



Cryoactivation, CRESS and the Importance of Preanalytics

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“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

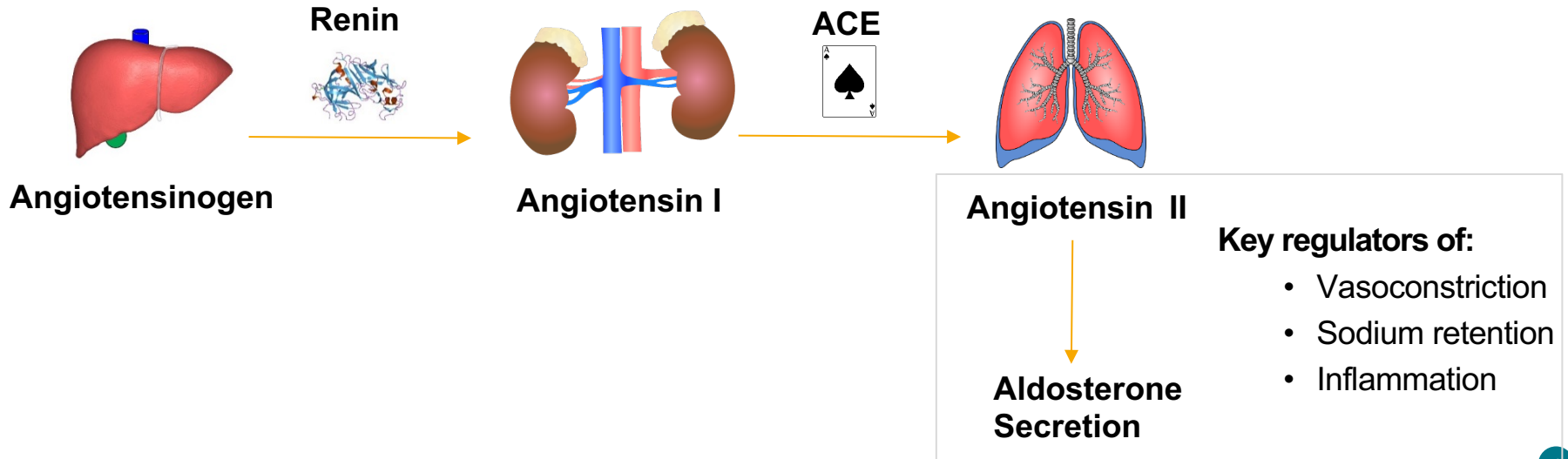


Plasma renin



Rate limiting enzyme in the renin-angiotensin-aldosterone system

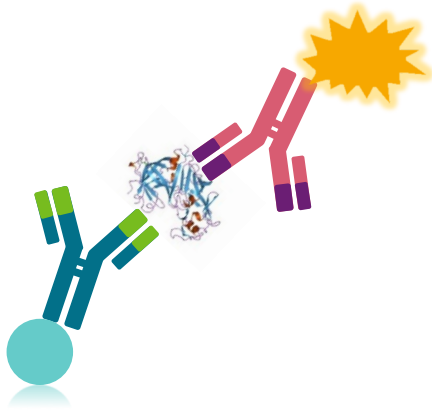
– The RAA system regulates blood volume, blood pressure and osmoregulation



