



# Cryoactivation, CRESS and the Importance of Preanalytics

Coral Munday, Senior Scientist, Drug Development Solutions, LGC

“Currently, pre-analytical errors account for up to 70% of all mistakes made in laboratory diagnostics”

\*“Quality Indicators to Detect Pre-Analytical Errors in Laboratory Testing” Mario Plebani

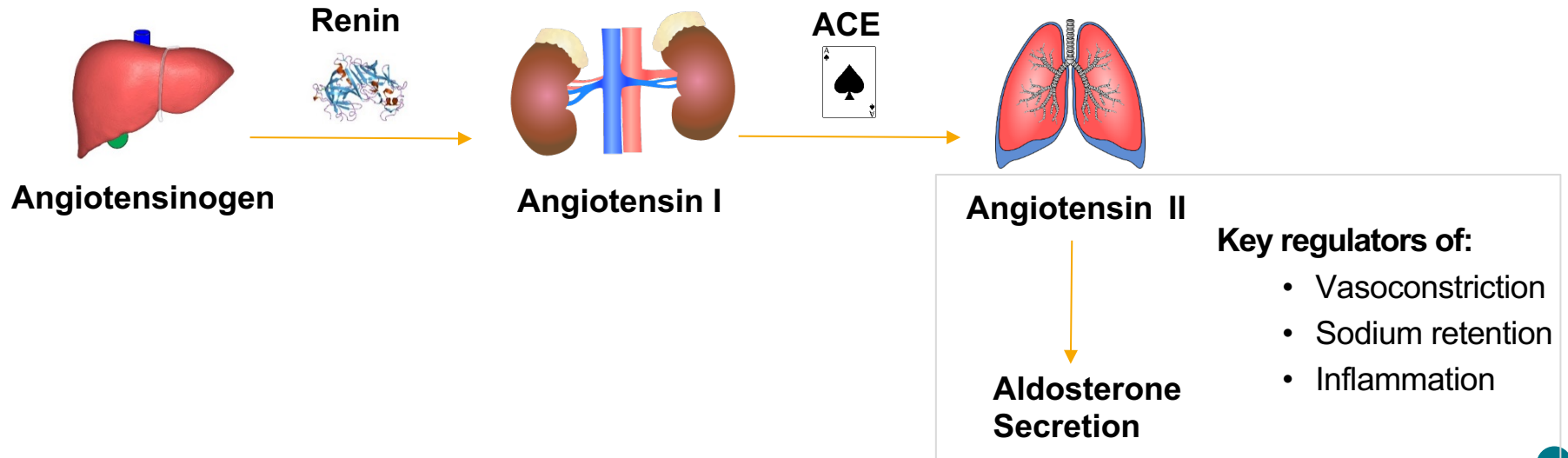
# The biomarker



# Plasma renin

## Rate limiting enzyme in the renin-angiotensin-aldosterone system

- The RAA system regulates blood volume, blood pressure and osmoregulation

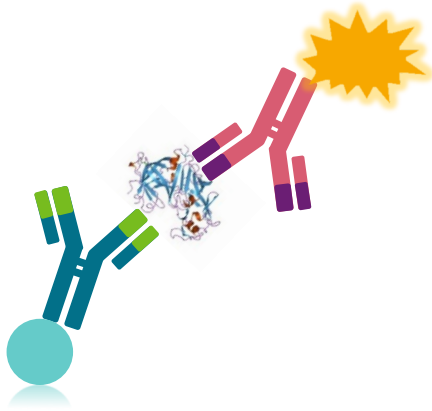


# Renin measurement



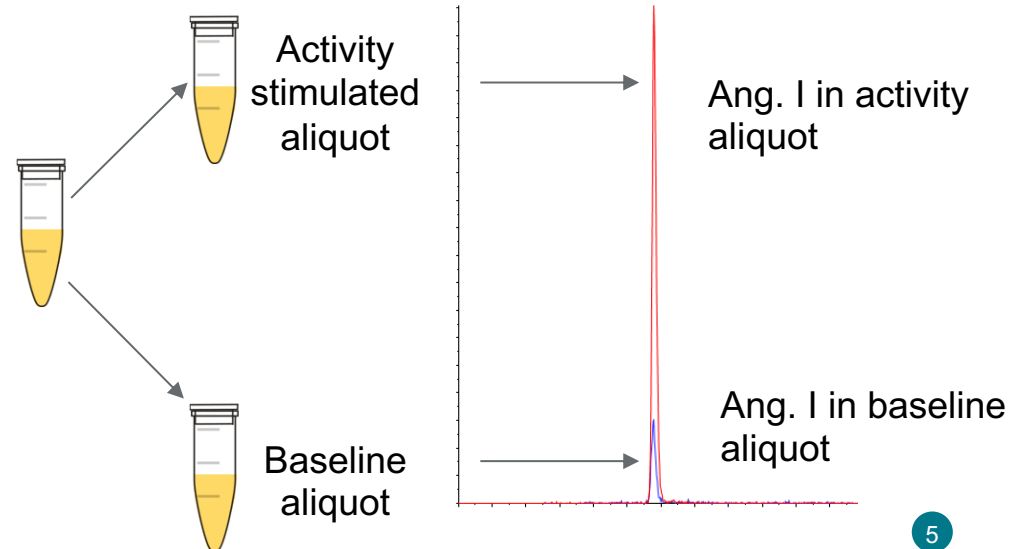
## Direct renin measurement

- Measures active and inactive renin
- Immunoassay



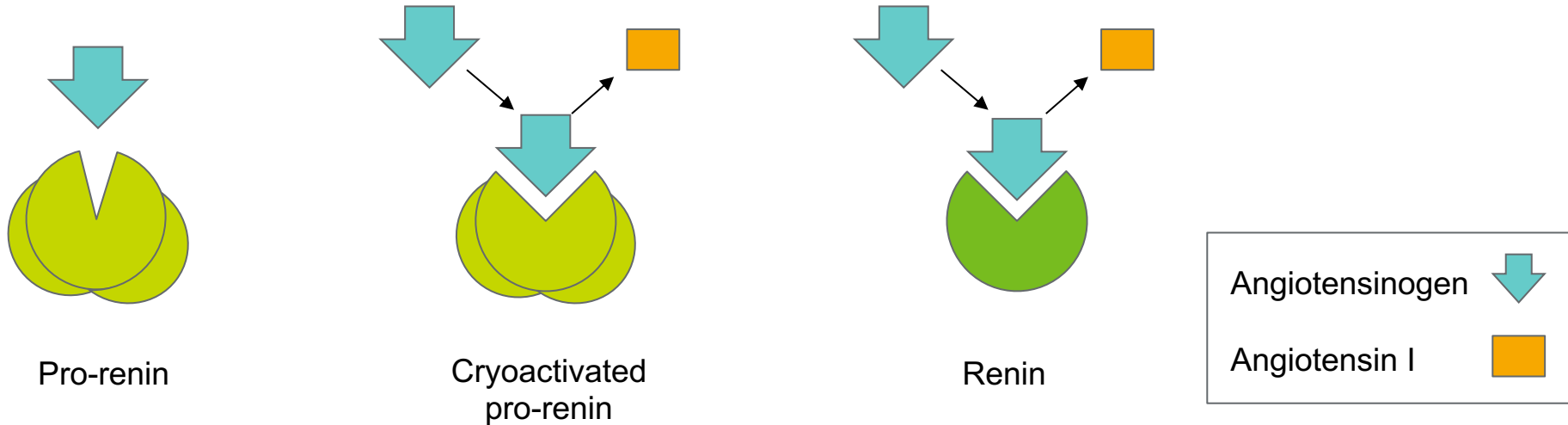
## Plasma renin activity

- Measures only the biologically active renin
