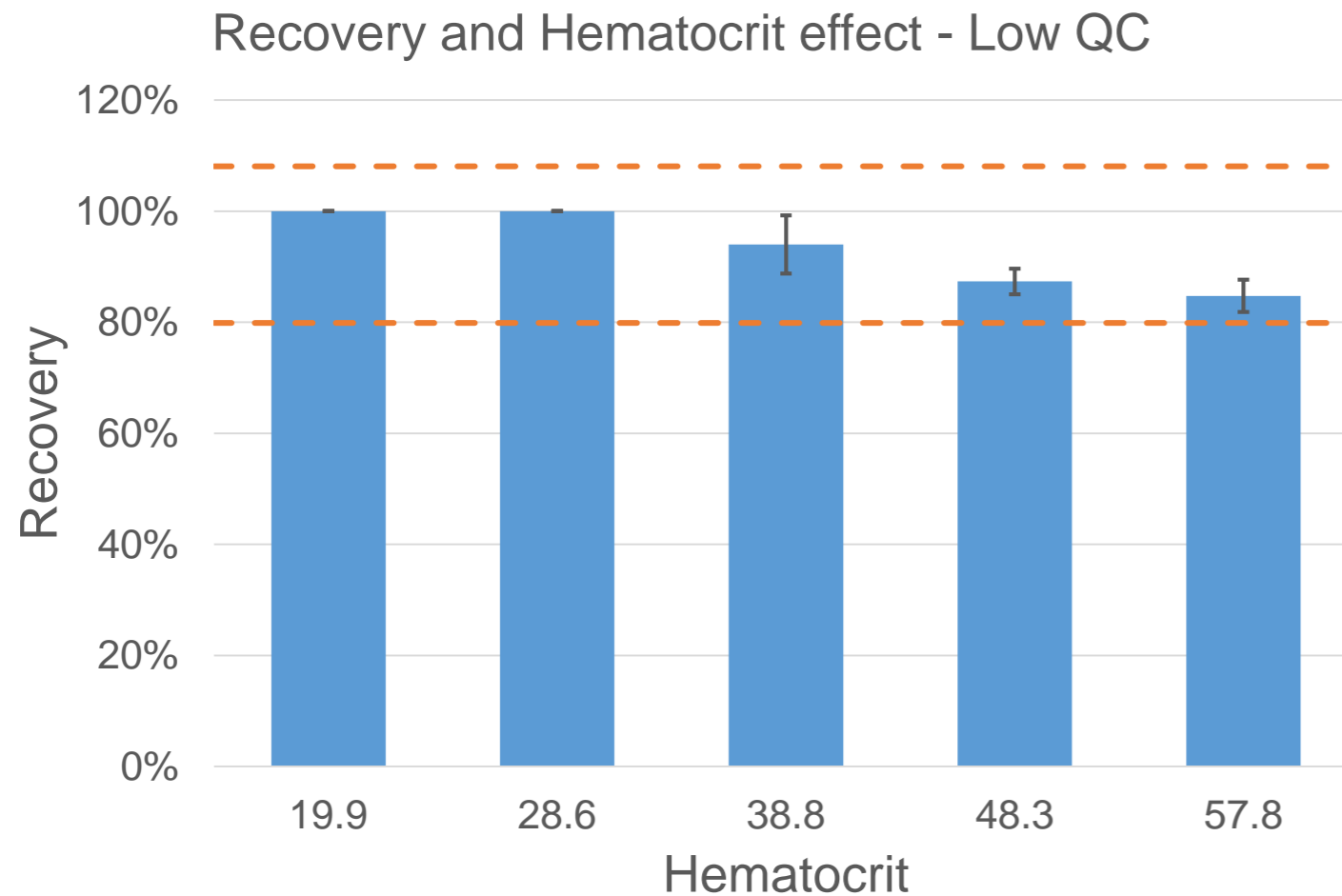
👉 Mix positive patient serum sample with blank blood with adjusted Hcts



VALIDATION: RECOVERY AND HEMATOCRIT EFFECT

Reperformed recovery/hematocrit experiment

👉 Mix positive patient serum sample with blank blood with adjusted Hcts



Recovery decreases with hematocrit, however, not more than 15% compared to Hct 40%

👉 No relevant effect of the Hct on the recovery for a Hct range of $\pm 20\%$ to $\pm 60\%$

CONCLUSION & FUTURE PERSPECTIVES

- 👉 A validated VAMS-based method was set up for the determination of APAP-Prot adduct-derived APAP-Cys in blood
- 👉 APAP in blood results in additional presence of APAP-Protein adduct (in RBC fraction of blood)

In Future, we will:

- 👉 Compare blood vs. plasma of patient samples to evaluate usefulness of blood as a matrix for the determination of APAP-Protein adducts
- 👉 Evaluate the kinetics of APAP-Protein adducts in blood
- 👉 Analyse patient samples to gain information on the presence of APAP-Prot adducts in specific patient populations

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