Renin measurement

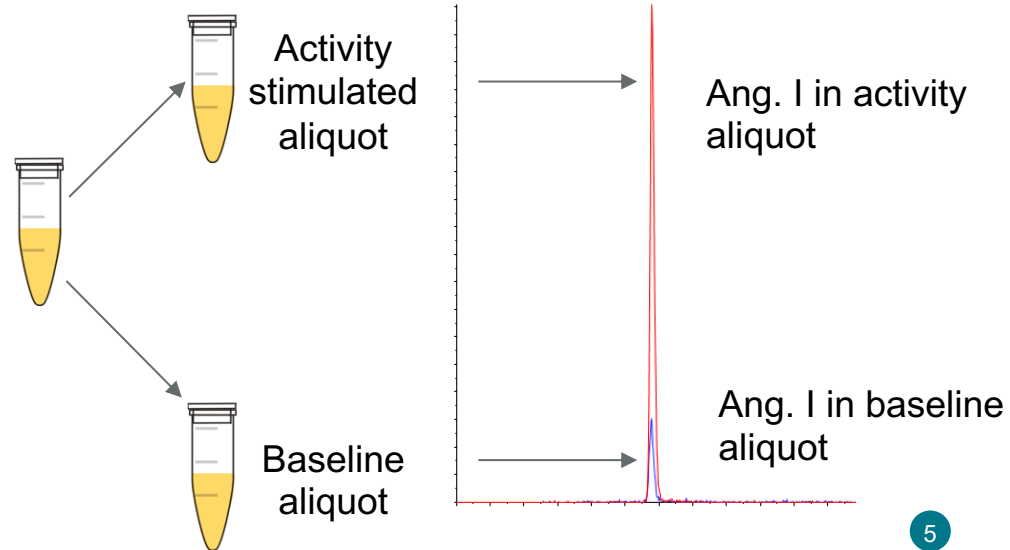
Direct renin measurement

- Measures active and inactive renin
- Immunoassay



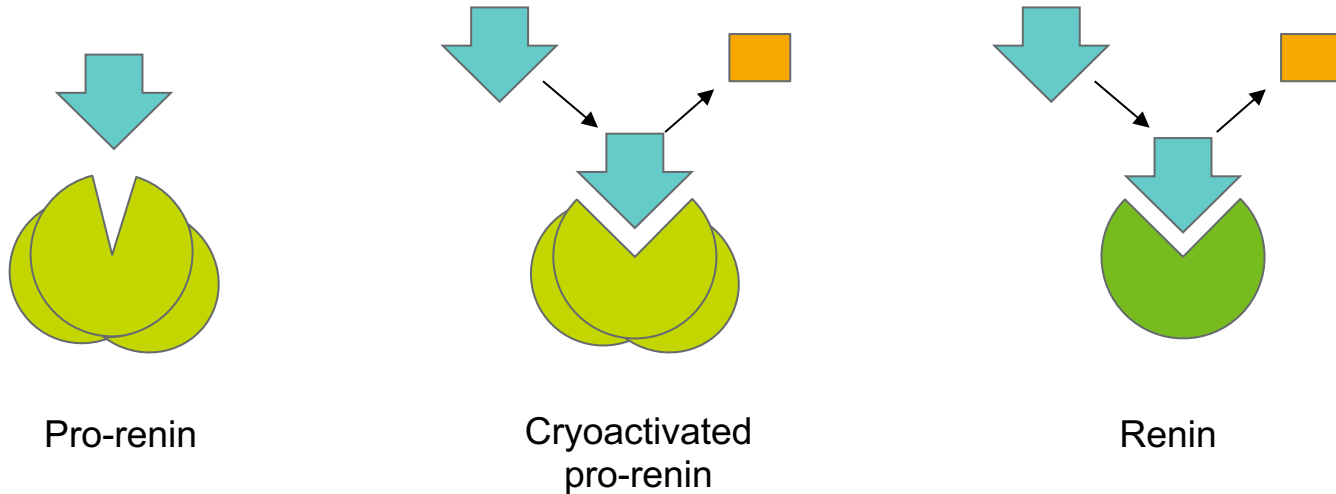
Plasma renin activity

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



Cryoactivation

Activation of renin pre-cursor at cold temperatures

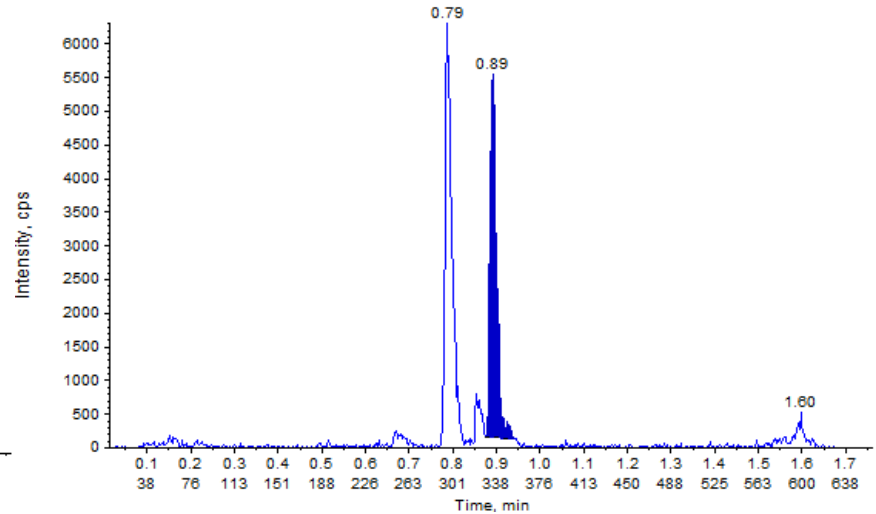
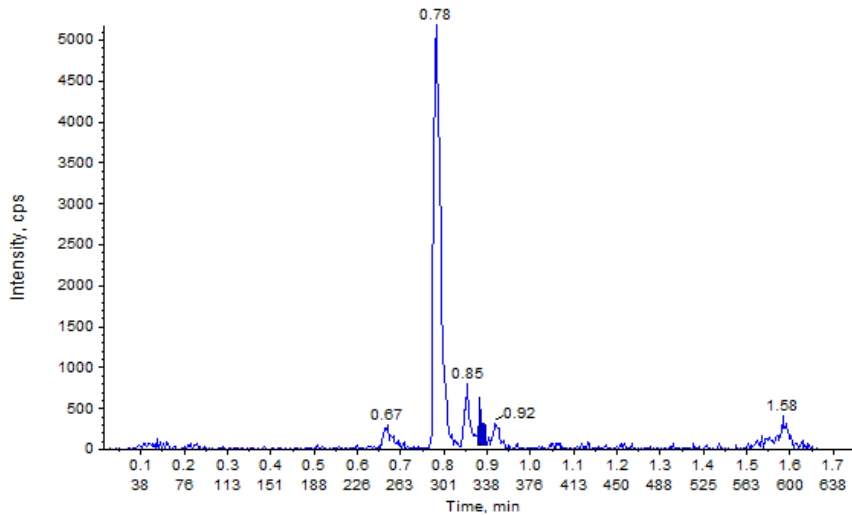


- Can contribute to overestimation of renin/renin activity measurement
 - 96% of PubMed results for 'Cryoactivation'
 - Contradictions in the literature

Substrate stability



Activity assay is reliant on angiotensinogen concentration



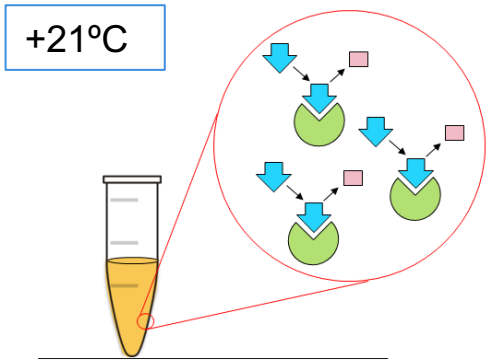
Angiotensin I levels in unstressed (left) vs stressed (right) baseline samples

Initial stability assessment



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

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

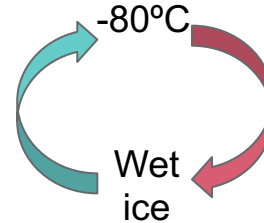


RT enzyme activity observed

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



EUROPEAN FEDERATION OF CLINICAL CHEMISTRY
AND 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 \(CCLM\)](#)