- Immunoassay or LC-MS



# Cryoactivation

## Activation of renin pre-cursor at cold temperatures

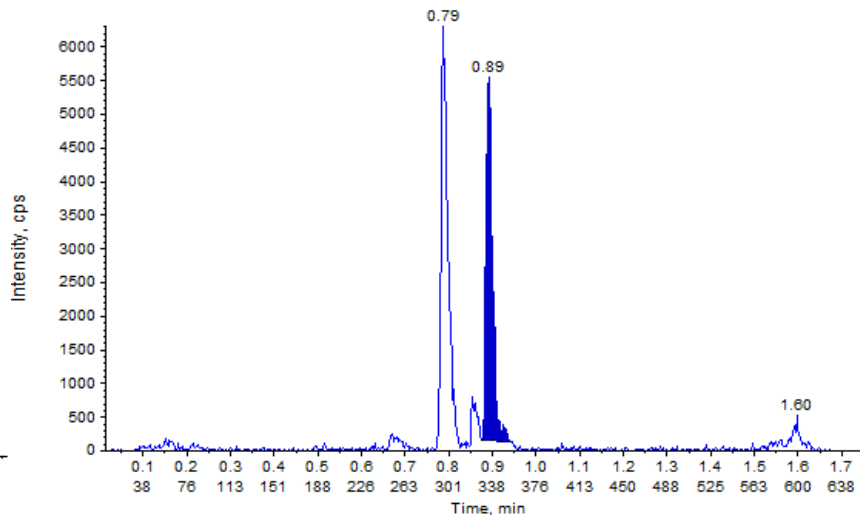
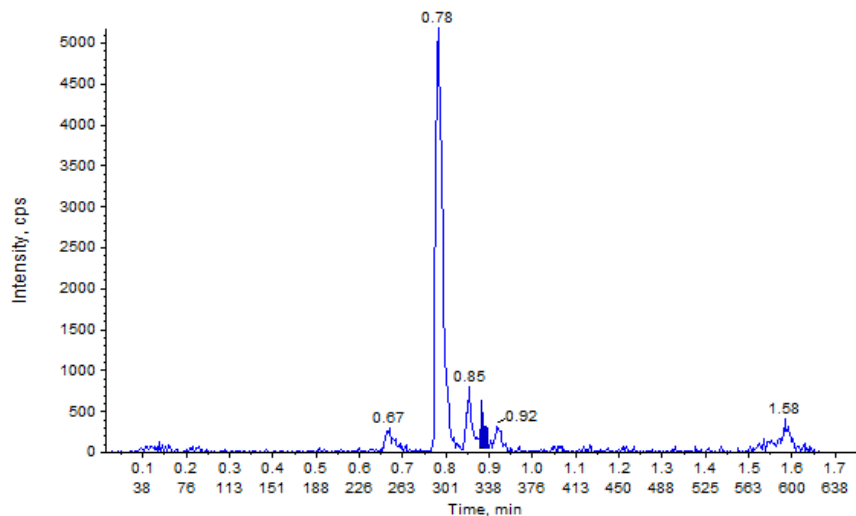


- Can contribute to overestimation of renin/renin activity measurement
  - 96% of PubMed results for 'Cryoactivation'
  - Contradictions in the literature

# Substrate stability



Activity assay is reliant on angiotensinogen concentration



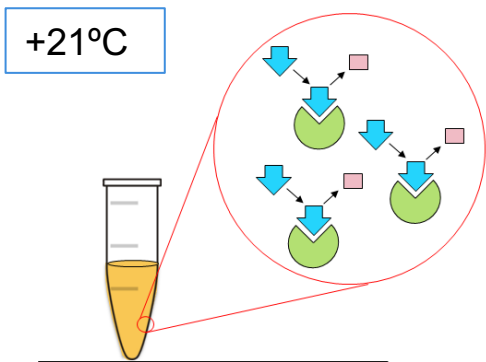
Angiotensin I levels in unstressed (left) vs stressed (right) baseline samples

# Initial stability assessment



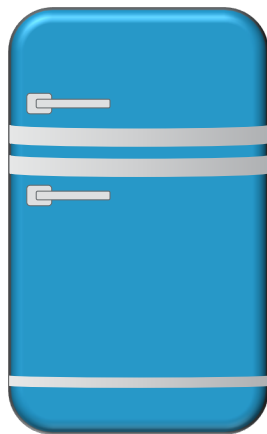
## Assessed 24 hours room temperature, +4°C, 4 F/T

- Initial assessment in pooled plasma
- Follow-up assessment in three individuals

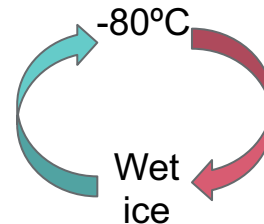


RT enzyme activity observed

Decrease in PRA (14%-29%)