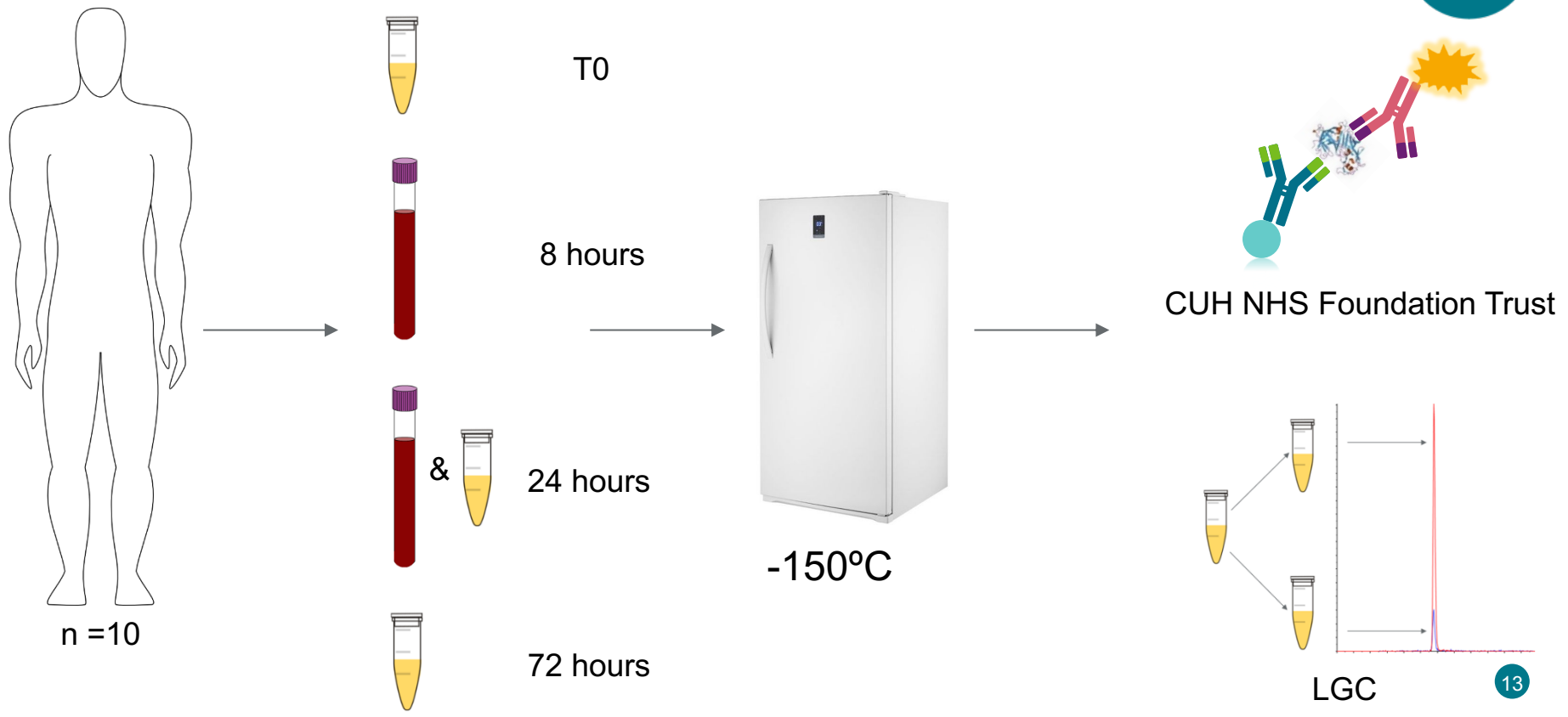
- 20 essential items for reporting stability studies
- Encourage use in the planning stages
- Encourage reviewers/editors use to evaluate stability studies

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

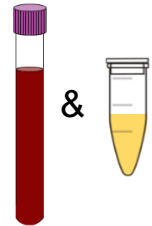
The study



Study design



Pre-Analytical conditions

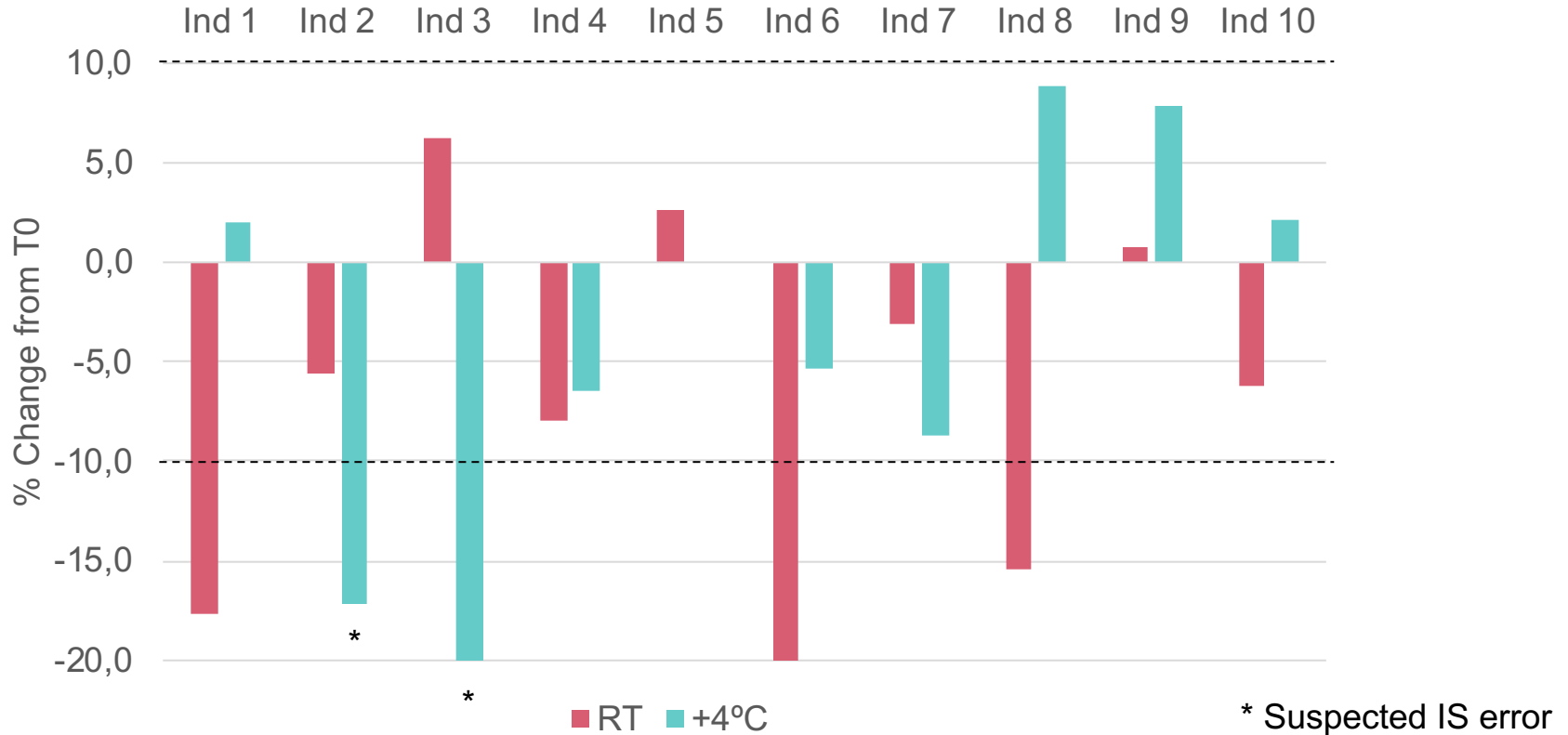


Donor collection position	Light conditions	Final storage container size, material
Blood tubes (anti-coagulant, size, manufacturer, number collected)	Container open/closed	Storage after centrifugation
Collection date & time	Centrifugation date & time	Time, date, temperature of storage
Storage conditions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