No evidence of  
cryoactivation



No cryoactivation in pool

≈13% increase in PRA in  
2/3 individuals



# The checklist



# European Federation of Laboratory Medicine



- A federation of clinical chemistry and laboratory medicine societies
- Currently made up of 40 national societies across Europe

Their vision:

“enhance patient care and improve outcomes by promoting and improving the scientific, professional and clinical aspects of clinical chemistry and laboratory medicine”

# Checklist for Reporting Stability Studies



## The CRESS checklist for reporting stability studies: on behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE)

*Michael Cornes, Ana-Maria Simundic, Janne Cadamuro, Seán J. Costelloe , Geoffrey Baird , Gunn B. B. Kristensen, Alexander von Meyer, Mads Nybo and Rubén Gómez Rioja *

From the journal [Clinical Chemistry and Laboratory Medicine \(CCLM\)](#)

- 20 essential items for reporting stability studies
  - Encourage use in the planning stages
  - Encourage reviewers/editors use to evaluate stability studies
- 

Title/keywords

Measurand/analyte

Spiking studies

Acceptability criteria

Abstract

Samples

Length of studies

Results

Introduction

Participants

Storage conditions

Discussion

Aim

Preanalytical conditions

Statistical analysis

Funding

Materials and methods

Analytical procedure

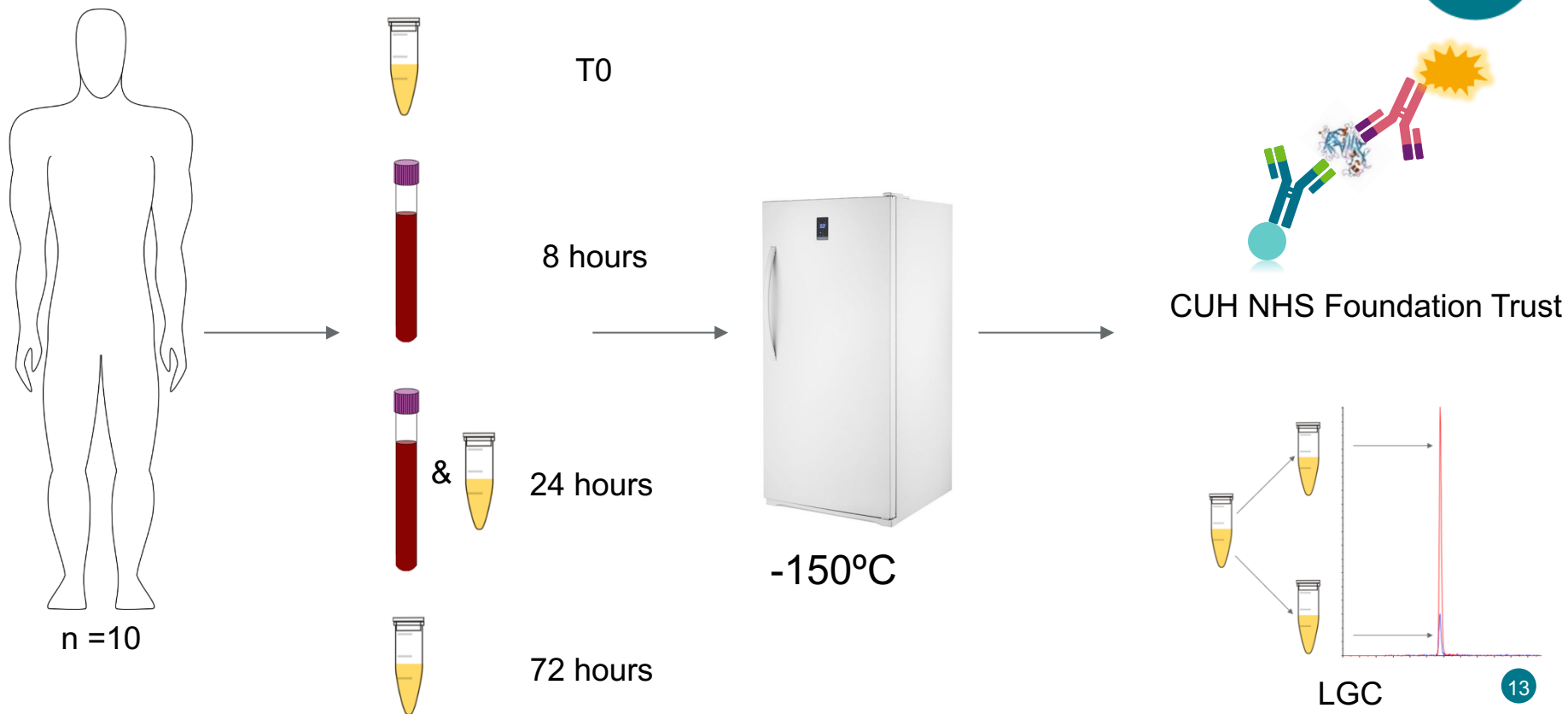
Outliers

Ethics

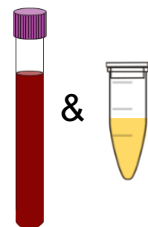
# The study



# Study design



# Pre-Analytical conditions

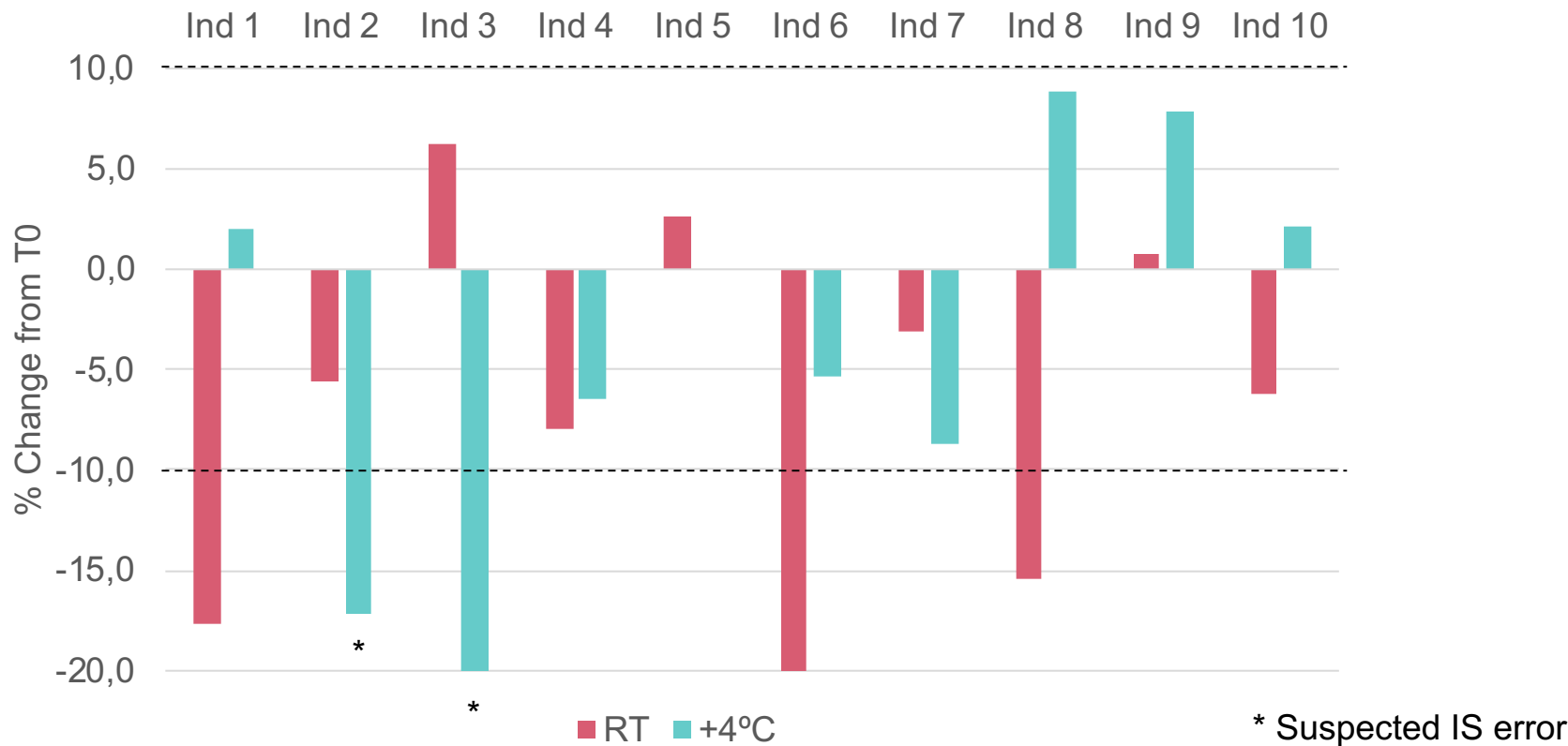


Donor collection position	Light conditions	Final storage container size, material
Blood tubes (anti-coagulant, size, manufacturer, number collected)	Container open/closed	Storage after centrifugation
Collection date & time	Centrifugation date & time	Time, date, temperature of storage
Storage conditions before centrifugation	Centrifuge speed, temperature, braking	Thawing conditions