PRA whole blood stability



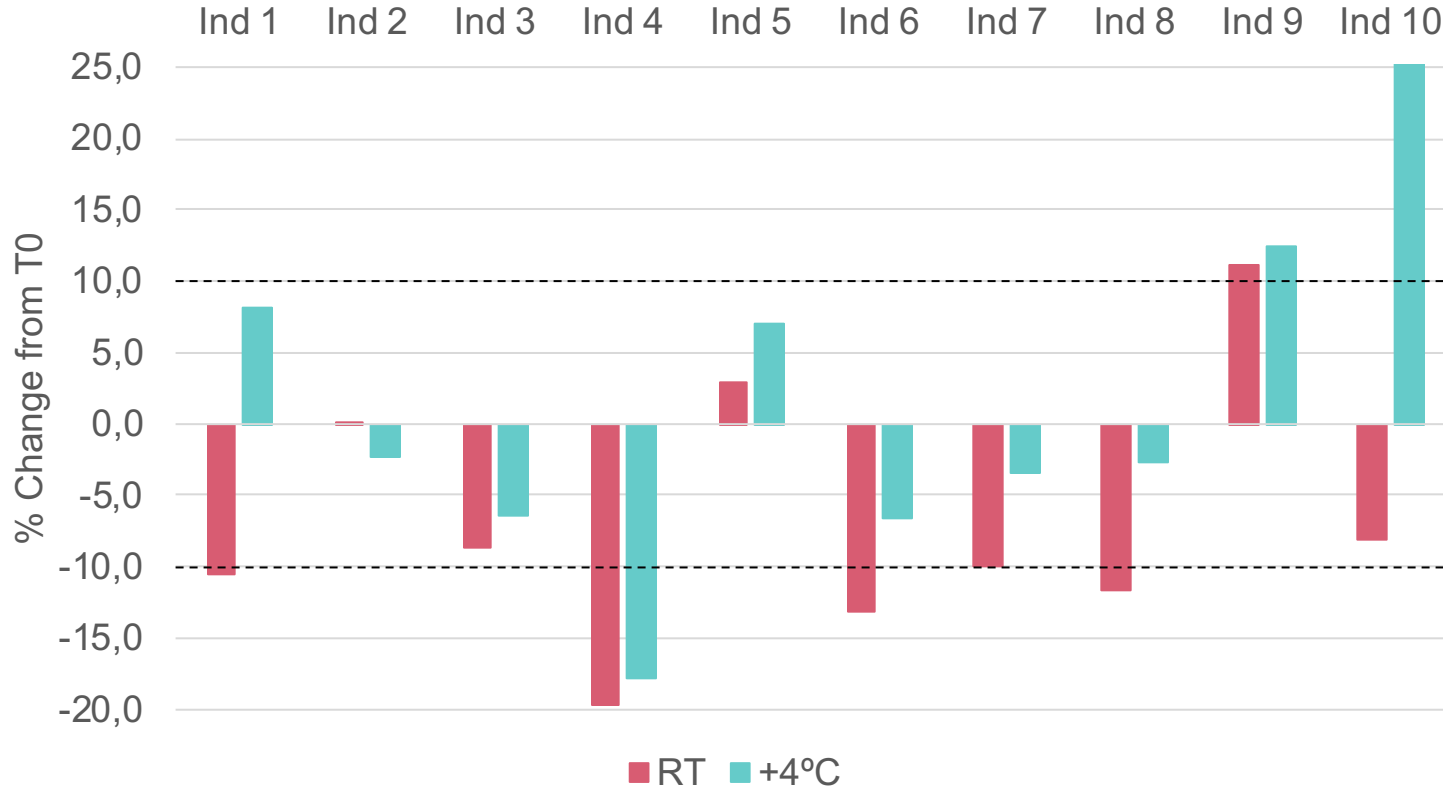
PRA stability in whole blood - 8 hours



PRA whole blood stability



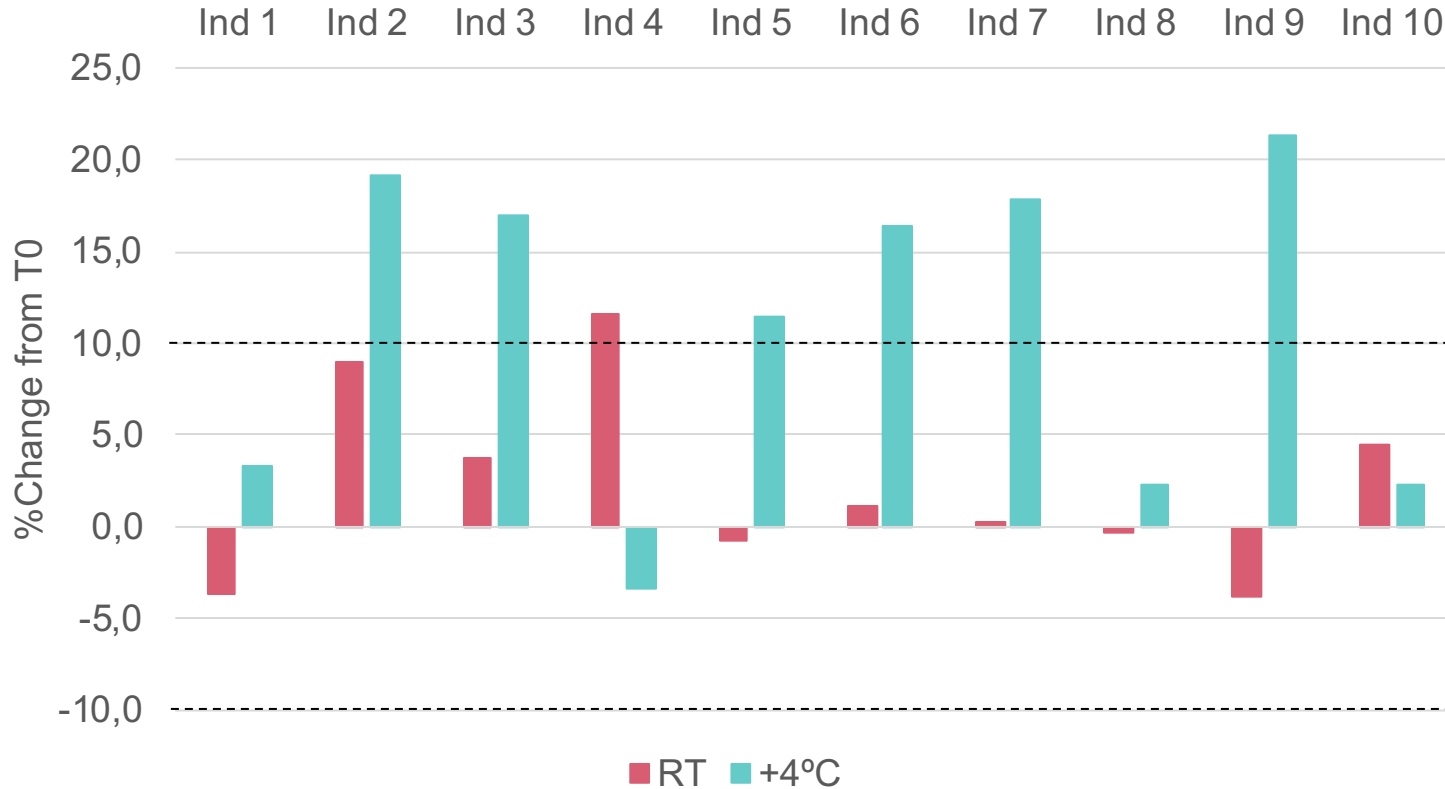
PRA stability in whole blood - 24 hours



DRC whole blood stability



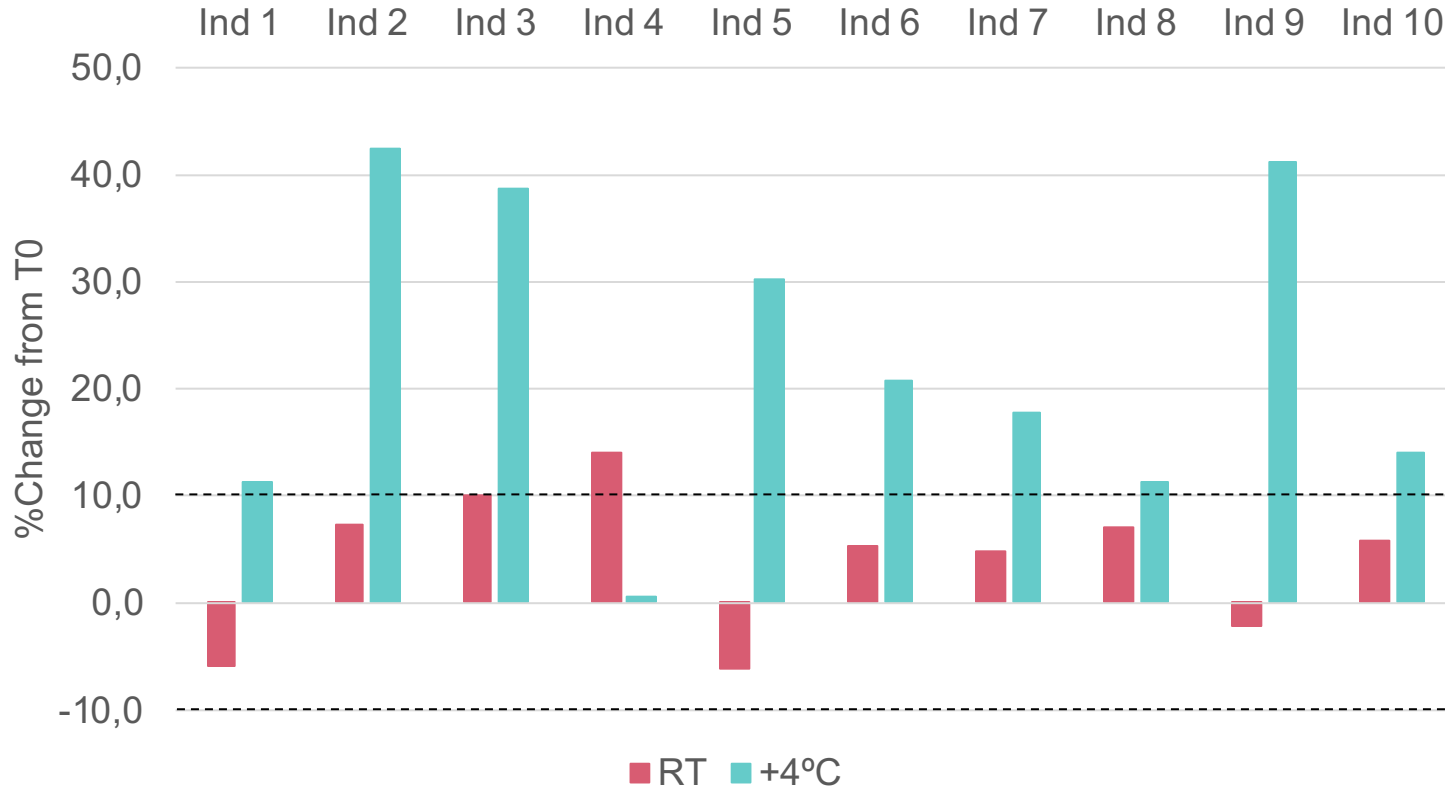
DRC stability in whole blood - 8 hours



DRC whole blood stability



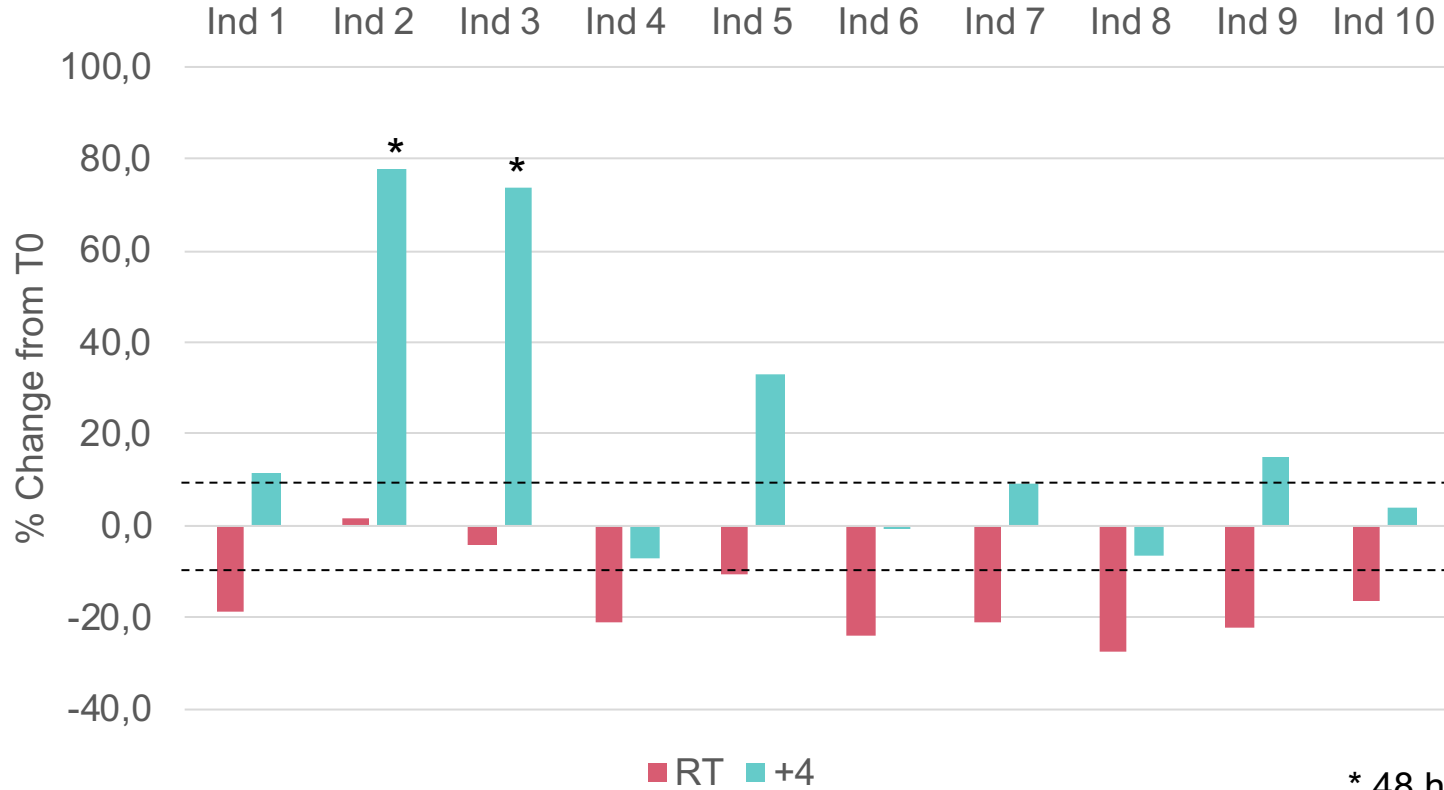
DRC in whole blood - 24 hours



PRA plasma stability



PRA stability in plasma - 24 hours

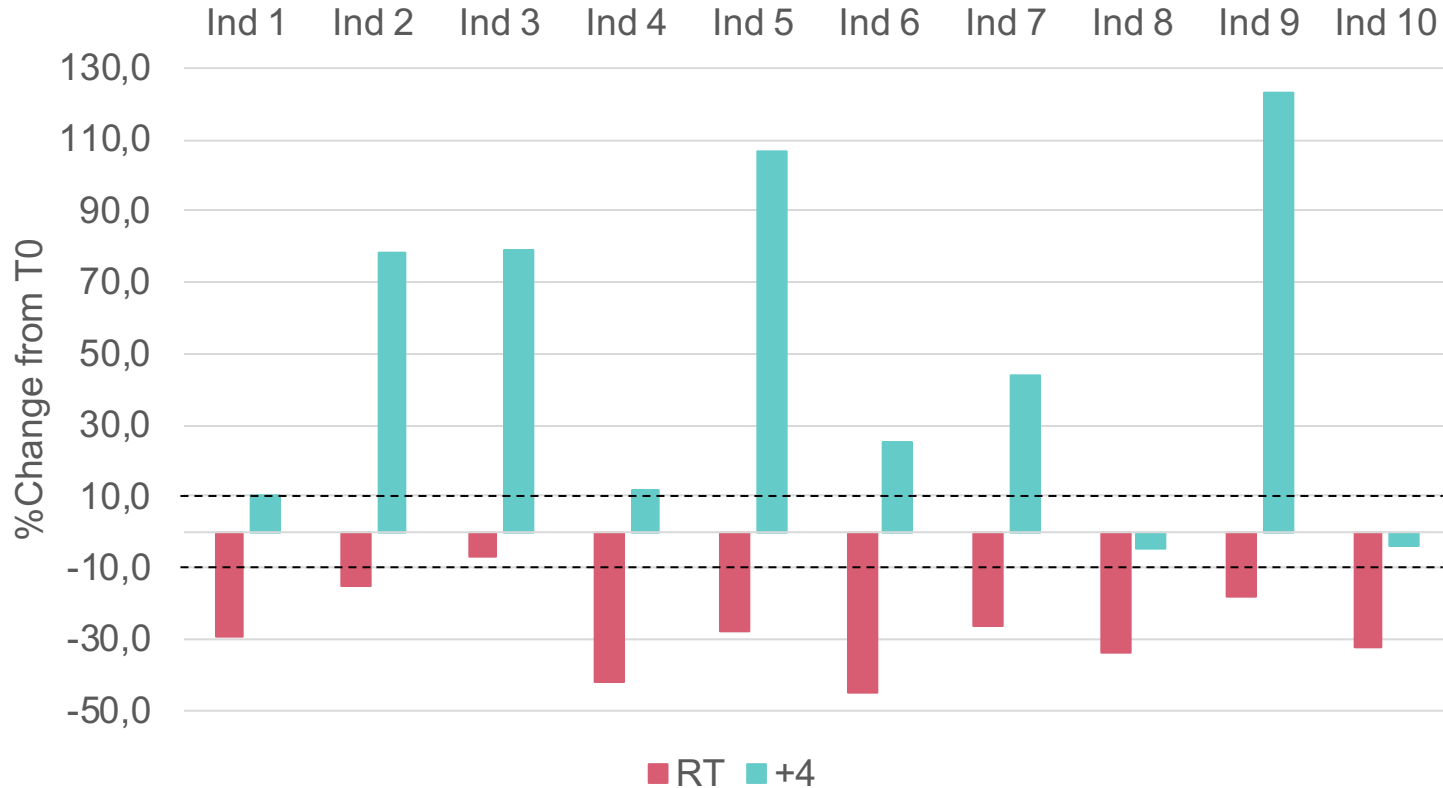


* 48 hours at +4°C

PRA plasma stability



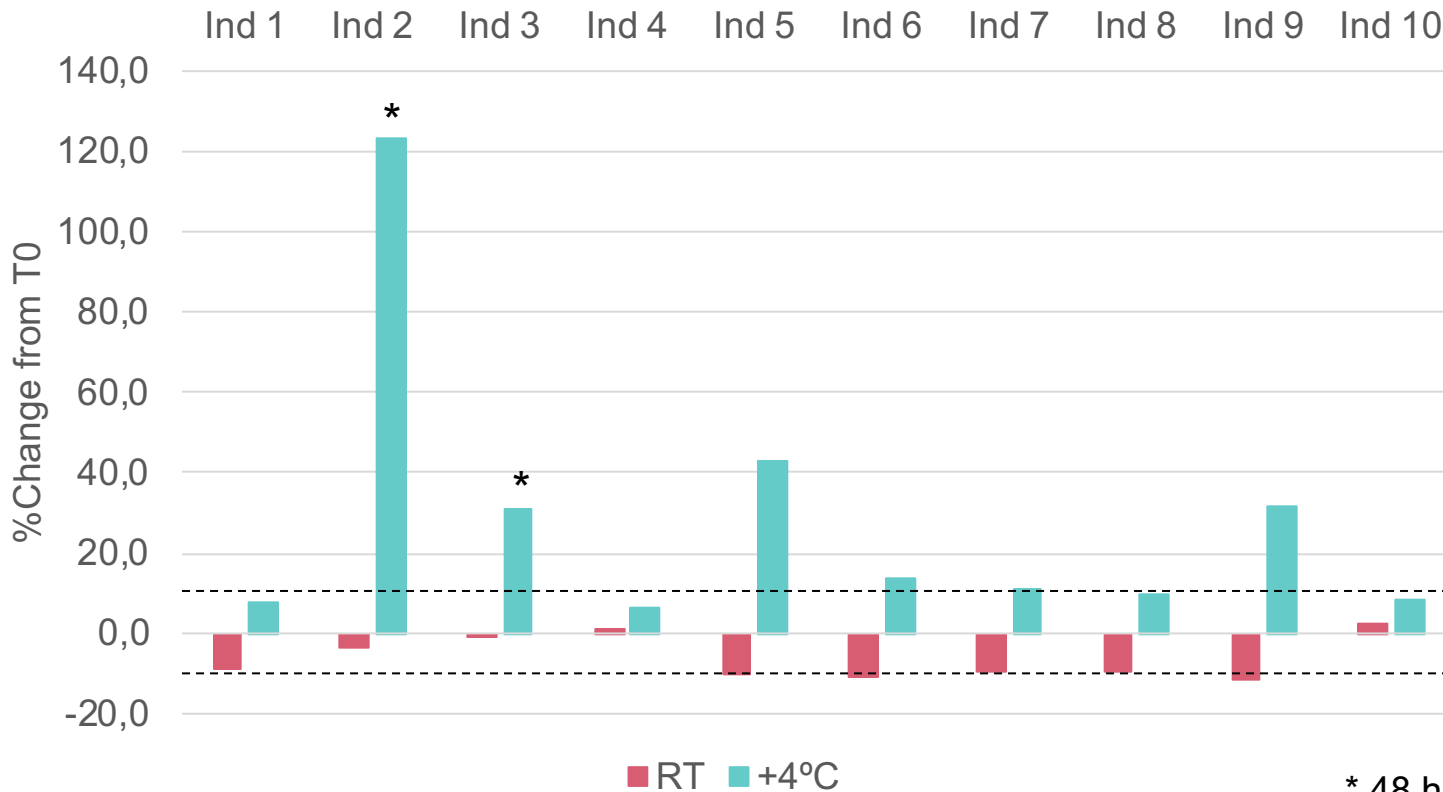