# PRA whole blood stability



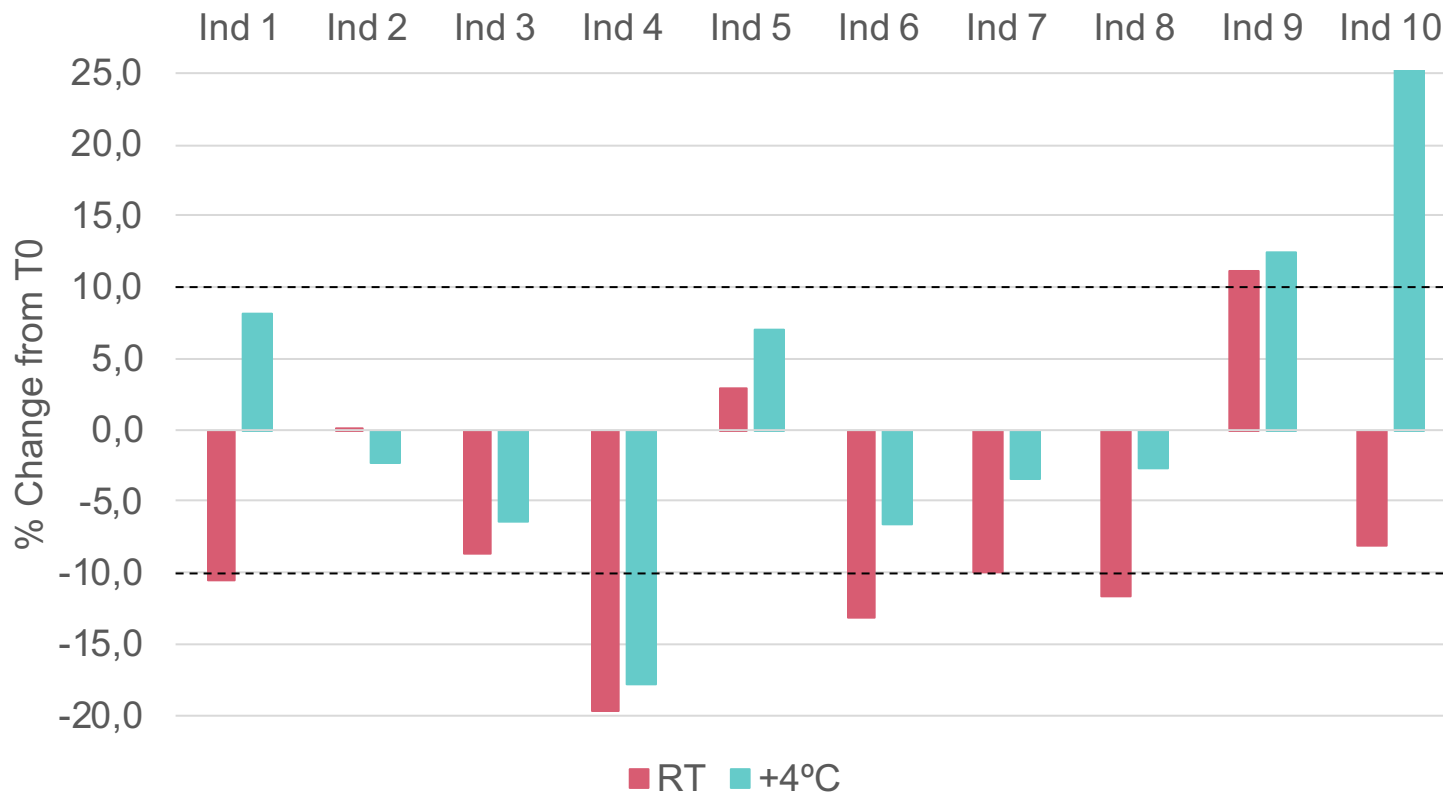
PRA stability in whole blood - 8 hours



# PRA whole blood stability

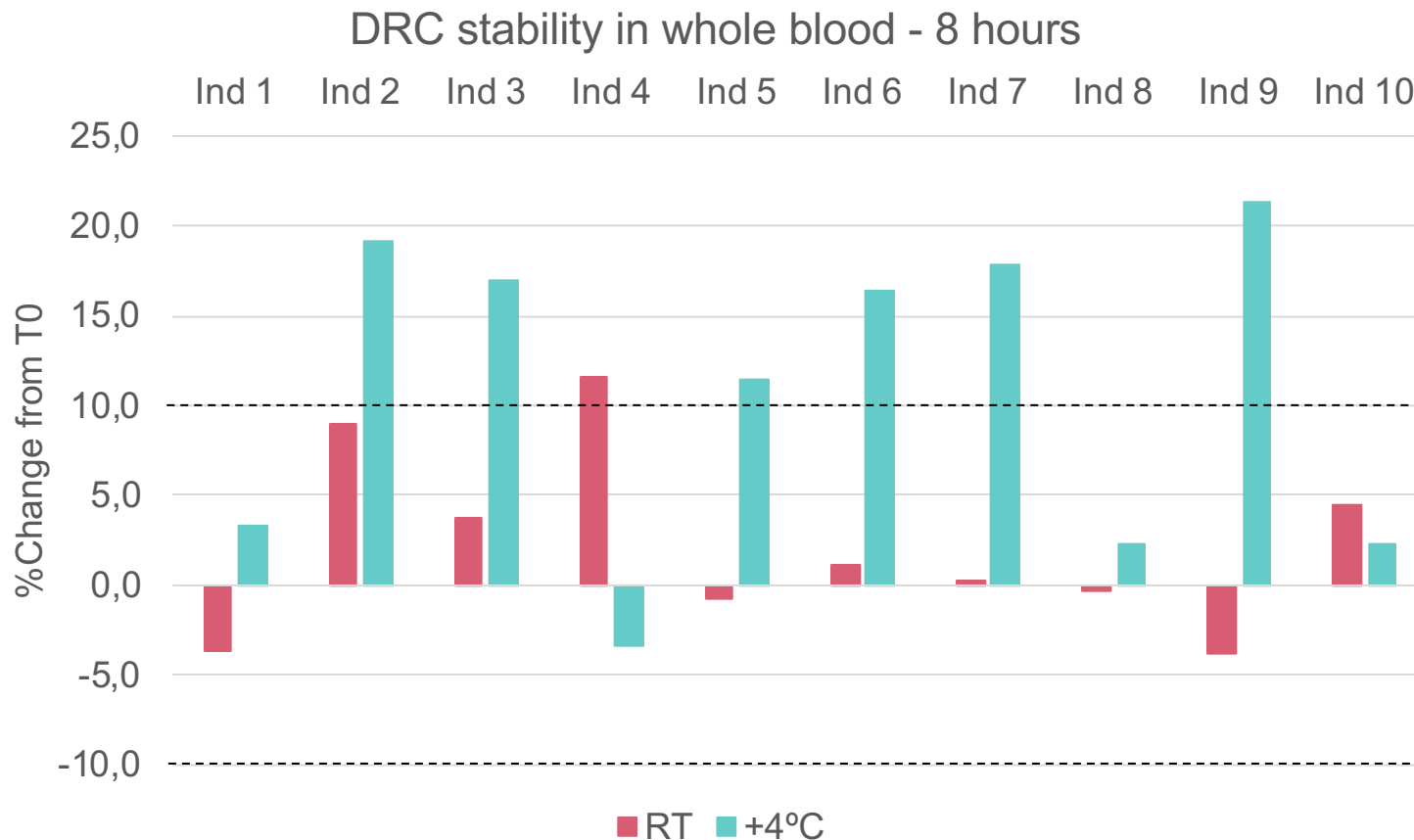


PRA stability in whole blood - 24 hours

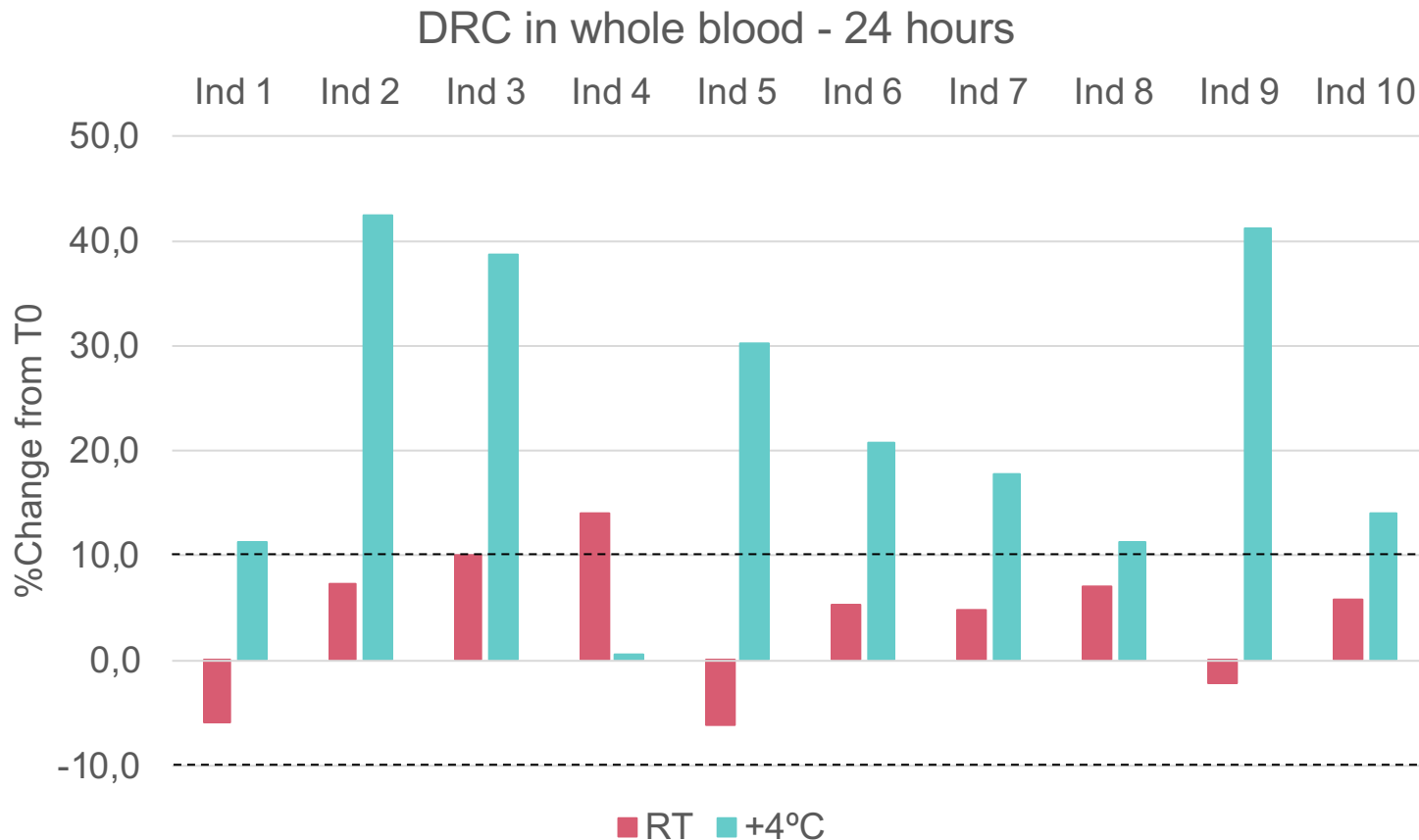




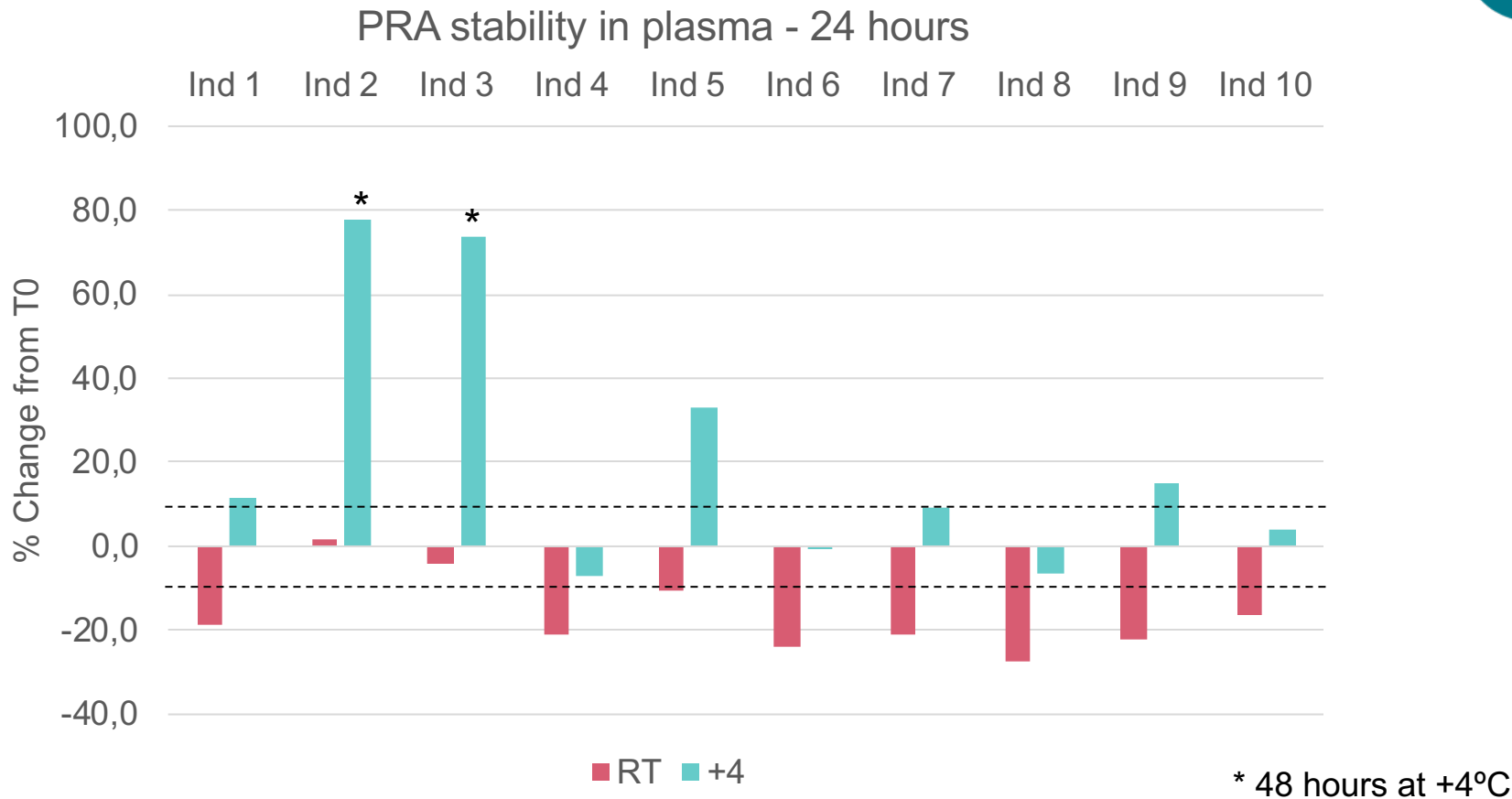