PRA stability in plasma - 72 hours



DRC plasma stability



DRC stability in plasma - 24 hours

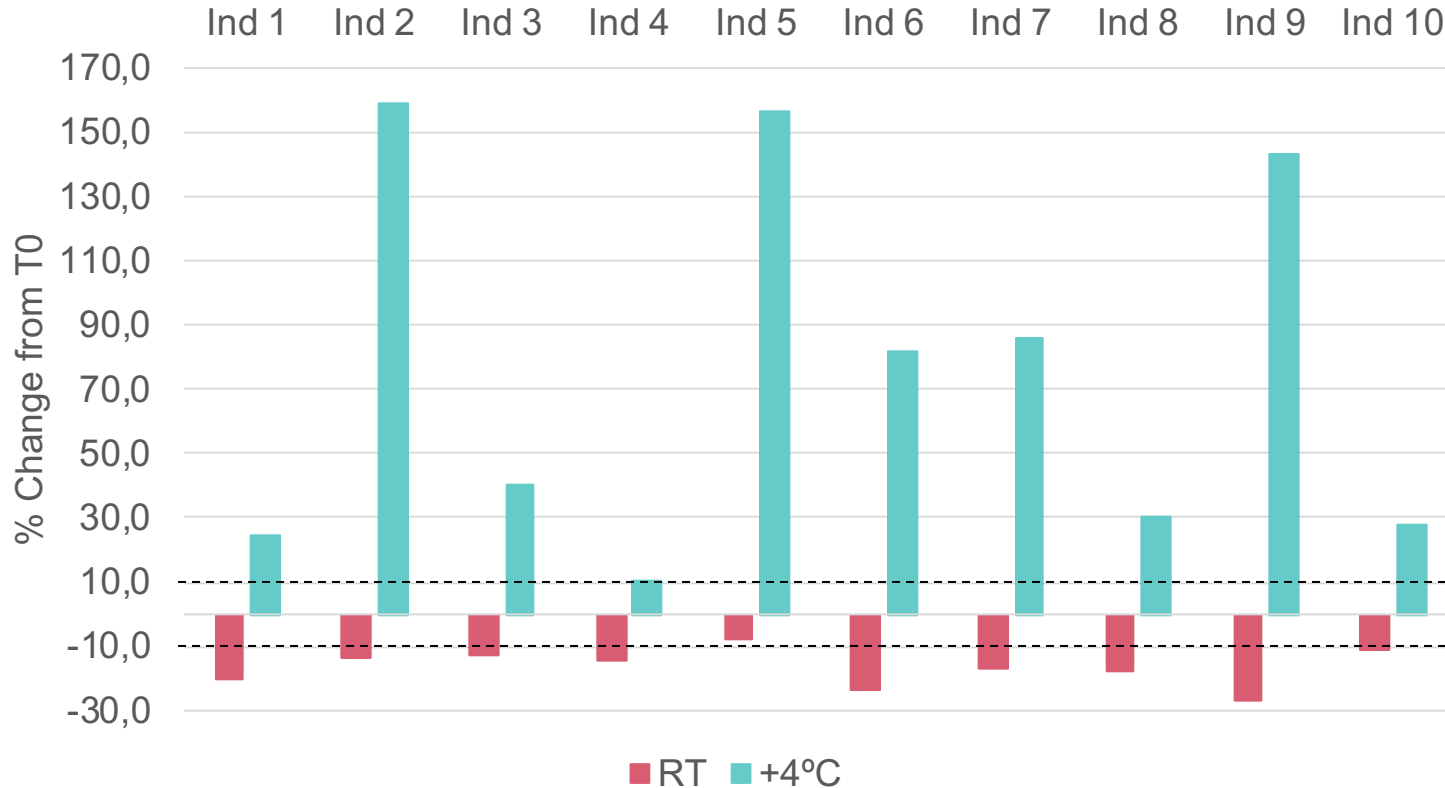


* 48 hours at +4°C

DRC plasma stability



DRC stability in plasma - 72 hours



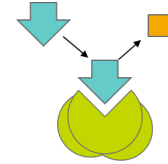
Conclusions

- **Confirmed cryoactivation of pro-renin**

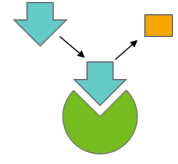
- Similar results between PRA and DRC in plasma
- More significant issue in DRC measurement in blood
- Individual variability



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



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