# DRC whole blood stability



# DRC whole blood stability



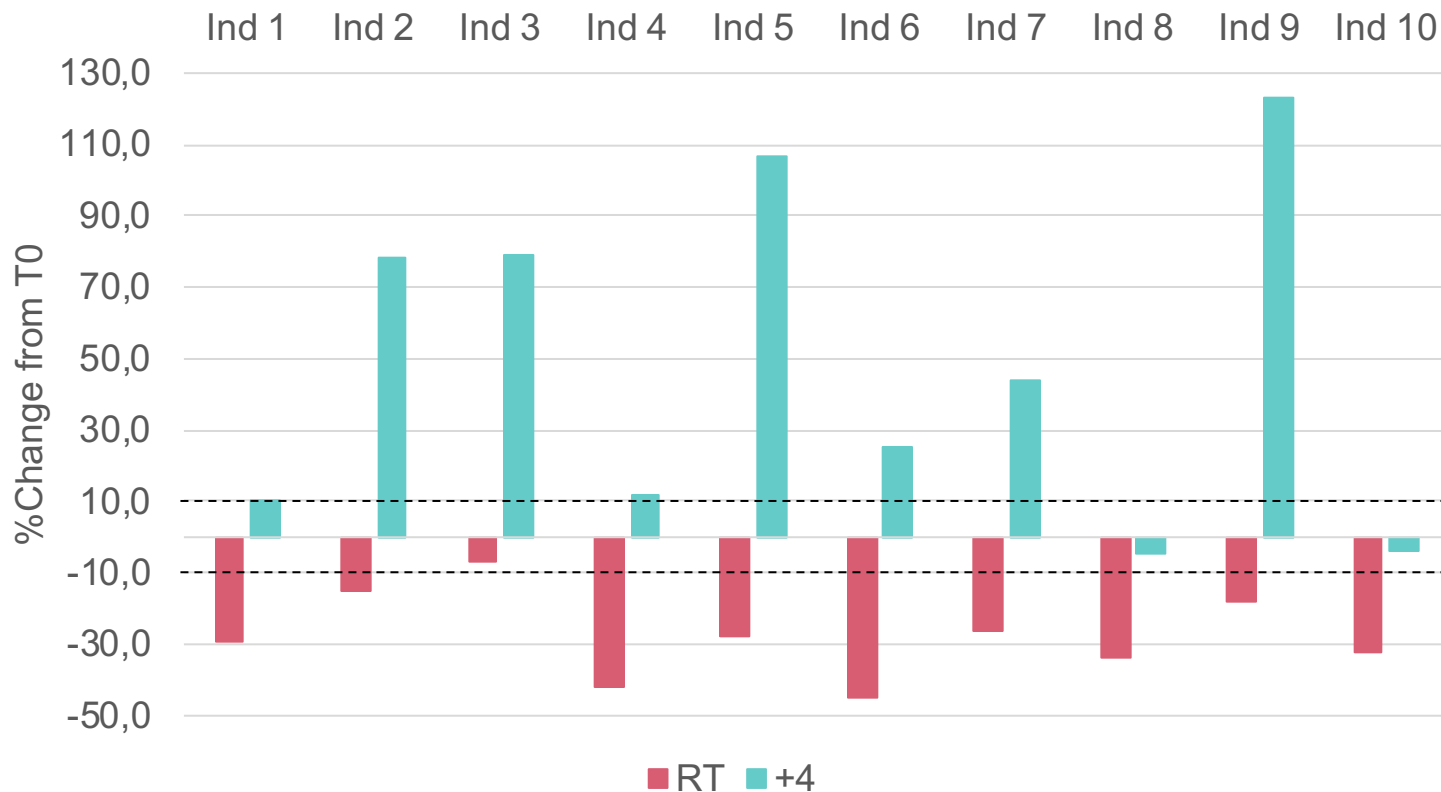
# PRA plasma stability



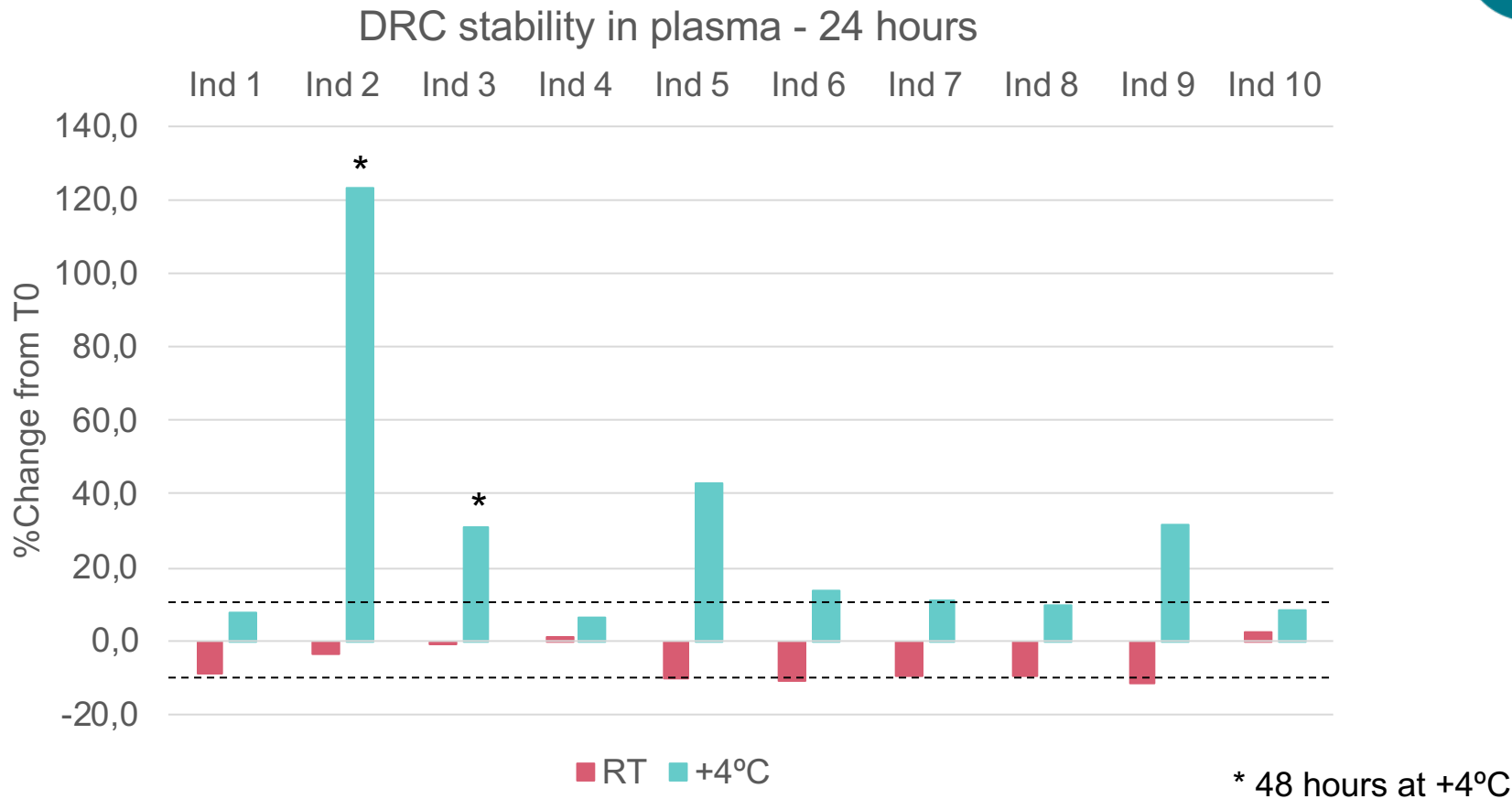
# PRA plasma stability



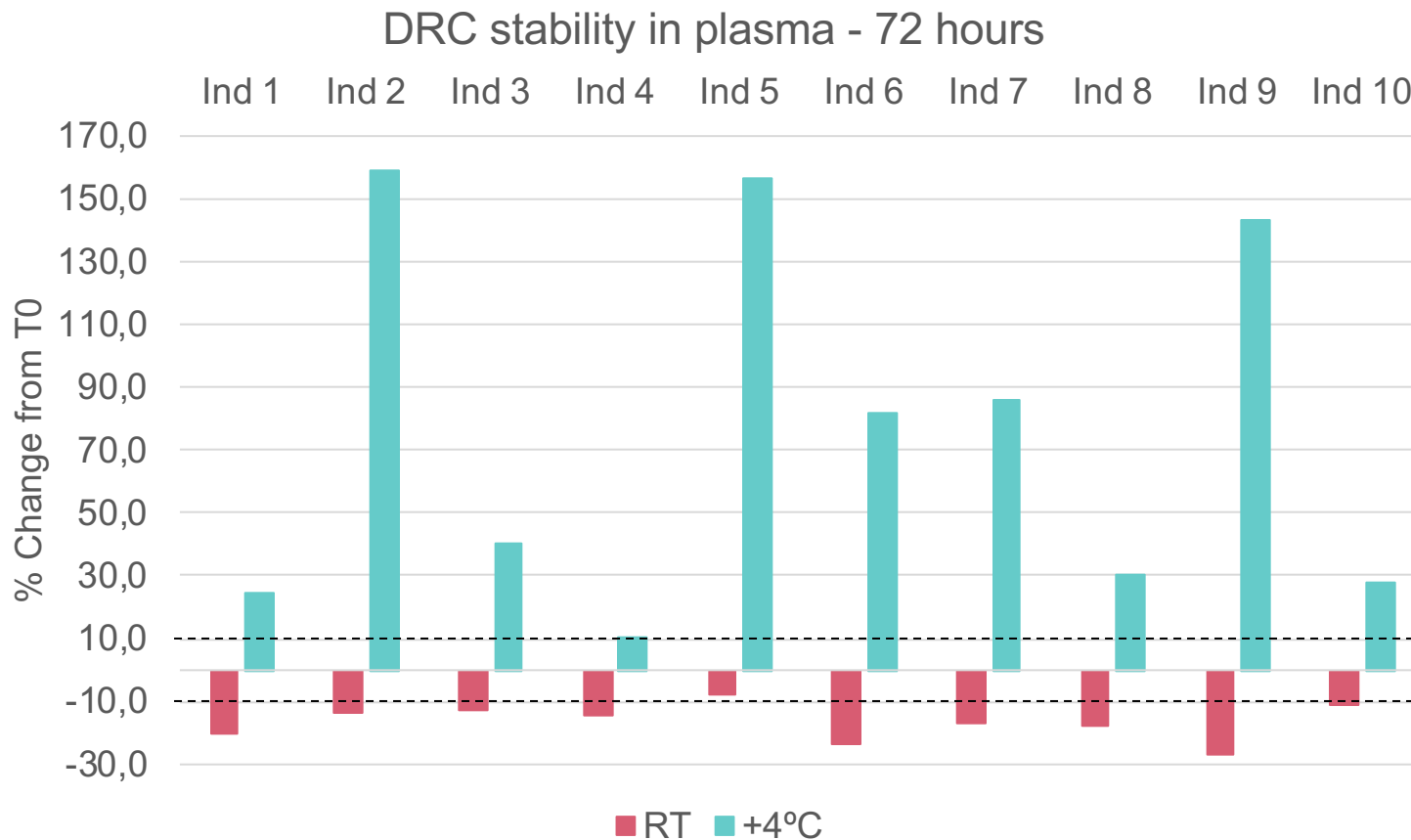
PRA stability in plasma - 72 hours



# DRC plasma stability



# DRC plasma stability



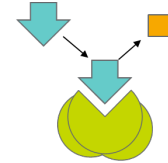
# Conclusions

- **Confirmed cryoactivation of pro-renin**

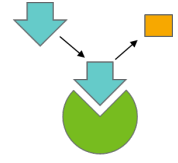
- Similar results between PRA and DRC in plasma
- More significant issue in DRC measurement in blood
- Individual variability



Pro-renin



Cryoactivated  
Pro-renin



Renin



- **Difference in stability at ambient temperatures**

- DRC not greatly affected by storage at RT
- PRA impacted... substrate depletion/instability?
- Individual variability

- **What next for renin?**

- Handle at RT
  - increase incubation period for PRA assay

# Acknowledgements



**Kevin Taylor – Cambridge University Hospitals NHS Foundation Trust**

**David Halsall – Cambridge University Hospitals NHS Foundation Trust**

**Sophie Hepburn – East Suffolk and North Essex NHS Foundation Trust**